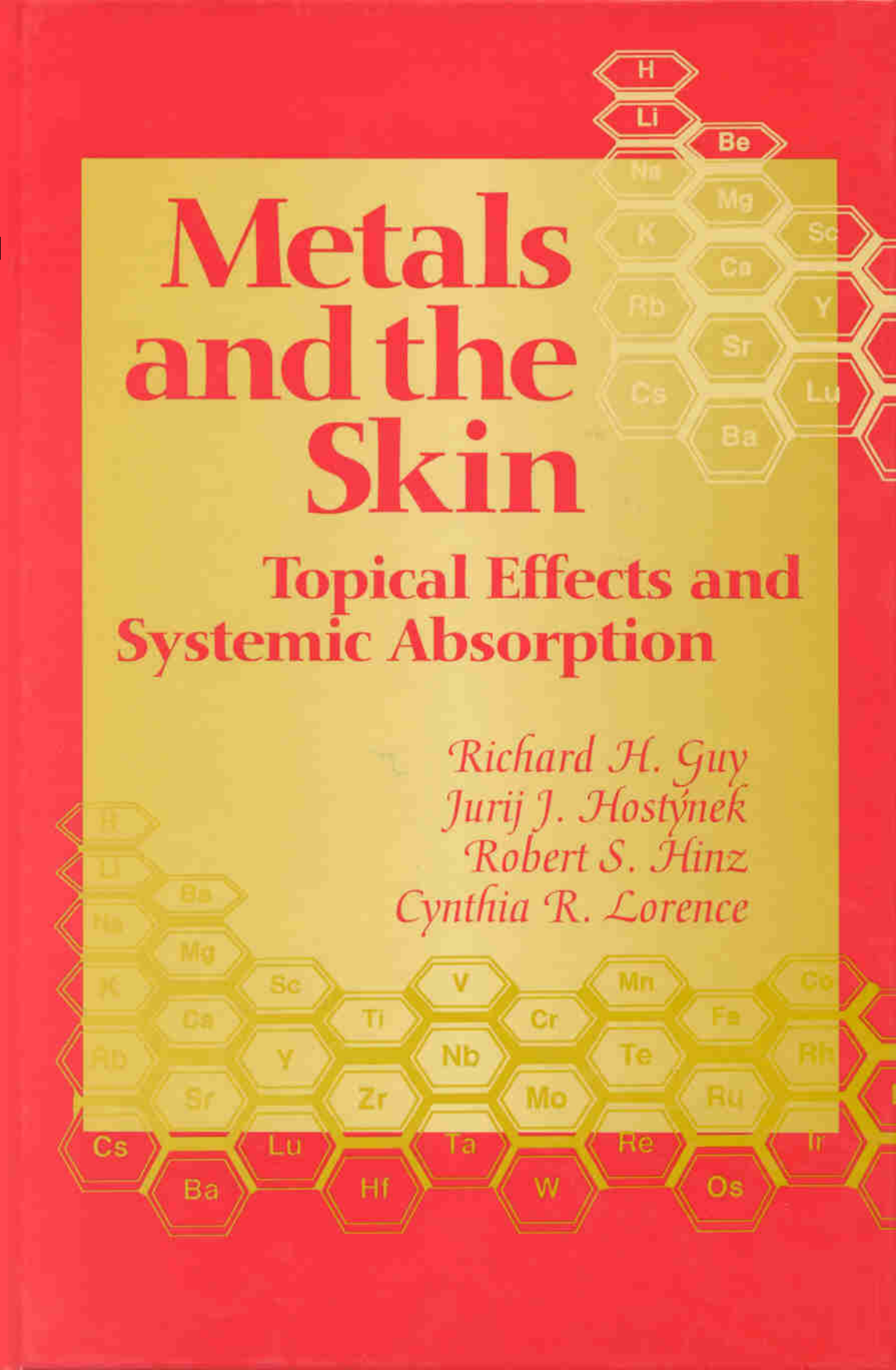

Metals and the Skin

Topical Effects and Systemic Absorption

Richard H. Guy
Jurij J. Hostýnek
Robert S. Hinz
Cynthia R. Lorence

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Richard H. Guy
Centre Interuniversitaire de Recherche et d'Enseignement
Archamps, France
University of Geneva
Geneva, Switzerland
University of California, San Francisco
San Francisco, California

Jurij J. Hostýnek
Euroamerican Technology Resources, Inc.
Lafayette, California
University of California, San Francisco
San Francisco, California

Robert S. Hinz
University of California, San Francisco
San Francisco, California

Cynthia R. Lorence
University of California, San Francisco
San Francisco, California

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On the occasion of their 70th birthdays, and in recognition of the advances in clinical dermatology that their endeavors made possible, we dedicate this book to Georg Klecak, M.D., and Howard I. Maibach, M.D.—two scientists and teachers who have devoted their professional lives to the better understanding of toxicant action in the skin, particularly with respect to the practice of occupational medicine.

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Preface

The interactions of metals and metal-based compounds with the human organism are complex and often detrimental. Protecting the public from such toxic effects is a principal objective of various regulatory agencies throughout the world. Risk assessment forms an important part of this exercise and requires, above all, reliable methods by which to determine exposure; that is, how much of a particular chemical enters the body and at what rate?

Dermal exposure—the absorption of chemicals across the skin—represents a route of entry that is ubiquitous and currently well-recognized for its significance. The ''rules'' that govern the percutaneous penetration of organic compounds (such as drugs, pesticides, and cosmetics) are becoming clear. Knowledge of the nature of the skin's barrier function, coupled with the development of useful ''structure-permeation'' relationships, has led to considerable advancement in the area of dermal risk assessment following exposure to such compounds.

In contrast, for metal-based chemicals, one enters *terra incognita*. While there is considerable and incredibly diverse literature, no comprehensive attempt to organize this information, nor any rationalization of the data, has been accomplished. Thus, it is our objective to respond to these challenges with this text.

The reader will find herein an accessible summary of most of the literature pertaining to the absorption of metal-based substances across the skin; pertinent aspects of the interactions between these species and the cutaneous biology are also presented whenever possible. However, space and time (and intellectual capability, it must be admitted) have not yet permitted us to provide simple algorithms with which to assess dermal exposure to metal compounds from ''first principles.'' This next, crucial step requires that one carefully examine and compare the large quantity of data that we have made available here in this single and unique source.

This book would not have been possible without the help and encouragement of many individuals. In particular, we thank Dr. Kim Hoang of the U.S. Environmental Protection Agency for her support and perseverance through this long and arduous project. We are most grateful to Professor Annette Bunge of the Colorado School of Mines for her energetic and always positive suggestions.

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Countless colleagues throughout the skin permeation field have offered advice, provided data, and hunted down references for us. In particular, we thank Professor Howard Maibach of the University of California, San Francisco, for unlimited and generous access to his encyclopedic knowledge of the field. Several people gave us invaluable aid by translating work (especially from the former Eastern Bloc) and sending us information; specifically, we thank Tatiana Gogoleva, Malgorzata Sznitowska, and Sviatoslaw Bozhenko. James Ganong tremendously facilitated the handling of large volumes of printed material (how did we survive before?). We are also most grateful to Drs. Aarti Naik and Yogeshvar Kalia for the conception and design of the cover graphic.

Financial support was provided by a cooperative agreement (CR-816785) with the U.S. Environmental Protection Agency. We are indebted to Christine Fernandez, Lauren Araiza, Matthew Price, and Dr. Christopher Cullander for their invaluable input and assistance with this work. Finally, it is a great pleasure and honor for us to dedicate this book to Drs. Georg Klecak and Howard Maibach in recognition of their sustained and formidable contributions to the science that lies behind the successful application of clinical dermatology.

Jurij J. Hostýnek
Robert S. Hinz
Cynthia R. Lorence
Richard H. Guy

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Introduction

It is the intention of the authors to present an overview of the health hazards posed to humans by metals and metal compounds present in the environment, as well as to delineate the benefits attributable to those essential for the organism. Particular focus is directed to the endogenous and exogenous interaction of metals with the skin, an interactive, semipermeable membrane that is well designed to moderate or inhibit penetration of xenobiotics and, to a large extent, is also involved in homeostasis of essential elements. Of the 80 metals and metalloids in the periodic table, only those for which significant relevant literature was retrievable and those that are nuclear-stable have been reviewed.

Compiling a significant compendium of relevant data on percutaneous absorption of metals has been challenging due to the paucity of robust experimental results. In gathering data on skin penetration of metals, we learned that most attempts to deal with this phenomenon in a quantitative manner have met with serious technical obstacles that have frequently discouraged investigators from further pursuits. This is evident from the fact that experimental data are extremely limited and, with really only one exception (1–9), the studies performed have followed very disparate procedures. Recently, though, some of this difficulty has been overcome via the availability of many radioactive metal isotopes—a prerequisite for quantitative experimentation.

In contrast, investigations dealing with the immunology and immunotoxicity of metals have steadily increased, and this research has surpassed skin penetration in volume and significance. The trend is reflected in the literature quoted for many of the metals reviewed in this book. Mercury was recognized as the first immunogenic metal 100 years ago (10). Nickel and chromium also were soon characterized as hazards in the work environment with their increasing industrial use. After World War II, a greater number of metals, including beryllium, platinum, and cobalt, were shown to be causal agents in the etiology of hypersensitivity, mainly contact dermatitis and systemic allergy. At present, enough evidence, albeit some of it anecdotal, has been collected on the immunogenicity of

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metals to demonstrate that virtually all metals known to be contact allergens are also capable of causing immediate-type urticarial reactions.

Since antiquity, a small number of metal compounds have been used therapeutically. With the advent of the science of toxicology, metals of obvious toxicity, as manifest from their chronic occupational exposure, were investigated in greater depth: beryllium, mercury, lead, cadmium, chromium and nickel. Yet, to date, the metals as dermatotoxins constitute a relatively small number, despite the explosive growth of nuclear and semiconductor technologies that use exotic alloys. Seven metals have been identified as carcinogens by the National Institute of Occupational Safety and Health: arsenic, beryllium, cadmium, chromium, palladium, nickel, and thorium. The most recent, principal publications in the field of fundamental and applied toxicology do not reveal any significant additions to this list.

Until a short while ago, a significant body of toxicological, pharmacodynamic, and immunological research on metals was in the former Eastern Bloc, such as the Soviet Union, Poland, and Czechoslovakia. This work was for the most part inaccessible to the scientific community in other parts of the world, due either to language barriers or to the security classification of the information. It is remarkable that the metals investigated, i.e., beryllium, cesium, manganese, molybdenum, rubidium, vanadium, tungsten, platinum and the platinum group metals, ruthenium, rhodium, palladium, iridium and osmium (with particular attention to the hazards of occupational exposure), were for the most part unaddressed by the Western scientific community. This work is now gradually becoming available in the West in translated form, mainly through the assistance of the United Nations, and is included in this book.

Considerable general knowledge on the mechanics of skin transport has been gathered. The laws governing passive diffusion of nonelectrolytes through the epidermis have been systematically investigated and adequately defined in mathematical terms. As the skin is a typical example of a barrier membrane, Fick's laws of diffusion are applicable, especially when using in vitro models. Furthermore, the body of skin penetration data has grown rapidly, particularly since transdermal drug delivery can offer unique pharmacodynamic advantage. This sizable database has been analyzed for quantitative relationships between percutaneous penetration rate and solute physicochemical properties, and quantitative structure-activity relationships (QSAR) models to predict skin penetration have been validated, thereby significantly reducing the need for in vitro and in vivo experimentation (11).

Insofar as metals form stable organic compounds with covalent bonds, their skin penetration characteristics are similar to those of common organic compounds. However, the concept of metal salts as penetrants, i.e., in their ionic form or as water-soluble charged complexes, is generally met with skepticism. Nevertheless, most of the metal salts in their dissociated form that have been

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investigated to date traverse the stratum corneum (SC) barrier with apparent permeability coefficients (K_p) on the order of 10^{-5} to 10^{-4} cm/hr (K_p for water is about 10^{-3} cm/hr). A QSAR model that can predict the skin penetration of metal salts has yet to be formulated and tested. While size, polarity, and hydrogen bonding ability are the principal factors governing diffusion of nonelectrolytes through the intercellular, lipid-filled pathway across the stratum corneum (skin's principal barrier to chemical absorption) (12), many other criteria may determine the diffusion of metal ions into and through the epidermis. For example, the route of permeation may not be uniquely intercellular; transcellular and appendageal pathways may also play a (significant) role. In the epidermis, strong interactions between electropositive metals and nucleophilic residues on, say, proteins may lead to the creation of significant deposits and/or reservoirs (as has been observed, for example, for nickel and chromium). Cadmium, zinc, and copper form complexes with, and are immobilized by, metallothioneins.

More subtle parameters, such as oxidation state, ionic charge and radius, electropositivity, redox potential, hydration state, counterion, pH, polarity, and polarizability, may play a role in determining the rate at which metal-based compounds cross the skin. With few exceptions the permeation route(s) of these compounds is(are) essentially undefined. It is thus unlikely that analysis of the data will be all-encompassing so as to allow expression of the involved processes by a single predictive algorithm; and it is therefore very unlikely, in our opinion, that predictions of metal compound absorption across the skin will be possible until a systematic body of experimental work is developed.

For those metals whose skin penetration has been investigated, the data rarely are adequate for the calculation of flux or permeability coefficient; those that are amenable to quantitative analysis often follow diverse experimental protocols, which lead to results that are not necessarily comparable. Although a permeability coefficient (K_p) is ideally determined under steady-state conditions, the percutaneous absorption of metals has rarely met this criterion. However, because K_p is a convenient parameter for comparison of percutaneous flux, estimated values are presented here based on available data and necessary assumptions. Whenever possible, data that are useful for the estimation of skin absorption have been abstracted and, as necessary, transformed so as to make them adequate for purposes of risk assessment. They are presented under a separate heading for each metal, together with a brief summary of the pertinent experimental details and mode of calculation. In this context, the terms *absorption* and *penetration* are used interchangeably as they apply to the process of penetrating the outermost skin layer (the stratum corneum), and to all of the associated and subsequent events, including distribution to the different strata and appendages of the skin, or the eventual uptake into the cutaneous blood and lymph vessels.

While retrieval of published material and its review was as comprehensive as possible with current on-line literature searching methods, it was not our intent

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to abstract and present all published data in this synopsis. From the total body of literature retrieved on any particular metal, only that part which is most relevant to human health and safety is discussed. Mostly, studies involving normal, healthy, and intact skin, not preconditioned in any way, have been considered. In some instances references may appear outdated, but this is due to the fact that for certain metal species pivotal investigations were conducted in the earlier part of the 20th century. A synopsis of the interactions of each of the metals with the skin from either direct or systemic contact is also provided because the skin is sensitive to the action of such agents.

Concerns with mechanisms of uptake and distribution of metals, particularly of toxic nonessential metals in the body, lead to the recommendation that agreement be reached on a standard protocol in measuring their dermal absorption. Such a protocol would include the establishment of carefully controlled, steady-state conditions; correct skin membrane selection and preparation, use of appropriate permeant concentrations, vehicles, exposure areas, and times of exposure; and identification of a suitable receptor medium. The need for advances in this field is made urgent by the continuing increase of highly toxic heavy metals in the environment.

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2
Special Aspects of Metal Reactivity

A. GRANULOMATOGENIC METALS

The presence of foreign bodies such as metals or poorly soluble compounds in the skin and other body tissues can cause an inflammatory reaction and damage. The phagocytic and hydrolytic capacity of degradative enzymes are usually sufficient to destroy or effectively sequester the foreign material and no granuloma will form. Normal healing processes can give rise to inflammation that can be of 3–4 weeks duration, representing chronic, nongranulomatous inflammation. Benign proliferation of blood vessels, perivascular mononuclear cells, and fibroblasts involved in the process can produce the aspect of granuloma or subepidermal fibroma with or without inflammatory component, but the classification of such clinical aspects as granuloma would be a misnomer (1).

However, only when the normal elimination mechanism fails, as in cases where the foreign body has low biological activity and resists the action of the body's degradative enzymes, and when the skin lesion persists for months or years does significant tissue destruction result and the term granuloma become appropriate. Granuloma is characterized by a collection of cells of the mononuclear phagocyte series, with or without the presence of other inflammatory cell types, and the inflammation typically heals with scarring.

Granulomas in general, including those caused by certain metal compounds, can be immunogenic or nonimmunogenic (the latter also being known as foreign body granuloma). It could be shown that most granulomas originate as the foreign body type, and only secondarily do the deposits of certain materials such as metal compounds involve the immune system once prolonged or massive exposure to the foreign agent has induced an altered tissue reactivity in the organism (2). Immunogenic granuloma cannot be distinguished from foreign body granuloma based on clinical appearance alone, but histologically the presence of monocytes, epithelioid cells and giant cells will identify the immunogenic nature of the focal lesion (3). Immunogenic granuloma formation results in granulomatous hypersensitivity, seen as a distinct form of delayed hypersensitivity, also

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distinguishable from the latter only by histopathological examination that reveals the presence of large epithelioid cells with a propensity to organize into tubercles. (1,4).

Most frequently seen was granulomatous hypersensitivity to zirconium, when deodorant sticks based on zirconium were widely used and also when zirconium lactate was applied therapeutically in the treatment of urushiol contact dermatitis. A small percentage of those affected also developed delayed hypersensitivity to zirconium compounds. Both types of products have since been removed from commercial products by order from the U.S. Food and Drug Administration (FDA). A zirconium oxychloride–aluminum chlorohydrate complex, on the other hand, also proved to be effective as a deodorant, without releasing zirconium into the skin, and can be used without the risk of granuloma formation (1,5).

Cutaneous and pulmonary beryllium granulomas are seen as a consequence of occupational exposure in industrial workers, with the particular effect that delayed hypersensitivity to beryllium salts occurs at the same time as granulomatous hypersensitivity. Beryllium oxide in particular is a potent and pernicious inducer of immunogenic granulomas, such lesions persisting as a rule until the foreign body is excised (6).

Most recently, persistent intracutaneous nodules appearing on ear lobes following ear piercing were seen to be associated with concomitant immunological gold dermatitis. Upon histological examination, the tissue appeared to contain giant cells at the site of contact, characteristic of hypersensitivity granuloma of the tattoo type (7; W. L. Epstein, private observation).

Also mercuric sulfide, cobalt, and chromium pigments, used in tattoos, have been reported to cause focal lesions of an immunological nature (1,3). Zinc was seen to cause granulomatous reactions at sites of injection of insulin preparations contaminated with the metal; intradermal testing with zinc confirmed the immunological nature of the dermatitis (8,9).

Nonimmunological granulomas, which contain only phagocytosing macrophages, without evidence of an associated hypersensitivity reaction, can be induced by metal compounds such as colloidal iron or aluminum oxides and hydroxides, by barium sulfate, and by oxides of mercury, selenium, and thorium (10). Such granulomas are induced intentionally in the skin with certain aluminum compounds to act as adjuvants in the process of vaccination.

B. METAL COMPOUNDS IN ADJUVANTS

A prophylactic and predictive tool in immunotoxicology is the use of adjuvants to potentiate the immunologic response to intrinsically weak antigens. Toward that end, the antigen is suspended in a water-in-oil emulsion, which is termed ''complete'' if it includes heat-killed tubercle bacilli (11). The two main applica-

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tions for adjuvants in human and veterinary medicine are to boost the response to vaccines in immunization, and to compensate for individual (variable) responsiveness to the antigen. Such formulations are also used in predictive testing with animal models (12), promoting an immune reaction to weaker allergens that might otherwise fail to sensitize and lead to potentially false-negative readings.

1. The Freund's Complete Adjuvant Test (FCAT) is a semiquantitative, intradermal screening method used to determine the sensitizing concentration and the minimal epicutaneous eliciting concentration of chemicals (13).
2. In the Guinea Pig Maximization Test (GPMT), the test agent is applied intradermally and topically, the injections made both with and without FCA. The GPMT is considered the most sensitive animal predictive test for contact sensitization, with a high degree of correlation with clinical experience in humans (14).
3. With Maguire's Split Adjuvant Technique, where allergen and FCA are administered separately and together in the guinea pig, even weak contact allergens in humans can be identified. The method is based on the observation that intradermal injection of FCA beneath the site of topical application of the allergen in the challenge phase highly potentiates the sensitization reaction (15). Often, certain metal compounds, particularly aluminum salts, are also used to provide this desired adjuvant (depot) effect.

The role of the various adjuvants used to potentiate an immune response covers a range of actions and properties at both the molecular (antigen) and cellular (host) levels (16):

1. If an antigen is retained in a focal granuloma or a particulate depot for a period of time, thereby controlling its gradual mobilization (and, hence, stimulation of the immune response), the course of its metabolism may be modified and its effect potentiated. Antigen metabolism is also modified depending on localization in tissue, which may result in a lowering of the rate of catabolism, i.e., the rate of phagocytosis and digestion. The term adjuvanticity is used in this context to describe the act of modifying (and augmenting) the antigenic properties of a molecule through a change in its steric configuration.
2. At the host level, adjuvants can have a stimulating effect both on immunocompetent cell transformation and proliferation, and on the release of active mediators. This is supported, particularly in reactions involving the delayed sensitivity mechanism, by the observation that injecting an allergen and an adjuvant simultaneously (or sequentially) into separate sites can be as effective as, or even more effective than,

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combining both in a single injection (17–19). The occasionally inferior effect of combined administration may be due to trapping of the hapten in the oil phase, preventing optimal combination with skin protein.

Both cell-mediated responses (delayed hypersensitivity) and humoral (immediate type) hypersensitivity with liberation of anaphylactic antibody can be induced selectively, depending on the choice of adjuvant, which also appears to promote the preferential formation of specific classes of antibodies (20). Freund's Complete Adjuvant stimulates both antibody formation and cell-mediated immune response (11). In contrast, the action of $Al(OH)_3$, the most widely used metal salt adjuvant, is limited only to promotion of antibody. A distinction is made between so-called pure adjuvants that lack the capacity to increase nonspecific immune responses, and are used for vaccine administration, and those agents that will generally increase resistance to infections and tumors (21,22).

The inflammatory and depot effects associated with adjuvant function, and also precipitation of antigen from solution, can be obtained with a number of metal compounds. Some protein precipitants based on metal salts that were used early on are cerium nitrate, zinc oxide and colloidal iron hydroxide. Those presently licensed for human use are limited to $Al(OH)_3$, $Al(OH)_3 + Mg(OH)_2$, $AlPO_4$(23), and $Ca_3(PO_4)_2$(24). Two beryllium compounds, $Be(OH)_2$(25) and $BeSO_4$(26), are currently approved only for experimental use (27). Also, powdered ferric oxide has been reported to give an adjuvant effect (28), while lead acetate enhances the reactivity of interferon inducers (29). By far the most widely used adjuvants, both in human and veterinary medicine and in predictive testing, are various formulations of aluminum hydroxide (27). Their uses and mode of action are discussed in detail in the chapter on aluminum.

C. MULTIPLE ALLERGIC RESPONSES TO METALS

Allergic reactions to chemicals as they involve the skin are broadly categorized into two distinct classes: (a) allergic contact dermatitis of the delayed type, which is mediated by allergen-specific T lymphocytes and expressed as a wide range of cutaneous eruptions following dermal contact, or systemic exposure to a hapten in individuals with cellular immunity to that hapten [type IV allergic reaction in the Coombs-Gell classification (30)]; and (b) immunological contact urticaria or immediate-type hypersensitivity, which involves IgE antibody and most notably results in respiratory allergy, but can also manifest in separate stages collectively described as ''contact urticaria syndrome''(31). This syndrome is characterized by local or generalized urticaria, urticaria with extracutaneous reactions such as asthma, rhinoconjunctivitis, and gastrointestinal involvement; and, ultimately, anaphylaxis (type I reactions after Coombs-Gell (30,32).

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A rationale has been proposed that explains how certain chemicals with the potential to induce immediate hypersensitivity may also cause delayed-type hypersensitivity (33). The difference in clinical manifestation is attributed to the preferential activation of different subpopulations of T-helper cells (TH), TH1 and TH2 (34–36). Activation of TH1 cells results in secretion of soluble cytokines that promote the cell-mediated response, e.g., interleukin-2 (IL-2) and interferon-γ (IFN-γ); activated TH2 cells, on the other hand, secrete IL-3 and IL-10, promoting antibody-mediated, immediate-type hypersensitivity. In the mouse model, T-cell activation involves only TH1 or TH2, the cytokines released by these two subpopulations being mutually antagonistic. In humans, however, the situation is less clear-cut and T-cell clones secrete both TH1- and TH2-type cytokines. This nonexclusive activation of T cells results, therefore, in a mixture of biological response modifiers being released, causing both IgE production (from TH2) and contact sensitivity (from TH1) (37).

Organic compounds infrequently cause both immediate-type reactions (anaphylactoid or immunological contact urticaria reactions) and delayed-type reactions (cell-mediated or contact allergy). Examples are trimellitic anhydride and diphenylmethane-4,4′-diisocyanate (38). However, such a dual immune response appears more common for metals and metallic compounds, with some being reactive to protein and, hence, resulting in a complete antigen, triggering both IgE production and cellular immune reactions. Immunogenic effects that result from exposure to metals can be attributed to the same factors that determine their toxicological and biological effects. Metal ions in general, and certainly those belonging to the transition group of elements, such as Cr, Co, Ni, and Cu, have an ionic radius too small to be antigenic. These metals, containing a partially filled d shell, oxidize to highly electropositive cations or oxoanions (e.g., chromate), which can act as haptens interacting with tissue protein. They form bonds that range from the fully ionized to the fully chelated and have the ability to modify the native protein configuration recognized as nonself by hapten-specific T cells in the host immune system (39), leading to allergic reactions of the different types described above.

Certain metals that are common topical sensitizers causing allergic contact dermatitis in humans and experimental animals are increasingly described as being immunogenic in the broader sense, presumably based on the factors mentioned above. Exposure to metals such as copper, mercury, nickel, and platinum can lead to both immediate-type immunological contact urticaria, which is often associated with respiratory hypersensitivity, and delayed-type cutaneous hypersensitivity. In some cases, concurrent existence of the two types has been observed in the same individuals. Such simultaneous presence of both immediate and delayed-type hypersensitivity to the same agent is a phenomenon barely recognized so far in dermatology due to lack of diagnostic acumen (40).

Upon skin challenge, contact urticants penetrate the epidermis and react

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with preformed, specific IgE molecules encountered on the surface of basophils and mast cell membranes, causing subsequent release of histamine and other cell-bound mediators of inflammation. Immediate hypersensitivity to a number of metals (e.g., Pt, Ni) could thus be diagnosed in vitro by the radioallergosorbent test that identifies the presence of IgE antibodies against specific causative agents in the patient's serum, or in vivo by the skin prick test that assesses immediate allergy in the patient's skin (32).

Several metals, especially nickel, cobalt, and chromium, can sensitize de novo following ingestion, inhalation, or implantation, as well as on skin exposure in the case of strong allergens. The dermatosis resulting from induced delayed-type hypersensitivity is included in the term *systemic contact dermatitis* (41). Such haptens are also known to induce skin reactions upon systemic challenge in the previously sensitized organism (42).

Particularly in the industrial setting, volatilization of metals and their compounds presents a respiratory occupational risk. In contrast to dusts generated in mining and construction, highly dispersible and respirable aerosols are formed during smelting and pyrometallurgical processes (43). Besides provoking chronic inflammatory response in the respiratory tract, inhalation of immunogenic metals can potentially inhibit tissue immunity, increasing susceptibility to infection. Airborne immunogenic metal compounds with sensitizing effects involving the skin following initial respiratory exposure include beryllium, chromate fumes, cobalt, mercury, nickel, and silver (44). Of particular concern in the industrial environment is the potential for anaphylactic vascular shock caused by inhalation of contact urticaria-generating metals and their derivatives (45,46). Long-term occupational exposure of complex platinum salts in particular can result in life-threatening respiratory symptoms as well as contact urticaria (47,48).

While much of the exposure risk occurs in the industrial setting under conditions of intense, long-term exposure to high concentrations of metals and metal compounds, some risk has also been identified for the general population. For example, unremitting hypersensitivity to common contact allergens, with nickel foremost among the metals, through constant systemic reexposure has been documented (49). The widespread occurrence of such immunogenic metal compounds, especially in food or as aerosols in the air, may contribute to the persistence of such allergic states.

Over the past 15 years there has been considerable effort to replace the use of traditional materials such as nickel and mercury in dental restorative work. Nickel, of course, is notoriously allergenic, and immunotoxicity due to mercury release from amalgams also has been documented. The increase in the price of gold on the world market also has become an added incentive to develop alternative alloys for restorative devices. Other than nickel, mercury, and gold, the major types of dental casting alloys now also contain cobalt, chromium, molybdenum, platinum, palladium, and silver (50). However, introduction of various substitute,

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metal-based materials has proceeded without the necessary corollary knowledge of their irritant and allergenic potential. Reports of contact stomatitis (contact allergy of the oral mucous membrane), lichen planus (51), and asymptomatic contact hypersensitivity (dental alloy contact dermatitis) are increasingly being linked with oral exposure to materials used in dental fillings, orthodontic appliances, or dentures: Ni, Co, Au, Cr, Pd, Be, and Hg (52–56). A recent study of professionals involved in the making and handling of such materials (dental technicians, orthodontists, and their assistants) reveals that they also run the risk of developing hypersensitivity to allergenic materials, as on the order of 40% among them complained of work-related skin problems (57).

Table 1 gives an overview of the different allergic reactions attributed to the metals reviewed without differentiating between specific types of metal compounds. While the literature cited only identifies confirmed, unambiguous case reports, sometime limited to isolated occurrences, no attempt is made to further define the frequency of sensitization or allergenic potency. For many metals, assignment of sensitization prevalence appears exceedingly subjective at this stage. The increased occurrence frequency of certain metal allergies (such as those ascribed to gold, palladium, or tin) is due to changing use patterns in fashion, medicine, or industry. The historically low incidence of reported reactions to certain antigens is at least partially due to the fact that allergy to such agents was not suspected and appropriate materials were not included in the routine dermatological testing program at the time.

D. METALLOTHIONEINS

Metallothioneins (MTs) are low molecular weight metal-binding proteins, mainly of intracellular origin, generally elevated during fetal development and exhibiting a significant degree of polymorphism. Since the discovery of the first MT in 1957 as a cadmium-carrying protein in horse kidney (342), and since the realization that production of this type of polypeptide can be induced by various heavy metals, other similar molecules contained in a variety of tissues, including skin and cell cultures, have been identified in many vertebrates, invertebrates, higher plants, and microorganisms. Using immunohistochemical techniques, various human organs and tissues other than skin have been found to contain substantial amounts of MT: mainly the liver and kidney, but also thyroid, thymus, pancreas, prostate, and retina (343), and, most recently, the central nervous system (CNS) (344). Research into the structures, biochemistry, and many functions of MTs in these diverse organisms has been rapidly expanding and has been the subject of frequent reviews (344–350). Species- and organ-specific (351), these single-chain polypeptides in the range of 6000–7000 Da have a remarkable amino acid composition, consisting of one-third cysteine (20 cysteines in mammalian MT)

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Table 1 Multiple Allergic Reaction Types Attributed to Metals

	ICU	ACD	SAR	STO	GRA
Ag	n.f.	(44,58–61)	n.f.	(62)	n.f.
Al	n.f.	(59,63–72)	(70,73,74)	n.f.	(68,72,74–76)
As		(77–82)	(83)	n.f.	n.f.
Au	(84,85)	(39,60,84,86–112)	(86,87,91,100, 109–111,113–117)	(88,92,96–98,109,113, 114,118–121)	(7,100, 110)
Be	n.f.	(4,44,55,122–128)	(43,55,124,126,129)	(55)	(4,5,130–134)
Bi	n.f.	(135)	(136,137)	(136)	n.f.
Cd	n.f.	(138)	n.f.	n.f.	n.f.
Co	(31,134,139–156)	(44,99,105,134,138, 146,153,157–174)	(152,159,167,173, 175–177)	(120,174,177,178)	(3)
Cr	(134,140,154,179–184)	(44,60,99,108,134,138, 140,166–168,173,176, 183,185–192)	(167,173–177,179, 185–187,189,193,194)	(174,178,188,195–197)	(3)
Cu	(154,198–201)	(59,60,94,105,108, 125,191,200,202–208)	(198,201,203,206, 209)	(210,211)	n.f.
Fe	n.f.	(99,191, 212–216)	n.f.	n.f.	(217,218)
Hg	(104,219–226)	(44,104,105,107,108, 110,111,134,138,165, 168,224,226–237)	(107,229,232,235,236, 238–242)	(120,121,196,197,210, 243–245)	n.f.
In		(99,246)	n.f.	n.f.	n.f.
K	n.f.	(247)	n.f.	n.f.	n.f.
Li	n.f.	n.f.	n.f.	(248,249)	n.f.
Mn	n.f.	(191,250)	n.f.	n.f.	n.f.
Mo	(154,251)	(125, 251-253)	(177,254,255)	n.f.	n.f.

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Ni	(134,148,152–154,182,224,256–273)	(39,44,52,95,99,105,134,153,159,162–170,173,189–191,252,254,256–259,269,270,273–283)	(159,167,173,175,177,196, 265,269,277,284–288)	(52,120,178,197,286,289)	n.f.
Pb	n.f.	(246,290–292)	n.f.	n.f.	n.f.
Pd	(293–298)	(52,99,165,171,278,293,299–307)	(306)	(52,121,306,308,309)	n.f.
Pt	(47,48,94,223,294,298,310–320)	(293,310,318,321–323)	(47,48,311)	(196)	n.f.
Sb	(324–326)	(78,94,174,191)	n.f.	(174)	n.f.
Se	(327)	(327–329)	n.f.	n.f.	n.f.
Sn	(330)	(105,174,331,332)	n.f.	(174)	n.f.
V	(148)	(191,246)	(175)	n.f.	n.f.
Zn	(333–336)	(8,9,105,337,338)	(8,9,338)	n.f.	(8,9)
Zr	n.f.	(339,340)	n.f.	n.f.	(5,134,341)

n.f. = none found.

ICU: Immunological contact urticaria. Allergic, IgE-mediated skin, nasal and bronchial (type I) reactions, appearing any time within a few minutes to several hours following exposure, and lasting up to 24 hr. Symptoms can include wheal and flare, generalized urticaria, and anaphylactoid reactions (contact urticaria syndrome).

ACD: Allergic contact dermatitis. Covers immunological type IV reactions (although in some cases type III mechanisms may also be involved) and includes asymptomatic sensitization.

SAR: Systemic allergic reactions. Generalized systemic eruptions or flare-up reactions, also associated with intradermal testing or oral challenge; sensitization following endogenous (oral, respiratory, parenteral, or implantation) exposure, also resulting in cutaneous manifestations: both contact urticaria as well as symptomatic or asymptomatic dermatitis (short term) and eczema (chronic).

STO: Contact stomatitis. Mucosal allergy: stomatitis, gingivitis, cheilitis, or oral lichenoid lesions, with episodic skin involvement.

GRA: Granulomatous hypersensitivity. Type IV reactions, coinciding with persistent nodular tissue reactions.

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and no histidine, aromatic, or heterocyclic components. This surfeit of thiol groups imparts to MTs (among other characteristics) an important (reversible) metal binding capacity and the ability to scavenge free radicals. The metal content of MTs is highly variable and depends on organism, tissue, and history of heavy metal exposure. Induction of MT in the animal organism through preadministration of certain specific metals was found to protect against what normally would be a lethal dose of that metal, and such tolerance would persist over several generations (352–354). Also, components of the immune system respond to insult from metals by producing MTs. Cadmium (Cd^{2+}) and other heavy metals added to human B and T lymphocytes and monocytes stimulated synthesis of MT, thus demonstrating that immune-competent cells also have a protective mechanism against such metals (355,356).

The observations that MTs are inducible (Cd^{2+} being the most potent inducer), that MTs will bind to heavy metal ions, and that they occur predominantly in the liver led first to the assumption that MTs have a protective function by detoxifying heavy metals, thereby increasing the organism's resistance to these agents encountered in the environment. Later, also their role as storage proteins and regulators in homeostasis became evident. They not only bind to such obvious potential toxins as Cd, Hg, Pb, or Ni, but also form complexes with essential micronutrients, including Zn, Cu, Fe, and Mn. When present above specific threshold levels, these essential trace elements exhibit acute toxicity. By removing, storing, and releasing these minerals on demand, MTs play an important role in homeostasis.

In its naive state, the vertebrate organism (e.g., the human fetus) comes equipped with significant levels of MT in the liver, complexed exclusively with zinc. MT thus serves as a source of Zn, which is vital for normal early development, growth, and for withstanding stress conditions. Presumably, MTs originally function as a dynamic Zn (and Cu) reserve in homeostasis and in controlling metal uptake in fetal and neonatal life. Detoxification of cadmium and other heavy metals is only a carryover of this fetal mechanism into later stages of life (357). Subsequent exposure to environmental toxins, such as heavy metals, results in MT release of zinc in favor of metal complexes with greater stability constants. In fact, of the metals known to complex with MTs, zinc has the lowest stability constant of all, being 10^4 times lower than that of cadmium (358).

By parenteral or enteral routes, three heavy metals—Cd, Cu and Zn—are the most potent inducers of MTs. In addition, other chemical, biological, and physical factors, such as mitogens (phorbol esters), hormones, organic solvents (chloroform, ethanol, carbon tetrachloride), bacterial infection, inflammation, IFN and IL-1, or acute stress due to exercise, heat or cold, trauma, fasting, starvation, or actinic and ionizing radiation will stimulate MT biosynthesis as well (345).

The occurrence of MT in normal human skin was studied in frozen sections

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using monoclonal MT antibody derived from human fetal liver. Immunohistochemical staining revealed strong reactivity in basal keratinocytes of epidermis and outer hair root sheath, hair matrix cells, and the secretory coil, but not the exocrine portion of eccrine glands (359). Of the major isoforms (MT-I, II, III, and IV) occurring in mammals, expression of MT-IV is restricted to cornified and stratified squamous epithelium of skin, tongue, and upper part of the alimentary tract (344).

Several reports also describe the role and involvement of dermal tissues in MT induction. Following single topical application of 0.1% $HgCl_2$ to human skin in vivo, histochemically observable densities were noted in the epidermis below the stratum corneum in both intracellular and extracellular sites. Mercury associated with MT was detected in keratinocytes, melanocytes, Langerhans cells, fibroblasts, and mononuclear cells of the dermis (360). The effect of repeated (three times) dermal application of 5% $ZnCl_2$ in vivo on the MT level of rat liver was a ten-fold increase from the preexposure level. Topical application of corticosteroids or zinc oxide on the skin of hairless mice was also found to induce MT (361,362). Both of these examples indicate significant skin penetration of topically applied zinc compounds. An MT-like protein was found in normal diploid human skin fibroblasts cultured in Zn- or Cu-supplemented medium (363). Human skin epithelial cells in vitro were made tolerant to otherwise lethal concentrations of Cd^{2+} following MT induction in the cells by a Cd-containing growth medium (352). By intraperitoneal $ZnSO_4$ injection, MT was induced in hairless mouse skin in vivo in a dose-dependent manner (364). Also in mouse skin in vivo, mRNA of the MT gene was induced after oral administration of 1-α-hydroxyvitamin-D_3, known to be metabolized to 1-α-25-dihydroxyvitamin-D_3, a hormonally active form of vitamin D_3. In culture, the dihydroxyvitamin also induced RNA in epidermal keratinocytes (365).

Ultraviolet (UV) radiation–induced skin damage is largely determined by the formation of free radicals, particularly the reactive oxygen species (ROS). ROS leads to an imbalance of free radicals in skin causing oxidative stress, resulting in the oxidation of biomolecules such as lipids, proteins, and DNA, as well as depletion of endogenous skin antioxidants. Cd-induced, intracellular MT in mouse skin in vivo has been suggested to have a protective effect against radiation damage caused by exposure to UVB, with such protection being due to the oxygen radical-scavenging properties of MT (366,367).

MT was identified in the basal cells of the interfollicular epidermis after the tissue was rendered hyperplastic either by a phorbol ester or cholera toxin, suggesting that MT is involved in the proliferation of epidermal keratinocytes, supplying zinc to enzymes involved in keratinocyte synthesis (368).

Thus, MTs encountered in most mammalian tissues appear to have a multiplicity of physiological functions, three of which have been substantially investigated and described in the literature: (a) MTs are involved in a wide range of

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protective mechanisms against noxious stimuli of endogenous or environmental origin, e.g., binding and detoxification of metal contaminants or preventing toxicity caused by actinic radiation or γ irradiation administered for therapeutic purposes. (b) Being predominantly zinc-containing proteins, MTs are intimately involved in ameliorating inflammation and in healing injuries. (c) Lastly, MTs are involved in homeostatic control of the absorption, storage, and transfer of the interdependent essential trace elements zinc and copper.

The regulatory signals for MT gene expression are complex, and the total scope and import of MTs for physiological processes in different organisms and tissues has not been fully clarified. Diverse stress factors trigger an array of physiological processes involving many mediators, and MT gene expression leading to the biosynthesis of that polypeptide is but one step in a critical sequence of events.

E. SKIN AND APPENDAGES AS SECRETORY ORGAN FOR METALS

1. General Comments

While urinary and biliary excretion are the primary routes of elimination of xenobiotics, for certain agents such as metals and their compounds, the skin with its excretory pathways and appendages also represents an important elimination route. As a general rule, metals with affinity for sulfhydryl groups, and thereby keratin-rich tissues, also find significant excretion via skin, hair, sweat, nails, and ear wax. The skin thus plays a role in detoxification as well as in maintaining an appropriate balance of certain essential elements. Integumentary losses of body minerals occur on a continuing basis and can be useful in the assessment of essential nutrient stores, environmental exposure to toxics, or disease. For instance, copper and iron, eliminated excessively under certain conditions through sweat or desquamation, respectively, elude the kidney's homeostatic control; under extreme conditions, such losses can result in untoward effects such as heat stroke or a state of iron deficiency.

Sampling of skin, along with its appendages and excretions, for elemental analysis potentially simplifies the process of assessing health and nutritional status, and could assist in the detection of adverse environmental exposure. However, both hair and sweat, the most abundant and accessible substrates for diagnostic purposes, present severe limitations because they are subject to metal-specific characteristics of absorption, storage, and elimination. As an example, for most metals, analysis of sweat cannot be used to directly estimate the body's elemental stores. Different tissues and organs store trace elements in a differential manner, and the components of the appendageal system exhibit highly characteristic modes of incorporating and transporting xenobiotics; therefore, analytical

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data are of little diagnostic value unless considered in relation to well-established control values.

2. Skin

Elimination of several bulk and trace elements by the natural process of desquamation has been calculated, based on their level of occurrence in normal epidermis. Metal content of skin obtained from different sites was determined with the aid of neutron activation analysis. While the daily loss of calcium and potassium appears negligible, that of iron reaches one-fourth of the daily urinary excretion; more importantly, the relative fraction for copper, cobalt, and zinc approaches one-tenth (369).

3. Hair

In some cases, the metal content of hair constitutes a permanent, longitudinal record of the organism's relative metal status, and within certain limits this information can also be used to assess trace metal exposure from nutritional or environmental sources (370), and bioaccumulation (371). Assays on hair are easy to run with modern analytical methods. Some 30 elements, most of them metals, have been assayed so far, ranging in concentration from less than 1 ppm to several hundred ppm, with the physiologically important elements Na, K, Ca, and Zn being the most abundant. The methods have been elaborated to a high degree of sophistication and now include atomic absorption and emission, neutron activation, energy dispersive x-ray fluorescence and emission, and particle-induced x-ray emission (PIXE), which yield accurate and reproducible results. While these techniques shed light on the dynamics of growth, structure, and composition of hair, the analytical data thus acquired are often seriously limited in diagnostic value with respect to metal status or environmental exposure.

Problems associated with hair analysis have been the subject of numerous reviews (372–377). In the malnourished organism, hair growth slows markedly, such that even while plasma levels are depressed in essential trace elements, the metal levels may nevertheless appear normal in the hair (372). According to data obtained on the cross-sectional and longitudinal localization of trace elements in the human scalp hair shaft by micro-PIXE, elemental deposition varies along the length and the cross section in characteristic manner for certain metals (378). In general, elements originating from blood and the root matrix can be incorporated into the hair strand via the radial or the longitudinal route through the cells of the outer and inner root sheaths. Endogenous trace elements are not necessarily distributed evenly across a section of the hair shaft but will follow characteristic patterns of distribution.

Other variables that impact on the elemental distribution and localization

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in the hair shaft are the source and route of incorporation (379). The following are examples of the endogenous sources of metals that become incorporated into hair:

1. Matrix (the lower, vascularized part of the hair root) extending into the follicle's internal root sheath and papilla; a source of trace elements in hair's formative stage
2. External root sheath, an extension of the epidermis
3. Sebaceous excretion
4. Eccrine sweat (containing mainly Na and K, but also Ca, Cu, Mn, Mg, Fe)
5. Apocrine sweat
6. Desquamating epidermis, incorporated into the hair shaft together with sebum

As various elements adhere to the hair matrix with varying tenacity, no standard sample preparation procedure can be applied to separate exogenous from endogenous materials prior to analysis. Metal affinity for keratin, as measured experimentally as binding tenacity to human scalp hair, decreases in the sequence Al, Cd, Cu, Pb, Zn, with the range extending over an entire order of magnitude (380). Further compounding the problems inherent in elemental analysis evaluation, hair composition and metal uptake vary as a function of growing versus resting phase, age, and developmental status of the individual; race; sex; hair color; pregnancy status; anatomical site; distance from the follicle; and, within samples of scalp hair, the site of collection on the scalp.

With the above list of concurrent phenomena and confounding factors in mind, a compilation of predominant elemental levels in the hair of adult humans, obtained from a literature survey, is presented in Table 2. Essential as well as toxic elements have been included (381).

In summary, trace metal determination from hair is applicable for only a limited number of heavy metals, namely, Pd, Cd, As, and Hg, for which precise sample preparation techniques have been elaborated. Hair will reflect long-term exposure to these toxicants with characteristic concentrations that give a measure of time course and degree of exposure, useful in the diagnosis of clinical poisoning. Elemental hair analysis has not been found useful in determining the essential trace element status of the organism, or its nutritional status, as those metals are subject to short-term homeostatic control and fluctuations (372).

4. Sweat

Sweat is the most copious bodily secretion after urine and feces; it can also be an important pathway in metabolism, maintaining the balance of minerals and other substances critical for normal physiology, as well as providing a detoxifica-

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Table 2 Concentration Range (mg/kg) of Trace Metals Occurring in Adult Human Hair

Metal	Conc.
Arsenic	0.15–0.30
Cadmium	0.40–1.0
Cobalt	0.05–0.30[a]
Chromium	0.30–0.80
Copper	0.25–0.40
Iron	0.35–0.60
Lead	2.0–20
Manganese	0.50–1.5
Mercury	0.50–2.0
Molybdenum	0.05–0.20[a]
Nickel	0.02–0.20[a]
Selenium	0.50–1.0
Zinc	150–250

[a] Value uncertain in cited source.
Source: Adapted from Ref. 381. Data (in part) for 13 trace metals collected from several countries. Values that cover a wide range (e.g., Hg, Pb) appear to indicate a lack of homeostatic control.

tion route. This role has been investigated for a number of metals, using different methods of sweat collection; however, for purposes of diagnosis, the interpretation of data is associated with certain problems. Furthermore, solute concentration was found to change as a function of sweat rate, and as a function of environmental humidity, local skin temperature, muscular activity, and pharmacological stimulation. The rate of elimination follows element- and individual-specific patterns, such as acclimatization of the organism (382). Sweat composition also varies in function of collection method (gauze pad, filter paper disks, arm bag, or total wash-down technique), as well as of the site of collection (arm, back, chest, abdomen, forehead, or total body). The mean total concentration of components routinely measured—sodium, potassium, chloride, nitrogen, calcium, and magnesium—was found to be lowest in total body sweat (383), and thus the total body wash-down method appears to be most representative when measuring total solute eliminated from the skin surface. The collection of sweat limited to discrete body sites and under occlusion, such as the most frequently used arm-bag sweat collection method, yields variable results, and the values thus obtained cannot

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Table 3 Composition of Thermal versus Exercise-Induced Sweat (mEq/L)

Metal	Thermal	Exercise
Na	84 ± 31	123 ± 33
K	14 ± 4	11 ± 3
Cl	67 ± 31	104 ± 31

Source: Adapted from Ref. 386.

be extrapolated to predict total body losses due to sweat (384). The validity of electrolyte levels measured in sweat is also often questioned due to their particular susceptibility to methods of analysis and sweat stimulation (physiological, physical, or pharmacological). Literature values define normal levels for sodium in sweat as less than 50 mmol/L, equivocal as 50–70 mmol/L, and abnormal as more than 70 mmol/L (385). Differences in composition between sweat induced by thermal stress and physical exercise were determined in seven healthy male volunteers and are presented in Table 3.

The main cause of corrosion of metal surfaces from skin contact in individuals referred to as rusters is not due to elevated electrolyte concentration, as generally assumed, but rather seems to coincide with palmar hyperhidrosis in those individuals. When the sodium concentration measured in normal subjects was compared to that of ''rusters,'' in fact no significant difference could be observed (mean values of 49.6 versus 49.1 mEq/L, respectively) (387). Mean macroelement concentrations recorded in sweat collected from normal subjects ($n = 10$) by iontophoretic stimulation with pilocarpine are given in Table 4.

Elevated levels of sodium and chloride concentrations in sweat in particular are a hallmark of cystic fibrosis. Their routine evaluation, also known as the ''sweat test,'' is a standard method and cornerstone of the diagnosis of that dis-

Table 4 Composition of Pilocarpine-Induced Sweat

Metal	Conc. (mM)
Na	46.1 ± 24.5
K	11.5 ± 4.7
Cl	45.6 ± 24.5
Mg	0.10 ± 0.09
Ca	0.45 ± 0.08

Source: Adapted from Ref. 388.

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ease, and data on those two elements are the most commonly encountered values found in the literature descriptive of electrolyte concentration in sweat. Elevated concentrations have also been associated with disorders other than cystic fibrosis and are seen as a common sign of malnutrition (389).

Human studies conducted at sustained elevated temperatures underscore the significance of sweat secretion to essential trace element balance, as extreme losses of Na, K, Mg, and Fe can result (390). The average sodium content of sweat is given as 60 mM and that of potassium as 8 mM, but these values fluctuate significantly as a function of sweat rate, hormonal control, diet (391), and, particularly in the case of sodium, reabsorption (382). Also for zinc and copper, sweat can be a critical excretory pathway, possibly resulting in significant depletion leading to adverse acute or even chronic manifestations such as heat stroke and iron deficiency anemia, respectively. Whole-body sweat collected during a 90-min exercise period from 6 male and 3 female volunteers by the whole-body wash-down technique was analyzed for trace metal concentration by atomic absorption spectroscopy (384). The range of values is given in Table 5.

In a different study, for nickel the concentration excreted in sweat was higher than that in urine, whereas for lead and cadmium the values were approximately equivalent (392).

In elemental analysis of sweat, an allowance must also be made for the different levels of desquamated cells present versus data obtained from cell-free sweat if the data are to be correctly interpreted. As an example, ''cell-rich'' sweat averaged 1.15 mg/mL iron, compared to 0.34 mg/mL for the cell-free sweat (393).

The effect of exercise on the content of zinc, iron, and copper was investigated in sweat from 12 healthy athletes. Samples were collected from different body sites for analysis, and concentrations were found to be different in sweat samples from different sites of the same subject; considerable variations were

Table 5 Trace Metals in Exercise-Induced Sweat

Element	Range
Zn	400–1200 (mg/L)
Cu	860–1600 (mg/L)
Fe	40–550 (mg/L)
Ni	40–80 (mg/L)
Pb	40–120 (mg/L)
Mn	10–30 (mg/L)
Na	10.6–28.6 (mEq/L)
Cl	8.5–26.3 (mEq/L)

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also seen between subjects in that study. Thus no general conclusion can be drawn about the significance of metal ion losses observed in sweat induced by physical exercise (394).

5. Nails

Consisting of α-keratin, formed by the keratinization of epidermal cells, nails represent a relatively extensive longitudinal record of xenobiotics present in the skin. The application of atomic absorption spectroscopy to metal analysis in nails provides information on metal stores chronically present in the body. By virtue of the growth process, nail analysis can be used as an indicator of chronic exposure, i.e., a period of at least 3 months is required from the time of exposure to the point at which the nail can be used for such noninvasive analysis. Toenails are the preferable material for such analysis, as they are less exposed to exogenous contamination compared to fingernails or scalp hair.

A statistically significant difference in data obtained on a number of trace elements from exposed industrial workers versus controls shows that the levels of Pb, Cr, and Mn present in toenails give a reliable record of occupational exposure (395). Nail nickel levels also increase significantly commensurate with intensity of exposure (396). The effect of vanadium on the cystine content in integumental appendages, nail material in particular, has been recognized as a sensitive, early indicator of systemic exposure and has been developed as a diagnostic test for occupational safety purposes (397).

6. Ear Wax

More recently, ear wax (cerumen) has been investigated as a medium for the biological monitoring of xenobiotics (398), including metals (399). Elemental analysis of samples of human cerumen, a combination of secretions from sebaceous and ceruminous glands and of exfoliated epidermal cells, showed the presence of certain metals that also occur in sweat. Representing 1.6–4.8% of the total dry weight of ear wax, the major metals found were K, Na, Ca, and Mg at the level of approximately 1 mg/g dry weight. A number of heavy metals of potential toxicological significance were also identified in smaller but still quantifiable amounts: As, Au, Cd, Cr, Fe, In, Pb, Pt, Sb, Sn, Sr, Ti, and Tl. As an alternate noninvasive method, and because the ear canal is less subject to environmental contamination than skin, analysis of ear wax appears to be a potentially useful monitoring medium for occupational exposure to those elements.

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3
Silver

A. GENERAL COMMENTS

There is no conclusive evidence that silver performs any essential function in living organisms. Similar to other heavy metals, the silver ion reacts with nucleophilic amino acid residues in proteins, attaching to sulfhydryl, amino, imidazole, phosphate, and carboxyl groups of membrane or enzyme proteins, generally resulting in protein denaturation (1). As for a number of other transition metals, this accounts for silver's antibacterial activity. The only significant therapeutic use of silver [as the nitrate or its sulfonamides (2,3)] is the disinfection of the skin of thermal injury patients, where the antiseptic effect is primarily due to the metal's reactivity with viral and bacterial proteins (4). This protein-denaturing action of the silver ion makes the metal an effective sterilant for water and other beverages, an effect that has been recognized and put to use over centuries in one form or another (e.g., in the use of silver drinking and storage vessels). Currently, finely divided silver metal is incorporated in commercial filters for domestic water purification, or in cosmetic formulations as a silver chloride/titanium dioxide composite, where at levels of 50–500 ppm it is an effective preservative against microbial contamination in the pH range 3–11.5. The only limiting factor there is the reducing potential of the medium (5,6).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Independent of the route of entry, silver preferentially accumulates in the mucosa, nail lunulae, hair, and superficial layers of the skin (7). Once deposited, silver particles remain immobile and accumulate throughout the aging process. Skin excretion of silver is insignificant; consequently, impregnation of dermal tissues, even through the trivial and habitual use of silver-plated tableware, potentially results in graying of the skin, most pronounced on the face and neck—a condition that is termed *argyria.* Two types of this phenomenon are described: generalized

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argyria, stemming mainly from chronic systemic medicinal application or external occupational exposure, and localized argyria, persistent bluish macules caused by, say, dental crowns or implanted needles (acupuncture) (7,8). Discoloration of the oral mucosa, tongue, sclera, and conjunctiva has been described, caused by silver-containing medications in the management of burn wound sepsis and in the treatment of sore throat, gastric discomfort, or oral ulcers (9–11). The cutaneous expression usually manifests (above body burden levels of 1 g of silver) as a permanent hyperpigmentation, particularly of sun-exposed areas of the skin. Both forms are only associated with esthetic effects. Also, discoloration of scalp and body hair has been noted, resulting in a steel gray tint (12). However, industrial exposure to concentrated silver nitrate solutions in metal plating, photography, or as a germicide and antiseptic has resulted in severe burns and permanent damage to eyes and skin. Electron microscopic and x-ray microanalytical studies reveal the deposition of electron dense (silver) granules on elastic fibers and around the basal lamina of the secretory portions of eccrine glands (8). Intentional dietary intake of silver, as practiced in certain parts of the world (in the form of extremely thin foil with some foods), results in markedly elevated levels of the metal in hair. Neutron activation analysis of precleansed human scalp hair showed a geometrical mean of 1.927 ± 1.1 μg/g silver in the Punjab population, which compared with values of 0.39 ± 2.58 μg/g seen in other parts of India or 0.16 ± 2.11 μg/g in Japan, where such dietary habits are not known to exist (13).

Silver ions are not significantly absorbed through unbroken human skin, probably due to silver reactivity with skin proteins, resulting in its accumulation in the horny layer (14). Chemical and electron probe techniques have revealed that complex silver salts do penetrate through the epidermal portion of the sweat duct, cross over into the adjacent epidermal cells, and enter the papillary bodies, precipitating as silver sulfide (15). Histology of involved areas also shows silver granules on the membrana propria of sweat glands, on sebaceous gland connective tissue membrane, and in collagen tissue below the basal membrane of the epidermis. However, no generalized deposits of silver are found in the epidermis (16). While no single pathway for the elimination of incidental silver from the mammalian organism has been identified, urinary excretion of the metal was elevated over 1000-fold above normal rates (>1000 μg/24 hr versus <1 μg/24 hr) in thermal injury patients when silver sulfadiazine, an effective topical antimicrobial agent, was applied to the burn areas, the missing epidermis evidently facilitating systemic absorption through the denuded tissues (3).

C. QUANTITATIVE ABSORPTION DATA

Resorption of silver through the human forearm skin in vivo, applied as 2%, 1%, and 0.5% $^{111}AgNO_3$ as ointment and aqueous solution, was determined under occlusion over 24 and 48 hr (Table 1). A fourth aqueous solution, 2×10^{-4}%,

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Table 1 Silver, as $^{111}AgNO_3$

Reference: O. Nørgaard, Investigations with radioactive Ag^{111} into the resorption of silver through human skin, *Acta Derm. Venereol. (Stockh.) 34*: 415–419 (1954).

Species: human in vivo ✓
in vitro

Area: from a 10-μL drop or a ''trifle of the ointment'' (about 0.5 mg) applied to the center of a 1.5-cm-diameter circle.

Vehicle: Solution not specified but probably water; evaporated right after application but the site of application was then covered with plastic film. Ointment-*unguentum argenti nitratis,* i.e., silver nitrate (2.5 parts per thousand), water (247.5), olive oil (250) and ''adeps lanae'' (wool fat, anhydrous lanolin; 500).

Analytical method: Geiger-Müller counter

Notes:

Time (hr)	Cpm measured	Initial cpm, corrected for decay
Experiment 1:	1 drop of 2% $AgNO_3$	
0	441[a]	441
22	388[a]	397
Experiment 2:	1 drop of 2% $AgNO_3$	
0	292[b]	292
6	272[b]	285
24	243[b]	263
49	236[b]	237

[a] Average from four 6-min counting periods.
[b] Average from four 8-min counting periods.

1. Solutions of 1% and 0.5% $AgNO_3$ on other subjects gave the same results, i.e., there was no significant absorption. In addition, results were the same for a carrierless solution of $AgNO_3$ which contained only about 0.0143 mg of silver in 10 mL of water.

Time (hr)	Cpm measured	Cpm corrected for decay
Experiment:	$AgNO_3$ ointment	
0	207[a]	207
22	186[a]	187

[a] Average from four 12-min counting periods.

2. The authors conclude that ''in the course of 24 hours there is no decrease in the radiation, i.e., that, at most, 4% of the silver may have been resorbed through the skin'' from solution or ointment.

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which contained no carrier, was also applied. No decrease in radioactivity on the skin was noted in any of these cases, with a standard deviation of ±2% of the amount of silver applied. The maximum absorption therefore could not have exceeded 4% (17).

For silver and seven other metals, Wahlberg and coworkers monitored the decrease in γ radiation that was measured above solutions of the radioactive metal ions on the skin. There were both in vivo and in vitro investigations. The parameters of the experiments were unchanged from metal to metal, and these experiments of 30 years ago still provide the largest body of data with which one can compare the percutaneous absorption of several different metals.

In Wahlberg's procedure (18), a scintillation detector (with a thallium-activated NaI crystal) was mounted over a collimator that was positioned above a 3.14-cm^2 chamber glued to skin. Hair was clipped from the skin and then, to demonstrate that the area to be exposed was not damaged, the electrical conductivity of the area was measured at 1 V. The volume of solution placed into the chamber was always 1.0 mL. From the time the solution was dispensed, the radioactivity was measured continuously with a rate meter, usually for 5 hr. The slope of the rate meter recording, therefore, was proportional to the rate of disappearance of γ radiation, i.e., to the percutaneous penetration of radioactive metal ions and their translocation out of the view of the collimator.

The results were reported as a percentage of the applied dose that disappeared during a given time interval [% lost/(time)] and/or as a first-order disappearance constant, k (min^{-1}), for the interval. Assuming steady-state conditions, either of these parameters may be converted to the permeability coefficient, K_p. That is,

$$K_p = \frac{J}{\Delta C} = \frac{(\%\ \text{loss})}{[\text{time(hr)}]} \cdot \frac{C_A \cdot V_A}{100 \cdot A \cdot C_A} = \frac{(\%\ \text{loss})}{[\text{time}]} \cdot \frac{V_A}{100 \cdot A} \tag{1}$$

where J (mol/cm^2/hr) is the chemical flux, ΔC (mol/L) is the chemical concentration gradient across the skin (which is reasonably assumed to be equal to the applied concentration C_A), V_A (mL) is the volume of chemical solution applied, and A (cm^2) is the area of application. It follows, therefore, that, for a 5-hr application of 1 mL of solution to a 3.14-cm^2 area of skin,

$$K_{p,5} = \frac{(\%\text{loss})_5}{1570} \tag{2}$$

$$K_p = \frac{J}{\Delta C} = \frac{(\text{fraction lost})}{[\text{time(hr)}]} \cdot \frac{C_A \cdot V_A}{A \cdot C_A}$$

where, assuming first-order disappearance kinetics (characterized by rate constant, k min^{-1})

$$(\text{fraction lost}) = 1 - \exp\{-60 \cdot k \cdot [\text{time(hr)}]\}$$

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Thus, for a 1-hr application of 1 mL of solution to 3.14 cm^2 of skin:

$$K_p = (1 - \exp(-60 \cdot k))/3.14$$

For most of these experiments, the amount of chemical lost was reported for only one time point, i.e., the loss of radioactivity from the chamber in 5 hr. As such, the assumption of steady-state conditions cannot be supported. There is, in fact, one in vitro report in this series that contains evidence to the contrary (19). The average disappearance constant (k) for the first 5 hr was larger than that over the first 12 hr, and the average k for the second 12 hr was smaller than that for the first 12 hr. These observations are consistent with the non-steady-state period prior to the attainment of a constant flux. Nevertheless, with the aims of reducing the results to a simple, uniform parameter and of enabling ready comparisons between as many data from as many diverse sources as possible, the assumption of steady state has been made in calculating K_p values.

The disappearance technique was used with seven concentrations of aqueous $^{110m}AgNO_3$ (0.00048–4.87 M) in contact with guinea pig skin in vivo for 5 hr (Table 2) (14). Overall, the average absorption of silver was less than 1%, i.e., less than the analytical limit for quantitative determination ($K_p < 6.4 \times 10^{-4}$ cm/hr). Additionally, in most animals at each concentration (i.e., for 71 of 90 attempts to measure absorption of silver) the rate of absorption was below the limit of quantitation. The highest measurable K_p for the 5-hr period was in the range 1.9–2.5 $\times 10^{-3}$ cm/hr.

D. IMMUNOLOGY

Patients with lichen planus, a particular disease of the mouth, showed evidence of sensitization to silver used in dental filling materials, confirmed through positive patch tests to silver nitrate (20).

In a study of 118 Finnish patients with such oral lichenoid lesions the majority were positive to mercury compounds. One mercury-positive patient also reacted to a silver nitrate patch; and one patient related to silver nitrate only (21). As in most similar cases, remission occurred following replacement of the fillings by other materials.

E. SUMMARY

Silver does not pose a notable hazard from dermal exposure, as binding to keratin-rich, superficial layers of the skin appears to prevent absorption into the deeper layers. Thus skin penetration rates of water-soluble salts are considered to be toxicologically insignificant. No data exist to allow calculation of average flux or

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Table 2 Silver, as $^{110m}AgNO_3$

Reference: E. Skog and J. E. Wahlberg, A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110m}Ag, ^{115m}Cd, ^{203}Hg, *J. Invest. Dermatol. 43*: 187–192 (1964).

Species: guinea pig, M & F — in vivo ✓ / in vitro

Area: 3.1 cm^2

Vehicle: water

Duration of exposure: 5 hr

Analytical method: disappearance of γ radiation from the application site; scintillation counting of isolated organs.

Notes:

1. No average values are given; no exact absorption values are reported; the absorption was reported as <1% of the dose in 71 out of the total of 90 experiments; in the remaining 19 cases the absorptions were reported as being in the ranges 1.0–1.9%, 2.0–2.9%, or 3.0–3.9%.
2. In organ analyses, liver, kidney, intestines, and feces were ''most suitable,'' but no data were presented. ''After the disappearance experiments the organ contents were low, . . . '' Organ contents were measured again after other absorption experiments wherein there was much more radioactivity, but again no data were reported. There is only the statement that ''[the compounds] could be found in most of the organs.''

				Absorption	
				% Ag/5 hr <1% Disappearance constant $k < 3.4 \times 10^{-5}$ (min^{-1})	% Ag/5 hr = 1–4% Disappearance constant $k = (3.4–13.5) \times 10^{-5}$ (min^{-1})
Conc. (M)	Time (hr)	N	pH	$K_p < 6.4 \times 10^{-4}$ (cm/hr)	$K_p = (6.4–26) \times 10^{-4}$ (cm/hr)
0.00048	5	10	6.0	9/10	1/10
0.005	5	10	5.7	6/10	4/10
0.08	5	15	5.5	9/15	6/15
0.118	5	10	5.2	9/10	1/10
0.239	5	14	4.7	12/14	2/10
0.398	5	10	4.6	8/10	2/10
0.753	5	11	4.4	8/11	3/11
4.87	5	10	3.3	10/10	0/10

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permeability coefficients, but in individual guinea pigs permeability coefficients ranged from below the limit of detection (6.5×10^{-4} cm/hr) for most animals to as high as 19×10^{-4} cm/hr. In rare instances, allergic reactions of the immediate and delayed type due to contact with the metal or its salts have been reported.

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4
Aluminum

A. GENERAL COMMENTS

Ubiquitous in the biosphere, aluminum is the most plentiful of metals. Physical contact with elemental aluminum or aluminum salts, whether topical, respiratory, or oral, is unavoidable. The skin, lung, and gastrointestinal tract are very effective barriers to aluminum absorption; there is no conclusive evidence that the metal performs any essential function in the animal organism, and in healthy human subjects the kidneys effectively excrete the amounts absorbed through the diet. Consequently, the only population at risk from aluminum toxicity are patients with chronic renal failure, where excessive accumulation leads to skeletal weakness. Traditionally, aluminum was considered relatively nontoxic, but increasing evidence suggests some negative interactions with important biological processes, such as the formation of DNA crosslinks (1), modification of enzyme activity, including those vital for neurotransmission (2,3), and competitive combination with calmodulin (4).

Recently, aluminum has been implicated in the etiology of several neurological disorders, most prominently in premature senile dementia, or Alzheimer's disease (AD). Experimental induction of changes in the central nervous system (CNS) was provoked in animals by injection of aluminum salts in brain tissue. Aluminum was detected in neuritic plaques isolated post mortem from the brain of patients affected by AD, and based on a number of epidemiological studies, a correlation was found between the incidence of AD and geographically associated increased levels of aluminum in drinking water. Whether alum, a widely used flocculant in municipal wastewater treatment, is an incremental risk factor has not been established by epidemiology. Chelation therapy slowed the progression of Alzheimer's disease in patients affected (5). However, a newer, more sensitive analytical technique failed to demonstrate the presence of aluminum in neuritic plaque cores, casting doubt on the validity of previous analytical data (6). Also unexplained is the access mechanism by which aluminum would gain entry into the CNS since existing data indicate insignificant aluminum absorption via the

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gastrointestinal tract, the only significant route of entry in the organism (7). Moreover, the blood–brain barrier represents a significant second obstacle to aluminum access to the CNS. Thus, factors leading to the onset of Alzheimer's disease may be failing natural barrier functions, compromised either by genetic predisposition or etiology, and until more is known about the aluminum connection to AD, individuals recognized to be at risk are best advised to minimize further exposure to aluminum in all its forms. As an alternative to the implication of aluminum as the cause of that degenerative condition, neurobiologists propose the abnormal deposition of amyloid beta protein in the brain, leading to the nerve cell degeneration underlying the hereditary form of AD (8,9). Excess formation of protein leading to fibrillar deposits in senile plaques is an event that was observed to precede by years the symptoms of premature senile dementia.

Most recent developments in the efforts to shed light on the etiology of AD now point to the action of zinc as the causative factor in that neurodegenerative disorder. Details are given in the section discussing zinc (10).

B. SKIN ABSORPTION AND ANTIPERSPIRANT ACTIVITY

Information concerning the interaction of aluminum with skin has emerged primarily from investigations addressing the antiperspirant and deodorant (bacteriostatic) effect of its water-soluble salts. Like several other metal salts, those of aluminum have protein precipitating and astringent properties. Relative to other, similar metals, the inorganic salts of aluminum were found to be the most effective in suppressing eccrine sweating; therefore, they have the longest history of use and form the basis of all commercial antiperspirants.

Aluminum salts have been shown to penetrate the skin by shunt diffusion through appendages; thus, the most widely accepted mechanism of eccrine sweat inhibition by aluminum and other metal salts is emphraxis (ductal closure). Two mechanisms have been proposed: (a) gradual neutralization of the acidic salts during their diffusion down the sweat duct, which results in the formation of a gelatinous or flocculent hydroxide precipitate; and (b) denaturation of the keratin in the cornified layer surrounding the ostium (opening) of the sweat duct, which was also observed in the substratum corneum. The obstruction gradually resolves as epidermal regeneration takes place. The duration of antiperspirant effect depends on the length of application and the concentration of applied compound (which determine the depth and degree of ductal obstruction). The long term axillary application of an Al/Zr-containing salt has been reported to result in a half-life of effect of 19.4 days (11).

Ductal diffusion and precipitation at varying depths have been demonstrated histologically. A comparative study of different aluminum salts showed that the more acidic salts migrate further down the sweat duct and produce a

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longer-lasting effect (12). Although aluminum chloride shows the best efficacy (13), it is the most damaging salt to skin and clothing. As a result of risk–benefit evaluation, the most commonly used salt in formulated antiperspirants currently is aluminum chlorohydrate [ACH; $Al_2(OH)_5Cl \cdot 2H_2O$] (14).

The organic salts of aluminum, in which the metal is strongly complexed, are ineffective antiperspirants, indicating that the metal is not precipitated as the hydroxide in the presence of strong ligands (15,16). Precipitation of hydroxide gels as the principal mechanism of sweat inhibition is further supported by the observation that metal chloride salts with pK_1 values >8 are ineffective antiperspirants, being unable to form gels at physiological pH (11).

The site of action of antiperspirant activity and the penetration depth of Al salts have been investigated in vitro using human skin. Metal hydroxide masses have been observed as far down as the secretory coil. Histological examination by transmission electron microscopy and fluorescence microscopy of skin treated with aluminum chlorohydrate revealed an obstructive, Al-rich, electron-dense mass within the sweat gland duct, typically at the level of the stratum granulosum, and occasionally as deep as the upper layers of the viable epidermis and the Malpighian layer (17).

In a different study, three Al salts [aluminum chlorohydrate (ACH), aluminum zirconium chlorohydrate glycine complex (AZAP), and aluminum chloride ($AlCl_3$)] were applied in vivo under occlusion. Both axillary skin and forearm skin were treated. Subsequently, the sweat glands were restored to firing by successive tapestripping, thereby permitting assessment of the relative depth of Al salt penetration. ACH and AZAP inhibited eccrine gland function near the skin surface, whereas $AlCl_3$ resulted in inhibition at a deeper level with a prolonged antiperspirant effect. The inhibitory differences presumably reflect the individual hydroxide precipitation kinetics of the various salts (18). The above pattern was observed in both forearm and axillary skin; however, penetration depth in axillary skin was greater (19).

Sweat duct plugs isolated from ACH-inhibited sweat glands of human forearm skin have been analyzed using Fourier transform infrared spectroscopy. This experiment established that epidermal cells in the sweat duct migrate toward the ductal lumen, keratinize and desquamate into the lumen, binding irreversibly to ACH. Complex formation between aluminum and stratum corneum was confirmed in vitro by spectral comparison of ACH complexed with carboxylate and nitrogen-containing groups (20).

Considerable experimental data indicate that both the depth of penetration, which determines duration of antiperspirant effect, and the relative antiperspirant efficacy are significantly dependent on ionic mobility (i.e., size) and charge state. Prevailing pH is a crucial factor, therefore, since different hydrolysis products are formed at different pH values, thus affecting $[Al(OH)_3]_n$ polymerization.

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Salts of Zr, Zn, Cr, Fe, Ti, In, Ga, Sn, Mg, Cu, Be, and Sc also show effective antiperspirant activity (16,21), albeit less than that of aluminum compounds. With the exception of Zr, these other compounds are not used in consumer products.

C. SKIN REACTIVITY AND ELIMINATION

The substantivity of aluminum ions for human keratin decreases in the order of callus > stratum corneum > hair cuticle. The binding of Al ions to guinea pig epidermis is pH-dependent, indicating that carboxylate is the primary sorption site. Binding increases with increasing pH from <1 up to 3.5 in relation to increasing carboxylic acid group ionization; thereafter, further increase in pH results in decreased binding due to formation of insoluble hydroxide gels (22).

In vivo aluminum ion interaction with mammalian skin depends upon the associated counterion. Profound pathological changes were evident in mouse, rabbit, and pig skin upon contact with chloride and nitrate, but not with sulfate, hydroxide, acetate, or chlorohydrate, all applied at identical concentrations. The degree of pathological effect has been attributed to the differential penetration ability of the various salts through the stratum corneum. The toxic effects are manifest upon reaching the epidermis, where Al salts have been shown to denature keratin (12,13).

A number of aluminum compounds were shown to be damaging to animal tissues, inducing granuloma formation containing phagocytosing macrophages, but without evidence of immune system involvement (23).

Injection of aluminum lactate into rabbits resulted in a significant, dose-dependent increase in aluminum content in the animals' hair proximal and contralateral to the injection site (determined by atomic absorption spectroscopy) (24).

D. IMMUNOLOGY

Aluminum is considered a weak allergen, and case reports of contact sensitization are rare. Such occurrences usually involve occupational exposure, e.g., aluminum production (25) or aircraft manufacture (26). More common are (a) reactions to the sustained use of antiperspirants and deodorants containing water-soluble aluminum salts (27); (b) hypersensitivity subsequent to immunization by subcutaneous injection of aluminum-bound vaccine (28), sometimes resulting in persistent granuloma at the injection site, usually in children (29,30) and occasionally in adults (31,32); and (c) sensitization in the course of allergy desensitization therapy with aluminum-bound pollen extracts (33–35). The majority of childhood

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granulomas caused by Al sensitization dissipated prior to adulthood (30). Also, in a cohort of 118 patients with oral lichenoid lesions patch-tested for possible allergy to metals used in dental filling materials, one patient reacted to aluminum chloride in petrolatum (36).

In cases where adults exhibited nodular reactions following hyposensitization with aluminum-precipitated antigen extracts, delayed-type sensitivity to aluminum was found when such reactions persisted for more than 6 months (32). In those patients, the aluminum patch tests showed the strongest reaction at 4 and 5 days, rather than the usual 2–3 days. Cases have been reported where allergic reactions at the site of vaccination based on aluminum hydroxide were exacerbated by the use of toothpaste containing a high percentage of aluminum oxide. This would demonstrate systemically aggravated contact dermatitis caused by aluminum (37). Reactions to aluminum are occasionally observed as secondary phenomena in allergy patch testing using Finn chambers and can result in false-positive test readings (38).

Systematic attempts to induce delayed hypersensitivity in the rabbit using aluminum chlorohydrate were unsuccessful, leading only to the induction of foreign body granulomas (39).

E. ALUMINUM IN ADJUVANT FORMULATIONS

Since the observation was made 65 years ago that a toxoid (an antigenic but nontoxic derivative of a toxin) complexed with aluminum hydroxide gel is more immunogenic than the free toxoid (40), aluminum salts have found widespread application in immunoprophylaxis; in fact, they are currently the only metal-based preparations authorized for human use. To prepare the aluminum-potentiated antigen, either a solution of alum is added to the antigen solution, precipitating the protein aluminate, or preformed aluminum gel (hydroxide, phosphate, mixed hydroxide-phosphate, or oxide) is used as the supporting matrix, on which the antigen is adsorbed.(41) The specific adjuvant effect achieved with such a preparation depends on the physicochemical properties of the gel used. Aqueous aluminum hydroxide gel has a pH of approximately 6, at which the gel is positively charged and will absorb to the negative charge of most proteins. Aluminum hydroxide gel is unique among adjuvants, being effective with a wide range of antigens and inducing in animals a broad isotype profile of antibodies that brings a high degree of vaccine protection (42). While aluminum adjuvants increase antibody levels, only in the rarest of cases are they themselves immunogenic, or able to act as haptens (43). A limitation in the use of aluminum salt adjuvants is their ineffectiveness in stimulating cell-mediated immunity (44).

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F. SUMMARY

Aluminum and its compounds constitute no notable hazard under normal, or even exaggerated (industrial), conditions of exposure, and only in individuals with chronic renal failure can aluminum accumulation reach toxic levels. Certain aluminum salts can cause irritation of the skin, and occasionally allergic contact sensitization, mostly in children following reactions to aluminum-adsorbed vaccines. Such hypersensitivity in early childhood appears to be a temporary state that disappears in adulthood. Injected into tissue, aluminum ion induces formation of IgE in animals but does not elicit immediate-type sensitivity in humans when used as adjuvant. The impact that aluminum may have on certain biological processes and, most notably, the causal role of Al in neurotoxic disorders remain to be resolved at this time. Evidence on hand supports the position that dermal contact with aluminum compounds poses no systemic hazard. The avidity with which aluminum forms complexes with skin protein precludes all but its most superficial penetration into the epidermis. The available data do not allow calculation of meaningful flux or permeability coefficient values.

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5
Arsenic

A. GENERAL COMMENTS

A nonessential toxic element, arsenic is a metalloid that exists in four valence states, (-3, 0, +3, and +5), with the principal ones being arsenite (As^{3+}) and arsenate (As^{5+}), occurring in trace amounts in both organic and inorganic compounds in the environment. Toxicity of the element is dependent on its chemical form. The inorganic compounds are of particular health concern as they are more acutely and subacutely toxic than the environmentally occurring organic ones (e.g., dimethylarsinate), and arsenites have been shown to be more toxic than arsenates (1). Specifically, a comparison of the ED_{50} values of the two oxidation states (as sodium arsenite and arsenate) in mouse embryos showed that arsenite was about three times more potent than arsenate with respect to both malformations and lethality (2). Present in all living organisms, As is found widely distributed in most human tissues. In the normal human body, total amounts of arsenic range from 14 to 21 mg, and individual tissues contain from 0.01 ppm (liver and bone) to 4 ppm (nails) (3).

Inhalation of inorganic arsenic compounds in smelters, agriculture, and pesticide manufacture can result in an increased incidence of lung cancers (4–6).

Arsine, the hydride of arsenic (AsH_3), is a gas and the most acutely toxic form of arsenic, and can be fatal to mouse and man (7,8). Such effect is due to rapid and severe hemolysis, an action associated with erythrocyte membrane disruption that is not yet understood in detail (9). Inhalation of arsine is an occupational hazard and exposure can occur in the manufacture of semiconductors in the electronics industry. Alkyldihaloarsines, alkyldihydroxyarsines and arsenosoalkanes are compounds of arsenic that exert their cumulative and potent toxicity by reacting with thiols and forming stable covalent bonds.

Depending on duration and level of exposure, arsenic can cause acute effects, e.g., death, gastroenteritis, neurological/hematological/hepatic effects, convulsions and paralysis (10), or chronic intoxication, affecting the integument, as well as internal organs. Chronic exposure induces hyperkeratosis and neoplasia.

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Skin is the most common cancer site, but also internal neoplasia is associated with arsenic exposure, comporting the risk of cancer involving the respiratory, gastrointestinal (GI), and genitourinary systems (11). No mechanism for such activity is presently known, but studies demonstrating growth-promoting cytokines and induction of growth factors in keratinocytes following treatment with arsenic are an indication of arsenic's role in the induction of skin cancer (12).

Arsenic is carcinogenic only in its inorganic form (arsenic trioxide; salts of arsenous acid). The trivalent form in particular binds preferentially to sulfhydryl groups, explaining As inhibition of enzymes such as pyruvate oxidase. The most toxic form, trivalent arsenic, affects the skin, respiratory tract, liver, and nervous system (13,14).

In certain geographic regions arsenic occurs at toxic levels in natural mineral deposits, mostly as inorganic, pentavalent arsenates. High arsenic concentrations in well and surface waters have resulted in well-documented cases of chronic poisoning and skin cancer throughout the local populations, e.g., in Taiwan (15,16), England and Wales (17), Central and South America (18–20), Oregon (21), Canada (22), and England (23). Data from Taiwan have been used by the U.S. Environmental Protection Agency (EPA) for cancer risk assessment, and the current maximum permissible arsenic level in drinking water in the United States is set at 50 μg/L. Thereby, the lifetime risk of developing skin cancer from 1 μg/L of arsenic in water is estimated to be 5×10^{-5}, i.e., if 10,000 people drank 2 L of water containing that concentration for an entire lifetime, one could expect 5 excess skin cancer cases due to arsenic (24). In the European Community the current drinking water directive also limits the arsenic level to 50 μg/L, but the World Health Organization (WHO) has introduced a provisional guideline value of only 10 μg/L (25).

Industrial processes, metal smelting in particular, can result in substantial local emissions and in accumulation of the more toxic trivalent form of arsenic in the environment (23). Concentration of the element as As_2O_3 in mine tailings can bring arsenic levels up to 10% (26). Cohort mortality studies demonstrate a dose-dependent correlation between respiratory cancer and occupational exposure to arsenic (27).

The heaviest and most widespread exposure of humans to arsenic, however, results from nutritional intake. In particular, marine organisms may contain high arsenic concentrations (up to 50 ppm) (28), most commonly as arsenobetaine (29). Furthermore, arsenic occurring in soil (e.g., lead arsenate) is readily translocated into plants (28), with the degree of uptake being a function of the level of soluble arsenic in the soil and of the particular plant species (30).

In the so-called arsenic cycle, in an enzymatically catalyzed process microorganisms can transform inorganic forms of arsenic to organic arsenicals (e.g., by methylation); in humans, methylation is a detoxifying mechanism, enabling efficient renal excretion of arsenical compounds (31–37). Besides biomethylation

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in mammalian tissues, metabolism by the microflora of the GI tract appears as a significant aspect of arsenic detoxification in mammals (38). Conversely, inorganic arsenic can also be biologically regenerated from methylated arsenicals (39).

Like mercury, arsenic (as the oxide and sulfide) has been used since antiquity to treat bacterial and parasitic diseases. Although the toxicity of arsenic has been known for centuries, various arsenicals continue to be used, primarily in veterinary medicine. Until recently, arsenic was indicated for internal use in humans as Fowler's solution (7.6 g As/L as potassium arsenite) for the treatment of leukemia and chronic bronchial asthma (40), and also in topical applications, e.g., for the treatment of psoriasis, lichen planus, acne, dermatitis herpetiformis, skin cancer, and other conditions. Chronic doses of Fowler's solution, also given internally for the treatment of psoriasis or asthma, could lead to angiosarcoma and death (41). As Donovan's solution (arsenous and mercuric iodides), arsenic was used internally to treat syphilitic skin conditions (40).

The principal biological action of As^{3+} as results from average environmental exposure is inhibition of enzyme activity via binding to enzymes containing sulfhydryl groups (14). Arsenic differs from certain other complexing metals, such as mercury, the toxicity of which involves the denaturation of cellular proteins and alteration of their structural integrity (42).

On a relative scale of carcinogenicity of metals based on human, animal, and short-term predictive data, arsenic compounds were ranked second only to those of nickel and chromium in activity, and comparable to beryllium and cadmium (43). Review of human data conducted by a body of experts in the field of reproductive and developmental toxicity on behalf of the U.S. General Accounting Office tentatively associated arsenic exposure during pregnancy with decreased birth weight and increased spontaneous abortions (44).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Direct dermal uptake of arsenic contributes to total body burden only when As derivatives are applied topically for therapeutic purposes. However, regardless of the route of exposure, the skin is critically sensitive to arsenic toxicity due to the attraction of arsenic to the skin's sulfhydryl group–containing proteins. Chronic arsenic accumulation increases the susceptibility of the skin to ultraviolet light and is associated with an increased incidence of skin tumors; skin cancer as a result of arsenic poisoning is characterized by multifocal lesions over the entire body (45,46). Because of the pronounced affinity of arsenic for keratinizing structures, including hair and nails, acute intoxication becomes primarily manifest in a variety of skin eruptions, alopecia, and characteristic striation of the nails.

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Manifestations of prolonged arsenic exposure are hyperpigmentation, keratosis, and neoplasia.

Arsenic has been causally linked to the development of cutaneous malignant melanoma (17). Chronic exposure to arsenic (as in industrial settings) has resulted in dermal lesions such as arsenical epithelioma, verrucous hyperkeratosis, warts and melanosis (11,47,48), Bowen's disease (precancerous dermatitis), and various other forms such as lung, lymphatic, and skin cancer (49,50). Available evidence also supports a causal role in the etiology of squamous cell cancers and basal cell carcinomas from exposure to either arsenical medications or high levels of arsenic in drinking water (23,28). Skin lesions can become manifest long after As exposure has ceased and systemic levels have returned to normal (46). The time of latency (i.e., the delay between exposure and the appearance of malignancy in the skin) can be as long as 30 years (51). Accumulation of arsenic in the organism, and thereby in the skin, giving rise to keratoses and carcinoma appears to be due to an individual predisposition. Patients with arsenical carcinoma were given arsenic preparations at 8-h intervals, and the concentration in the blood and total arsenic excretion in urine were monitored over 8–10 days. Compared to normal control subjects who received the same doses, the carcinoma-prone patients appeared to retain up to twice as much As in their tissues as did the controls (52).

Arsenical melanosis, another manifestation of elevated systemic arsenic levels, is viewed as the abnormal stimulation of normal melanin biosynthesis by arsenic (13,53).

While epidemiological evidence clearly points to arsenic as the causative agent for various forms of cancer in humans, presently no such evidence exists for animal species other than humans. The systematic study of arsenic carcinogenicity in an animal model has not been performed (54).

No controlled studies determining the quantitative rate of arsenic absorption through intact human skin are available. Evidence from occupational accidents indicates that skin absorption of arsenic is a significant route of systemic entry; extensive skin contact with aqueous solutions of inorganic arsenic compounds results in systemic toxicity and increased urinary arsenic excretion (13,55). Skin contact with arsenicals by professional pesticide applicators has been reported to result in hyperkeratosis, necrosis, and ulceration of the skin; absorption appears to be greatest for the lipophilic organic arsenicals (56,57).

Dichloro(2-chlorovinyl)arsine (lewisite) and other homologs of this class of compounds are vesicants, i.e., chemicals that cause severe skin burns on contact, even in relatively low concentrations. In contact with water they form the corresponding arsenoxide, which has a great affinity for sulfhydryl groups. By reacting with these groups in proteins, enzymes, and coenzymes, the trivalent arsenic compounds (arsenites) form alkylbis(organylthio)arsines (58).

Autoradiographic studies on arsenic distribution in animals indicate that

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long-term retention is most pronounced in hair and skin, a phenomenon ascribed to their high keratin contents (59). Diagnosis of arsenic exposure by analysis of skin tissue, however, is inconclusive due to the cumulative nature of the levels found (60,61). In hair, on the other hand, spectrophotometric analysis shows that mean arsenic levels accurately reflect acute systemic absorption. This element is incorporated into the hair matrix during growth and retained indefinitely (62,63). (The reader should refer to section, ''Skin as a Secretory Organ for Metals: Hair.'')

A case of extreme arsenic retention and accumulation in the skin has been reported as a consequence of therapeutic oral intake of the element as Fowler's solution (potassium arsenite). General hyperkeratosis resulted that subsided with appropriate treatment (64). As arsenic preferentially accumulates in the keratin-rich integumental system, desquamation and hair loss become significant modes of arsenic excretion (65).

C. QUANTITATIVE ABSORPTION DATA

Significant absorption of inorganic arsenic through animal skin has been measured in vivo by immersion of rat tails in solutions of radiolabeled sodium arsenate, $Na_2H^{74}AsO_4$ (Table 1). An initial accumulation of arsenic was observed in the skin, followed by slow, continuous diffusion from the skin into the bloodstream as determined by the increase of arsenic concentration in erythrocytes. These levels did not change over an observation period of 10 days following exposure. The rate of skin absorption was 1.14–33.1 μg/cm^2/hr from 0.01–0.2 M aqueous solutions. From a 0.1 M solution, applied for 1 h, the average flux was 20.3 μg/cm^2/hr and the apparent K_p was 2.71×10^{-6} cm/hr (66).

Percutaneous absorption of $H_3{}^{73}AsO_4$ applied both in soil and in a small volume of water has been measured in vivo in Rhesus monkeys and in vitro through dermatomed human skin (Table 2) (67). In vivo absorption in 24 hr ranged from 2.0 ± 1.2% (SD) to 6.4 ± 3.9% of the applied dose with 19 and 42 ng/cm^2 absorbed from the higher doses. Even when the post-washing ^{73}As content of the skin is included as absorbed arsenic, absorption through dermatomed human skin was less than that in monkeys: 0.8% from soil and 1.9% from water.

D. IMMUNOLOGY

Arsenicals are primary irritants that can also act as contact allergens due to their reactivity with skin proteins. Interaction of trivalent arsenic and thiol-containing residues in proteins is primarily responsible for the effects which this element

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Table 1 Arsenic, as $Na_2H^{74}AsO_4 \cdot 7H_2O$

Reference: T. Dutkiewicz, Experimental studies on arsenic absorption routes in rats, *Environ. Health Perspect. 19*:173–177 (1977).
Species: rats in vivo ✓
in vitro
Area: tails, 10 cm^2 (Dutkiewicz, personal communication)
Vehicle: water
Concentration: 0.01, 0.1, 0.2 M
Duration of exposure: 1 hr
Analytical method: ^{74}As content of all tissues, organs, and excreta for 10 days.
Notes:
1. ''The rate of skin resorption was 1.14–33.1 μg/cm^2/hr for 0.01–0.2 M concentrations.''
2. Almost all data are for kinetics of distribution and kinetics of excretion of arsenic subsequent to administration via four routes.

Molarity	Absorption following skin exposure for 1 hr (μg As/cm^2)	$10^4 K_p$ (cm/hr)
0.01	Not specified	—
0.1	20.3	0.027
0.2	Not specified	—

exhibits on the structure and function of biomolecules, disrupting their normal function (68,69). Following an initial exposure, even low, nonirritating concentrations will later result in allergic reaction in the sensitized organism. Hypersensitivity due to arsenic is uncommon and is mainly observed in industry workers, in glass and pesticide manufacturing, and in metal mining and refining (48). Both tri- and pentavalent arsenic oxides and their salts have been characterized as definite sensitizers, giving rise to eczematous and follicular types of lesions (47). In one study, when both sodium arsenate and arsenite were included in routine testing of 379 dermatitis patients, two allergic contact dermatitis positives were identified (70). In a separate examination, a crystal factory worker was found to be sensitized specifically to sodium arsenate which, he routinely handled on the job (71).

E. SUMMARY

Arsenic is a ubiquitous element, and exposure to inorganic arsenate and arsenite by consumption of contaminated water and food is unavoidable. Chronic expo-

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Table 2 Arsenic, as $H_3{}^{73}AsO_4$

Reference: R. C. Wester, H. I. Maibach, L. Sedik, J. Melendres, and M. Wade. In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil, *Fund. App. Toxicol. 20*: 336–340 (1993).

Species: human, monkey in vivo ✓
in vitro ✓

Area: 1, 12 cm^2

Vehicle: 5 µL water/cm^2; 40 mg soil/cm^2

Concentration: from water, 0.000024 µg/cm^2, 2.1 µg/cm^2 of skin
from soil, 0.0004 µg/cm^2, 0.6 µg/cm^2 of skin

Duration of exposure: 24 hr

Analytical method: scintillation counting

Notes:

1. In vivo: Monkeys were exposed for 24 hr to the arsenic under a nonocclusive cover. The skin application site was then washed. Urine was collected for seven days including the exposure period. Total percutaneous absorption was calculated with a correction for the urinary excretion of arsenic following an intravenous dose.

In vivo percutaneous absorption of $H_3{}^{73}AsO_4$ on monkeys

	Water		Soil	
	Low dose	High dose	Low dose	High dose
Percent of applied dose	6.4 ± 3.9	2.0 ± 1.2	4.5 ± 3.2	3.2 ± 1.9
Nanograms/cm^2[a]	0.0015	42	0.018	19.2

[a] Total area = 12 cm^2.

2. In vitro: Human cadaver skin dermatomed to 500 µm was exposed for 24 hr with phosphate-buffered saline as the receptor in flow-through diffusion cells.

In vitro percutaneous absorption of $H_3{}^{73}AsO_4$ by human skin

Vehicle	Amount of as applied (µg/cm^2)	Skin		Receptor		Surface wash
		% of dose	ng/cm^2	% of dose	ng/cm^2	% of dose
Soil	0.0004	0.33 ± 0.25	0.0013	0.43 ± 0.54	0.0017	99.2 ± 27.3
Water	0.000024	0.98 ± 0.96	0.00024	0.93 ± 1.1	0.00022	69.8 ± 16.4

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sure from these sources is associated with the incidence of cancer of the skin, bladder, and lung, and inorganic arsenate and arsenite are also recognized as teratogens and developmental toxicants. Arsenic is an important occupational carcinogen, and inhalation exposure during the refining and industrial manufacturing processes results in an elevated incidence of pulmonary carcinoma. The metalloid displays particular affinity for keratinizing structures, and amounts systemically absorbed thus preferentially accumulate in the skin, nails, and hair, making the integument the principal organ at risk for arsenic toxicity. Epidemiological evidence suggests that cancer of the skin can result due to such accumulation; inordinate retention of arsenic in the organism appears to be an idiosyncratic trait and risk factor. Arsenic does not act as a sensitizer upon casual skin contact due to the poor skin penetrating ability of its naturally occurring compounds. However, occupational exposure can be hazardous because corrosive, synthetic arsenic compounds can disrupt the skin's barrier. Arsenic can then form conjugates with skin proteins, resulting in hypersensitivity. Also, as with other heavy metals, lipophilic organoarsenicals used as pesticides and other agricultural products, can penetrate intact skin at significant rates. Acute and chronic arsenic intoxication can result following such exposure. Through rat tail skin, the apparent K_p for sodium arsenate was 2.71×10^{-6} cm/hr.

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6
Gold

A. GENERAL COMMENTS

Elemental gold is extremely stable and generally regarded as inert; very few gold salts are used either in industry or in pharmaceutical applications. Ionized to its mono- and trivalent forms, gold avidly combines with electron donors forming stable coordination complexes, precluding the occurrence of free gold ions. Monovalent gold, in particular, binds strongly with sulfhydryl groups, presumably accounting for its therapeutic value as an antibacterial.

Given therapeutically for their antiinflammatory properties, gold compounds are used to treat (a) juvenile and adult rheumatoid arthritis via intramuscular injection of the water-soluble gold sodium thiomalate (GSTM), thioglucose (GSTG), or thiosulfate (GSTS) (1), and (b) certain skin diseases, such as pemphigus and psoriatic arthritis, all conditions that feature an immunological component (2). Ever since the inception of systemic therapy using gold compounds, the incidence of allergic skin reactions to the metal has been remarkably high, attributed to systemically induced hypersensitivity (3).

Given orally as Auranofin, in which it is complexed with trialkylphosphines, gold has a marked modulatory effect on both cellular and humoral immunity (2,4). Both proteinuria and nephrotic syndrome are the most serious side effects associated with parenteral administration of gold compounds, commonly believed to be caused by circulating immune complexes (5,6). In cases of nephropathy, renal biopsy specimens revealed the presence of elemental gold granules within the proximal tubular cells (7).

B. SKIN REACTIVITY AND ELIMINATION

Following parenteral treatment of several diseases such as rheumatoid arthritis, gold persists in the organism for several months, being distributed to virtually every tissue, including skin, hair, and nails (8).

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Excessive tissue accumulation of gold can cause toxic effects on the skin and mucous membranes, usually preceded and accompanied by pruritus, with generalized exfoliative dermatitis being the most serious manifestation (1). In a prospective study of 444 patients in Sweden, the risk of cutaneous drug reactions (CDR) seemed to be highest for gold compounds (compared to other prescription drugs) and was associated with macular and maculopapular eruptions (9). A slate-like, bluish discoloration, termed *chrysiasis*, can appear following exposure of involved skin to ultraviolet light (10) and is due to the presence of gold in dermal tissue in the elemental state (11). Metallic gold particles deposited in the dermis remain in place permanently and have been located around blood vessels and in dermal macrophages (12,13). Gold cyanide solutions, used in industry, are highly irritating, can cause discoloration of fingernails, and are also suspected sensitizers (14).

C. IMMUNOLOGY

1. An Overview

Systemically, gold compounds have modulatory effects on immediate and delayed immune responses, both in vitro and in vivo (4), causing type I hypersensitivity (contact urticaria syndrome), cell-mediated (type IV) sensitization or allergic contact dermatitis (ACD), as well as type III or Arthus reactions involving antigen–antibody complexes. Hypersensitivity reactions to both the monovalent and trivalent forms of the metal have been described. While ACD is not uncommon due to the widely practiced systemic therapy using gold compounds or the intimate skin contact with metallic gold, urticarial reactions are extremely rare. The relatively high incidence of delayed skin reactions has been noted since the introduction of gold compounds for the treatment of rheumatoid arthritis and is seen in more than 50% of patients so treated (3). Although not clinically relevant in the most part, the rate of sensitization to gold seems to be on a steady increase, with comparable prevalence in the United States, Europe, and Japan because routine screening for gold allergy was started in those countries (15–19). It has been postulated that trivalent gold ion as the reactive metabolite generated by mononuclear phagocytes from monovalent gold alters self-proteins, leading to the activation of T cells. Such Au^{3+}-specific T cells may then be the releasing factor in contact allergy to Au^{+} as well as in systemic exposure to Au^{+} (20–22).

In the etiology of ACD, water soluble gold salts proved to be strong (grade 4) skin sensitizers in the human maximization test (23). Furthermore, it could be demonstrated that elemental gold dissolves in aqueous solutions of various amino acids, particularly cysteine or glutathione, which contain thiol groups capable of complexing and thus solubilizing the metal. Accordingly, the presence of gold was noted in human skin samples taken from areas of prolonged contact with the metal, indicating absorption of the soluble complexes formed (24). Salts of

trivalent gold, such as the trichloride, are strong primary irritants, that damage the skin, facilitating penetration and provoking a lymphoid response (25).

2. Allergic Contact Dermatitis

The proposed cell-mediated mechanism for allergic reactions of the delayed type is supported by positive lymphocyte transformation and proliferation tests (26,27). Histochemical evidence shows that gold (applied as a $HAuCl_4$ solution), like other metals, is selectively taken up by Langerhans cells in whole, viable human epidermis (28). Earlier, the occurrence of cell-mediated gold allergy was considered unlikely, particularly due to skin contact, because of the unreactivity of the metal in general; indeed, overt allergic contact dermatitis to the metal was seen so rarely as to be virtually unknown (29). Consequently, patch testing for gold hypersensitivity was not practiced routinely, and this may have resulted in serious underreporting of the condition (30). Also, late onset of symptoms characteristic of gold sensitivity may have led to false-negative patch tests readings, leaving cases of allergy unrecognized.

Long-term activity in dental laboratories and dental offices produces significant human exposure to dust, mist, and fumes of various metals, including gold, during prosthesis manufacture and application (30). Nonsymptomatic, delayed-type hypersensitivity to gold has also been reported in dentistry students (31). Since gold sodium thiosulfate is now part of the routine patch standard series in dermatological practice, the prevalence of gold hypersensitivity is increasingly coming to the attention of both dental and dermatological practitioners, and has attained surprising proportions. In a Swedish study involving 823 dermatology patients, the incidence of positive reactions to gold was 8.6%, without any signs of an irritant etiology (32). Recent confirmation of this result now makes gold the second most common contact allergen (in the Swedish population), after nickel. In routine patch testing of dermatitis patients in Sweden, the rate of positive readings now approximates 10%. In a large number of asymptomatic patients, allergy of a subclinical nature was frequently noted, i.e., prior to patch testing, few patients had reason to suspect gold hypersensitivity. The occurrence of accidental active sensitization as a consequence of test exposure was positively excluded in this well-controlled study (17).

3. Late-Onset Reactions

Recent studies have shown that individuals sensitized to gold can nevertheless remain nonsymptomatic or manifest reactions with uncharacteristic delays; such cases may remain unrecognized in the course of routine testing because the patch test reactions can follow such unconventional patterns. Skin reactions appear later and last longer, sometimes as long as several months. In a number of cases,

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positive test sites remained negative after 3 days but became positive by day 7 (16,21,33). Elsewhere, it was observed that reaction to a gold sodium thiosulfate (GSTS) patch remained negative after 1 week, becoming positive only 10 days following application, and then persisting for more than 2 months (16). In dermatological practice, gold sodium thiomalate (GSTM) is also applied as a patch test reagent to diagnose ACD (34). The pattern of late-onset and extended duration of skin reaction distinguishes this allergen from sensitization patterns usually observed; the phenomenon of the delayed reaction pattern described may be attributed to the general antiinflammatory properties of gold salts and persistence of reactions to the slow elimination pattern of the allergen from the site of reaction (16).

4. Systemic Sensitization

Although gold in the elemental state is generally regarded as unreactive and sensitization from casual contact of the metal with intact skin is unlikely, a sustained topical or more intimate tissue contact with elemental gold, such as occurs in pierced ears, the wearing of jewelry, or the contact of dental gold with mucous membranes, does comport a certain potential to sensitize (35). Divergent forms of allergic response to gold and its compounds have also been noted, such as stomatitis of the oral mucous membranes from restorative dental work (36), and orbital discharge and systemic contact allergy from a gold orbital implant (37), besides the occasional contact dermatitis from gold jewelry (the latter most often occurring secondarily to systemic sensitization). Gold compounds administered parenterally for the treatment of rheumatoid arthritis exhibit a modulatory action on humoral as well as cell-mediated immunity, and it has become apparent that injection of water-soluble gold complexes brings with it a high risk of sensitization. The incidence of specific, delayed-type contact sensitivity as a side effect of such therapy has been known for several years (38–41). Repeated exposure to gold salts in those sensitized does not invariably lead to a recurrence of dermatitis, suggesting a gradual buildup of tolerance (42).

Gold is frequently used in restorative materials in dentistry in the form of alloys most frequently containing copper, silver, zinc, platinum, and palladium. Oral fluids slowly dissolve elemental gold, a process that can be accelerated by galvanic reactions with other adjoining restorative metals, and the salts thus formed may provoke allergic responses when absorbed through the mucous membranes. Oral lesions are seen as a consequence: erythema, mucosal erosions, lichen planus, stomatitis, etc (43). Involvement of the oral mucosa and the pharyngeal and laryngeal tissue has also been reported, due to dental gold. Delayed-type sensitization was confirmed by positive patch test. Involved tissues returned to normal after removal of the gold (44).

An increase in the incidence of type IV hypersensitivity to gold has been

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recorded in recent years, in accord with the increasing practice of ear piercing, but most of all due to greater acumen on the part of the testing medical practitioner. Piercing of the ears to accommodate gold jewelry as an important contributor to the increasing prevalence was documented in Japan. Dermatology patients wearing gold earrings patch-tested routinely for gold sensitivity with 0.2% chloroauric acid were positive in 34.6% of cases (37/107), as compared to 0.7% (2/270) of such patients without pierced ears.

In a number of cases, hypersensitivity to gold was associated with the formation of intracutaneous nodules in the earlobes at the sites of piercing, that did not resolve over time and had to be removed surgically (45,46). Histological examination of biopsies of such nodules showed a collection of large macrophages, lymphoid cells, and eosinophils, confirming the immunological nature of the reaction; at least one of the cases examined featured organized collections of giant cells. This is characteristic of immunological foreign body granuloma seen with beryllium and zirconium, and is the first such observation attributed to intimate contact with metallic gold (47; private observation by W. L. Epstein).

Serial monitoring of eosinophil counts and IgE levels by radioallergosorbent test (RAST) in 47 nonatopic patients on gold therapy demonstrated that cutaneous side effects and eosinophil count as well as IgE levels rose and fell repeatedly, in concordance with therapy onset and cessation (49). Elevated IgE levels were also noted there in patients without symptoms of allergy. While this is suggestive of type I hypersensitivity, 2 of 12 patients with hematological side effects to gold also had positive lymphocyte transformation responses, indicative of a delayed (type IV) hypersensitivity reaction (48). One patient on chrysotherapy showing symptoms of hypersensitivity had a specific RAST score 10 times higher than control values.

5. Flare-Up Reactions

The organism that has been rendered hypersensitive through systemic exposure to an allergen such as gold, e.g., through enteric exposure to gold salts for antiinflammatory therapy, can react with flare-up reactions upon mucosal, dermal, or enteric challenge at a later point in time. Chrysotherapy in particular involves injection of relatively high doses of gold in form of GSTM or GSTS, which can lead to so-called systemic drug reactions (SDRs) in the organism. They can result in a variety of clinical symptoms such as anaphylaxis, pompholyx, photosensitivity, urticaria and others, and flare-up reactions are among those more commonly occurring. Besides drugs, gold and a number of other metals are known to result in SDR also, e.g., nickel, cobalt, chrome, and mercury (50,51). Flare-ups in the form of eczema or lichenoid reactions involving the skin or mucosa are two of several clinical manifestations attributable to SDR . Oral, dermal, or parenteral exposure of the sensitized organism to the allergen can elicit such erythematous

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flares anywhere on the body; they take the form of generalized eruptions, or can reactivate areas of previously involved but clinically healed skin, such as patch test sites (52–56). In a study involving 20 patients known to have allergy to gold, clinical and histological flare-up of healed patch test sites was provoked by intramuscular injection of GSTM. After only 4–6 hr following parenteral provocation, the former patch test sites were reactivated with an itch, followed by dermatitis or eczema at 48 hr (57). These clinical features are shared with classic ACD resulting from exogenous allergen contact. Patients with contact allergy to GSTS also react to GSTM, epicutaneously as well as intradermally (16).

The activation and elicitation mechanisms regarding the cellular components seem to be similar in both exogenous and endogenous types of ACD. Only the route of elicitation is thereby described, however; the pathophysiological process is not yet thoroughly understood (58).

6. Cross-Sensitization Reactions

A high correlation between gold and mercury hypersensitivity, observed in dermatology patients allergic to gold, leads to the suspicion that mercury cross-reacts with gold. Patch testing of patients with mercuric chloride, GSTM, and chloroauric acid in fact resulted in positive reactions to all three reagents. Moreover, the intradermal test with 0.1% GSTM sodium thiomalate also caused generalized eruptions, the apparent result of systemic hypersensitivity. Cross-sensitization by the two metals was confirmed in animal experiments, when guinea pigs sensitized with a mercuric compound developed positive patch test reactions to both mercuric and gold compounds (46). Possible differences in immunochemistry between the two forms most often involved in hypersensitivity to mercury, Hg^{2+} and Hg^{0}, were investigated by comparing the patch test reactions in (a) a group of patients with gold dermatitis due to ear piercing but no apparent history of mercury hypersensitivity, and (b) a group with systemic contact dermatitis due to mercury. All subjects were patch tested with 0.2% aq. chloroauric acid and 0.05% aq. mercuric chloride ($HgCl_2$), and 0.5% metallic mercury in petrolatum. There was a statistically significant difference between the positive reactions to ionized mercury (68%) versus metallic mercury (14.3%) in patients with gold dermatitis but no previous involvement with mercury. On the other hand, all patients with systemic hypersensitivity to mercury reacted equally to ionized and nonionized mercury, but none to Au^{3+}. All patch test reagents were checked for cross-contamination by electron microprobe analysis. It thus appears that patients originally sensitized to mercury carry markers that do not react with Au^{3+}, but that those sensitized to Au^{3+} preferentially cross-react to mercuric ion. A dual path leading to sensitization for the two valence states of mercury has therefore

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been deduced. In order to examine possible cross-reactivity with another common metal sensitizer, nickel, gold-positive patients were also patched with nickel sulfate but showed no reactions (19,59).

7. Immune Complex Formation

The most prevalent cause of sensitization to gold or its compounds is systemic exposure to soluble gold compounds administered for therapeutic purposes, for which hypersensitivity of the various types has been described as the side effects of such treatment. Nephrotic syndrome and glomerulonephritis, in particular, have been reported following parenteral or oral administration of gold in its organic form. Certain gold compounds such as Auranofin, in which gold is complexed to trialkylphosphines, are thus suspected when given orally to give rise to circulating immune complexes (type III hypersensitivity) (4). As a hapten reacting with protein, the metal forms a complete antigen that induces specific antibody formation, in turn giving rise to the glomerular injury observed (5–7,60). In patients treated parenterally with a variety of gold compounds, the metal was retained in significant amounts (over 80% of amount injected over 39 days of administration) and was bound to the plasma protein, α-globulin, as a gold-protein complex. Excreted amounts (primarily in urine) were not quantitatively related to the injected dose (1), and it is hypothesized that such retention may be the causal factor in the incidence of immune complex glomerulonephritis. In addition, the occurrence of glomerulonephritis appears linked to a genetic predisposition involving the major histocompatibility complex (61).

8. Diagnostic Methods

To avoid overlooking urticarial reactions as well as contact allergy to gold, the recommended diagnostic procedure is an open skin test or a skin prick test with aq. chloroauric acid ($HAuCl_4$) preceding the closed patch test with GSTS; the latter should involve weekly readings of up to 3 weeks after patch application (17). The reactivity of GSTS was compared with that of potassium dicyanoaurate in routine patch testing and found to be the more sensitive agent (15). To improve the diagnostic accuracy of contact allergy patch tests, an alternative in vitro test has been developed, that relies on gold-stimulated cytokine secretion in peripheral blood obtained from patients with gold contact dermatitis (62). The lymphocyte transformation test has proven valuable in predicting, before the development of overt toxicity, the sensitization potential to gold compounds by reacting GSTM (Myocrisin), a standard antirheumatic agent, with peripheral lymphocytes from patients on antirheumatic therapy (40).

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D. SUMMARY

In its metallic state, gold is relatively inert and, in contact with tissue, poses few health risks. However, as a therapeutic agent administered parenterally, soluble gold complexes exert modulatory effects on immune responses. Accumulation in the elemental state in skin tissue as a result of such exposure may lead to adverse cutaneous reactions. Occurrence of immediate- and delayed-type hypersensitivity to gold was rarely recognized in the past. Since its introduction into the patch test standard series, however, gold gives rise to a surprisingly high number of positive reactions, albeit with a low degree of clinical relevance. When cases of such asymptomatic sensitization are evaluated statistically, gold ranks as the second most common allergen after nickel. Water-soluble gold salts are irritating to the skin and on penetration cause allergic sensitization, mostly of the cell-mediated type. Contact with the metal in dental restorative materials can cause allergic stomatitis.

No data were found regarding the rate of skin absorption of any gold compounds.

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7
Boron

A. GENERAL COMMENTS

Boron, a metalloid, occurs widely in surface waters, plant and animal tissues. As sodium borate or boric acid, it is readily absorbed into animal organisms via food and is promptly excreted again via urine without measurable toxicity at naturally occurring concentrations.

B. SKIN ABSORPTION AND REACTIVITY

Boron as boric acid and borax is used therapeutically as a mild topical antiseptic. Absorption through intact human skin, from dilute aqueous solutions (5–10%), is too slight to result in systemic toxicity (1). However, when applied as a saturated solution to irrigate wounds, or on large areas of injured skin and burns in the form of ointments, boric acid is absorbed in toxic quantities.

Absorption through unbroken human skin was first reported in 1924 when boric acid was detected in urine after the soaking of both feet in ethanolic 5% boric acid for over 2 hr (2). Subsequent investigations in rats and rabbits indicated increased concentrations of boron in blood and urine following dermal exposure to solutions of boric acid, sodium borate, and calcium metaborate (3,4). Compared to the observations with normal skin, the exposure of scratched or burned skin to borate or boric acid produces much greater penetration.

Occasionally, boron absorption has resulted in depressed central nervous system (CNS) function and even death. Repeated doses result in accumulation in the brain, liver and fat (5). Applied to burns covering as little as 4% of the body surface, USP 10% boric acid ointment produced pathological changes in the CNS (6). The minimal dermal lethal dose for humans is 8600 mg/kg (7).

On the skin of infants, boric acid and borax can be irritating (6). Infants who were powdered with dusting talc containing boric acid showed no significant boron increase in their blood, although they did display minor skin irritation (8).

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Table 1 Boron, as boric acid, borax and disodium octaborate tetrahydrate (DOT)

Reference: R. C. Wester, personal communication.
Species: human in vivo ✓
in vitro ✓
Area: 900 cm^2 in vivo (dorsal)
Vehicle: water, 1 mL/cm^2, 2 $\mu L/cm^2$
Concentration: boric acid, 0.05%, 0.5%, 5%
borax, 5%
DOT, 10%
Duration of exposure: 24 hr
Analytical method: mass spectrometric analysis of the receptor fluid and of the skin

Notes:

Percutaneous absorption of boric acid in vitro (1 mL/cm^2)

Conc.	Absorbed boron % of dose	Recovered in washes (%)	Flux ($\mu g \cdot cm^{-2} \cdot hr^{-1}$)	$10^4 \cdot K_p$ ($cm \cdot hr^{-1}$)
0.05%	1.2	72	0.25	5
0.5%	0.28	86	0.58	1.2
5%	0.7	82	14.6	2.9

1. When tested at 1 mL/cm^2, in vitro results were much higher than those in vivo where the solutions were applied at just 2 $\mu L/cm^2$. When the in vitro dose was also 2 $\mu L/cm^2$ the results were closer to the absorption in vivo. In this case, the flux from 5% borax was 0.07 $\mu g/cm^2/hr$, a 200-fold decrease compared to the ''infinite'' dose, and the permeability coefficient decreased to 1.7×10^{-6} $cm \cdot hr^{-1}$.

Percutaneous absorption of borax and DOT in vitro (1 mL/cm^2)

Compound/conc.	Absorbed boron (% of dose)	Recovered in washes (%)	Flux ($\mu g \cdot cm^{-2} \cdot hr^{-1}$)	$10^4 \cdot K_p$ ($cm \cdot hr^{-1}$)
Borax, 5%	0.14	88	8.5	1.7
DOT, 10%	0.19	91	7.9	0.8

Percutaneous absorption of boric acid, borax and DOT in vivo (2 $\mu L/cm^2$)

Compound/conc.	Absorbed boron (% of dose)	Flux ($\mu g \cdot cm^{-2} \cdot hr^{-1}$)	$10^4 \cdot K_p$ ($cm \cdot hr^{-1}$)
Boric acid, 5%	0.23 ± 0.12	0.0094	0.0019
Borax, 5%	0.21 ± 0.19	0.0088	0.0018
DOT, 10%	0.12 ± 0.11	0.010	0.0010

Eight volunteers/group
2. Approximately one-half of the in vivo topical dose was recovered after 24 hours in the clothing covering the dosed area and in the skin washes.
3. Pretreatment with 2% sodium lauryl sulfate had no effect on the boron absorption for any of the three applied compounds.

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The various forms of boron hydride (boranes), used industrially and as high-energy fuels, are highly toxic (9), and the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values bear the ''skin'' notation, indicating appreciable skin absorption potential [See Appendix for comments on ''skin'' notation (10)]. By cutaneous application, the rabbit LD_{50} of decaborane ($B_{10}H_{14}$) was 113 mg/kg (11).

C. QUANTITATIVE ABSORPTION DATA

The use of the isotope ^{10}B has allowed the unambiguous in vitro and in vivo measurement of percutaneous absorption of boric acid, borax, and disodium octaborate for a 24-hr exposure period (12). For all of these three materials when they were administered in vitro at ''infinite'' doses (1 mL/cm^2), permeability coefficients were 0.8×10^{-4} cm/hr to 5×10^{-4} cm/hr (Table 1). In vivo the applied volume was much less (2 μL/cm^2) and the apparent permeability coefficients were three orders of magnitude smaller, from 0.001×10^{-4} cm/hr to 0.0019×10^{-4} cm/hr. In vitro results from the smaller finite dose were closer to the in vivo results.

D. SUMMARY

Casual skin exposure to naturally occurring boron salts and minerals (borax) poses no toxicological risk. Only exaggerated exposure to borate solutions of high concentration may lead to toxic levels of absorption, especially through damaged or burned skin. Injury to the CNS and possibly death can result. Boranes, which are used in industry, represent a significant occupational hazard because they are highly toxic and are readily absorbed through the skin. Especially the cutaneous absorption of decaborane has been recognized by the ACGIH as presenting a significant risk of systemic toxicity, and particular care is recommended to avoid direct skin contact in the work environment. The apparent permeability coefficient in vivo in humans is on the order of 10^{-7} cm/hr.

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8
Beryllium

A. GENERAL COMMENTS

Elemental beryllium and its salts are highly toxic, and immunotoxicity represents the greatest risk factor in industrial exposure. In beryllium-using industries (metal extraction, refining and machining, electronics, nuclear weapons manufacture, aerospace, and high-tech ceramics), inhalation of dust containing the metal triggers an immune reaction, potentially resulting in a condition termed chronic beryllium disease (CBD), with a prevalence of 2–5% in beryllium-exposed individuals (1,2).

Having a small atomic and ionic radius (1.11/0.31 Å for Be/Be^{2+}, respectively), beryllium can fit into positions on the surface of macromolecules too small for most other atoms; and its capacity for forming strong covalent bonds that displace other divalent cations may be the basis for its toxicity. Highly reactive with protein substrate, it is strongly retained by tissues and difficult to remobilize as its cytotoxicity interdicts normal phagocyte action (3–5). Albeit an alkaline earth element, beryllium can exist in the form of positive or negative ions depending on the pH of the environment (existing, respectively, as Be^{2+} or $Be(OH)_4^{2-}$ in acidic and basic conditions). Over the pH range 4–8, Be compounds contacting the skin, lungs or lower GI tract typically decompose to form the poorly soluble, amorphous oxide BeO or hydroxide $Be(OH)_2$, both of which are involved in granuloma formation. Once lodged in tissue, these amorphous beryllium precipitates are very slowly excreted, with granulomas persisting as long as the metal remains present. As a rule, removal is only accomplished by excision.

Major areas of concern regarding beryllium-related diseases and the questions still to be answered were the object of a conference in 1994 (6). From the review of several longitudinal data and population-based studies it became evident that, following exposure, beryllium disease progresses in discrete stages that in turn seem to depend on individual predisposition. Sensitization in individuals suspected of beryllium exposure, not necessarily showing symptoms or prog-

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ressing into clinical disease, can be diagnosed in vitro by the beryllium lymphocyte proliferation test. Such sensitization can progress into clinical CBD, potentially followed by beryllium-specific immunity, chronic inflammatory response mostly within the lung, measurable physiological derangement, disability, and, ultimately, death (7).

A series of adverse reactions to beryllium at the cellular and molecular level have been noted. Among these are enzyme induction, inhibition of magnesium- and potassium-activated enzymes, cell transformation, infidelity of DNA synthesis, and inhibition of protein phosphorylation and cell division (8).

The carcinogenic action of beryllium is well established in several animal species but is questionable in humans (9). On a relative scale of carcinogenic metals based on a model combining human, animal, and short-term predictive data, beryllium compounds were ranked behind only those of nickel and chromium in activity, comparable with arsenic and cadmium (10).

B. SKIN ABSORPTION AND REACTIVITY

The absorption of beryllium through intact skin is insignificant. Due to the strong irritant nature of a number of beryllium salts, however, contact with such compounds results in a rapid breakdown of skin barrier function, allowing substantial penetration through injured skin, e.g., 12–21% and 17–27% after 1 and 24 hr exposure, respectively, to aqueous $^{7}BeCl_2$ (11).

In vivo topical application of $^{7}BeF_2$ in guinea pigs resulted in binding of beryllium to alkaline phosphatase and nucleic acids in the epidermis, precluding further systemic diffusion (12).

Lesions, stemming from the action of beryllium on the skin, vary depending on the reactivity of the specific compound. When beryllium in solution penetrates the skin, contact dermatitis and ulceration can result (13,14). Similarly, dermal granulomas can result when this absorbed beryllium precipitates in the tissue under physiological pH, or when beryllium particles penetrate and become lodged in the skin (15–17). These granulomas do not heal until all traces of the metal have been excised (18,19). Subdermal injection of beryllium lactate in human volunteers resulted in intracellular granulomas (20).

C. IMMUNOLOGY

Beryllium, a reactive metal, is a hapten that readily combines with tissue protein producing a complete antigen leading to cell-mediated immune response with delayed type-hypersensitivity reaction in the skin. Beryllium sulfate, as a hapten complexed with guinea pig serum albumin, for example, gives rise to a stronger

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cutaneous reaction than beryllium sulfate itself (21). The early method for determining beryllium hypersensitivity involved patch testing, itself comporting the risk of sensitization and untoward reactions, however; more recently, in vitro blood lymphocyte proliferation tests were developed and are routinely used to confirm a history of beryllium exposure (22). Demonstration of hypersensitivity and occurrence of pathological changes such as skin or lung granulomas serve to diagnose CBD before the onset of clinical symptoms. Early detection of CBD is critical because appropriate treatment can achieve regression of signs and symptoms and prevent further progression of the disease. The basis of CBD management using corticosteroids is the assumption that suppression of the hypersensitivity reaction will prevent the development of fibrosis and thus irreversible damage later (23).

Beryllium exhibits its antigenic reactivity (a) in the solid state, as the insoluble oxide or hydroxide, (b) as a soluble ion in an acidic environment, or (c) as a complex (e.g., covalently bound as citrate or aurintricarboxylate) (1). Beryllium salts are able to stimulate lymphocyte proliferation and to nonspecifically activate B cells (24).

Beryllium causes granuloma formation in lung and skin, with the form of tissue response depending on the type of exposure (1). Inhalation of soluble beryllium compounds in relatively high concentrations, as they occur in beryllium extraction plants, results in acute beryllium pneumonitis, a disease involving all aspects of the respiratory tract. That condition is not associated with immune system involvement.

The deposition of insoluble beryllium compounds (e.g., zinc beryllium silicate, formerly used in fluorescent lamps) in the lung leads to granulomatous hypersensitivity (berylliosis or chronic beryllium disease, CBD), an inflammatory reaction distinct from ordinary delayed hypersensitivity. This chronic pulmonary granulomatosis of undefined pathogenesis is mediated through the accumulation and proliferation of reticuloendothelial cells (15–17).

A genetic predisposition for sensitization to beryllium and subsequent CBD, identifiable via a genetic marker, has recently been discovered, i.e., individuals can be classified as responders and nonresponders, depending on their levels of reactivity (25). The marker is characterized as an aberration in the genetic sequence in the major histocompatibility complex. Although at present the predictive value of this marker is uncertain, in the future it may prove valuable for prospective screening of the work force in risk assessment for CBD, identifying those most likely to develop the disease upon exposure to beryllium dust (26). This confirms earlier observations made in animals that the adverse reaction to beryllium exposure is genetically controlled (27).

In the skin, beryllium preferentially binds to epidermal tissue (28,29). Little or no binding occurs in the stratum corneum and dermis (12). The penetration of soluble salts through injured skin leads to adsorption of ionic beryllium by

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protein, causing denaturation; this process results in delayed hypersensitivity or beryllium dermatitis (30). Its cell-mediated character was demonstrated by animal experiments with guinea pigs. Sensitization was successfully transferred from sensitized to nonsensitized animals by injection of lymphoid cells; serum, however, produced no sensitization (31). Hypersensitivity did not lead to general anaphylaxis or involve humoral antibody formation (32). Hypersensitivity to beryllium is permanent, and even minimal re-exposure after long periods will result in recurrence of dermatitis (19).

Beryllium is added in low percentage to copper, to yield an alloy of enhanced formability, conductivity, and corrosion resistance. That occupational skin contact with such alloys harbors the risk of sensitization was demonstrated in the animal model: delayed hypersensitivity reaction was elicited in guinea pigs that were presensitized to beryllium sulfate (33). The use of beryllium in dental restorative work is recent, motivated by the tendency to replace materials such as nickel and mercury. The dissolution of beryllium from dental alloys is remarkably high. After incubation of dental alloy samples in human saliva at pH 6 for 120 days at 37°C, between 0.3 and 3.48 mg/L beryllium was found in solution (34). Stomatitis and asymptomatic contact hypersensitivity to beryllium have recently been reported following the use of beryllium containing prosthesis materials (dental alloy contact dermatitis) (35).

Inhalation exposure to beryllium can be correlated to cutaneous immune response in humans, guinea pigs, and rats. Individuals with chronic beryllium lung disease, resulting from occupational inhalation of airborne reactive compounds (e.g., BeF_2), tested positive for Be hypersensitivity. In similar controlled studies, experimental animals also tested positive for Be hypersensitivity; these data confirm a delayed-type reaction following inhalation exposure to Be compounds (36).

D. SUMMARY

Beryllium metal and its salts are immunotoxic, representing primarily an occupational risk factor for genetically predisposed individuals. Inhalation of beryllium-containing aerosols can induce acute beryllium pneumonitis, without involvement of the immune system. Inhalation of insoluble beryllium-containing dust can result in berylliosis (i.e., chronic pulmonary granulomatosis or chronic beryllium disease), a respiratory condition that occurs almost exclusively following chronic exposure in the workplace and that can also involve the skin due to an antigen-dependent response by beryllium-specific T cells. In contact with intact skin, beryllium minerals do not pose significant toxicological risk. Soluble beryllium salts, however, are corrosive, capable of causing lesions and, in reaction with proteins, sensitization resulting in allergic contact dermatitis. Also, when particles

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of insoluble beryllium compounds penetrate the skin, they remain embedded causing granulomas and ulcers, as well as associated hypersensitivity, until surgically removed. Inhalation of volatile, highly reactive beryllium compounds can ultimately result in allergic contact dermatitis or systemic contact allergy. Although a confirmed carcinogen in animals, the carcinogenic action of beryllium in humans is only suspected at this stage. No data were located on the rate of skin penetration of any beryllium compounds.

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9
Bismuth

A. GENERAL COMMENTS

The only significant human exposure to bismuth involves pharmaceutical uses. A number of trivalent and, rarely, pentavalent salts of bismuth have been used orally or intramuscularly over the past two centuries for the treatment of a number of conditions: syphilis, malaria, hypertension, warts, stomatitis, upper respiratory tract infections, amebiasis, dyspepsia and diarrhea. Bismuth compounds have also been used as radiocontrast agents in diagnostic testing and as topical astringents having slight antiseptic action (1). With the introduction of more effective therapeutic agents, primarily antibiotics and antimicrobials, the internal use of certain trivalent bismuth salts (subnitrates, subcarbonates, subgallates, tartrates, subcitrates, and subsalicylates) is now limited primarily to oral preparations for the prevention and treatment of gastric and intestinal disorders, such as ulcers and diarrhea. Available as over-the-counter products, the (presumed) insoluble bismuth salts were long considered safe, being poorly absorbed from the GI tract. Recently, however, long-term, unmonitored, and indiscriminate use of certain Bi salts, particularly lipid-soluble subgallates and subnitrates, has been associated with a number of toxic effects in humans, primarily (reversible) encephalopathy and nephropathy (2). At present, use of colloidal bismuth subcitrate (CBS; De-Nol) and bismuth subsalicylate (BSS; Pepto-Bismol) is considered safe and effective for the treatment of diarrhea; both salts have antimicrobial activity against causative pathogens. More controversial (3) are the claims of their therapeutic activity on both gastric and duodenal ulcers, based on the combination of bismuth's specific activity against the etiologic agent (*Helicobacter pylori*) and the formation of a protective coating by colloidal bismuth compounds in the ulcer crater, thus providing a diffusion barrier to HCl and pepsin (4,5). Low-level absorption of bismuth, resulting in nontoxic blood levels (below 50 μg/L), was noted upon oral administration of tripotassium dicitratobismuthate to both healthy volunteers and patients with gastroduodenal ulcers (6).

Upon absorption, bismuth equilibrates between bone, soft tissues, and

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blood with the highest concentration found in the kidney. Bismuth is excreted in urine. In rat and rabbit kidney tubules, electron probe x-ray analysis identified intranuclear inclusions of the metal in the elemental state of up to 5 μm in size only days following administration of bismuth salts (7). Also, animal experiments indicate that ingestion exposure to elemental Bi induces biosynthesis of low molecular weight metal-binding proteins in the kidney, similar to hepatic metallothioneins (8). While bismuth is rapidly excreted in the urine, residual amounts were shown to persist for weeks in the kidneys of patients treated with bismuth compounds, suggesting tissue accumulation and slow mobilization (6). Thus, the significant accumulation and retention of bismuth observed in the human kidney (2) may be explained by the binding of the metal to sulfhydryl (thiol) groups in an inducible metal-binding protein.

Organometallic bismuth compunds show strong biological activity similar to that of organomercurials, and are effective against bacterial and fungal infections. Organobismuth(III)thiolates were shown to exhibit antitumor activity similar to that of certain platinum group metal compunds (9).

No adverse effects from industrial exposure to bismuth or its compounds have been reported.

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Topical bismuth compounds include medicinal powders, as well as salves and ointments used as a treatment for skin lesions or as emollients, astringents or disinfectants. Bismuth compounds also have cosmetic applications, being used in skin-lightening creams and as a pearlescent pigment in makeup.

High Bi concentrations in cerebral venous blood and spinal fluid were measured in patients suffering from an organic brain syndrome who had been using oil-based cosmetics containing Bi (10). This may indicate significant skin absorption rates for the metal or accumulation in tissues over an extended period of exposure.

Comparison of normal and urticarial skin biopsies from patients receiving intramuscular bismuth therapy indicated a direct relationship between bismuth concentration and degree of urticaria (11). Systemic bismuth is apparently deposited selectively in skin affected by eruptions.

The parenteral use of bismuth, now largely discontinued, sometimes resulted in permanent, generalized hyperpigmentation of skin and conjunctiva, a condition referred to as *bismuthia*. Similar to the blue-gray discoloration of argyria, bismuthia is characterized by a blue-black gingival line (12). Histological examination reveals the presence of metallic granules in the papillary and reticular layers of the dermis (13).

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C. IMMUNOLOGY

Lichen planus-like skin eruptions were observed repeatedly in syphilis patients after therapeutic intramuscular injections of bismuth compounds. These rashes, occurring at various distal sites of the body, usually appeared as verrucous plaques densely infiltrated by lymphocytes, and rarely as stomatitis. They would subside on discontinuation of treatment and reappear when bismuth was readministered. With recurrence, however, the rash severity decreased, and scaling, infiltration, and time to recovery were reduced. In some cases, desensitization progressed to the point that bismuth therapy could continue without much discomfort to the patient (11,14). Characterized as lichen planus or pityriasis rosea, the above clinical observations suggest an immune factor in the etiology of bismuth induced eruptions.

In a systematic surveillance program involving several clinics and thousands of patients receiving oral CBS therapy for peptic ulcer, only two cases of allergic skin eruptions due to bismuth were recorded over a period of 13 years (15).

D. SUMMARY

In the use of bismuth compounds to treat a variety of conditions, a number of adverse effects have been noted. The toxic action observed varies with the subject, and the targeted organ depends on the particular bismuth salt. At present, medicinal use of mainly the subcitrate and subsalicylate salts for the prevention and treatment of gastric and duodenal ulcers continues.

Certain lipid soluble bismuth salts (e.g., subgallate) penetrate biological membranes, including human skin, potentially causing neurotoxicity and nephrotoxicity. Oral administration of certain bismuth compounds can result in absorption in both the diseased and healthy GI tract. Symptoms can occur at bismuth blood concentrations in the range of 100–500 μg/L but, as a rule, the toxic effects observed are reversible. No quantitative information on the skin penetration of bismuth compounds could be retrieved.

During the intramuscular bismuth injections for the treatment of syphilis, rare cases of systemically induced allergic contact dermatitis and stomatitis have been reported.

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10
Calcium

A. GENERAL COMMENTS

Calcium is the most abundant metal among the elements and the fifth most abundant element in the body. It holds a special place in animal physiology and cell biology. Together with phosphate, Ca is the major constituent of the endoskeleton; about 99% of body Ca^{2+} resides in bones and teeth. The balance, as mobile Ca^{2+}, is found throughout the body fluids, and is the most important mineral for general cellular function and for the regulation of a wide variety of biological activities. Calcium is an intracellular messenger in signal transduction, e.g., in the regulation of muscle contraction, insulin secretion, and cell growth and differentiation; all cells feature specific Ca^{2+} transport systems. Many of these activities involve interactions with proteins to which calcium binds tightly and exerts a stabilizing, activating or modulating effect. These strong associations can lead to crosslinking and conformational changes (1). A versatile Ca-regulating protein present in every eukaryotic cell is calmodulin, a multipurpose intracellular Ca^{2+} receptor possessing four calcium binding sites. Calmodulin mediates most Ca^{2+}-dependent processes in the skin including enzyme regulation, histamine release, T-lymphocyte activation, and platelet aggregation, critical factors in the process of wound healing of the skin (2). A specific Ca-binding protein has been identified in the human epidermis (3). A large Ca^{2+} concentration gradient exists across the cell membrane, serum calcium being approximately 1 mM, while free Ca^{2+} in keratinocytes is approximately 100 nM (4).

Calcium and phosphate enter the body via the diet and are maintained in dynamic equilibrium at a ratio of 2:1 between bone and extracellular fluid (5). Milk and other dairy products, certain fruits, and leafy green vegetables are the richest sources of calcium. Vitamin D, also present in milk, ensures optimal Ca absorption; alcohol, on the other hand, inhibits it.

When dietary intake of calcium is insufficient to meet the body's needs, increased amounts are mobilized from the bones to maintain an adequate supply in the bloodstream. Abnormally high, as well as low, serum calcium levels can

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result in severe disturbances. Caused primarily by hyperparathyroidism or cancer, hypercalcemia is potentially lethal. Hypocalcemia, caused mainly by hypoparathyroidism, vitamin D deficiency, renal failure or malabsorption, can lead to tetany, muscle cramps, and convulsions (6).

From a toxicological perspective, calcium is most hazardous as its highly alkaline hydroxide, which upon contact damages the skin and cornea of the eye with the potential to cause blindness.

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

The in vitro permeability of human skin to Ca^{2+} ions shows a marked dependence on anatomical site. Consistent with data observed for nonelectrolytes, penetration decreased in the following order: foreskin > mammary > scalp > thigh. Mouse and guinea pig skin show comparable permeability to that of human scalp. Ca^{2+} transport was also measured in the opposite direction, i.e., application was made to the dermal side of excised tissue, resulting in significantly higher permeation (7,8).

Calcium penetrates human skin, forming deposits of insoluble precipitate in dermal connective tissues, as shown by ion capture cytochemistry. Such ultrastructural localization demonstrated significant intercellular Ca^{2+} precipitates in the lower stratum granulosum and intracellular Ca^{2+} in mitochondria (9,10). However, no changes in serum Ca^{2+} and phosphate levels are found that would reflect such accumulation. Long-term occupational exposure to high levels of dissolved calcium, as experienced by oil field workers (11), miners (12), and agricultural laborers (13), can result in calcinosis cutis, a benign and reversible hardening of the exposed skin.

Current analytical techniques (using, respectively, fluorescence and cytochemistry) allow precise localization of free ionic calcium and organelle-bound calcium in epidermal substructures. Such techniques further illuminate the role of inter- and intracellular calcium gradients, particularly as they appear to regulate epidermal cell differentiation and maintenance of the skin barrier function (14,15). Acute barrier disruption by tape stripping or solvent was shown to eliminate that gradient (16).

The concentration profile of Ca^{2+} across normal human skin has been determined by particle-induced x-ray emission (PIXE) analysis. Calcium levels in the basal layer of the epidermis are lower than those seen in the dermis (16). Concentration decreases from a level of 400 ppm in the basal layer to approximately 200 ppm at the stratum granulosum–stratum corneum interface (17). Using ion capture cytochemistry (a semiquantitative analytical method), relatively small quantities of Ca^{2+} were observed in the intercellular domains of the lower stratum corneum (becoming undetectable toward the superficial layers) in mouse (9) and

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human skin (10). These data suggest that, prior to the terminal differentiation of keratinocytes at the stratum granulosum–stratum corneum interface, Ca^{2+} is recycled and is not lost in the subsequent, normal desquamation process. Notably, such a Ca^{2+} gradient is not present in psoriatic skin, such that some Ca^{2+} is lost by desquamation in this disease condition (9).

In a number of studies, Ca^{2+} (like Mg^{2+} and K^{+}) has been demonstrated to play a critical role in the regulation of cell homeostasis, in maintenance and repair of the skin's barrier function, and in the control of epidermal differentiation. Data from these experiments facilitate the interpretation of observed Ca^{2+} concentration gradients in human epidermis in vivo (14). Perturbation of the cutaneous barrier function by removal of skin lipids with organic solvent, or removal of the stratum corneum by tape stripping, leads to the loss of normal Ca gradients, which in turn stimulates the secretion of preformed lamellar bodies instrumental in barrier repair (15). Large Ca aggregates appear in the intercellular space of the stratum corneum where none are normally present, leading to a decrease in Ca^{2+} in the stratum granulosum. With barrier recovery after solvent delipidization, the normal calcium gradient is re-established within 24 hr (9); however, when the skin is occluded, the normal water (and Ca) flux across the basement membrane is halted and barrier recovery is blocked (18). Conversely, the return of normal lipids to the stratum corneum, critical in re-establishing normal barrier function, can also be prevented by the presence of exogenous calcium (or potassium), as such repair mechanisms depend on decreased Ca^{2+} in the upper epidermis (19). Intracellular Ca^{2+} gradients determine differentiation of keratinocytes (20); furthermore, the absence of normal calcium gradients is associated with differentiation defects such as those occurring in psoriasis (10). Calcium concentration abnormalities can also be detected in dystrophic nails of psoriatic patients; the affected nails show a significantly higher Ca level (11–13 wt%) than those unaffected, or those from normal controls (4–5 wt%) (21).

Divalent cations including calcium are necessary for intercellular adhesion and stratification. Adequate ion concentrations play an important role in maintaining the structural integrity of the mammalian skin. This role was demonstrated experimentally by first inducing epidermolysis in mouse skin with EDTA solution, then reestablishing normal Ca^{2+} concentration, thus reversing cohesion loss (22).

Cross-sectional and longitudinal localization of Ca in human scalp hair reveals that Ca occurs in significant concentration in the internal and external root shaft, indicating longitudinal and radial input, respectively. At increasing distance from the root end, calcium concentration increases and is homogeneously distributed throughout the diameter, peaking in concentration at hair ends. The fact that calcium can be readily eluted from the hair by various treatments indicates that it is principally of exogenous origin (23). Scalp hair of young children up to 12–14 years of age shows an age-dependent, gradual increase in

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calcium levels. Such age dependence in the excretion of this structurally important metal is related to the changing requirements for Ca during skeletal growth and development in children and adolescents (24).

Calcium loss via the sweat is an important factor in the overall balance of this metal ion in the body. Amounts lost per day by this route have been reported to be on the order of 120 mg [ranges quoted 20–365 (25), 72–230 (26), and 70–164 mg (27)]. Elemental analysis of human cerumen (ear wax) samples (28) showed the presence of dry weight calcium in the range of 0.69–2.2 mg/g dry weight (29).

C. SUMMARY

In the skin, calcium has an important role in regulating epidermal keratinocyte physiology and maintaining skin structural integrity. A Ca^{2+} concentration gradient appears to regulate growth and differentiation of epidermal cells, and thereby maintain an effective permeability barrier. Disturbance of the normal calcium gradient is observed in serious functional abnormalities of the skin, such as psoriasis and pemphigus. Dermal exposure to naturally occurring calcium levels in water has no notable physiological effect. Chronic occupational exposure to elevated concentrations, however, can lead to a transient hardening of the epidermal tissue, presumably due to deposition of absorbed calcium as calcite ($CaCO_3$) crystals.

Calcium oxide (quicklime) and calcium hydroxide are corrosive to epithelial tissue and may cause blindness upon eye contact. No quantitative data were located regarding the rate of skin absorption of any calcium compound.

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11
Cadmium

A. GENERAL COMMENTS

Cadmium is a ubiquitous, highly toxic element that is present at low levels throughout the environment, including food, water, and soil (1), and in most biological substrates, although it has no known biological role. Thus the primary routes of exposure to the metal are dietary in the general population and dermal contact upon sustained occupational exposure. Persistent in the environment and living organisms, the toxicology of the metal has been well documented (2). In accord with its relatively high ionization energy, Cd^{2+} is highly electropositive and Cd complexes have a considerably covalent character. This accounts for the antagonistic effect that cadmium has on other essential trace metals in the mammalian organism, such as copper, iron, zinc and magnesium (3–5). There is also growing evidence that cadmium interferes with bone formation (6) and increases bone resorption (7) in vivo, and that it decreases osteoblastic accumulation of calcium in vitro (8).

Due to its relatively high vapor pressure and ease of thermal volatilization, especially significant in metal smelting and refining processes, and the release of highly water-soluble cadmium salts into industrial wastewater, environmental levels of the metal are continually rising and present a considerable public health concern. The metal is seen to concentrate in certain plants, e.g., in tobacco leaves, and to accumulate in animal soft tissue, reaching potentially toxic levels (9). Dietary intake and smoking of tobacco thus are the principal sources of human exposure to cadmium. That among the general population smokers are particularly at risk was demonstrated by significantly elevated cadmium levels measured in their blood and seminal fluid, as compared with the corresponding values seen in nonsmokers (10).

The metal has been associated with malignant neoplasms in humans (11), and it is predicted that Cd-induced carcinogenicity might be mainly due to oxidative DNA damage (12). In a model, on a relative scale of carcinogenicity based on human, animal, and short-term predictive data, cadmium compounds were

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ranked second only to those of nickel and chromium, and were at par with arsenic and beryllium (13). In the Seventh Annual Report on Carcinogens (1994), cadmium has been officially classified as a human carcinogen by the National Institute of Occupational Safety and Health (NIOSH), based on sufficient evidence gathered from environmental exposure. Occupational exposure to the metal itself, and to a number of its salts, has been associated with increased incidences in cancer of the prostate, respiratory, and urogenital tracts (14–19). Review of human data conducted by a body of experts in the field of reproductive and developmental toxicity on behalf of the U.S. General Accounting Office identified cadmium as a reproductive toxicant: the data showed that male exposure to cadmium is associated with testicular toxicity, altered libido, and infertility (20).

An outstanding adaptive phenomenon exhibited by virtually all living organisms, including plants and microbes, is an efficient cadmium-sequestering system based on the metallothioneins, which immobilize incidental cadmium. In mammals, the metal thus sequestered accumulates in liver and kidneys. With continued significant exposure, cadmium metallothionein is eliminated from the liver by two mechanisms: (a) biliary excretion in the short term and (b) transfer from liver to kidney in the long term (21). When a critical level of cadmium is reached in the renal cortex, metallothionein binding of cadmium is no longer adequate and the metal becomes toxic, primarily to the renal tubular cells; the kidney thus becomes the organ most susceptible to damage from long-term exposure to this metal (22–24). Nephrotoxicity expresses in defects in resorption of solutes such as proteins, glucose, amino acids, phosphate, and calcium (25–29). In the developing organism, the nervous system is thought to be the most vulnerable to cadmium-induced damage (30).

The half-life of cadmium in the body is 10–30 years; therefore, even at low levels of environmental exposure, tissue concentrations of cadmium tend to increase continuously over a lifetime. Such long retention times in the organism are at least partially due to Cd binding to metallotheonein (MT) in tissues. The role of MT on distribution and retention of Cd was investigated by exposing normal mice versus MT-null mice to $^{109}CdCl_2$ intraperitoneally (31). While the absence of MT in MT-null mice did not affect initial tissue distribution of Cd, the elimination of Cd was much faster in MT-null mice versus control mice. Twenty-four hours after administration, 40% of Cd was present in the liver of controls, mostly bound to MT, while only 20% was detected in the liver of MT-null mice, which was not bound to MT. Also, Cd was eliminated more rapidly from MT-null mice than controls: 28% of the dose was retained in MT-null mice 15 days post dosing, versus 56% in control mice. A corresponding difference in elimination was seen in individual organ tissues, particularly in the pancreas which stores the highest Cd levels, but also in liver and kidney, spleen, and heart. This confirms earlier findings that increased MT concentrations induced by Cd pretreatment also resulted in a longer biological half-life for the metal (32,33).

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Immunotoxicity of cadmium has been demonstrated in various animal species (34–37).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Applied topically, cadmium appears to bind tightly to epidermal keratin, which may explain the insignificant amounts seen to penetrate human skin in vitro (38,39). The standard protocol of skin tape stripping was implemented to examine the surface penetration of cadmium salts through human stratum corneum in vivo following single open application at levels of 0.001–0.1% of the metal as the sulfate and chloride (40). The cadmium concentration gradient across the stratum corneum declined to nondetectable levels (<5 ppb) beyond the fifteenth strip, confirming the observation that diffusion of cadmium through the stratum corneum occurs minimally and only after considerable lag times (38,39). A mass balance calculation points to the fact that, particularly at higher concentrations, a significant part of the applied amount remains unaccounted for. This seems to indicate that cadmium chooses the alternate, shunt pathway for diffusion and deposition to a significant degree. Thus, dermal absorption is not a significant route of entry for cadmium compounds in the general population due to the still low concentrations occurring in the environment and apparent low absorption rates (41). However, its potential to be a percutaneous hazard has been demonstrated in animals. Guinea pigs were exposed to cadmium (0.24 M on 3.1 cm^2) for a 6-week period. Six of 20 guinea pigs died as a result of cadmium poisoning. The absorbed amount was unknown (42). Although a known carcinogen, cadmium is not known to cause cancer in the skin. Cadmium sulfide, used as a tattoo pigment, can cause persistent localized, nonimmunological skin reactions to UVA radiation (43).

Sweat is an important excretory pathway for cadmium. Under excessive exposure, such as in the industrial environment, cadmium excretion in sweat becomes more important than urinary excretion (44).

Mean cadmium levels in hair accurately reflect systemic absorption due to environmental and dietary exposure. The metal is incorporated into the hair matrix during growth and is lost only by mechanical removal (45).

C. QUANTITATIVE ABSORPTION DATA

Although cadmium can exert serious health effects, only one report of percutaneous absorption through human skin has been published. In vitro experiments (39) using dermatomed human cadaver skin (Table 1) were used to assess the 16 hr exposure to ppb concentrations of cadmium in water and in soil. When aqueous

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Table 1 Cadmium, as $^{109}CdCl_2$

Reference: R. C. Wester, H. I. Maibach, L. Sedik, J. Melendres, S. DiZio, and M. Wade, In vitro percutaneous absorption of cadmium from water and soil into human skin, *Fund. Appl. Toxicol. 19*:1–5 (1992).
Species: human, dermatomed to 500 μm in vivo ✓
in vitro ✓
Area: 1 cm^2
Duration of exposure: 0.5, 16 hr
Vehicle: 2.5, 5 μL water/cm^2, 116 ppb (1.0 μM);
4, 20, 40 mg soil/cm^2, 6.5, 13, 26, 65 ppb (0.06, 0.12, 0.24, 0.6 μM).
Analytical method: Liquid scintillation counting of ^{109}Cd (specific activity = 0.561 mCi/μg Cd)
Notes:
1. Example of an aqueous dose of 5μl/cell, 5 μL @ 116 μg/L = 5.8×10^{-4} μg (0.58 ng). Then, 5.8×10^{-4} μg × 0.561 mCi/μg × 2.2×10^9 dpm/mCi = 700,000 dpm/cell.
2. To remove unabsorbed cadmium, the skin was washed once with liquid soap and twice with distilled water.

Percutaneous absorption (percent of applied dose) of cadmium from water (5 μL/cm^2) after 16 hr exposure

Skin sample	Receptor fluid (human plasma)	Skin	Surface wash	Total
A	0.5 ± 0.2	8.8 ± 0.6	93 ± 3	103 ± 3
B	0.6 ± 0.6	12.7 ± 11.7	74 ± 11	88 ± 201

Percutaneous absorption (percent of applied dose) of cadmium from water (2.5 μL/cm^2) after 16 hr exposure

Skin sample	Receptor fluid (human plasma)	Skin	Surface wash	Total
C	0.2 ± 0.2	2.4 ± 1.6	86 ± 3	89 ± 2
D	0.1 ± 0.04	3.2 ± 4.4	88 ± 13	92 ± 12

Percutaneous absorption of cadmium from water (volume was not reported) after a 30 min exposure

	Cadmium content, percent of dose	
Experiment	Skin	Receptor (plasma)
Exposure period only	2.3 ± 3.3	0.0 ± 0.0
Exposure period followed by washing and 48 hr of perfusion	2.7 ± 2.2	0.6 ± 0.8

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Table 1 Continued

3. Because the concentration of the applied Cd was very low, the amount of Cd absorbed was also very low; when 0.5% of the dose was absorbed, the weight of absorbed cadmium was only 2.9×10^{-6} μg. The corresponding average flux is then <1 pg/cm^2/hr (2.9×10^{-6} μg/cm^2 ÷ 16 hr = 0.18×10^{-6} μg/cm^2/hr).

Percutaneous absorption (percent of applied dose) of cadmium from soil (13 ppb Cd) after 16 hr exposure

Skin source	Applied dose mg soil/cm^2	Receptor fluid (human plasma)	Skin	Surface wash	Total
A	40	0.02 ± 0.01	0.06 ± 0.02	102 ± 17	102 ± 17
B	40	0.07 ± 0.03	0.13 ± 0.05	82 ± 33	83 ± 33
C	20	0.02 ± 0.02	0.08 ± 0.06	106 ± 2	106 ± 2
D	20	0.02 ± 0.02	0.08 ± 0.06	88 ± 13	92 ± 12

$CdCl_2$ (116 ppb $CdCl_2$ applied as 2.5 μL/cm^2 and 5 μL/cm^2, i.e., 0.29 and 0.58 ng/cm^2) was applied, only 0.1–0.6% of the applied cadmium was found in the receptor solution after 16 hr. Much more, 2.4–12.7% remained in the skin after the surface had been washed.

To mimic exposure such as might occur during swimming or bathing, skin was also exposed to cadmium solution for 30 min before washing the surface. No cadmium was found in the receptor after 30 min; after 48 hr 0.6% of the applied dose reached the receptor and 2.7% remained in the skin.

When skin was exposed for 16 hr to soil spiked with cadmium (13 ppb Cd, 20 and 40 mg soil/cm^2), small fractions of the applied cadmium, less than those from solution, were detected in the receptor fluid (0.02–0.07% of the applied dose) and, after it had been washed, in the skin (0.06–0.13%).

Wester et al. also found that exposing skin to soil with increasing cadmium concentrations (6.5–65 ppb) resulted in increased cadmium in the skin, but the cadmium recovered in the receptor was relatively constant. The trend was similar when the soil loading was increased (4, 20, and 40 mg/cm^2) at a fixed concentration of cadmium (13 ppb), i.e., the amount of cadmium in the skin increased but the amount in the receptor was relatively constant.

Cadmium was also found to accumulate in pulverized stratum corneum; its partition coefficient between powdered stratum corneum and water was 36.

The only other quantitation of cadmium's percutaneous absorption allows an estimate of the in vivo permeability coefficient in guinea pigs (46). Tested at six concentrations (0.005–4.87 M), cadmium chloride's absorption was above the limit of detection for all replicates only at one concentration (0.239 M $CdCl_2$)

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corresponding to an apparent permeability coefficient was 11×10^{-4} cm/hr (Table 2). At other concentrations, the percentage dose absorbed was generally smaller.

D. IMMUNOLOGY

Although human patch tests to $CdCl_2$ are occasionally positive (25 of 1502 dermatology patients tested), such evidence of cadmium contact sensitization in humans is inconclusive since most of the patients showing a positive patch test reaction had no evidence of contact with any materials that may have contained the metal. Also, on retesting, most positive reactions could not be reproduced (47). The same resulted from a survey of dental technicians and patients for intolerance to denture materials, when the cohort was tested with the denture material series recommended by the German Contact Dermatitis Research Group: Although most frequently positive reactions were seen to cadmium chloride, there was no evidence for relevance of these reactions as none were more than borderline positive (48). The possibility of human sensitization to the metal arises from previous practice of cadmium pigments being used as coloring agents in dental prosthesis materials, where the final product contained on the order of 200 ppm of the metal (49). In fact, yellow cadmium sulfide pigment as it is used in tattooing has been reported to cause photoallergic reactions; upon exposure of the tattooed skin to sunshine, the yellow areas developed wheals (50).

Animal studies also failed to induce cadmium hypersensitivity by the guinea pig maximization test (51). The immunotoxicity of cadmium was demonstrated in mice, with cadmium augmenting (36) or inhibiting (37) an immediate-type response to an allergen in a dose-dependent fashion. Chronic oral gavage and inhalation of subtoxic doses of cadmium salts in various animal species led to immunotoxic effects in vivo, as noted in significantly depressed host defense mechanisms (34,35). Neutrophil, macrophage and lymphocyte functions were also reduced in vitro (52).

E. SUMMARY

Cadmium is a ubiquitous metal, occurring at low levels throughout the environment and in most biological tissues. A carcinogen, cadmium is one of the most toxic metals, having an extremely long biological half-life which results in gradual accumulation. Chronic exposure can lead to reproductive toxicity in males. The risk of acute overexposure only exists in the industrial setting, primarily through inhalation of volatile cadmium fumes. A correlation between occupa-

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Table 2 Cadmium, as $^{115m}CdCl_2$

Reference: E. Skog and J. E. Wahlberg, A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110m}Ag, ^{115m}Cd, ^{203}Hg, *J. Invest. Dermatol. 43*: 187–192 (1964).
Species: guinea pig in vivo ✓
in vitro ✓
Area: 3.1 cm^2
Vehicle: 1 mL distilled water
Duration of exposure: 5 hr
Analytical method: disappearance of γ radioactivity from the donor chamber; scintillation counting of isolated organs.
Notes:
1. An average absorption value is given for only one of six concentrations; no exact absorption values are reported.
2. ''After the disappearance experiments the organ contents were low.''
3. Organ contents were also measured after other absorption experiments wherein there was much more radioactivity (about 100-fold more); no data were reported but ''the compounds could be found in most of the organs.'' For organ analyses liver, kidney and urine were ''most suitable.''

				Absorption of cadmium	
				% Cd/5 hr <1%	% Cd/5 hr = 1–4%
				Disappearance constant $k < 3.4 \times 10^{-5}$ min^{-1}	Disappearance constant k = $(3.4–13.5) \times 10^{-5}$ min^{-1}
Conc. (M)	Time (hr)	N	pH	$k_p < 6.4 \times 10^{-4}$ cm/hr	$K_p < (6.4–26) \times 10^{-4}$ cm/hr
0.005	5	10	5.6	2/10	8/10
0.08	5	10	5.6	2/10	8/10
0.239[a]	5	10	5.5	0/10	10/10
0.398	5	10	5.4	4/10	6/10
0.753	5	10	5.1	7/10	3/10
4.87	5	10	2.9	10/10	0/10

[a] Mean values for 0.239 M Cd: absorption of cadmium = 1.8%/5 hr and disappearance constant $k = 5.8 \times 10^{-5}$ min^{-1}. For this concentration, $K_p = (1 - e^{-60k})/(3.14) = (1 - e^{-60 \times 0.000058})/(3.14) = 1.1 \times 10^{-3}$ cm/hr (see Chap. 3).

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tional exposure and various forms of cancer, pulmonary carcinoma in particular, has been established. Under circumstances of chronic exposure, cadmium excretion in urine and sweat increases markedly, indicating that metallothionein binding sites have been saturated.

As indicated in skin penetration experiments on animals, a permeability coefficient in vivo has been estimated to be 10^{-3} cm/hr. In vitro, much of the penetrated metal ion is retained in the skin tissue, with only a small portion emerging into the receptor phase. High-level skin exposure in the work environment can result in irritant dermatitis, but no evidence of allergenicity has been found.

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12
Cobalt

A. GENERAL COMMENTS

Cobalt is an essential trace element. It occurs in vitamin B_{12}, which is required by all mammals for hemoglobin synthesis. In humans and all other monogastric species, B_{12} is the only bioavailable form of cobalt, since these organisms are unable to synthesize the vitamin from dietary cobalt. Uptake of cobalt from the diet, therefore, must be in the form of vitamin B_{12} (1), which is also suspected to exacerbate, if not induce, cobalt allergy (2).

Cobalt compounds can pose significant dermal and respiratory toxicity problems, primarily in industry, due to their allergenic potential (3,4) and as causative factors for hard metal disease, a particular form of pneumoconiosis (5). Occurrence of the latter, ranging in form from intense alveolitis to end-stage pulmonary fibrosis, appears to require concomitant exposure to other agents, including tungsten metal dust (6,7). It is prevalent among workers exposed to metal-containing, respirable dust generated in the production and use of hard metal cutting tools. Evidence of cobalt carcinogenicity in humans is inadequate and cannot be evaluated quantitatively (7).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

The human stratum corneum appears to be an effective barrier to cobalt penetration. In guinea pigs, in vivo, on the other hand, percutaneous toxicity of cobalt was considerable, based on the observation that 11 of 20 animals died within 2 weeks of continuous dermal exposure (8). Skin absorption of the metal also depends on the respective counterion with chloride > tartrate > hydroxide (9).

Hair analysis is seen as a suitable method for biological monitoring of exposure to the metal in the work environment, as atomic absorption analysis revealed geometrical mean values for cobalt in exposed workers to be signifi-

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cantly higher than those seen in nonexposed individuals matched by age (96.81 μg/g versus 0.38 μg/g) (10).

In an open patch test study, in which cobalt chloride in isopropanol was applied to the forearm skin of human volunteers, the majority (7/10) showed an immediate (20 min) response that was classified as nonspecific (irritant) urticaria, disappearing within 30 min (11).

C. QUANTITATIVE ABSORPTION DATA

The earliest attempt to measure the quantitative absorption of cobalt across human skin in vivo showed no detectable uptake, i.e., less than about 15% or 13 μg of the applied amount, over an 8-hr period (Table 1) (12). In rabbits and guinea pigs, however, measurable cobalt was absorbed in as little time as 1 hr.

The in vivo absorption of cobalt chloride by guinea pigs was measured for

Table 1 Cobalt, as ''cobalt chloride labeled with Co^{56}'' and as ''a solution of . . . Co^{56}''

Reference: O. Nørgaard, Investigations with radioactive nickel, cobalt and sodium on the resorption through the skin in rabbits, guinea-pigs and man, *Acta Derm. Venereol.* (*Stockh.*) *37*:440–445 (1957).
Species: rabbits, guinea pigs, humans in vivo ✓
in vitro ✓
Area: ''the center'' of a 25-cm^2 area on an animal's back; on the human forearm ''the center'' of an area corresponding to the window (area = 1.77 cm^2) of the Geiger-Müller tube (59)
Vehicle: 10 μL evaporated to dryness and then covered with plastic film.
Concentration: 0.3%, 5% $CoCl_2$
Analytical method: ^{56}Co measured in blood, in urine, and on skin with a Geiger-Müller counter

Notes:
1. In the human experiments, there was no measurable loss of ^{56}Co in 8 hr from 10 μL of either 0.3% or 5% cobalt chloride deposited on the dorsal forearm.
2. In a similar experiment with a rabbit, half of the radioactivity from 10 μL of 5% cobalt chloride was gone from the site of application in 2 hr.
3. The experiments with rabbits and guinea pigs showed that ^{56}Co appeared in the blood and urine of all five animals—as quickly as within 1 hr in a rabbit. The animals were prepared for the experiment by clipping the hair and then applying a depilatory to the area of exposure. The skin pretreatment renders the value of the data difficult to assess.
4. Flux and K_p values cannot be calculated from the data.

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seven concentrations from 0.005 M to 3 M by Skog and Wahlberg (Table 2) (13). For all concentrations combined, only a third of the individual values were above the sensitivity limit, which corresponds to a permeability coefficient of 6.4×10^{-4} cm/hr (see Chap. 3). The absorption rate for some animals at each concentration fell below the limit, so that no average rate could be calculated accurately, but overall for the cobaltous ion the apparent permeability coefficient was below 6.4×10^{-4} cm/hr. Even at 0.239 M $CoCl_2$, the concentration with the highest absorption percentage, 4 of 10 values were below the minimum.

Subsequently, both from crystalline cobaltous chloride in petrolatum and

Table 2 Cobalt, as $^{58}CoCl_2$

Reference: E. Skog and J. E. Wahlberg, A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110m}Ag, ^{115m}Cd, ^{203}Hg, *J. Invest. Dermatol. 43*: 187–192 (1964).

Species: guinea pig in vivo ✓
in vitro ✓

Area: 3.1 cm^2 on the back of each animal

Vehicle: water, 1 mL

Analytical method: disappearance of γ radioactivity from the donor chamber as measured by scintillation counting

Note: No average values are given; no exact absorption values are reported; the absorption was reported as <1% of the dose in 48 of the total of 71 experiments; in the remaining 23 cases the absorptions were reported as being either in the range 1.0–1.9% or 2.0–2.9%.

			Absorption	
			% Ag/5 hr <1%	% Ag/5 hr = 1–4%
			Disappearance constant, $k < 3.4 \times 10^{-5}$ min^{-1}	Disappearance constant, $k = (3.4–13.5) \times 10^{-5}$ min^{-1}
Conc. (M)	Time (hr)	pH	$K_p < 6.4 \times 26\ 10^{-4a}$ cm/hr	$K_p = (6.4–26) \times 10^{-4a}$ cm/hr
0.005	5	5.7	6/10	4/10
0.080	5	5.5	7/10	3/10
0.154	5	5.4	7/11	4/11
0.239	5	5.4	4/10	6/10
0.398	5	5.2	9/10	1/10
0.753	5	5.1	5/10	5/10
3.00	5	3.8	10/10	0/10

[a] For the methods of calculating K_p, see Chap. 3.

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from two aqueous solutions, Wahlberg (14) again found in vivo guinea pig permeability coefficients for cobalt in the range 10^{-4}–10^{-3} cm/hr subject to the limit of detection (Table 3). The differences in absorption between vehicles were not statistically significant.

In spite of low permeability coefficients, the in vivo percutaneous toxicity of cobalt in guinea pigs was considerable; 11 of 20 animals died within 2 weeks of continuous exposure (through 3.1 cm^2 of skin) to 2 mL of 0.239 M $CoCl_2$ (Table 4) (8). By intraperitoneal injection of the same amount, the mortality was 60% in 6 hr and no greater in 7 days. The flux reported, <157 nmol/hr/cm^2 for 0.239 M $CoCl_2$, was taken from the earlier work (13) and corresponds to the permeability coefficient at the limit of detection.

Table 3 Cobalt, as $^{58}CoCl_2$

Reference: J. E. Wahlberg, Vehicle role of petrolatum. Absorption studies with metallic test compounds in guinea pigs, *Acta Derm. Venereol.* (*Stockh.*) *51*:129–134 (1971).
Species: guinea pig in vivo ✓
in vitro ✓
Area: 3.1 cm^2 on each animal's back
Vehicle: petrolatum; distilled water
Analytical method: disappearance of γ radioactivity from the donor chamber as measured by scintillation counting
Notes: In $CoCl_2$-petrolatum mixtures, the $CoCl_2$ crystals were less than 0.3 mm.

Conc. of $CoCl_2$	Time (hr)	N	Disappearance constant $k \cdot 10^5$ min^{-1}	Flux of Co (μg/cm^2/hr)	$K_p \cdot 10^{4a}$ cm/h From k	$K_p \cdot 10^{4a}$ cm/h From flux	Vehicle
2% (0.154 M)	≥5	10	(3.1–5.2)[b]	(3.0–5.0)[b]	(5.9–9.9)	(3.3–5.5)	1 mL distilled water
5.69% (0.438 M)	≥5	10	(3.3–4.7)[b]	(9.0–12.8)[b]	(6.3–9.0)	(9.9–14.1)	1 mL distilled water
2%	≥5	10	(1.3–4.0)[b]	(0.6–1.9)[b]	(2.5–7.6)	(0.7–2.1)	0.5 mL petrolatum
2%	≥5	10	(1.1–3.8)[b]	(1.1–3.7)[b]	(2.1–7.3)	(1.2–4.1)	1 mL petrolatum
5.69%	≥5	10	(3.2–5.3)[b]	(8.7–14.5)[b]	(6.1–10.1)	(9.6–16.0)	1 mL petrolatum

[a] See Chap. 3; values from some guinea pigs were below the limit of sensitivity; the average value would be within this range.
[b] For this reference only, K_p values calculated from k are 79–81% larger than those calculated from flux although, of course, both kinds of data should yield the same value. For the methods of calculating K_p, see Chap. 3.

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Table 4 Cobalt, as $CoCl_2$

Reference: J. E. Wahlberg, Percutaneous toxicity of metal compounds, *Arch. Environ. Health 11*:201–204 (1965).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 2 mL water, 0.239 M
Analytical method: bioassay ($N = 20$)
Notes:
1. Of 20 animals, 11 died within 2 weeks and no more died in the next 4 weeks; an intraperitoneal administration of the same amount of the same solution caused 6 deaths out of 10 guinea pigs in the first 6 hr after injection and no more in the remainder of the week.
2. Considering the mean in vivo rate of percutaneous absorption of $CoCl_2$ (reported as <0.157 μmol/hr/cm^2) and the toxicity of $CoCl_2$ administered intraperitoneally, the authors found the 11 deaths following percutaneous absorption ''somewhat surprising,'' i.e., higher than might have been expected.
3. If the above mean in vivo absorption rate is used, then the in vivo $K_p = <157/0.239 \times 10^9 \times 10^3 = <657 \times 10^{-6}$ cm/hr $= <6.6 \times 10^{-4}$ cm/hr.

In vitro experiments using 0.085 M $CoCl_2$ found human skin to be only one-half to one-third as permeable as guinea pig skin (Table 5) (15). There were higher permeabilities for both tissues in the first 5 hr of exposure (4.4×10^{-4} cm/hr for human and 12.2×10^{-4} cm/hr for guinea pig) than in any later interval up to 48 hr. In the 24- to 36-hr period permeabilities were less than one-twenthieth those of first 5 hr. The average in vitro permeability for guinea pig skin was higher than in vivo but within a factor of 2.

The hazards of cobalt contamination in the nuclear power industry prompted what may be the last, albeit not recent, published investigation of cobalt's percutaneous absorption (9). A much larger fraction of cobalt, deposited as microgram or submicrogram amounts of different salts, was absorbed in vivo by damaged rat and guinea pig skin than by intact skin. Three cobalt compounds were administered in vivo to rat and guinea pig skin following various pretreatment conditions (Table 6). Guinea pigs with irradiated skin but nearly recovered epidermis renally excreted only 1–2% of tracer amounts of cobalt chloride in 24 hr. For rats with normal skin, absorption of cobalt chloride was similar; only 1–3% of about 50 μg/cm^2 appeared in urine in 2 days. That was about the same as the absorption of cobalt tartrate through the intact skin of guinea pigs. Most cobalt tartrate was absorbed in 24 hr through stripped guinea pig skin and an intermediate fraction was absorbed through skin from which the hair had been plucked. Although stripping the skin resulted in the renal excretion of about 24%

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Table 5 Cobalt, as $^{58}CoCl_2$

Reference: J. E. Wahlberg, Percutaneous absorption of sodium chromate (^{51}Cr), cobaltous (^{58}Co), and mercuric (^{203}Hg) chlorides through excised human and guinea pig skin, *Acta Derm. Venereol. (Stockh.) 45*:415–426 (1965).
Species: guinea pig, human in vivo
in vitro ✓
Area: 3.1 cm^2
Vehicle: 1 mL distilled water
Concentration: 0.085 M $CoCl_2$
Analytical method: disappearance of γ radioactivity from donor chamber and/or analysis of receptor solution
Notes: No data were reported for the analysis of Co in receptor solution.

	Disappearance constant, $k \cdot 10^5$ min^{-1}		$K_p \cdot 10^{4a}$	
Interval hours	Guinea pig fresh	Human abdominal stored[b]	Guinea pig	Human
0–5	6.4 ± 0.6	2.3 ± 0.5	12.2	4.4
0–12	4.0 ± 0.4	1.6 ± 0.3	7.63	3.06
12–24	1.2 ± 0.3	0.2 ± 0.1	2.29	0.382
24–36	0.2 ± 0.1	0.1 ± 0.1	0.382	0.191
36–48	0.5 ± 0.2	0.2 ± 0.1	0.955	0.382
0–24	2.6 ± 0.3	0.8 ± 0.1	4.96	1.53
24–48	0.2 ± 0.1	0.1 ± 0.1	0.382	0.191
0–48	1.3 ± 0.2	0.4 ± 0.1	2.48	0.764

[a] For the methods of calculating K_p, see Chap. 3.
[b] Stored at 4°C; isotope was applied 42 ± 3 hr after death.

and 54% of applied cobalt tartrate and cobalt chloride, respectively, in the first 48 hr, cobalt hydroxide was relatively poorly absorbed under the same conditions; only 0.4% of the cobalt from its hydroxide, a rather insoluble compound, appeared in the urine over the same time period.

D. IMMUNOLOGY

Metallic cobalt as respirable dust or in the form of water-soluble ionized salts is allergenic (3,6,16) and photoallergenic (17,18) causing immediate as well as delayed-type skin sensitization, rhinitis, and asthma (19). IgE and IgG antibodies specific to cobalt have been demonstrated (16). A distinct pattern of change in

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serum immunoglobulin levels was seen in response to cobalt-exposed workers: IgA levels were elevated, with a concurrent drop in the level of IgE. These changes may have an impact on the susceptibility of exposed individuals to infection (10). Isolated cases of sarcoidal granuloma induced by hypersensitivity to blue cobalt pigments deposited in tattooed skin have also been described (20).

Cobalt hypersensitivity usually involves occupational exposure, e.g., in metal fabricating, etching, and cement work (21,22). The occurrence of so-called hard metal disease, reactions in the lung parenchyma potentially leading to end-stage pulmonary fibrosis, is associated with such exposure. It is presumed to include an immunological component, possibly in synergy with concomitant exposure to tungsten carbide. Inhalation of cobalt metal dust, particularly among diamond cutters using polishing disks made of highly refined cobalt, has been reported to result in severe asthma through a type I immunological mechanism; cobalt-specific antibodies were observed in workers so affected (16,23–25).

Cobalt salts are formed readily upon skin contact with the metal present in alloys; subsequent absorption of these compounds may cause sensitization. Standard patch testing indicates that the incidence of contact allergy to cobalt (tested with cobalt chloride) reaches 4.2% in the female population (26). Once sensitized, individuals can have an allergic reaction even from simple contact with metallic cobalt (27). Ingestion and injection of cobalt-containing preparations, e.g., vitamin B_{12}, can result in systemic sensitization and generalized cobalt dermatitis (2,28).

In humans, cobalt chloride is a class 3 (moderate) sensitizer on the Magnusson-Kligman scale (29). In the guinea pig, however, cobalt is classified more strongly (class 5) (30). The relatively high incidence of cobalt sensitization reported in the medical literature may reflect false-positive diagnoses. For example, cobalt dermatitis resembles that caused by nickel. Furthermore, cobalt and nickel are closely associated in nature, and because the complete separation of cobalt from nickel is difficult and costly, cobalt patch test materials may likely be contaminated with nickel (31). In a survey of commercial patch test materials, the nickel contaminants found in cobalt test reagents were sufficient to produce false positive reactions in subjects highly sensitive to nickel (32).

In cobalt-allergic subjects, the minimum cobalt level capable of eliciting a positive patch test reaction was 1% $CoCl_2$ (2270 ppm Co) in petrolatum (33). However, such relative insensitivity may reflect poor cutaneous penetration of the salt under the conditions used and it is suggested that for diagnostic purposes intradermal testing may be more appropriate (3).

Preexisting sensitization to chromium or nickel augments cobalt sensitivity, and cobalt dermatitis frequently accompanies nickel and chromium reactions (34). Such occurrence is not thought to be due to cross-reactions, however, but to distinct sensitization from simultaneous exposure to the individual metals, all three of which are routinely incorporated into common metal alloys. Data analysis

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Table 6 Cobalt, as ^{57}Co tartrate, as ^{58}Co tartrate, as ^{60}Co tartrate, as $^{57}CoCl_2$, as $^{58}CoCl_2$, as $^{60}CoCl_2$, as ^{60}Co hydroxide

Reference: M. Suzuki-Yasumoto and J. Inaba, Absorption and metabolism of radioactive cobalt compounds through normal and wounded skins, *Diagnosis and Treatment of Incorporated Radionuclides,* International Atomic Energy Agency, Vienna, 1976, pp. 119–136.

Species: rats, guinea pigs in vivo ✓
in vitro

Vehicle: aqueous

Concentration: in single-isotope ^{58}Co experiments, carrier-free solutions at ~30 μCi/mL, i.e., ~16 nM; 1 mg Co/mL in double-isotope experiments.

Area: 1 cm^2 except for a 1-cm-diameter area (approximately 0.8 cm^2) in the case of irradiated skin

Analytical method: ''animal counter'' to count radioactivity in urine, feces, and whole body

Duration: 1–18 days

Notes:

1. Absorption of Co from the GI tract is poor; this causes orally administered Co to be rapidly excreted mostly in feces, whereas Co absorbed through the skin was mostly excreted in the urine. In guinea pigs 64% of an intravenous dose was excreted in urine in the first day.
2. Although the treated area of skin was initially protected, many of the animals at some time chewed or otherwise disrupted the plastic cover.
3. In the case of irradiated guinea pigs, the applied solution of ^{58}Co was dried before the treated area was covered. For all other experiments this step was unspecified.

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Animal	Skin condition	Compound applied to skin	Dose μCi[b]	Area cm^2	Absorption
Rat $N = 3$	Intact	^{58}Co tartrate	1.5–2	≤3 (est.)	Not reported
Rat $N = 4$	Scratched	^{58}Co tartrate	1.5–2	≤3 (est.)	Not reported
Guinea pig $N = 5$	Semi-skin[a]	^{58}Co chloride	1.2–1.9	0.8	0.9% (av.) in urine for day 1
Guinea pig $N = 4 + 6$	Stripped	^{58}Co tartrate	1.3	1	Rapid, 40% in urine in day 1
Guinea pig $N = 2$	Intact, lightly shaved	^{58}Co tartrate	2.5	1	9.72%[c] in 24 hr (10.42[d]); 1.72% in urine/24 hr
Guinea pig $N = 2$	Stripped	^{58}Co tartrate	2.5	1	73.08%[c] in 24 hr (73.36[d]); 41.4% in urine/24 hr
Guinea pig $N = 2$	Plucked	^{58}Co tartrate	2.5	1	25.33%[c] in 24 hr (24.91[d]); 6.12% in urine/24 hr
Rat $N = 3$	Intact, lightly shaved	$^{60}CoCl_2$	40–60 μL at 1.8 μCi/50 μL and 50 μg Co/50 μL	1	1.1, 2.7, 3.2% in urine in 2 days[e]
Guinea pig $N = 3$	Stripped	$^{57}CoCl_2$ and ^{60}Co tartrate	≈ 50 μL each at 1.8 μCi/μL and 50 μg Co carrier /50 μL	1 cm^2 per chemical	Cl > tartrate 54% and 24% in urine in 2 days, resp.
Guinea pig $N = 2$	Stripped	$^{60}Co(OH)_2$ and ^{57}Co tartrate		1 cm^2 per chemical	Tartrate > hydroxide, 23% and 0.4% in urine in 2 days, respectively

[a] Twenty days after irradiation of skin with 3000 rads by ^{90}Sr β rays. Epidermis but not deeper layers had almost recovered.
[b] ''Carrier-free,'' 5.46×10^{-13} moles Co/μmCi.
[c] Whole-body count after washing + urine + feces.
[d] Sum of feces + urine + GI tract + liver + kidneys & spleen + treated skin + remaining carcass.
[e] Compared to 64–70% for stripped skin, 51–58% after intracutaneous injection, and 43–50% for scratched skin.

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from 1006 patients tested for allergic contact dermatitis (ACD) resulted in 92 (9.1%) positives to cobalt. Of these, 14 were allergic to cobalt only, 52 to cobalt and chromate or cobalt and nickel, and 26 to all three metals (35). Most recently, combined sensitization to cobalt, nickel, and chromium was noted from occupational handling of construction materials manufactured from waste fly ash containing the three metals (36). High incidence of cobalt dermatitis was noted in underground workers employed in the construction of the Channel Tunnel, indicating that cobalt may also be a significant allergen in cement, besides chromium (37).

Systemic contact allergy to cobalt has been observed in both chromium- and nickel-sensitive individuals. A number of systemic sensitization reactions have been ascribed to trace concentrations of cobalt being dissolved from orthopedic implants fabricated from stainless steel or Vitallium, all containing varying percentages of cobalt, nickel, chromium, and molybdenum. Placebo-controlled oral challenge with cobalt resulted in a flare of dermatitis 2 days after ingestion of the metal, confirming the cause of such reactions (38,39). Leaching of metal into the tissues has been identified as a risk factor for sensitization, especially in patients fitted with stainless steel prostheses for total joint replacement, and is described by the term orthopedic dermatitis (40–43). When 50 patients who had received such joint replacements were patch-tested with various metal salts, 8 showed a cell-mediated immune response to cobalt alone (2% cobalt chloride in white paraffin), and 6 to both nickel (5% nickel sulfate in white paraffin) and cobalt. None of the patients sensitive to cobalt alone had a history of metal sensitivity. However, a majority of, but not all, early cases of metal sensitization were correlated with loosening or mechanical failure of the prosthesis (44). Risk of sensitization to cobalt, nickel, chromium, molybdenum, and other heavy metals used in artificial joints appeared to be heightened in the initial stages of prosthetic procedures. However, the development of improved materials and safer alloys has now minimized mechanical failure and abrasion through metal-to-metal contact (45).

Similarly, corrosion products that are released from metal-based dental restorative materials can penetrate into hard and soft oral tissues, and also reach the gastrointestinal tract via the saliva to be absorbed across the intestinal mucosa. Cobalt and various other metals used in dentures and fillings have been reported to cause oral lesions (46) as well as generalized dermatitis in sensitized individuals (47). In epimucosal tests in patients with eczema, an allergic reaction only appears likely when the allergen concentration is 5–12 times higher than that routinely applied in skin tests (48). Patients with a cobalt-containing dental framework developed skin sensitivity to cobalt, even though the oral mucosa appeared normal; when the metal dentures were removed, the eczema regressed and disappeared (49,50). The release of cobalt from alloy constructions in the oral cavity has been investigated by analyzing concentrations of the metal in saliva and

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tongue scrapings of volunteers who had been fitted with a restorative alloy containing the metal. Within 2 days, the median cobalt concentration increased from nondetectable levels prior to insertion to peak values of 6 ng/g wet weight, with a maximum value of 200 ng/g in saliva, and rose from 8 ng/g preinsertion to 47 ng/g to 396 ng/g in tongue scrapings. When compared to the amount of cobalt ingested normally in the diet, the relatively small quantities released from denture materials may represent an allergic risk only for patients already sensitized to cobalt (51).

The persistence or loss of patch test reactivity to cobalt was investigated in cement workers. After a lag time of 2–6 years after the initial finding of hypersensitivity, 40% of those initially sensitized had become negative to patch test challenge with cobalt chloride. Remarkably, in those subjects with persistent reactions, the T cells were identified as memory cells, whereas in the group that became desensitized they were of the suppressor-inducer type. This would seem to indicate that immune selection of memory versus suppressor-inducer T cells affects the course and persistence of contact dermatitis (52).

Allergy due to exposure to cobalt naphthenate, a mixture of cobalt salts of naphthenic acids, which is a catalyst used in polyester resin and rubber manufacture, has been repeatedly observed in industry workers (53,54). Atomic absorption spectrophotometry demonstrated the presence of free residual cobalt in the commercial material, and concomitant positive reactions to cobalt naphthenate as well as cobalt chloride would point to a preexisting sensitivity to cobalt rather than to the naphthenate itself as the primary sensitizer.

In viable human skin, cobalt is selectively taken up by Langerhans cells upon penetration into the epidermis (55). The local lymph node assay (LLNA), developed on mice for the detection of contact allergens (56), has been modified to test for metal hypersensitivity. Under conditions designed to facilitate diffusion to the viable epidermis, cobalt chloride significantly increased lymph node cell proliferation (57,58).

E. SUMMARY

An essential trace element, cobalt is one of the least toxic heavy metals. Primarily in industry, however, exposure to the metal and its salts can result in contact dermatitis and urticaria with late-stage reactions; inhalation of cobalt-containing dusts can lead to pathological changes in the lung parenchyma, ranging from intense alveolitis to pulmonary fibrosis, the so-called hard metal disease. Cobalt salts are among the more important cutaneous and systemic allergens that can also lead to anaphylactoid reactions in sensitized individuals, and the prevalence of such disease in the industrial environment is only exceeded by that caused by platinum salts. While cobalt sensitivity often occurs simultaneously with allergy

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to other metals, most notably nickel and chromium, this is not a cross-sensitization phenomenon. Certain cobalt salts can also cause irritant dermatitis. Quantitative in vitro penetration experiments using human skin indicate an apparent K_p in the range of 10^{-4} cm/hr.

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13
Chromium

A. GENERAL COMMENTS

An essential trace metal in its trivalent form, chromium in low concentrations is environmentally ubiquitous and vital for several important biological processes. At high concentrations, on the other hand, particularly in the hexavalent state, chromium is toxic, genotoxic, and carcinogenic in animals and humans. Many barriers exist, however, that limit the uptake and distribution of the element in its more toxic hexavalent form. The most effective detoxification process is its reduction to poorly absorbed Cr^{3+} in the reducing environments found in body tissues and fluids such as saliva, gastric juice, gastrointestinal bacteria, blood plasma, or the liver (1). Direct inhalation of hexavalent chromium compounds resulting from certain industrial activities appears to comport the highest risk of tumor formation (2). As observed with lead, chromium can exchange between plasma and contacting bone surfaces to be ultimately incorporated into actively mineralizing bone (3). Normally, intake of the element occurs through the diet: highest levels are found in thyme (10 mg/g), black pepper (3.3 mg/g), and cloves (1.5 mg/g); the mean level seen in fish and red meat is 0.11 mg/g. Most hexavalent chromium taken in with food is reduced in the acid medium of the stomach to the trivalent form (4,5), severely limiting its ultimate uptake [average, 6.9% (6)]. Trivalent chromium increases glucose tolerance, acting as a cofactor with insulin to promote glucose utilization (7), and enhances lipid synthesis (8). Of the many oxidation states of chromium, Cr(III) and Cr(VI) are the most stable and prevalent in living organisms. Trivalent chromium is the prevalent form of the metal occurring in mineral deposits (chromite ores). An industrial hazard, Cr(VI) is absorbed by oral, respiratory, and skin routes. Many hexavalent chromium compounds, mostly oxides or oxohalides, are corrosive, primary irritants (9), sensitizers (10), and respiratory carcinogens (11,12). In vitro cytotoxicity studies using human keratinocytes show that Cr(VI) is more toxic than Cr(III), attributed to its greater membrane diffusivity and oxidizing potential. Cr(VI) can be reduced to the less damaging Cr(III) species by cysteine or glutathione, and

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most notably by ascorbic acid (13). Dependent on pH, Cr(VI) exists as either the chromate (pH > 6) or dichromate form (pH = 2–6). On a relative scale of metal carcinogenicity, based on human and animal data, and calculations from acute exposure predictive models, chromium compounds were ranked highest in activity, comparable to nickel (14).

Significant industrial exposure to chromium occurs in metal plating, wood preservation, leather tanning, chromium pigment production, and stainless steel manufacture. Such processes can generate local, artificially high, and hazardous concentrations of chromium. The average levels occurring in nature are 200 ppm on land and 1–2.5 ppb in seawater (15). The presence of large chromium residues at several sites in the US near chromite-processing facilities has prompted a risk assessment study regarding chromium bioavailability following oral, dermal, and inhalation exposures. Although high levels of chromium were found in soils from these areas (Cr^{6+}, 180 ppm; Cr^{3+}, 2879 ppm), the calculated human health risks associated with such deposits were assessed to be negligible. Also, the risk of both nonspecific dermatitis and allergic sensitization from contact with these levels was determined to be insignificant (16).

B. SKIN ABSORPTION AND REACTIVITY

Chromium adheres to the skin and traces of the metal are tenaciously retained following even the most casual contact with common, everyday objects. Elevated levels are seen in the skin up to 3 weeks following a single inunction of potassium dichromate (0.5%) in petrolatum (17). The baseline chromium content of 0.62 ppm measured in control skin was seen to increase to a mean value of 163 ppm after a single contact with a door handle made of a chromium-containing alloy (18).

Both Cr(III) and Cr(VI) penetrate the skin, with hexavalent chromium (as CrO_4^{2-}) usually the better penetrant. From a dermatotoxicological perspective, Cr(III) compounds are the least problematic because of their poor aqueous solubility and the apparent inability of Cr(III) ultimately to penetrate biological membranes. Cr(VI), present as chromate $(CrO_4)^{2-}$ and dichromate $(Cr_2O_7)^{2-}$, is the most injurious to human health. Certain hexavalent chromium compounds appear both toxic and carcinogenic because the chromate oxy anion is rapidly taken up by the cell through an anion transport system. Particulate hexavalent species are the most carcinogenic, which may be due to their persistence in tissues. To a degree, Cr(VI) applied to the skin is converted to Cr(III) during passage through the skin. On sustained application, however, Cr(VI) passes through the skin unchanged, an indication that a given tissue mass has only a limited capacity to reduce the chromate ion (21).

Trivalent chromium salts with different counterions exhibit different pene-

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tration rates when topically applied in vivo in humans at equivalent concentrations (22). Undamaged human skin was found to be virtually impenetrable to Cr(III) sulfate and only marginally permeable to Cr(III) nitrate. When the stratum corneum is removed, all Cr(III) salts penetrate the skin equally well.

Chromate and dichromate ions (Cr^{6+}) do not complex with organic substances, whereas Cr^{3+} shows a strong affinity for epithelial and dermal tissues, forming stable complexes (24). When fresh guinea pig serum was incubated with radiolabeled chromium trichloride and then dialyzed to remove nonconjugated material, 1 mL serum contained 1.5 mg chromium chloride. When the serum was incubated with hexavalent chromium (as the dichromate), under the same conditions, no measurable amount of chromium was bound to proteins (25). This helps to explain the observation that the flux of Cr(III) compounds through protein-containing membranes is low.

Percutaneous absorption of chromium compounds is also pH-dependent. Above pH 7, absorption of Cr(III) salts decreases, as does their solubility. With increasing pH, chromate permeation increases to the point where it corresponds to that of water. This is also consistent with the decreased barrier properties of skin that have been observed with increasing pH (21,26,27).

t-Butyl chromate, a hexavalent chromium compound used in the chemical and petroleum industries as a catalyst, represents a particular occupational hazard due to the ease of its skin penetration; as such it has an ACGIH ''skin'' notation [see Appendix for comments on ''skin'' notation (29)]. In vitro distribution of chromium in human skin following topical application has been assessed by x-ray emission analysis. After exposure for 18 hr to a dichromate solution at 4°C, the highest level of Cr (40 ± 12 ppm) was seen at a depth of 30 μm (30).

C. QUANTITATIVE ABSORPTION DATA

Percutaneous absorption data for chromium encompasses in vivo and in vitro data, nearly all published in the 1960s and 1970s, for both Cr(III) and Cr(VI) salts in three species: humans, guinea pigs, and rats.

In the earliest report (31), guinea pigs clearly absorbed chromium, both Cr(III) and Cr(VI), since radioactivity was found in various tissues after application of ^{51}Cr solutions. The permeability coefficients could not be calculated because the total amount of absorbed chromium and other experimental parameters were not reported (Table 1).

Guinea pigs exposed to sodium chromate for 24 hr (27) absorbed ^{51}Cr with an average permeability coefficient for the entire period of at least 2.3×10^{-4} cm/hr (Table 2). After 24 hr chromium-51 that was still in the skin at the site of application was not included in the ''absorbed'' amount although some of that chromium may have passed beyond the stratum corneum.

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Table 1 Chromium, as ^{51}Cr(VI) and ^{51}Cr(III)

Reference: E. Schwarz and H. W. Spier, Die percutane Resorption von 3- und 6-wertigem Chrom (Cr^{51}). Zur Pathogenese des Kontaktekzems, *Archiv Klin. Exp. Dermatol. 210*:202–215 (1960).
Species: guinea pigs in vivo ✓ application
in vitro
Area: 3.8 cm^2
Vehicle: aqueous, with and without 0.5–1% sodium lauryl sulfate
Concentration: 0.25–2.5 mg%
Duration of exposure: 18 hr to 8 days
Analytical method: gamma counting of ^{51}Cr
Notes:
1. The exact amount of Cr applied was not reported.
2. The exact concentration of Cr applied was not reported.
3. The exact volume of Cr solution applied was not reported.
4. The total amount of Cr absorbed was not reported.
5. The Cr content of some organs but not of the whole animal was reported. The tissues with the most radioactivity absorbed from undamaged skin were lymph nodes, particularly the node closest to the application site.

To test the influence of other factors, skin of other guinea pigs in three groups of three animals was pretreated before exposure to ^{51}Cr-chromate. Quantitation of absorption was not complete in these groups and many standard deviations were 50–125%, but some comparisons can be made with the control group. Pretreatment with sodium hydroxide resulted in more absorption of chromium. Pretreatment with chromate to sensitize the skin resulted in less absorption. Treatment with a protective cream led to ambiguous results; the concentration of ^{51}Cr in blood was lower than for controls but the amount of ^{51}Cr in excreta was higher.

Based on analyses of the dermis, early in vivo experiments (32) of 0.5–12 hr duration with human volunteers exposed to aqueous potassium dichromate led to permeability coefficients for chromium that ranged from 0.1×10^{-4} cm/hr to 9×10^{-4} cm/hr (Table 3).

In a series of reports wherein any decrease of measured ^{51}Cr radioactivity from solutions in contact with skin was considered to be the result of percutaneous absorption (see also Chap. 3), many of the average permeability coefficients covering the first 5 hr of contact with Cr(III) and Cr(VI) were 10×10^{-4} to 20×10^{-4} cm/hr (Tables 4–9). This was the case for both in vivo and in vitro experiments with guinea pigs and for in vitro experiments with human skin as well. Some of these data were included in another report (33) for comparison with data for other metals. For concentrations at both the low and high ends of

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Table 2 Chromium, as $Na_2{}^{51}CrO_4$

Reference: A. Czernielewski, K. Brykalski, and D. Depczyk, Experimental investigations on penetration of radioactive chromium (Cr^{51}) through the skin, *Dermatologica 131*:384–396 (1965).
Species: guinea pigs in vivo ✓
in vitro
Area: 4 cm^2, dorsal
Vehicle: 0.9% NaCl, 0.1 mL (covered)
Concentration: 0.00015% Cr, i.e., 0.0029 M (15 μg/dose)
Duration of exposure: 24 hr
Analytical method: Uptake was based on scintillation counting of the radioactivity of the applied dose and 24 hr later the radioactivity of the skin and site-covering material. Urine, feces, blood, and eight other tissues were also analyzed.
Notes:
1. The animals were shaved 24 hr before the chromium was applied.
2. Of the applied dose (2,353,000 impulses/min), the average amount absorbed, i.e., not recovered at the site of application, by nine control animals (no pretreatment of the skin) was 23% (530,000 impulses/min) in 24 hr. No similar accounting of the dose was made for the treated animals for which there was only analysis of the excreta and some organs to indicate absorption relative to controls.

Pretreatment	Permeability coefficient $10^4 \cdot K_p$ cm/hr	Number of animals
None	2.3	9
0.5 N NaOH, 3 min/day, 7 days	>2.3	3
Protective cream	≥2.3[a]	3
Sensitized with 0.5% $Na_2Cr_2O_7$, 2 min/day, 14 days	<2.3[b]	3

[a] Total chromium was less in organs but more in excreta than for controls.
[b] Without considering that binding and retaining chromium in skin at the site of application may be greater after sensitization.

the concentration range, permeability coefficients were often below the limit of sensitivity, e.g., less than 6.4×10^{-4} cm/hr.

Over a 10,000-fold range in concentration, in the first publication of the series (34), (0.00048–4.8 M), the average permeability coefficient for sodium chromate absorption in vivo in guinea pigs for the first 5 hr peaked at 26×10^{-4} cm/hr for 0.261 M chromate (Table 4). For concentrations of 0.005 M or lower and 0.753 M or higher the 5-hr permeability coefficients were $<6.5 \times 10^{-4}$ cm/hr, but they could not be calculated exactly because some data were below

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Table 3 Chromium, as $K_2{}^{51}Cr_2O_7$

Reference: J. W. H. Mali, W. J. van Kooten, F. C. J. van Neer, and D. Spruit, Quantitative aspects of chromium sensitization, *Acta Derm. Venereol.* (*Stockh.*) *44*: 44–48 (1964).
Species: human in vivo ✓
in vitro ✓
Area: in vivo, 1-cm^2 patches on forearm and 0.28 cm^2 cylinders (54) at unspecified location
in vitro, 8 cm^2 from the back (cadaver).
Vehicle: water 0.02 mL 0.25% Cr (0.048 M) on covered patches;
0.2, 0.3 mL in covered cylinders
3 mL 0.004% (0.00077 M) Cr in vitro
Analytical method: gamma counting of ^{51}Cr with a Geiger-Müller counter
Notes: The outer layers of the stratum corneum, i.e., the stratum corneum that was removed by four tape strippings, contained 4–16% of the radioactivity applied by patches in vivo. Of the 0.35 μg ^{51}Cr remaining in the skin, only 0.02 μg was present after 1 day, but 0.001 μg or more was measurable for about 6 weeks.

Cr conc. M	In vivo/in vitro	Time period (hr)	Penetration constant[a] $10^4 \cdot K_p$ cm/hr
0.048	in vivo	12	0.11[b]
0.048	in vivo	12	0.22
0.00077	in vitro	120	0.54[c]
0.081	in vivo	6	9[d]
0.081	in vivo	12	7.2[d]
0.081	in vivo	0.5	4[d]
0.040	in vivo	1	5.0[d]
0.006	in vivo	6	1.1[d]
0.081[P]	in vivo	0.5	13[d]
0.040[P]	in vivo	1	6.1[d]
0.013[P]	in vivo	3	4.0[d]
0.006[P]	in vivo	6	0.4[d]

[a] These values reported by Mali et al. are based only on the amount of chromium in the dermis at the end of the experiment.
[b] Mali et al. also reported that 22 μg Cr ''permeated into the inner body'' and on that basis they calculated a penetration constant of $\leq 20 \times 10^{-4}$ cm/hr ($\leq 5.5 \times 10^{-7}$ cm/sec).
[c] Mali et al. also reported 0.017 μg Cr in the dermis and 0.002 μg Cr in the saline beneath the dermis; the sum of those amounts (compare to the applied amount, 120 μg) would correspond, by our calculations, to a much lower K_p (0.005×10^{-4} cm/hr).
[d] The details of these experiments appear in an earlier paper (54) but without the ''penetration constants'' presented in this subsequent report.
[P] After pilocarpine iontophoresis to provoke sweating.

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Table 4 Chromium, as $Na_2{}^{51}CrO_4$

Reference: J. E. Wahlberg and E. Skog, The percutaneous absorption of sodium chromate (^{51}Cr) in the guinea pig, *Acta Derm. Venereol*. (*Stockh*.) *43*:102–108 (1963).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 1 mL distilled water
Duration of exposure: 5 and 19 hr
Analytical method: (a) decrease of γ radiation in 5 hr from the donor chamber detected by Geiger-Müller counter and (b) measurement of ^{51}Cr in organs after 5 and 19 hr exposure.
Notes:

Absorption of sodium chromate

		Absorption, # animals/data range			
	a.|	% Cr/5 hr			
	b.|	Disappearance constant, $10^5 \cdot k$, min^{-1}			
	c.|	Permeability coefficient, $10^4 \cdot K_p$, cm/hr			
	a.|	<1%	1–2.9%	3–5.9%	
	b.|	<3.4	3.4–10.1	10.2–20.5	$10^4 \cdot K_p$ cm/hr
Conc. (M)	pH	c.| <6.5	6.5–19.2	19.4–38.9	mean
0.00048	7.0	8	2	—	<6.5
0.005	7.9	7	3	—	<6.5
0.017	8.2	—	8	2	16.8
0.080	8.2	—	10	2	13.2
0.261	8.4	—	2	8	26.4
0.398	8.4	—	6	4	17.9
0.753	8.4	8	1	1	<6.5
1.689	8.5	7	3	—	<6.5
4.87	8.5	6	4	—	<6.5

1. The maximum chromium flux (690–725 nmol/hr/cm^2) was reached from 0.261 M and 0.398 M solutions; further increases in molarity did not further increase flux.
2. Radioactivity was found in largest amounts in blood, kidneys, urine, and lymph glands. Lesser amounts of ^{51}Cr were found in liver, lungs, heart, spleen, and bone. More ^{51}Cr was found after 19 hr than after 5 hr. No exact amounts were reported.

the limit of detection. Absorption of chromium was confirmed with the detection of radioactive chromium in various tissues after 5 and 19 hr of exposure. These data also appear in a comparison of the absorption of salts of various metals (33).

Chromium chloride, in an extension of these experiments (19), was absorbed only slightly more slowly than chromate. Compared at eight concentra-

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Table 5 Chromium, as $Na_2{}^{51}CrO_4$ and $Cr^{51}Cl_3$

Reference: J. E. Wahlberg and E. Skog, Percutaneous absorption of trivalent and hexavalent chromium. A comparative investigation in the guinea pig by means of ^{51}Cr, *Arch. Dermatol. 92*:315–318 (1965).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 1 mL water
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:

Mean absorption of Cr[a]

	$CrCl_3$			Na_2CrO_4		
Conc. (M)	$10^5 \cdot k$ min^{-1}	%/5 hr	$10^4 \cdot K_p$ cm/hr	$10^5 \cdot k$ min^{-1}	%/5 hr	$10^4 \cdot K_p$ cm/hr
0.00048	[b]	[b]	[b]	[b]	[b]	[b]
0.005	[b]	[b]	[b]	[b]	[b]	[b]
0.017	7.4	2.2	14	8.8	2.6	17
0.080	5.4	1.6	10	6.9	2.1	13
0.126	6.1	1.8	12	—	—	—
0.239	6.8	2.0	13	7.6	2.3	15
0.239[c]	[b]	[b]	[b]	—	—	—
0.261[d]	6.5	1.9	12	13.7	4.0	26
0.398[d]	[b]	[b]	[b]	9.4	2.8	18
0.753	[b]	[b]	[b]	[b]	[b]	[b]
1.689	—	—	—	[b]	[b]	[b]

[a] 10–12 animals for each concentration of each salt.
[b] Some individual values were below the sensitivity limit and no average was calculated (see also Chap. 3.
[c] After the solution had been stored for 1 month. As their aqueous solutions age, trivalent chromium compounds frequently form less soluble polymeric species.
[d] Differences between the salts were statistically significant at these two concentrations.

1. For the two highest and the two lowest concentrations the detection of ^{51}Cr in various organs demonstrated that absorption of chromium had occurred.
2. The pH values of the solutions were quite different: chloride, 1.8–3.8; chromate, 7–8.5.
3. From chromate, the maximum flux of chromium was found to be about 690–725 nmol/hr/cm^2 at 0.261–0.398 M, and from the chloride the maximum was about 315–330 nmol/hr/cm^2 at 0.239–0.261 M $CrCl_3$. Higher concentrations of either compound did not increase chromium flux.
4. Some of the chromate data appeared earlier (34).

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tions, its absorption was statistically different only at two concentrations (Table 5). For 0.261 M solutions, where both K_p values could be calculated, chromium was absorbed about half as fast from Cr(III) ($K_p = 12 \times 10^{-4}$ cm/hr) as from chromate ($K_p = 26 \times 10^{-4}$ cm/hr).

The same basic technique was applied to in vitro chromate absorption with human as well as guinea pig skin (35). The in vitro permeability of human skin (abdominal) to 0.034 M chromate was only 13–33% that of guinea pig skin, depending upon the interval compared (Table 6). In the first interval, 0–5 hours,

Table 6 Chromium, as $Na_2{}^{51}CrO_4$

Reference: J. E. Wahlberg, Percutaneous absorption of sodium chromate (^{51}Cr), cobaltous (^{58}Co), and mercuric (^{203}Hg) chlorides through excised human and guinea pig skin, *Acta Derm. Venereol.* (*Stockh.*) *45*:415–426 (1965).
Species: guinea pig, human in vivo
in vitro ✓
Area: 3.1 cm^2
Vehicle: 1 mL distilled water
Concentration: 0.034 M $Na_2{}^{51}CrO_4$
Duration of exposure: 48 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes: For the in vivo percutaneous absorption of 0.034 M chromate by guinea pigs (gp) the permeability coefficient was 12×10^{-4} cm/hr.

Absorption of chromium from sodium chromate

	Disappearance constant, $10^5 \cdot k$, min^{-1} (Mean ± SE)		Permeability coefficient $10^4 \cdot K_p$ cm/hr	
Interval (hr)	Guinea pig fresh	Human abdominal stored (48 hr, 4°C)	Guinea pig	Human
0–5	5.7 ± 0.5	1.6 ± 0.4	11	3.1
0–12	3.9 ± 0.3	0.9 ± 0.2	7.4	1.7
12–24	1.4 ± 0.6	0.2 ± 0.1	2.7	0.38
24–36	0.9 ± 0.2	0.3 ± 0.2	1.7	0.57
36–48	0.8 ± 0.2	0.1 ± 0.1	1.5	0.19
0–24	2.4 ± 0.2	0.5 ± 0.1	4.6	0.96
24–48	0.8 ± 0.2	0.2 ± 0.1	1.5	0.38

1. The average gp/human ratio for K_p values was 5.3 for 0.034 M Na_2CrO_4 over 8 time periods between 0 and 48 hr; this was the highest ratio among the three salts examined: $CoCl_2$, $HgCl_2$, and Na_2CrO_4. It was also a considerably higher ratio than those (0.8–2.2) reported for other concentrations in a later paper (36) for which the human skin had not been stored.

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the permeability coefficient for human skin was 3.1×10^{-4} cm/hr. For guinea pigs the permeability coefficient was about the same in vivo as in vitro, i.e., 12×10^{-4} cm/hr and 11×10^{-4} cm/hr, respectively, for 0–5 hr.

Subsequently, the in vitro comparisons were expanded to include human mammary skin and chromium chloride (36). There was no significant difference between absorptions of sodium chromate and chromium chloride for the four tested concentrations either through human or guinea pig skin (Table 7). Average permeability coefficients for were 9×10^{-4} cm/hr to 15×10^{-4} cm/hr for human mammary skin and 11×10^{-4} cm/hr to 20×10^{-4} cm/hr for guinea pig skin. While guinea pig skin was usually more permeable than human mammary skin, the differences were not consistent (guinea pig/human = 0.8–2.2) and not large (average ratio = 1.42). Extrapolation to predict the in vivo absorption for humans was considered impossible.

The pH value of chromate solutions influences the percutaneous absorption of chromium. For 0.017 M and 0.239 M solutions, chromate was absorbed slightly more rapidly by guinea pigs from solutions with pH values of 6.5 or higher at the time of application than from more acidic solutions (20). Permeability coefficients with the higher pH solutions were 12×10^{-4} cm/hr to 17×10^{-4} cm/hr (Table 8). At the lower pH values, six of seven average permeability coefficients, including uncertainty from values below the limit of sensitivity, were 6×10^{-4} to 12×10^{-4} cm/hr. The pH values of both unbuffered and buffered solutions in contact with skin changed during the 5 hr of the absorption experiments. As frequently happened with this technique at high concentrations of other metal salts, absorption of chromium on a percentage basis from 1.689 M chromate was often too low to measure precisely and, in this case, not noticeably dependent on pH.

Two factors may explain this observation. First, at the higher pH values the chromium-containing species is CrO_4^{2-}, whereas at the acidic pH values, chromium exists as the larger, less diffusible ion, $Cr_2O_7^{2-}$. The other factor to consider is a change in the skin structure. If alkalinity had caused permanent damage thereby rendering the skin more permeable, then the increase in absorption should be dependent on the degree of alkalinity; that was not the case.

With the aim of more reliable dermatological patch testing, Wahlberg incorporated sodium chromate crystals and solution into petrolatum and, again by the disappearance method in guinea pigs, measured and compared the absorption of chromium (37). Although absorption was higher from aqueous solutions than from petrolatum, the differences were not statistically significant (Table 9). Chromium flux increased with concentration (0.4, 1.87, 5.59%) and with increasing volume applied (0.25, 0.5, 1.0 mL). The permeability coefficients, with one lower exception, were in the range 9×10^{-4} to 17×10^{-4} cm/hr.

In a toxicological comparison (28) of solutions of three chromium compounds (0.239 M Cr) on guinea pig skin for 4 weeks, sodium dichromate caused

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Table 7 Chromium, as $Na_2{}^{51}CrO_4$ and $^{51}CrCl_3$

Reference: J. E. Wahlberg, Percutaneous absorption of trivalent and hexavalent chromium (^{51}Cr) through excised human and guinea pig skin, *Dermatologica 141*:288–296 (1970)
Species: human, guinea pig in vivo
in vitro ✓
Area: 3.1 cm^2
Vehicle: water
Duration of exposure: 5 and 24 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:

Absorption of chromium measured during 5 hr

		Human mammary skin			Guinea pig back skin			
Conc. (M)	Chromium salt	$10^5 \cdot k^a$ min^{-1}	Flux nmol $Cr/cm^2/hr$	$10^4 \cdot k^b$ min^{-1}	$10^5 \cdot k^a$ min^{-1}	Flux nmol $Cr/cm^2/hr$	$10^4 \cdot k^b$ min^{-1}	K_p ratio gp/human
0.017	Na_2CrO_4	4.5 ± 0.9	15	8.6	9.8 ± 3.0	32	19	2.18
0.017	$CrCl_3$	7.6 ± 1.7	25	14	10.5 ± 2.2	35	20	1.38
0.080	Na_2CrO_4	6.8 ± 0.9	105	13	7.8 ± 2.4	121	15	1.15
0.080	$CrCl_3$	6.6 ± 0.7	102	13	10.6 ± 0.8	164	20	1.6
0.239	Na_2CrO_4	7.2 ± 2.6	333	14	7.2 ± 1.4	333	14	1.00
0.239	$CrCl_3$	5.5 ± 1.5	254	10	11.1 ± 1.4	513	21	2.02
0.398	Na_2CrO_4	7.8 ± 2.2	601	15	6.0 ± 1.4	462	11	0.77
0.398	$CrCl_3$	5.8 ± 1.0	447	11	7.5 ± 0.9	578	14	1.29

[a] Disappearance constant, $k = (\log cpm_0 - \log cpm_1)/\{(0.4343)(t_1 - t_0)\}$ where cpm_0 and cpm_1 are counting rates at time t_0 and time t_1, respectively.
[b] $K_p = (1 - e^{-60k})/3.1$ = flux/concentration (See also Chap. 3)

1. Absorptions of chromium from chromate and from chloride were not significantly different.
2. The rate of disappearance gradually decreased over 24 hr.
3. The ratios between in vivo and in vitro absorption of chromium [in vivo data were reported in another paper (19)] were not constant at different concentrations for either salt in guinea pigs. Most differences between guinea pig in vivo and in vitro rates were not statistically significant.
4. Extrapolation to absorption in man in vivo was considered not possible.

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Table 8 Chromium, as $Na_2{}^{51}CrO_4$

Reference: J. E. Wahlberg, Percutaneous absorption from chromium (^{51}Cr) solutions of different pH, 1.4–12.8. An experimental study in the guinea pig, *Dermatologica 137*:17–25 (1968).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 1 mL water
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:

Percutaneous absorption of Na_2CrO_4

Conc. (M)	pH, buffered/unbuffered	Disappearance constant, mean ± SE[a] $10^5 \cdot k$ min^{-1}	Permeability coefficient, $10^4 \cdot K_p$ cm/hr
0.017	1.5 unbuffered	(5.0–6.1)[c]	(9.5–11.6)
0.017	1.5 buffered	(7.1–8.1)[c]	(3.5–15.4)
0.017	2.9 unbuffered	(5.0–6.3)[c]	(9.5–12.0)
0.017	5.6 unbuffered	6.3 ± 0.7	12.0
0.017	6.5 unbuffered	7.7 ± 1.1	14.7
0.017	8.0 unbuffered	8.8 ± 1.4	16.8
0.017	8.0 buffered	8.5 ± 1.4	16.2
0.017	12.0 unbuffered	6.5 ± 1.7	12.4
0.017	12.0 buffered	7.8 ± 1.2	14.9
0.239	1.4 unbuffered	(5.6–6.6)[c]	(10.7–12.6)
0.239	3.0 unbuffered	(3.4–4.8)[c]	(6.5–9.2)
0.239	5.6 unbuffered	(3.1–4.4)[c]	(5.9–8.4)
0.239	6.6 unbuffered	8.1 ± 1.0	15.4
0.239	8.4 unbuffered	7.6 ± 0.7	14.5
0.239	12.8 unbuffered	8.0 ± 1.4	15.2
1.689	1.4 unbuffered	(1.7–3.8)[c]	(3.2–7.2)
1.689	3.0 unbuffered	(4.4–5.8)[c]	(8.4–11.1)
1.689	5.6 unbuffered	(4.5–5.9)[c]	(8.6–11.3)
1.689	6.6 unbuffered	(5.5–6.1)[c]	(10.5–11.6)
1.689	8.5 unbuffered	(1.7–4.1)[c]	(3.2–7.8)
1.689	12.7 unbuffered	(4.6–6.0)[c]	(8.8–11.4)

[a] Ten animals per average.
[b] Calculated from relative absorption, *k*.
[c] Values from some guinea pigs were below the limit of sensitivity. See also Chap. 3.

1. For 0.017 and 0.239 M solutions, the permeability of chromate-dichromate was higher in the initial pH range 6.5–12.8 than at lower values (1.4–5.6). Chromium at the higher pH values exists as the smaller $CrO_4{}^{2-}$, ion whereas in the lower pH range the larger dichromate ion, $Cr_2O_7{}^{2-}$, predominates.
2. The pH values of both buffered and unbuffered 0.017 M chromate solutions changed toward neutral by 0.16–3.25 pH units during the period of contact with guinea pig skin.

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Table 9 Chromium, as $Na_2{}^{51}CrO_4$

Reference: J E. Wahlberg, Vehicle role of petrolatum. Absorption studies with metallic test compounds in guinea pigs, *Acta Derm. Venereol.* (*Stockh.*) *51*:129–134 (1971).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: petrolatum with crystals (crystals were <0.3 mm), petrolatum with aqueous solution, 1 mL water
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Duration of exposure: ≥5 hr
Notes:
1. Absolute absorption increased with increasing volume of petrolatum and with increasing concentration, whether in water or petrolatum.
2. Absorption was higher from water than from petrolatum but differences were not statistically significant.

Mean absorption (± SE) of Cr from petrolatum and from water

Conc. Na_2CrO_4 (%)	N	Relative absorption $10^5 \cdot k$ (min^{-1})	Flux (μg Cr/cm^2/hr)	Permeability coefficient[a] $10^4 \cdot K_p$ cm/hr	Vehicle
0.4	10	8.8 ± 1.4	1.5	17	1 mL water
0.4	10	(5.4–6.4)[b]	(0.2–0.3)	(10.3–12.2)	0.25 mL petrolatum
0.4	10	7.4 ±0.7	0.6	14	0.5 mL petrolatum
0.4	10	7.4 ± 1.2	1.3	14	1 mL petrolatum
0.4[c]	10	(5.9–6.6)[b]	(1.0–1.1)	(11.3–12.6)	1 mL petrolatum
1.87	12	6.9 ± 0.6	5.6	13	1 mL water
1.87	10	(4.8–6.2)[b]	(1.0–1.2)	(9.2–11.8)	0.25 mL petrolatum
1.87	10	(3.4–5.4)[b]	(1.4–2.2)	(6.5–10.3)	0.5 mL petrolatum
1.87	10	(5.4–5.8)[b]	(4.3–4.7)	(10.3–11.1)	1 mL petrolatum
5.59	10	7.6 ± 0.7	18.3	15	1 mL water
5.59	10	(1.5–3.6)[b]	(0.9–2.2)	(2.9–6.9)	0.25 mL petrolatum
5.59	10	(3.9–5.2)[b]	(4.7–6.3)	(7.4–9.9)	0.5 mL petrolatum
5.59	10	(5.1–6.1)[b]	(12.3–14.7)	(9.7–11.6)	1 mL petrolatum

[a] Calculated from relative absorption, *k*. [b] Values from some guinea pigs were below the limit of sensitivity. See also Chap. 3. [c] Prepared from a 50% aqueous solution of Na_2CrO_4.

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more deaths than sodium chromate, and chromium chloride caused the fewest (Table 10). Compared to control animals, chromium chloride had no effect on weight gain.

A very different technique in rats revealed that chromium from a potassium dichromate solution was absorbed with a permeability coefficient of 14×10^{-4} cm/hr (Table 11), a value that is very similar to those found by Wahlberg for guinea pigs (38). In this case, the rats' tails were immersed for 1 hr in dichromate solution; the absorption was determined by analyzing the urine for chromium and then applying an excretion correction factor derived following intravenous injection of chromium. Not addressed was the question of whether a correction factor derived from intravenous administration was valid for absorption through the skin.

Comparable results were obtained for sodium chromate in similar experiments using ^{51}Cr for quantitation (38). For 0.01 M, 0.1 M, and 0.2 M chromate, the apparent average permeability coefficients were 8×10^{-4} to 15×10^{-4} cm/hr (Table 12). The ratio of chromium excreted in urine to chromium excreted in feces was different following intravenous administration (3:2) compared to percutaneous absorption (1:7). It was not specified as to whether the fraction of chromium excreted in urine is smaller following dermal absorption or whether the fraction in feces is larger.

The permeability of human skin to chromium was investigated in later work. In a comparison of the permeability of human epidermis to four chromium compounds (23), chromate and three Cr(III) salts, the relative penetration from

Table 10 Chromium, as Na_2CrO_4, $CrCl_3$, $Na_2Cr_2O_7$

Reference: J. E. Wahlberg, Percutaneous toxicity of metal compounds. A comparative investigation in guinea pigs, *Arch. Environ. Health 11*:201–204 (1965).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 2 mL water, 0.239 M Cr (12.4 mg Cr/mL)
Duration of exposure: 4 weeks
Analytical method: death, weight change
Notes:
1. No absorbed amounts were measured.
2. Mortality ranking (20 animals/compound): $Na_2Cr_2O_7$ (12 deaths) > Na_2CrO_4 (4 deaths) > $CrCl_3$ (1 death). All deaths occurred within 3 weeks.
3. For animals exposed to $CrCl_3$, weight changes were ''parallel . . . with that of animals exposed to distilled water and controls.'' The effects of chromate and dichromate on growth were not reported. Body weights were measured for 8 weeks.
4. Minimum lethal dose of $CrCl_3$ in mice = 801 mg/kg i.v. (*Merck Index*, 9th ed.).

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Table 11 Chromium, as $K_2Cr_2O_7$

Reference: T. Dutkiewicz and M. Przechera, Estimation of chromium compounds absorption through the skin (in Polish, English summary), *Ann. Acad. Med. Lodz. 8*: 189–193 (1966).
Species: rat in vivo ✓
in vitro
Area: approximately 20 cm^2, tail
Vehicle: 9% solution, aqueous
Duration of exposure: 1 hr
Analytical method: Measurement of chromium excreted in urine during 10 days following exposure; values were corrected appropriately using a factor determined from the renal excretion of chromium following intravenous dosing.
Notes:
1. After the exposure period the tails were removed from the solution and the remaining solution was removed from the tails, but there are no details about washing or wiping the tails.
2. In the first 11 days after intravenous dosing 24% of the chromium dose was measured in the urine.
3. Four protective creams decreased the chromium flux to as little as 8% of the flux through unprotected skin.

Percutaneous absorption of chromium

Cr conc. (mg/mL)	Number of rats	Chromium flux $mg/cm^2/hr$[a]	Permeability coefficient $10^4 \cdot K_p$ cm/hr
31.8	8	0.043	14

[a] The unit of flux in Table 1 of this reference is incorrect (Dutkiewicz, personal communication).

0.2 M KCl at pH 7 was as follows: chromate $>$ $CrCl_3$ $>$ $Cr(NO_3)_3$ $>$ $Cr_2(SO_4)_3$. At pH 5 and 9, absorption of Cr(III) may be higher. For all compounds, the concentration of chromium in the receptor chamber increased nonlinearly and very rapidly after the first 2 hr. The apparent permeability coefficients (accurate calculations are impossible) for chromate are estimated to be 30×10^{-4} to 2000×10^{-4} cm/hr (Table 13). The latter value is so high as to suggest that there may have been damage to the epidermis.

Analyses of human skin biopsies after exposure to dichromate (39) indicated that the concentration of chromium in the skin had reached a steady state within 5 hr (Table 14). The concentration profile as a function of depth in the skin was the same after 5 hr as at 24 and 72 hr. For most biopsies there were two peaks of chromium concentration in the profile. One peak corresponded to the lower part of the epidermis, about 60–80 μm from the surface. The other

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Table 12 Chromium, as Na_2CrO_4

Reference: T. Dutkiewicz and J. Konczalik, The kinetics of Cr^{51} distribution and elimination in rats, *Proceeding of 15th International Congress on Occupational Health* (*Vienna*, *19–24 September 1966*), 281–284.
Species: rat in vivo ✓
in vitro
Area: about 10 cm^2 on tail (Dutkiewicz, personal communication).
Vehicle: aqueous
Concentration: 0.01–0.2 M
Duration of exposure: 1 hr
Analytical method: scintillation counting of ^{51}Cr in urine and feces ''as well as on the basis of chromium body retention.''
Notes:
1. The only percutaneous absorption data in the report are in a statement that ''the resorbed amount of chromium oscilated [sic] from 7.36 to 136.5 μg'' and in a graph of concentration vs. absorption rate in units of μg/cm^2/hr. While 7.36 μg absorbed by 10 cm^2 of tail skin in 1 hour from the most dilute solution, i.e., 0.01 M chromate, correlates well with the graph, the value of 136.5 μg is not found on the graph although it could be the value for an individual rat. There are no individual data points marked on the graph. The following is based on the graph:

Absorption of chromium

Conc. (M)	Flux μg/cm^2/hr	Permeability coefficient $10^4 \cdot K_p$, cm/hr
0.01	0.8	15
0.1	7.7[a]	15
0.2	8.0	8

[a] In a personal communication, Dutkiewicz reports 125 μg Cr absorbed from 0.1 M Na_2CrO_4; for 10 cm^2, this value corresponds to an apparent permeability coefficient of 25×10^{-4} cm/hr.

2. The ratio of chromium in the urine to chromium in feces depended on the route of administration of chromate. After intravenous administration, the ratio $Cr_{urine}:Cr_{feces}$ was 3:2, but following topical application most chromium was excreted in feces and the ratio was 1:7.

peak was at 180–300 μm deep in the skin. A chromium(III) complex with glycine also delivered chromium into the skin, but the resulting dermal concentrations were about 75% lower than those following dichromate application.

On the basis of chromium recovered after 15, 30, and 60 min from solutions placed on the forearms of human volunteers (40), apparent permeability coefficients for chromate were 10×10^{-4} to 35×10^{-4} cm/hr depending on the

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Table 13 Chromium, as chloride, sulfate, nitrate and chromate

Reference: M. H. Samitz, S. Katz, and J. D. Shrager, Studies of the diffusion of chromium compounds through skin, *J Invest. Dermatol. 48*:514–520 (1967).
Species: human (epidermis, autopsy) in vivo
in vitro ✓
Area: not reported. There is a photograph of the in vitro cell without a scale and without dimensions but, based on the relative proportions, the area of exposed epidermis appears to be about 1 cm^2.
Vehicle: unspecified volume of 0.2 M aqueous KCl; pH 5, 7, 9. Our estimate (based on the relative proportions in the photograph) is 6 ± 3 mL.
Concentration: 1, 5, 10, 50, 100 mM chromate; none reported for other salts.
Duration: 5 hr
Analytical method: scintillation counting of radioactivity in the receptor solution
Notes:
1. It is impossible to calculate flux or K_p without area and volume.
2. There is no volume reported for the lower chamber and the diffused Cr is reported as μmol/mL.
3. If we use the estimates for exposed area (A) and receptor volume (V), the concentration of Cr in the receptor chamber allows estimates of flux (J) ÷ CrO_4^{2-} concentration (c) with the limitation that the flux increases greatly after 3 or 4 hours. If $A \approx 1$ cm^2 and $V \approx 6$ cm^3, then the estimates of J/c for all concentrations range from 30×10^{-4} to 2000×10^{-4} cm/hr. The values are highly dependent on time and not so dependent on CrO_4^{2-} concentration.
4. Comparisons of percutaneous absorption of the Cr(III) salts (after 5 hr):
   pH 5, pH 9: $Cl^- > SO_4^{2-} > NO_3^-$
   pH 7: $Cl^- > NO_3^- > SO_4^{2-}$
5. Penetration of chromate was higher than the penetration of chromium from similar solutions of these trivalent salts. Depending on concentration and pH, chromium nitrate is ''3 to 30 times less diffusible'' than chromate. Chromic chloride ''is only 2 to 4 times less diffusible.''
6. For all four salts at pH 7, penetration (μmol/mL) increased with increasing concentration; however, the trivalent salts permeated 50–75% less than chromate. ''Diffusion [of trivalent Cr] from all solutions appeared to be lower at pH 7 than at pH 5 or 9.''
7. The temperature for the experiments was 37°C.
8. Samitz et al. remark that Cr(III) in 0.2 M KCl can form $CrCl_4^-$ complexes in solution and speculate that perhaps only these anionic species cross the epidermis. They present ion exchange chromatographic evidence to support the existence of such species.

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Table 14 Chromium, as $K_2Cr_2O_7$ and chromium(III)–glycine complex, mainly $\{Cr(gly)_{3-6}^{3+}\}$

Reference: S. Lidén and E. Lundberg, Penetration of chromium in intact human skin in vivo, *J. Invest. Dermatol*. *72*:42–45 (1979).
Species: human (back) in vivo ✓ exposure
in vitro
Area: 79 mm^2 was exposed; biopsies were 3 mm in diameter
Duration of exposure: 5, 24, 48 hr of exposure
Vehicle: yellow petrolatum (0.5% $K_2Cr_2O_7$), water (0.05% and 0.5% $K_2Cr_2O_7$, 0.5% Cr–glycine complex)
Analytical method: graphite furnace atomic absorption spectroscopy of tangential sections of punch biopsies of skin
Notes:
1. The Cr content of skin was measured after different periods of exposure but there is no measurement of flux. Although ''the curves [pg Cr/section vs. depth] representing the individual biopsies were largely congruent,'' the absolute Cr contents varied widely between biopsies.
2. Sections (25 μg average dry weight) parallel to the skin surface were cut from processed biopsies with a microtome set to 10 μm.
3. The Cr content seems to have reached a steady-state value by 5 hr; after each of the three exposure periods, the Cr content was similar.
4. The first section, corresponding to the stratum corneum, had the highest amount of Cr. Below the stratum corneum there were usually two peaks of Cr concentration, one 60–80 μm deep in the lower part of epidermis and the papillary layer of dermis, and a smaller second peak 180–300 μm deep in ''the upper part of the mid-dermis.''
5. With 0.5% $K_2Cr_2O_7$ the Cr levels in skin were 2–7 times higher (up to 9000 pg/section) from petrolatum than from aqueous solution.
6. Chromium amounts in the skin (about 100–2600 pg/section) were higher from 0.5% solutions than from 0.05% solutions, but by less than a factor of 10.
7. Chromium levels from chromium-glycine (0.5% Cr) were lower than levels from $K_2Cr_2O_7$.
8. In sections from the control skin, the chromium content was 0.4–2 ppm, i.e., 10–50 pg per section of average weight.

length of contact and on the concentration applied (Table 15). Longer periods of contact and/or lower concentrations led to lower permeability coefficients. These values are similar to results found in rats (Tables 11 and 12). The dependence of apparent permeability coefficients on time of contact and on concentration was also observed by Wahlberg and associates for guinea pigs and for other metals.

Human abdominal skin was less permeable in vitro to an unspecified Cr(III) salt than it was to chromate (41). Although it was reported that ''calculated solute

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Table 15 Chromium, as Na_2CrO_4

Reference: B. Baranowska-Dutkiewicz, Absorption of hexavalent chromium by skin in man, *Arch. Toxicol. 47*:47–50 (1981).
Species: human, forearm in vivo ✓ exposure
in vitro
Area: 20.4 cm^2
Vehicle: water
Duration of exposure: 15, 30, 60 min
Analytical method: spectrophotometry or atomic absorption spectrometry to find the difference between the applied dose and the recovered Cr
Notes:
1. The amounts absorbed were 8–220 μg of chromium(VI).
2. There were 27 experiments with seven volunteers.
3. The fraction absorbed was highest (7.7–23%) from the lowest concentration and lowest (3.4–10.6%) from the highest concentration.
4. ''The absorption rate decreased as time of exposure increased.''
Using the data in figure 1 of the paper, average flux values and apparent calculated K_p values (Cr flux ÷ Cr concentration) are as follows:

	15 min exposure		30-min exposure		60-min exposure	
Na_2CrO_4 (M)	Flux μg/cm^2/hr	$10^4 \cdot K_p$ cm/hr	Flux μg/cm^2/hr	$10^4 \cdot K_p$ cm/hr	Flux μg/cm^2/hr	$10^4 \cdot K_p$ cm/hr
0.01	1.8	35	1.5	29	1.1	21
0.1	10.6	20	8.2	16	6.5	12
0.2	15.9	15	13.4	13	10	90.6

5. These fluxes are similar to those in rats during exposures of 1 hr to the same applied concentrations (38).

steady-state flux values, J_s, were found to increase linearly for CrO_4^{2-},'' the flux did not increase in direct proportion to the concentration. Increasing the concentration of chromate by a factor of 420, i.e., from 0.005 M to 2.1 M, led to a flux that was multiplied 35-fold. The resulting apparent permeability coefficients, therefore, are 2.3×10^{-4} cm/hr for 2.1 M chromate and 27×10^{-4} cm/hr for 0.005 M chromate (Table 16). For Cr(III) the permeability coefficients are about an order of magnitude smaller, 4×10^{-4} cm/hr with 0.006 M Cr(III) and 0.13×10^{-4} cm/hr for 1.2 M Cr(III). This is another case for which K_p was dependent on concentration.

In the most recent in vitro determinations of skin's permeability to chromium (21), Cr(VI) was again absorbed about 10 times faster than Cr(III). However, this investigation, used full-thickness human abdominal skin for periods of

Table 16 Chromium, as $CrO_4^{2-}/Cr_2O_7^{2-}$ and Cr(III)

Reference: J. J. Fitzgerald and T. Brooks, A new cell for in vitro skin permeability studies–chromium(III)/(VI) human epidermis investigations (abstract), *J. Invest. Dermatol. 72*:198 (1979).
Species: human, abdominal epidermis in vivo
in vitro ✓
Area: not reported
Vehicle: aqueous; pH 2.43–7.00 for Cr(VI) in $NaClO_4$
Duration: not reported
Concentration: $CrO_4^{2-}/Cr_2O_7^{2-}$, 10^{-5}–2 M
Cr(III), 5×10^{-4}–1.5 M
Analytical method: atomic absorption
Notes: Steady-state flux values for CrO_4^{2-} at pH 7.0 were stated to increase linearly from 0.07 to 472 nmol · cm^{-2} · hr^{-1}, but for the only two or three reported concentrations flux did not rise in direct proportion to the increase in molarity.

Chromium ion	Conc. (M)	Flux, (steady-state) nmol · cm^{-2} · hr^{-1}	Permeability coefficient, $10^4 \cdot K_p$, cm/hr
CrO_4^{2-}	0.00001	0.07	70
CrO_4^{2-}[a]	0.005	13.4	27
CrO_4^{2-}[a]	2.1	474	2.3
Cr^{3+}[b]	0.006	2.4	4.0
Cr^{3+}[b]	1.2	16	0.13

[a] At pH 7.0.
[b] At varied pH.

48–190 hr and estimates of permeability coefficients were much smaller than those in Table 16. It is noteworthy that no chromium was detected in the recipient phase following the application of Cr(III) for 168 hr or following the application of dichromate for 48 hours (Table 17).

To estimate permeability coefficients, we have considered the absorbed chromium to be the sum of the chromium in the epidermis, the dermis, and the recipient phase. Most of the chromium in the skin was in the epidermis. From dichromate, permeability coefficients for chromium ranged from 0.15×10^{-4} cm/hr (0.17 M, 168 hr) to 4.3×10^{-4} cm/hr (0.034 M, 190 hr). At about pH 3, from chromium chloride or chromium nitrate, the range for Cr(III) was from 0.013×10^{-4} cm/hr (0.34 M, 168 hr) to 0.41×10^{-4} cm/hr (0.034 M, 190 hr). At pH values of 8.3 and 10.1, the solubility of Cr(III) is low and, though the flux of chromium is small, the apparent permeability coefficients were larger, i.e., about 2×10^{-4} cm/hr.

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Table 17 Chromium, as $K_2Cr_2O_7$, $CrCl_3 \cdot 6H_2O$, $Cr(NO_3)_3 \cdot 9H_2O$

Reference: B. Gammelgaard, A. Fullerton, C. Avnstorp, and T. Menné, Permeation of chromium salts through human skin in vitro, *Contact Dermatitis 27*:302–310 (1992).
Species: human abdominal, full-thickness (frozen) in vivo
in vitro ✓
Area: 0.7, 1.8 cm^2
Vehicle: aqueous, 556 µL/cm^2; various pHs
Concentration:

$K_2Cr_2O_7$:	0.125%, 0.25%, 0.5% (0.034 M Cr), 2.5% (0.17 M Cr)
$CrCl_3 \cdot 6H_2O$:	0.9% (0.034 M), 4.5% (0.17 M Cr)
$Cr(NO_3)_3 \cdot 9H_2O$:	1.36% (0.034 M Cr)

Duration: 48–190 hr
Analytical method: atomic absorption spectroscopy and, for some samples, ion chromatography
Notes:
1. Capacitance measurements were used to establish the integrity of the skin to be used.
2. All experiments were done in triplicate.
3. The permeability of skin to ^3H_2O changed only slightly during experiments, which lasted 160–180 hr. Permeability coefficients of ^3H_2O were somewhat dependent on pH: 8.8×10^{-4} cm/hr at both pH 5 and pH 6.8, 18.6×10^{-4} cm/hr at pH 8.8, and 24.6×10^{-4} cm/hr at pH 10.
4. Recipient phases were centrifuged and passed through a 0.22-µm ''Micropore'' filter (Millipore Corp.) before analysis. Whether this caused a loss of any chromium that had penetrated the skin is not discussed.

Chromium content after 190 hr of exposure to three chromium salts

Cr salt 0.034 M Cr	pH	Epidermis µg Cr/cm^2	Dermis µg Cr/cm^2	Recipient phase µg Cr permeated/cm^2	$10^4 \cdot K_p$[a] cm/hr
$K_2Cr_2O_7$	4.2	134	12.0	0.037	4.3
$CrCl_3$	3.0	12.5	1.30	None detected	0.41
$Cr(NO_3)_3$	2.8	9.6	0.33	None detected	0.30

[a] These values, determined by including the Cr in tissue, assume that none of the Cr in the epidermis would be lost by desquamation.

Effect of concentration on Cr content after 48 hr of exposure to $K_2Cr_2O_7$

$K_2Cr_2O_7$ conc. %	Epidermis µg Cr/cm^2	Dermis µg Cr/cm^2	Recipient phase µg Cr permeated	$10^4 \cdot K_p$[a] cm/hr
0.125	2.4	0.28	None detected	1.26
0.25	3.1	0.24	None detected	0.79
0.5 (0.034 M Cr)	5.7	0.89	None detected	0.78

[a] These values, determined by including the Cr in tissue, assume that none of the Cr in the epidermis would be lost by desquamation.

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Table 17 Continued

Chromium content after 168 hr of exposure to Cr(VI) and Cr(III)

Chromium salt	Conc. %	Epidermis μg Cr/cm^2	Dermis μg Cr/cm^2	$10^4 \cdot K_p$[a] cm/hr
$K_2Cr_2O_7$	0.5	11.1 ± 2.9	2.54 ± 0.33	0.46
$K_2Cr_2O_7$	2.5	21.1 ± 8.6	0.73 (N = 2)	0.15
$CrCl_3 \cdot 6H_2O$	0.9	1.54 ± 0.68	0.37 ± 0.16	0.064
$CrCl_3 \cdot 6H_2O$	4.5	1.53 ± 0.39	0.43 ± 0.24	0.013

[a] These values, determined by including the Cr in tissue, assume that none of the Cr in the epidermis would be lost by desquamation.

Effect of pH on Cr content after 168 hr of exposure to $CrCl_3$

pH	Conc. (M)	Epidermis μg Cr/cm^2	Dermis μg Cr/cm^2	$10^4 \cdot K_p$[a] cm/hr
3.0	0.034	1.54 ± 0.68	0.34 ± 0.16	0.063
8.3	0.00036	0.16 ± 0.06	0.60 ± 0.36	2.4
10.1	0.00014	0.16 ± 0.06	0.11 ± 0.06	2.2

[a] These values assume that none of the Cr in the epidermis would be lost by desquamation.

1. ''No detectable amounts of chromium could be found in the recipient phase after application of even large amounts of chromium(III) to the skin. . . .''
2. After application of 0.034 M dichromate at pH 10 to the skin, chromium(III) was found in the recipient phase. This represented a decreasing fraction of the total permeated Cr as time of diffusion increased.
3. Application to skin of buffered solutions (pH 5, 6.8, 8.8, and 10) of 0.5% $K_2Cr_2O_7$ for about 160 hr resulted, during the last half of the experiment, in an accelerating accumulation of chromium in the recipient phase from the alkaline solutions. Between 80 and 160 hr the percutaneous chromium rose from less than 2 to nearly 40 μg/cm^2 at pH 8.8 and from about 10 to over 80 μg/cm^2 at pH 10. In the same situation, the flux of tritiated water, while it was dependent upon pH, was relatively constant over the entire 160 hours. At pH 5 and 6.8 the passage of chromium into the receptor chamber was less than 3 μg/cm^2 over 160 hr.

Recently, an in vivo human experiment evaluated the percutaneous chromate absorption in perhaps the most realistic situation to date (42). Four volunteers were immersed below the shoulders for 3 hr in water containing 22 mg Cr/L (0.21 mM $K_2Cr_2O_7$) to simulate exposure that might occur by swimming in contaminated water. Chromium in the blood and plasma was elevated only on the first day after exposure. Based on the amount of chromium excreted in the urine during the 5 days beginning with the exposure, the absorption of chromium

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Table 18 Chromium, as $K_2Cr_2O_7$

Reference: G.E. Corbett, B.L. Finley, D.J. Paustenbach, and B.D. Kerger, Systemic uptake of chromium in human volunteers following dermal contact with hexavalent chromium (22 mg/L), *J. Exposure Analysis Environ. Epidemiol., 7*(2):179–189, 1997
Species: human in vivo ✓
in vitro
Area: entire body below the shoulders; 11,400–14,300 cm^2 (estimated)
Vehicle: aqueous, 450 gallons
Concentration: 22 mg Cr/L (0.21 mM $K_2Cr_2O_7$)
Duration of exposure: 3 hr
Analytical method: atomic absorption spectrometry of urine, plasma, red blood cells, and exposure solution
Notes:
1. There were four volunteers.
2. Based on the amount of chromium excreted in the urine in 5 days immediately following exposure to dichromate, ''some trivalent chromium [Cr(III)] may have penetrated the skin at a rate of about 3.3×10^{-5} to 4.1×10^{-4} μg/cm^2-h.''
3. Since Cr(VI) in the circulatory system would have produced a sustained elevation of chromium in the red blood cells and since that was not observed, this experiment did ''not result in measurable systemic uptake of Cr in the hexavalent form.''
4. No analysis was reported for chromium in the skin.

Subject	Chromium excretion before skin exposure (μg/day)	Chromium excreted above background[a] (μg/5 days)	Permeability coefficient $10^4 \cdot K_p$, cm/hr
H1	1.5	17.5	0.184
H4	0.5	1.4	0.015
H5	0.6	3.0	0.034
H7	1.0	2.4	0.031

[a] On the fifth day after exposure, the excretion of chromium by subject H1 had not yet decreased to the preexposure level.

across the skin occurred at fluxes of 3.3×10^{-5} to 4.1×10^{-4} mg/cm^2/hr (Table 18). The corresponding range of permeability coefficients is 0.015×10^{-4} to 0.19×10^{-4} cm/hr. There was no analysis for chromium in the skin.

D. IMMUNOLOGY

1. An Overview

Metallic chromium or chrome-plated objects are not immunogenic (43). When the metal comes in contact with skin, however, traces of the metal are easily

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dissolved by the salt content and acidic microenvironment of the skin. Certain of the derivative salts are allergens, capable of penetrating the skin. Transport via the stratum corneum, and via the hair follicles and the sebaceous glands, has been demonstrated whereas the sweat glands appear to play an insignificant role (31). In the viable epidermis, chromium ions can react with tissue protein to form a complete antigen or induce conformational changes in peptides. Such newly created nonself can cause delayed as well as immediate anaphylactoid-type allergic reactions. The latter is an immune response involving IgE antibody. Type I reactions are on record that have been characterized by late-onset urticaria, angioedema, and bronchospasm on challenge. Others do not conform with a classic IgE-mediated response, showing, for example, negative intradermal skin tests for immediate reaction (44–46). Sarcoidal granulomas due to green chromium pigments (hydrous chromium oxides) deposited in tattooed skin can lead to delayed-type, granulomatous hypersensitivity (47–49).

2. Allergic Contact Dermatitis

Delayed-type hypersensitivity to chromium is a common and growing health problem, resulting from the increasing use of the metal in alloys. Contact eczema caused by chromium compounds is the most common occupational dermatosis after nickel eczema. The voluminous literature dealing with chromium immunology and contact sensitization has been extensively reviewed (50,51). Skin contact with Cr(VI), as it occurs in chromate in alkaline solution [$(CrO_4)^{2-}$], is believed to present the greatest skin sensitizing potential, probably due to the ease with which it can penetrate the skin (52). Chromate or dichromate anion, however, does not complex with protein, a reaction that first requires reduction to the trivalent form. Cr(III) is unequivocally the true hapten responsible for allergic contact sensitization, as only antibodies to trivalent chromium are found in rabbits when the animals are exposed to human erythrocytes sensitized with both $K_2Cr_2O_7$ or $CrCl_3$ (52–56). Histochemically, trivalent chromium ($CrCl_3$ solution) is observed to be selectively taken up by Langerhans cells in viable human epidermis (57). In the epidermis, Cr(III) is believed to bind to tissue protein sulfhydryl groups, forming complete antigen. In vitro studies corroborate this hypothesis; cystine, cysteine, and methionine reduce Cr(VI) to Cr(III) (58–60). Evidence for the reduction of Cr(VI) in the skin was also obtained by measuring the disappearance of dichromate in the presence of various water-soluble skin components. Of the substances tested, lactic acid was the most reactive; chondroitin sulfate and glucose showed slower rates (54). Indeed, it has been estimated that 1 g of skin can reduce approximately 1 mg dichromate to trivalent chromium (58,59).

Due to its affinity for protein, chromium (like Ni) persists at the site of penetration and forms a reservoir (61). Eczematous skin takes up considerably more chromium than normal healthy skin (17). In patients allergic to chromium,

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the minimum elicitation threshold (MET) was 9 ppmbr Cr(VI), 8850 ppm for Cr(III) (62). In another well-controlled study, when 54 presensitized volunteers were patch-tested with serial dilutions of Cr(VI) and Cr(III) , the MET level that sensitized 10% of volunteers was 0.089 mg/cm^2 for Cr(VI) and was, at best greater than 33 mg/cm^2 for Cr(III) (63).

Environmental contamination risk assessment studies indicate that a level of 350–500 ppm Cr(VI) in soil is sufficiently low that elicitation of ACD in subjects (including children) sensitized to Cr(VI), will not occur. Experimentally, under environmental exposure conditions, human sweat does not extract Cr(VI) from the soil to generate concentrations on the skin surface that are sufficient to elicit dermatitis; in fact, it has been demonstrated that less than 0.1 ppm is available even when the soil concentration exceeds 500 ppm (64,65).

Overall, hexavalent chromium salts (chromates and dichromates) rank as the second most common metal-based contact allergens behind nickel, and as the most common inducers of occupational dermatitis (66). In terms of sensitizing potency, based on a repeated patch test protocol in humans, potassium dichromate produced 100% positive reactions, making the salt a class 5 (extreme) sensitizer on the Magnusson-Kligman scale (67). Employees engaged in metal fabrication and cement work are most commonly affected (10).

In construction work, the skin's barrier function can be compromised by the alkalinity and abrasive action of wet cement, thereby facilitating the penetration of chromate. The prevalence of chromium sensitivity in the general U.S. population is about 1.6% (64). Among patients manifesting industrial dermatitis, only 7–10% with chromium hypersensitivity completely cleared over a 3 year period, even after a change in occupation or complete discontinuation of work altogether (68).

The cause of persistence or loss of patch test reactivity to dichromate was investigated on a cohort of cement workers ($n = 180$). Two to six years after the initial diagnosis of hypersensitivity, the circulating T cells were analyzed on rechallenge. Of those initially sensitized, 63% remained positive on repatching with dichromate, whereas 17% of those initially sensitized had become negative. In 20% of the patients the reactivity had decreased. Remarkably, in patients with persistent reactions, the T cells were identified as memory cells, whereas in the group that had become desensitized, they were of the suppressor-inducer type. This indicates that idiosyncratic immune selection of memory versus suppressor-inducer T cells affects the course and persistence of contact dermatitis (69). In another study of so-called cement dermatitis, i.e., a cohort of predominantly chrome-sensitive construction workers, only 6% experienced complete remission over a follow-up period of >10 years (18). Due to the prevalence of chromate sensitivity among construction workers, Danish legislation limiting the content of water-soluble chromate in dry cement to a maximum of 2 mg/kg (2 ppm) was passed in 1983. Control of the composition is achieved by the addition of ferrous

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sulfate to the cement mix to reduce Cr^{6+} to Cr^{3+}. The success of this intervention can be measured in a dramatic decline in chromate dermatitis among cement workers in Denmark (70). To illustrate this point further, construction of the England/France tunnel used European cement, containing ''normal'' levels of Cr(VI), and hundreds of workers developed chromate dermatitis (71). By contrast, for the Denmark/Sweden tunnel, Scandinavian cement was used, in which chromium(VI) had been reduced by Fe^{II}, and no construction worker developed dermatitis (72,73).

While the source of chromate sensitivity in men can usually be identified, unexplained sensitivity among women is common (74). In the case of nickel hypersensitivity, constant reexposure through inhalation of metallic nickel particles suspended in atmospheric aerosols (levels that can reach 0.4 mg/m^3 in urban air) renders remission difficult (75). Atmospheric chromium or chromate has therefore, also been suspected, in the etiology of unexplained chromate allergy. In a comprehensive study, total chromium and hexavalent chromate were determined in air samples collected from metal fabrication sites, downtown city districts, suburban districts, steel mills, and construction sites. Hexavalent chromate was less than 0.3 mg/m^3 in all locations, an indication that it is probably not the source of unexplained chromate sensitivity and that sensitized workers may continue working in an industrial setting provided that skin contact with chromate-containing materials is avoided (76). To reduce the risk of chromate exposure to the general and occupational population, a limit of 0.3 mg/cm^2 has been proposed for hexavalent chromium release to synthetic sweat (77). Animal studies indicate that chromium sensitization has a genetic predisposition (78).

The potential to elicit an allergic reaction in guinea pigs sensitized to trivalent chromium ranks according to the counterion: chloride (highest) > nitrate > sulfate > acetate > oxalate (lowest). This reflects the concentration of free (noncoordinated) metal ion available to form complete antigen with skin proteins (79).

3. Systemic Sensitization

Delayed type eczematous reactions due to systemic exposure can occur following sensitization by the oral, parenteral, or inhalation route. Chronic inhalation exposure to chromium-containing dust can result in such systemic sensitization, and it should be noted that dermatitis occurred in a patient thus sensitized upon dermal reexposure (80). Conversely, flare-up reactions in sensitized individuals upon systemic re-exposure to the allergen have been documented for chromium and other haptens (81). This was also observed in chromate-sensitive guinea pigs, which, upon repeated oral exposure, also showed signs of desensitization (82). Chromium metal, used in alloys for dental restorative materials, has been reported to cause stomatitis and systemic contact allergy (83). The amount of chromium

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dissolving from an orthodontic appliance was determined to be 36 μg/day upon immersion in 500 ppm aqueous NaCl, the average chloride concentration in saliva. This value compares with the 50–200 μg recommended as the daily intake of chromium for adults by the National Research Council Committee on Dietary Allowances. Taken orally, however, this amount does not induce an immune response; in contrast, when steadily released in direct contact with injured mucosa, as can occur from an orthodontic appliance, a comparable quantity of chromium can sensitize a naïve organism or elicit a reaction (e.g., a flare of skin eczema) in sensitized individuals (84). Several systemic sensitization reactions have been ascribed to trace concentrations of chromium dissolved from orthopedic implants, fabricated from stainless steel or Vitallium, which contain varying percentages of chromium, nickel, cobalt, and molybdenum (85). Such leaching of metal into the tissues has been identified as a risk factor for primary sensitization, especially in patients fitted with stainless steel prostheses, and is known as orthopedic dermatitis (86–88). Earlier cases were usually correlated with loosening or mechanical failure of the prostheses (89). The risk of sensitization to chromium, nickel, cobalt, molybdenum, and other heavy metals, used in trace amounts in artificial joints, was greater in the early days of total joint replacement, prior to the development of improved materials and safer alloys, which now minimize mechanical failure and abrasion through metal-to-metal contact (90).

4. The Dietary Factor

Oral challenge of chromate-sensitive patients with as little as 0.05 mg chromate elicited positive skin reactions in all tested (85,91). Chromium hypersensitivity often yields a very poor prognosis, with chromium dermatitis never completely or permanently clearing because of constant reexposure through skin contact with commonplace chromium-plated materials or through systemic intake of chromium in the diet (17,92–94). Recurrence of allergic reactions can be somewhat controlled by avoiding foods known to contain significant amounts of chromium; levels of 0.5–3.4 mg/g have been reported in certain spices, tea leaves, nuts and dried fruits. An attempt to define an oral threshold challenge dose to provoke dermatitis in chromium-sensitive subjects was not successful because of the variable bioavailability and pharmacokinetics of chromium from different foodstuffs (6,94).

5. Cross-Reactivity

In view of the high prevalence of chromium hypersensitivity, possible cross-reactivity between the two principal oxidation states of the metal, and to the other common allergens, cobalt and nickel, has also been examined. In clinical tests, Cr^{3+} compounds failed to elicit a reaction in patients with proven sensitivity to

hexavalent chromium; thus, cross-sensitivity could not be demonstrated between the two different valence states of the metal (95). In mice, it has been shown that cobalt and nickel do not cross-react with chromium, indicating involvement of different antigenic epitopes in these metals (96). Similarly, guinea pigs sensitized to Cr^{6+} or Co^{2+} did not show simultaneous reactivity to the other metal upon challenge in the maximization test (97).

6. Diagnostic Models for ACD

In animals, epicutaneous sensitization with trivalent chromium has proven difficult. The concentration of chromium sulfate (Cr^{3+}) required to achieve the same degree of sensitization as with chromate (Cr^{6+}) is 20-fold higher (98). On the other hand, the same degree of contact sensitivity resulted from intradermal application of either potassium dichromate or chromium sulfate, demonstrating that the stratum corneum is the barrier preventing percutaneous diffusion of trivalent chromium (99). For this reason, barrier creams for metal-sensitive individuals, particularly in the occupational setting, have been designed to reduce the valence of chromate to the less soluble and less permeable trivalent ion (100).

The local lymph node assay (LLNA) was developed on mice for the detection of contact allergens, based on the fact that they induce T-lymphocyte proliferative responses (101,102). In addition, chemicals that cause respiratory allergy are usually found to elicit positive responses also (103). Under conditions designed to facilitate diffusion to the viable epidermis, potassium dichromate gave variable results in the literature (104,105). It significantly increased murine lymph node cell proliferation in a number of tests (106–108), but was only marginally positive in others with this reputedly potent human sensitizer (109,110).

In addition to the conventional in vivo patch test for the diagnosis of suspected chromate allergy in humans, the in vitro lymphocyte transformation and proliferation test has also been validated as a useful diagnostic tool for this purpose (111).

7. Immunological Contact Urticaria and Occupational Asthma

Chromium-sensitive patients showed immediate-type reactions on testing with $CrCl_3$ as well as Cr^{3+} bound to human serum albumin, suggesting the presence of antibody to the metal cation as well as the chromium-denatured protein. Several patients also demonstrated divergent immune responses: they reacted with a delayed-type eczema upon patch testing with dichromate, indicative of simultaneous activation of Th1- and Th2-type cells (112,113). It was notable that a patient giving a positive RAST result did not react with urticaria on prick testing with chromate (45). Inhalation exposure to chromate or chromium trioxide fumes

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or dust (hexavalent chromium), which are generated in industrial activities such as welding, is known to cause asthma, a result of allergy rather than a direct toxic effect on the airways (85,114–116). Bronchial provocation of patients, with a history of contact dermatitis and asthma due to occupational exposure to chromium salts, with dichromate solution showed immediate, dual, and late reactions; no reaction was seen in asthmatic controls previously unexposed to dichromate. Considering that the same patients were skin prick test–negative and that a bronchial challenge was uninhibited by sodium cromoglycate (cromolyn), a pathophysiological mechanism other than an IgE-mediated reaction is possible (117). More typically with respect to type I allergic reactions, pretreatment of a different cohort of asthmatic stainless steel welders with disodium cromoglycate and betamethasone mitigated the asthmatic reaction (118).

E. SUMMARY

Chromium is both an essential and a toxic trace element. The major factor determining metabolism and toxicity of chromium compounds is their oxidation state. Cell membranes, including the skin and digestive tract, have low permeability to Cr^{3+} but are readily penetrated by Cr^{6+} present in chromates and dichromates, which in turn are subject to intracellular reduction to Cr^{3+} by tissue proteins containing sulfhydryl groups.

Upon casual contact in the trivalent state, chromium is among the least toxic trace elements, in part because of its poor permeability and its limited ability to reach peripheral tissues in toxicologically significant amounts. As an essential mineral required for normal glucose metabolism, 50–200 μg/day of Cr^{3+} has been recommended by the National Research Council Committee on Dietary Allowances. A wide margin of safety exists between the amounts consumed in a normal diet and the levels that cause toxic effects in humans. Hexavalent chromium is significantly more toxic, and inhalation of chromate-bearing dust has been associated with lung cancer. Absorption of chromium salts from the digestive tract is low. Skin penetration experiments considering various chromium salts have given contradictory results. The worst case data on humans for the more toxic form, i.e., the hexavalent chromate, yields a K_p of approximately 10^{-3} cm/hr; other data, especially when the chromium content of the skin is disregarded, place the permeability coefficient one or more orders of magnitude lower. Cutaneous absorption of *tert*-butyl chromate has been recognized by the ACGIH as presenting a significant risk of systemic toxicity, and particular care is recommended to avoid direct skin contact in the work environment. Chromate solutions are potentially corrosive and are hazardous at concentrations encountered in the industrial setting.

Of greatest importance is the allergenicity of chromium salts, which can

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lead to both immediate and delayed hypersensitivity, and occupational contact dermatitis is a prevalent problem in industrial hygiene. While chromium metal does not act as a hapten, plasma, sweat, or saliva can transform metallic chromium into immunogenic chromium salts. Chromium pigments permanently deposited in the skin can also exhibit granulomatogenic activity. Experimental data indicate that the sensitization potential of chromium may be highly subjective, as large variations are observed between different studies. Furthermore, the allergenicity of chromium salts differs significantly between oxidation states: the salts of hexavalent Cr are the better penetrants, being more readily soluble, whereas trivalent Cr is the more potent allergen in accord with its greater protein binding capacity. Sensitization will also depend on the nature of the counterion, the pH of exposure, and the concentration. Recent evidence suggests that prevalence of chromium hypersensitivity is not due to cross-reactivity with other transition elements.

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14
Cesium

A. GENERAL COMMENTS

Cesium is an alkali group element, the most basic and electropositive metal known, and it reacts explosively in contact with oxygen or water. In mammals, cesium is a biologically important trace element due to its similarity and relationship with potassium in a number of biochemical and physiological processes. The amount detected in serum and packed blood cells was about 0.74 and 4.82 μg/kg net weight, respectively (1). Cesium also closely approximates the other biologically active alkali metals, e.g., Li, Na, and Rb, in function and toxicity, and its salts exhibit no acute or chronic effects that significantly depart from similar compounds derived from the other group IA elements. A number of the more common salts of cesium were screened for acute toxicity in standard animal tests (skin/eye irritation, cutaneous sensitization, oral LD_{50}) and were found to be of low toxicity, with the exception of the hydroxide, which was irritating to abraded skin (2). In animals, also in common with other alkali metals, Cs was shown to affect various CNS functions, mainly involving K^+ transfer, with which it competes for transport across cell membranes. ^{133}Cs nuclear magnetic resonance (NMR) studies carried out on suspended human erythrocytes showed that Cs^+ is taken up at approximately one-third the rate of K^+ (3). It is due to such metabolic similarities with potassium that cesium can be potentially harmful in mammals. Elevated cesium levels were observed in brain tissue of schizophrenic patients and depleted in those with Alzheimer's disease (AD) (4). Seen in conjunction with the persistent imbalances observed for Na^+, K^+, and Rb^+ in various brain tissues of AD patients, this supports the argument of membrane abnormality associated with AD (5). In that study it was also noted that sodium, with its relatively small ionic radius, showed elevated values in AD patients, whereas the other alkali metals studied, with larger ionic radii, are generally depleted in brain tissue.

All forms of cesium, both stable and radioactive, exhibit the same charac-

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teristics as to translocation in the environment and uptake and distribution in living organisms. Formerly, few investigations dealt with the toxicology of the stable Cs compounds but rather focused on cesium isotopes, primarily the four radionuclides cesium-134, 135, 136, and 137, which are components of fission products and occur in high amounts as fallout from arms testing and releases from nuclear reactors. Of these radionuclides, ^{137}Cs is the most important in fallout because of high fission yield, long physical half-life, and similarity to potassium in physiological processes (6). Particularly as a consequence of the Chernobyl incident in 1986, radioactive forms of Cs have been located in dust samples and grasslands ranging from Greece (7) to the United Kingdom (8). Dairy cows fed with Cs-contaminated feed excreted 5–8% of the Cs uptake with the milk (8).

Cesium is readily and almost quantitatively absorbed in the GI tract (9) and widely distributed throughout the human body, mainly in the soft tissues. ^{137}Cs absorbed from the GI tract was found to be taken up by erythrocytes from the plasma and concentrated within red cells to a similar extent as is potassium (10). With the observation that there exists a maximum threshold for cesium in red blood cells, it becomes possible to prevent binding of radiocesium stemming from nuclear contamination by maintaining saturation of erythrocytes with stable Cs, so that the nuclide is excreted again (8). Excretion appears to be bimodal: an average 10% of a single oral dose is excreted within 1–2 days, but the major part has a half-life of 50–150 days. ^{137}Cs was measured to have a physical half-life of 30 years and a biological half-life (the retention half-time corrected for radioactive decay) of 102 days (11). The distribution of radiocesium throughout the body and the β and γ radiation from its decay result in essentially whole-body irradiation. As a consequence, the acute toxicity of Cs radionuclides is related primarily to bone marrow destruction.

Recently, the stable form of this element has become the subject of growing toxicological interest as it is increasingly being used industrially in new materials, e.g., photoelectric cells, infrared lamps, semiconductors, and photographic emulsions. Although cesium has no known vital function in mammals, it was suggested that it may be essential to life in analogy with the other alkali metal rubidium, which occurs in similar concentrations in animals and was shown to possess unique neurophysiological characteristics in humans (12). Although cesium has been characterized as an antidepressant in humans, the results from different investigators are inconclusive (13,14).

The major source of cesium uptake in the human body are foodstuffs, where its occurrence is determined by a relatively easy transfer from soil to plants and ready absorption in the GI tract of animals. In fruits and vegetables concentrations generally fall below 1 μg/g dry weight (15).

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B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

The isotope ^{137}Cs is indicated in the treatment of skin disorders; better tolerated than x-ray therapy, especially by the sensitive skin of children and fair-skinned individuals, it is particularly advantageous for the treatment of subcutaneous epithelioma and Kaposi's sarcoma, giving rise to less erythema and to reversible skin pigmentation (16). Cesium was one of several trace elements identified in human scalp hair (17). The hydroxide, CsOH, is a nonirritant on intact rabbit skin at 5%, and only a mild irritant on abraded skin. It did not induce cutaneous sensitization when injected intracutaneously in guinea pigs (2).

C. QUANTITATIVE ABSORPTION DATA

Percutaneous absorption of cesium has been demonstrated in 1- and 2-hr in vivo exposures of rats (18). Only 0.1 mL of pH 1 solution was applied to 5 cm^2 and, since there is no mention of occlusion of the site, the concentration of the solution was subject to evaporation. The solution applied apparently contained only radiocesium, so that the initial concentration of cesium was extremely low. With these limitations, the estimated absorption coefficient from experiments lasting only 1 hr was not more than approximately 3×10^{-4} cm/hr (Table 1). With other groups of rats the absorption was measured after whole-body irradiation; the estimates of K_p ($0.1–0.2 \times 10^{-3}$) are lower in these groups; however, significantly, in 2-hr experiments with the same application technique, the blood concentration of cesium reached its maximum 38–45 min after its application.

D. SUMMARY

Toxicity of cesium and its compounds is of the same order as that of the other alkali metals. However, the isotopes ^{134}Cs and ^{137}Cs (the main long-term radioactive pollutants) are a significant source of whole-body α and γ radiation once they have been absorbed. Since translocation of cesium, either stable or as a radionuclide, occurs readily from soil to plants to animals to humans, the main risk of exposure to relatively high-energy radiation emitted by certain cesium isotopes is uptake through the food chain. From the only report of percutaneous absorption the estimated absorption coefficient in rats for cesium is $1–3 \times 10^{-4}$ cm/hr. No human data could be retrieved in the literature.

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Table 1 Cesium, as ^{137}CsCl

Reference: D. Stojanovic and K. Milivojevic, Effect of total body gamma irradiation on the percutaneous absorption of radiocesium, *Strahlentherapie 141*: 93–95 (1971).
Species: rats in vivo ✓
in vitro
Area: 5 cm^2, ventral side of the abdomen
Vehicle: not specified, probably water, 0.1 mL at pH 1.0
Duration of exposure: 1, 2 hr
Analytical method: measurement of radioactivity—in the whole body by scintillation counting of the Cs-treated area of skin after it was excised; in blood with extracorporeal circulation of blood
Notes:
1. ''The skin was 'fat-free' with soap before contamination [with ^{137}Cs].''
2. Occlusion of the site of application is not mentioned, therefore, the concentration of Cs may have increased by evaporation during the course of the exposure. The estimated permeability coefficient would be subject to such changes.
3. Applied radioactivity ~30 μCi. There is no mention of cesium concentration or of carrier, i.e., nonradioactive CsCl. The radioactivity corresponds to about 0.4 μg ^{137}Cs.
4. The radiation treatment of the whole body was 600 rads from ^{60}Co.

Treatment[a]	*N*	Absorption of ^{137}Cs, % of applied[b]	Permeability coefficient, K_p (est.) cm/hr
Control, not irradiated	15	1.33 ± 0.21	$\leq 2.7 \times 10^{-4}$
1 day after irradiation	15	0.78 ± 0.37	$\leq 1.6 \times 10^{-4}$
3 days after irradiation	15	1.00 ± 0.45	$\leq 2.0 \times 10^{-4}$
6 days after irradiation	15	0.69 ± 0.34	$\leq 1.4 \times 10^{-4}$

[a] The skin was exposed to CsCl for 1 hr.
[b] Mean ± standard deviation.

5. During a 2-hr exposure period in another experiment radioactivity in the blood peaked just 38–45 minutes after application of ^{137}CsCl. The reported radioactivity in blood is given in ''imp/min'' but there is no mention of volume of blood counted or any measure of total absorption.

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15
Copper

A. GENERAL COMMENTS

Copper is an essential trace element that is critical for a wide variety of biological processes, e.g., hemoglobin synthesis, as was experimentally observed in the rat (1); enzyme activation, particularly that of superoxide dismutase; and, more generally, as a key component of mitochondrial, cytoplasmic, and nuclear enzyme systems (2). Absorbed mainly from dietary sources (organ meats, shellfish, nuts, whole-grain cereals), copper absorption and utilization is interdependent with that of other metals, including zinc, iron, cadmium, and molybdenum. The most significant stores of copper are found in liver, muscle, and bone. It is required for bone formation, cardiac function, keratinization, and tissue pigmentation, where it binds to proteins, preferentially sulfur- and nitrogen-containing ligands. In mammals, ceruloplasmin, an endogenous plasma glycoprotein, is responsible for copper transport in the blood where it binds more than 90% of the metal present. Physiological factors requiring involvement of copper as a remedial factor, such as tissue injury, chronic tissue inflammation, infection, and hormonal action, as well as increased levels of the metal itself, induce ceruloplasmin production in the liver, thus assuring an adequate transport medium in response to increased translocation requirements (3).

As with zinc, another vital trace element, copper absorption and excretion are regulated by a homeostatic mechanism involving metallothionein (MT). Most intracellular copper binds reversibly to MT, a ubiquitous protein in the organism fulfilling a multifunctional role in absorption, transport, and detoxification. Because both are able to bind reversibly to MT, copper and zinc exist in equilibrium. Perturbation of that stasis results in metabolic disturbance. For example, increased dietary intake of zinc can contribute to such conditions as rheumatoid arthritis, characterized by excess zinc and a corresponding deficiency of copper. In addition to antiinflammatory activity Cu and Zn also share a protective function, scavenging free radicals through the copper-zinc enzyme superoxide dismutase (4–6). Involved in defense and repair mechanisms in response to infection

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and injury, plasma copper in the form of ceruloplasmin increases with local inflammation and arthritis (7).

Levels of free ionic Cu, a relatively toxic metal, are moderated to the minimum levels sufficient for physiological needs, 10^{-19} mol/L estimated in blood plasma via binding to ceruloplasmin and MT (8). The dynamic equilibrium between ceruloplasmin and metallothionein prevents toxic accumulation or deficiency of Cu in mammals (3).

Deficiency of copper is associated with characteristic integumentary and skeletal abnormalities, defects in growth and development, and abnormalities in sensory perception (9). (Integumentary abnormalities associated with copper deficiency are discussed in detail in the following section.) Copper status of the organism is reflected in ceruloplasmin levels. Plasma levels below 125 μg/dL are generally considered as indicative of copper deficiency (10). Wilson's disease is an inherited Cu metabolism disorder, impairing biliary tract Cu excretion which leads to excessive Cu levels in tissue, particularly in the liver. This Cu accumulation leads to hemolytic anemia, which over the years can result in progressive hepatic failure and ultimately death (11).

Increases in systemic copper via parenteral entry from a contraceptive intrauterine device (IUD) can lead to adverse effects, even though the amounts liberated from such a device are relatively low (determined at 90 μg/day). The toxic effect of copper ions thus released in the uterus appears responsible for the contraceptive result, i.e., inhibition of implantation (12).

A chlorophyllin copper complex (CCC), derived from chlorophyll by replacing the chelated magnesium with copper, has antiinflammatory and antimicrobial properties, as well as a marked stimulating effect on epithelial cell growth rates and cell regeneration. First established in tissue culture studies, these findings were confirmed clinically through wound healing and deodorizing characteristics observed in animals and humans (13). Administered orally, CCC is classified as a safe and effective internal deodorant by the U.S. FDA (14).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

In vivo application of copper oleate in a lanolin-petroleum base over 24 hr to human back skin resulted in a significant increase in urinary copper levels over several days (15).

Electron microscopy of skin treated topically with copper acetate reveals that copper initially localizes in the intercellular spaces. Subsequently, the cell membranes of viable cells are penetrated and the metal accumulates in and around the cell nucleus (16). By use of electron probe analysis and analytical electron microscopy, the abundance of copper was followed across human cadaver skin following application of $CuSO_4$ using Franz cells. Copper was observed to enter

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the outer stratum corneum cells at high concentrations; it then encounters an apparent barrier approximately halfway within the stratum corneum, falling below the detection limit (0.1% by weight), to reappear at the granular interface. There it was primarily seen traversing an intercellular route through the granular layer and into the spinosum layer (17).

Absorption of copper has been observed following the accidental lodging of the finely divided metal in skin dermatoglyphics. Serum levels were found to increase over 4 days and then gradually decrease with an apparent half-life of over 100 days (18).

Besides urinary excretion, copper is also excreted in sweat, nails, hair, and skin (19); sweat is the most important pathway. Strenuous exercise over a prolonged period therefore can result in significant losses that can ultimately lead to heat stroke. Exudative losses of copper appear particularly pronounced in patients with burns (20), potentially resulting in hypocupremia in severe cases, as a strong and persistent correlation was noted between serum copper concentration and extent of burn injury (21). The (inverse) correlation between burn surface area and plasma copper concentration may be explained by the cutaneous losses of ceruloplasmin due to injury, as 95% of plasma copper is bound to ceruloplasmin.

The normal copper concentration in human skin varied according to anatomical site from 1 to 7 μg/g dry weight by neutron activation analysis, as determined in biopsies from 15 individuals. This variance in Cu concentration also correlates to dendritic cell density (22).

Albinism, the striking absence of pigmentation in the skin, hair and eyes, is characterized by the absence of the copper enzyme tyrosinase, which converts tyrosine to melanin in the melanocyte (23). Menkes' kinky hair syndrome, a hereditary defect in intestinal copper absorption that causes hair to be abnormally sparse and brittle, becomes manifest in early infancy. Afflicted infants have low levels of copper and ceruloplasmin, dying usually within the first year of life. Although copper absorption and reabsorption are impaired, tissue copper levels of many epithelial tissues, including the skin fibroblasts, are elevated, and an increased production of metallothionein, the cysteine-rich protein that binds copper in cells, appears to be the cause of such accumulation (24). The biochemical defect underlying Menkes' syndrome, however, remains unknown.

Human scalp hair analysis is ineffective for the determination of environmental Cu exposure with hair values being inconsistent with those of serum (25,26) Micro-PIXE analysis further confirms this conclusion, indicating that Cu is incorporated into hair from both external and internal sources. Cu is transferred from blood to epidermis, internal and external hair root sheaths, hair cuticle and cortex by a transcellular migration process. The longitudinal Cu gradient down the hair shaft is reflective but not definitive of environmental exposure.

Copper metal is highly reactive with biological tissues (27). Topically ap-

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plied lipophilic copper complexes significantly penetrate the skin, producing anti-inflammatory and antiarthritic activity; the presence of Cu(II) in these complexes is considered essential for their efficacy (28,29). Copper as it is used in jewelry (e.g., bracelets and rings) will corrode upon contact with the skin, producing copper salts of amino acids. Mobilization of the metal from various copper articles in synthetic sweat was investigated over 24 hr at 35°C at pH 5.1; the range of Cu^{2+} dissolved was 80–100 μg/mL sweat (30). Topical applications of copper salicylate produced antiinflammatory effects in test animals and humans, where copper increased the therapeutic efficacy of salicylate (19,31,32). Based on such observations, in certain countries ethanolic Cu(II)salicylate-containing preparations are now available over the counter for human use to alleviate arthritic pain (33).

Table 1 Copper, as $^{64}CuCl_2$, copper salicylate, bis(glycinato)copper(II)–$^{64}CuCl_2$

Reference: W. R. Walker, R. R. Reeves, M. Brosnan, and G. D. Coleman, Perfusion of intact skin by a saline solution of bis(glycinato) copper(II), *Bioinorg. Chem. 7*:271–276 (1977).

Species: cat in vivo ✓
in vitro ✓

Area: 8.6 cm^2 in vitro; not reported for in vivo application

Duration: in vitro, 24 hr; in vivo, 20 hr

Vehicle: in vitro, 8.5 mL saturated bis(glycinato)copper(II) solution, i.e., 0.05 M, in physiological saline; in vivo, (a) 1 mCi $^{64}CuCl_2$ in physiological saline, (b) 1 mCi $^{64}CuCl_2$ in 5% w/v Cu salicylate tetrahydrate in 1:1 ethanol-glycerol, (c) a solution containing bis(glycinato)copper(II)

Analytical method: scintillation counting of ^{64}Cu radioactivity in blood and receptor solution; atomic absorption analysis of the receptor solution

Notes:

In vivo:

1. All three copper compounds were tested. Neither the volume of solution nor the area exposed nor the length of exposure were reported. No ^{64}Cu was detected in 0.5–1 mL blood samples even after 20 hr although with a portable monitor radioactivity was detected throughout the cat.

In vitro:

1. Electron micrographs of skin sections stained for copper revealed the metal in all layers of the skin in exposed samples.
2. ''In 24 hr, 3.3% of the applied copper(II) complex had completely penetrated the skin.'' This is equivalent to 3.0 mg of the complex. Atomic absorption analysis found 47 ppm Cu in the washing solution beneath the skin (20 mL isotonic saline). This corresponds to 0.94 mg of Cu which, in agreement with the radioactivity, is found in about 3.1 mg of complex.
3. The apparent K_p, after the lag time of about 9 hr, = flux/conc. = (0.94 mg/8.6 cm^2/15 hr)/0.05 mol/L = 24×10^{-4} cm/hr.

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C. QUANTITATIVE ABSORPTION DATA

Bis(glycinato)copper(II) complex was the first copper compound for which quantitative absorption data became available (31). A radioactive (^{64}Cu) 0.05 M solution in physiological saline applied to excised cat skin revealed, after a lag time, a steady-state transport rate described by a $K_p = 24 \times 10^{-4}$ cm/hr (Table 1). In vivo exposure of cat skin demonstrated qualitatively that copper was absorbed.

Table 2 Copper, as copper chloride and copper sulfate

Reference: F. Pirot, F. Panisset, P. Agache, and P. Humbert, Simultaneous absorption of copper and zinc through human skin in vitro. Influence of counterion and vehicle, *Skin Pharmacol*. *9*:43–52 (1996).
Species: human, from surgery in vivo
in vitro ✓
Area: 3.14 cm^2
Duration: 72 hr
Vehicles: white petrolatum, carboxypolymethylene gel (Carbopol) and hydroxypropylmethylcellulose gel (Metolose 60 SH); 20 mg/cm^2. Each also contains zinc chloride or zinc sulfate
Concentration: 1.27% and 1.86% copper
Analytical method: flame atomic absorption spectrometry of receptor fluid and skin layers
Notes:
1. Average thickness of the dermatomed skin = 410 μm.
2. Diffusion cells were Franz-type static cells. Receptor fluid was isotonic saline with antibiotics and 5% human albumin. It was kept at 33°C.
3. The copper fluxes always peaked between 1.5 and 6 hr.
4. The epidermis after the experiments contained about 2–6 times as much copper as the total accumulated in receptor fluid.
5. The dermis, however, contained less copper than was found in the receptor fluid except in the case of copper chloride–zinc chloride in gel. In that experiment the amount of copper in the dermis was 7 times greater than in the receptor and was, furthermore, 1.5 times greater than in the epidermis.

Salt	Formulation	Copper conc. (%)	Apparent permeability coefficient[a] $10^4 \cdot K_p$ cm/hr ± sd
$CuSO_4$ (& $ZnSO_4$)	Petrolatum	1.27	0.032 ± 0.031
$CuSO_4$ (& $ZnSO_4$)	Carbopol 940 gel	1.27	0.045 ± 0.057
$CuCl_2$ (& $ZnCl_2$)	Petrolatum	1.86	0.16 ± 0.12
$CuCl_2$ (& $ZnCl_2$)	Metolose 60 SH gel	1.86	0.023 ± 0.010

[a] Based only on the copper in the receptor fluid.

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Table 3 Copper, as copper 2-pyrrolidone 5-carboxylate (CuPC) and $CuSO_4$

Reference: F. Pirot, J. Millet, Y. N. Kalia and P. Humbert, In vitro study of percutaneous absorption, cutaneous bioavailability and bioequivalence of zinc and copper from five topical formulations, *Skin Pharmacol. 9*:259–269 (1996).
Species: human, dermatomed abdominal, 400 μm in vivo
in vitro ✓
Area: 3.1 cm^2
Vehicle: three emulsions (two commercial products and a custom-made variant of one of the former) and two commercial ointments. All contained both zinc and copper compounds. Each formulation was applied at the rate of 16 mg/cm^2.

Emulsion A: Cu/Zn d'Uriage, water/oil, contains ZnO, ZnPC, and CuPC.
Emulsion B: same as A except that $CuSO_4$ and $ZnSO_4$ replace CuPC and ZnPC.
Emulsion C: Dermalibour, water/oil, contains $ZnSO_4$, ZnO, and $CuSO_4$.
Ointment D: Dalibour Monot, contains $ZnSO_4$, ZnO and $CuSO_4$
Ointment E: Dermocuivre, contains ZnO and $CuSO_4$

Analytical method: atomic absorption spectrometry of receptor solution and of tissue
Duration of exposure: 72 hr
Notes:
1. The receptor solution was 0.9% NaCl and a water bath kept the diffusion cells at 33°C.

Percutaneous absorption of copper

Formulation	Copper compound	Copper conc. (mg/cm^3)	Permeability coefficient $10^4 \cdot K_p$ cm/hr 0–2 hours	24–48 hours	0–72 hours
		Experiment 1			
Emulsion A	CuPC	1	0.57	0.02	0.06
Emulsion B	$CuSO_4$	1.3	0.44	0.010	0.015
Emulsion C	$CuSO_4$	0.5	1.2	0.03	0.08
		Experiment 2			
Emulsion A	CuPC	1	1.2	0.06	0.13
Ointment D	$CuSO_4$	0.9	0.8	0.05	0.09
Ointment E	$CuSO_4$	0.5	0.9	0.10	0.12

2. Differences in percutaneous copper absorption between formulations were generally not statistically significant although absorption after 2, 6, and 24 hr from emulsion C was greater than from either A or B.
3. The absorption rate of copper generally decreases as the experiments progress, and for formulations B, C, and E there was little or no absorption in the final 24 hr.
4. After 72 hr exposure there was a significant increase in the average copper concentrations (140–430% of control values) in the epidermis for all the formulations. In the dermis, where control concentrations averaged just 2–3% of the epidermal control values, the treatments significantly increased on average only three of the six copper contents.

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Recently, the percutaneous absorption of copper from copper chloride and copper sulfate has been investigated in vitro with human skin (34). As is done for medical purposes, these salts were administered with zinc chloride and zinc sulfate also incorporated in the vehicles, which were petrolatum and two aqueous gels. For $CuSO_4$ from petrolatum or from Carbopol gel and for $CuCl_2$ from Metolose gel the apparent permeability coefficients were similar, i.e., 0.023–0.045×10^{-4} cm/hr (Table 2). For $CuCl_2$ from petrolatum the permeability coefficient was somewhat larger, i.e., 0.16×10^{-4} cm/hr.

Commercial products (emulsions and ointments) containing copper and zinc have also been studied under similar experimental conditions for 72 hr (35). Whether formulated with copper sulfate or the organic salt, copper 2-pyrrolidone 5-carboxylate, apparent permeability coefficients, over 72 hr, were in the range 0.015×10^{-4} to 0.13×10^{-4} cm/hr (Table 3). These values were not significantly different between products and were in the same range as the values of the previous report (34) for aqueous gels and petrolatum. The noteworthy differences in permeability coefficients were associated with the period of exposure; the values decreased over successive time periods from near 1×10^{-4} cm/hr (0–2 hr) to 0.1×10^{-4} cm/hr or less (25–48 hr) and finally to undetectable values in two cases (49–72 hr). A similar phenomenon has been observed elsewhere for different metals, e.g., cobalt, chromium, and mercury (36).

D. IMMUNOLOGY

The role of copper in immune function has been investigated in laboratory animals. Deficiency of this trace element in rats resulted in immunotoxic effects; the reticuloendothelial system failed to respond to infection and ultimately there was a significant increase in mortality rate (37,38)

Although rarely causing hypersensitivity, and that mostly from occupational exposure (39,40) consistent with the rating as a grade I allergen in the guinea pig maximization test (41), systemic as well as topical exposure to copper is known to cause both immediate- and delayed-type sensitization. Contact stomatitis from copper present in dental restorative work has also been reported and is suspected to be the cause of oral lesions of lichen planus. The patient's skin showed a delayed reaction upon contact with copper metal, which can be viewed as consistent with copper's antiinflammatory action observed in therapy (42,43). The local lymph node assay, developed on mice for the detection of contact allergens (44), has been adapted to test for allergenicity of metal salts also. Under such modified conditions, cupric ion significantly increased lymph node cell proliferation, and mice could be sensitized by application of copper(II) sulfate (45,46).

Copper-containing IUDs release measurable amounts of metal ion and in

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several cases have been reported to cause immediate-type hypersensitivity, manifest as urticarial eruptions (47,48). In a number of cases, delayed-type hypersensitivity was confirmed by positive skin patch test (39,41,49–56).

E. SUMMARY

An essential trace element for life, growth, and development in humans, copper is also noted for its strong antiinflammatory activity in both humans and animals. Deficiency as well as excess of the element are associated with biochemical disturbances. It is immunogenic on dermal as well as systemic exposure, capable of causing immediate as well as delayed-type sensitization. Metallic copper worn as jewelry is measurably oxidized by sweat, forming organometallic salts, which can penetrate skin and exert antiinflammatory activity in joints. In vitro quantitative measurements of the skin penetration of copper salts in humans revealed apparent permeability coefficients of 10^{-4} cm/hr or less. The cytotoxic property of the metal ion is kept in check by an efficient homeostatic mechanism involving metallothionein.

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16
Iron

A. GENERAL COMMENTS

Iron is the most abundant transition metal occurring in nature. This element also constitutes the most important of the required trace elements in human nutrition. The crucial role of iron in human physiology has been recognized since antiquity when the ancient Greeks prescribed iron salt supplements in the diet to improve muscular weakness in injured warriors (1). Iron is essential for DNA and RNA synthesis, electron transport, and oxygen metabolism in most microorganisms, plants, and animals. Absorption, storage, mobilization, and excretion of iron are delicately regulated by a number of interrelated control processes that have not been definitively identified (1).

Ionic iron has a great affinity for nucleophilic functional groups present in proteins, with the most important being the amino group of lysine, the sulfhydryl group of cystine, and the imidazole group of histidine. Certain carrier proteins function specifically in iron transport and storage. Sixty to seventy percent of iron present in a normal human adult occurs in hemoglobin, and the remainder, about 30%, is stored bound to a protein called ferritin, or its aggregated form, hemosiderin. Less than 0.1% of the total body iron circulates in plasma bound to the protein transferrin, and the percentage of transferrin that is saturated with iron is the best indicator of iron status in the body, routinely used for diagnostic purposes. Normal levels of saturation in the healthy human are about 30%. The largest stores of noncirculating iron are found in the liver, spleen, and bone marrow, where it is readily available for erythropoiesis. Once the storage capacity of ferritin is exceeded, iron becomes acutely toxic. Redundant or waste iron, mainly released from hemoglobin catabolism, is so efficiently recycled in the liver that its half-life in the human organism has been estimated to be more than 10 years (2).

In nature iron occurs mostly in the biologically unavailable trivalent state, as a highly insoluble hydroxide ($K_{sol} = 10^{-38}$ at pH 7, Fe^{3+} conc. $= 10^{-11}$ mM) (3). Consequently, several mechanisms have evolved to make iron assimilation

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possible. In mammals iron is predominantly absorbed in the duodenum in the ferrous rather than the ferric state. Iron bound to heme, a prosthetic group that is part of hemoglobin occurring in high concentrations in organ or muscle meats, is the most common absorbable source of Fe^{2+} available to the gastrointestinal tract. When iron stores are adequate, the body reduces the absorption of nonheme iron, the form that occurs in plants, but it continues absorbing heme iron, regardless of iron status. In the living organism, iron typically is present in the trivalent state also and must be reduced to the more soluble bivalent state before it can pass through membranes. The bioavailability of iron is also greatly influenced by the composition of the diet. For instance, ascorbic acid, is known to facilitate the absorption of nonheme iron by reducing the ferric to the ferrous state. Once it reaches its designated target organ, Fe^{2+} is enzymatically oxidized back to Fe^{3+} (4).

While adequate iron stores appear to be crucial for human and animal nutrition, particularly during growth, pregnancy, and lactation, both lack and overabundance may result in a number of untoward effects:

1. Iron deficiency can occur due to insufficient dietary intake of absorbable iron, as well as metabolic disorders. Serum and tissue iron deficiency (ID) can lead to anemia and adverse mucocutaneous effects, such as changes in hair growth and structure; hair loss; deterioration of the nails, mucosa, and tongue; chronically sustained inflammation, such as various forms of dermatitis and acne (5). Conversely, in the treatment of acne and alopecia, iron has been administered therapeutically since antiquity. Hematological iron balance does not directly reflect the state of iron stores in serum and tissues, i.e., ID can occur without anemia, and mild forms therefore are not readily detected (6). Upon iron replacement therapy, e.g., with oral ferrous sulfate, effects of ID are reversible, although return to normal iron status may require months (5).
2. While most iron in the diet is unavailable for absorption, therapeutic iron preparations, e.g., ferrous sulfate, designed for high bioavailability can sometimes cause acute toxicity. The oral LD_{50} of elemental iron lies in the region of 200–250 mg/kg. Children in particular are at risk for overdose from iron supplement tablets; as little as 130 mg of elemental iron may be fatal to an infant (7). According to a report from the American Association of Poison Control Centers, iron supplements were the single most frequent cause of pediatric oral poisoning, accounting for over 30% of such fatalities due to ingestion of pharmaceuticals (8). The pathophysiology of iron intoxication has yet to be completely clarified, but sequelae include hepatic cirrhosis and CNS damage (7). Recent evidence also suggests that excessive iron stores

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(reflected in degree of transferrin saturation) may increase the risk of cancer (9), colorectal cancer in particular (10), and also of coronary heart disease (11). A widely-known theory holds that iron causes oxidative damage to tissues by generating radicals, thus initiating the sequence of events abutting in cancer, among other effects, but no direct correlation has yet been established between body iron stores and cancer.

Iron excess can also result in increased risk of infection. Bacteria require iron for growth, metabolism, and replication, and appear to absorb the vital nutrient through gated channels in the outer membrane bilayer (12). In normal metabolism, iron is sequestered by lactoferrin and transferrin in extracellular body fluids; thus, iron available to invading microorganisms is severely limited. Excess iron given accidentally or intentionally to humans in a state of anemia or severe malnutrition may overwhelm the iron binding capacity of sequestering proteins, making the nutrient available for uptake by pathogens, and thus promote microbial growth (13). Normal or pathogenic microorganisms residing in the host organisms compete for iron and assume the role of complexing, solubilizing, and transporting dietary iron via hydroxamic or catecholic siderophores (14), such as the cyclic trimer of 2,3-dihydroxybenzoylserine enterobactin formed by enteric bacteria (15).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Percutaneous absorption of iron has been reported only for chelated forms administered in ointments to mice in vivo (16). For Fe-EDTA from both oil-in-water and water-in-oil ointments, the average absorption of iron in 20 hr was 55–80%. However, because of the low levels of iron in the ointments, this uptake corresponded to only nanogram quantities of the metal. For Fe-cupferron, a lipid-soluble chelate, absorption from both ointments averaged 10–15%.

In humans no known, conventional route of elimination for excess iron exists other than through skin and its appendages, intestinal epithelium, menstrual blood, and lactation (17,18). Skin and its appendages are therefore critical excretory routes for iron, with females excreting less than males, possibly reflecting the lower iron body stores seen in the former (19). Otherwise, the element is essentially recirculated among the various metabolic pools (20).

Subcutaneously injected ^{59}Fe (0.71–1.80 μg as ferric citrate) was found to clear triphasically. The first phase, with a half-life in the order of half an hour, was probably due to the clearance of unbound ferric ions from the injection site into systemic circulation. The second phase is related to lymphatic clearance of transferrin-bound iron, with a half-life of about 24 hr. During the final phase the

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remainder, approximately half the amount found in the second phase and seen to be retained by the epidermis, disappeared more slowly ($t_{1/2}$ = 66–69 days). This slow flux is presumably due to saturation of normal iron binding and transport mechanisms (21). The extended half-life of that epidermal component, retained in the epithelial cells, also exceeds by far the residence time anticipated if normal cell turnover and desquamation were the determining mechanism of elimination. Instead, it is seen as the result of a recycling process back to the interstitial fluid and a return to the intravascular iron pool, in analogy to the conservation process observed for epidermal DNA (22).

A large amount of transferrin-bound iron circulates through dermal tissue (23), and is eliminated from the skin by desquamation and sweating (18,24,25). The concentration profile of iron across normal human skin determined by micro-PIXE analysis indicates maximum levels in the stratum germinativum (200 ppm, ± 10 ppm), decreasing towards the skin surface (26,27).

The processes of iron elimination through the skin and its appendages also account for the occurrence of iron in hair, as the metal is incorporated there during the growth process. Micro-PIXE analysis reveals that iron is incorporated in scalp hair primarily through the root sheath. Beyond the scalp, iron is found primarily in the hair shaft periphery, indicating radial absorption. Longitudinal iron supply along the hair shaft is apparently absent (28). Iron binds to the hair cuticle, as demonstrated in various shampooing tests on human hair (29).

Levels measured in hair and nails are unreliable indicators of body iron status. Analysis by energy dispersive x-ray fluorescence of scalp hair and fingernails collected from normal children, from subjects with iron overload and from subjects suffering from iron deficiency anemia indicated no significant differences in iron concentrations in hair or in nails between various subject groups. Nails, consisting of keratinized epidermal cells, reflect only the iron content of the epidermis. Mean iron content of fingernails was measured at 67.6 μg/g dry nail (30) (see sections on sweat, nails, and hair).

In ''whole'' sweat, i.e., sweat not separated from its cellular components, iron content reached levels as high as 7.1 μg/mL. Under hot and humid conditions, where sweat volume can amount to 900 mL/hr (calculated as net body weight loss per hour) (17), iron loss can be significant. Cell-free sweat also was found to contain some iron; values ranged from 0.34 μg/mL for men to zero for anemic women (31). Low iron content in sweat was indicative of iron deficiency anemia, with iron levels increasing following iron supplement therapy (32).

Iron not eliminated by the aforementioned mechanisms is retained in the body and can gradually accumulate throughout one's lifetime. This retention can result in abnormally high body levels, particularly in older men. In such cases, acquired amounts of 20–69 g iron have been recorded for 50 year periods. High body burdens can result in hemochromatosis (HC), a condition clinically manifest as skin discoloration due to increased melanization, altered skin texture, and hair

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loss. More serious effects are liver cirrhosis, endocrine abnormalities, and cardiac disorders. Iron deposited in the skin can be located in the deeper dermis, especially in the macrophages of capillary endothelial cells and, in the form of ferritin, hemosiderin or lipofuscin in the sweat glands. HC may be attributed to a hereditary metabolic defect (primary HC), or to elevated intake and accumulation of iron (secondary HC), e.g., due to sustained transfusion therapy (20). Abnormal iron metabolism, leading to excessive formation or accumulation of porphyrins and porphyrin precursors, can result in a variety of untoward effects, cutaneous porphyrias among them. There, skin tissue porphyrin levels correlate with degree of cutaneous photosensitivity, hirsutism, hyperpigmentation, and other skin disorders (33,34). Porphyria cutanea tarda (PCT), one of the skin porphyrias, a photosensitive blistering disorder, is often associated with HC, the most common cause of severe iron overload. As ferritin levels often are indicative of both conditions, a simplified and accurate diagnosis for HC and PCT is possible by a plasma scan for ferritin levels, rather than by analysis of excreta (35).

Exposure of human and animal skin to sunlight (specifically UVB or UVC) resulted in significant increases of nonheme iron in both the epidermis and dermis (36). The iron content in human epidermis biopsies, representing sun-exposed (cheek, forehead, or neck) and nonexposed (buttock and thigh) areas, differs significantly ($p < 0.05$): 53 ppm (dry weight) versus 18 ppm. Since iron can catalyze the generation of reactive oxygen species, light-induced iron deposits in skin presumably contribute to photodamage. The fact that chelated iron exerts a photoprotective effect further supports the above statement (37).

Iron deficiency, in addition to occurring due to insufficient dietary intake of absorbable iron, can also occur due to metabolic skin diseases, such as erythroderma, exfoliative dermatitis, eczema, and several others, wherein iron is lost through the accelerated turnover of keratinocytes (5). In psoriasis iron loss can be significant, since the epidermis has both elevated iron levels and is also sloughed off at an accelerated rate (38,39).

C. QUANTITATIVE ABSORPTION DATA

Percutaneous absorption of iron has been reported only for chelated forms administered as ointments to mice in vivo (Table 1) (16). Fe-EDTA, a water-soluble chelate, was absorbed most readily. From both oil-in-water and water-in-oil ointments (each prepared with $CHCl_3$), average absorption of iron in 20 hr was 55–80%; these figures correspond to 70–100 ng of iron. Fe-tiron, also a water-soluble chelate, was incorporated into ointments without chloroform and its absorption averaged 16% for a water-in-oil system and 35% for an oil-in-water vehicle. From polyethylene glycol 1500, a hydrophilic ointment base, the absorption of

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Table 1 Iron, as ^{59}Fe chelates: Fe-EDTA, Fe-cupferron (ammonium nitrosophenylhydroxylamine), Fe-tiron (disodium pyrocatechol-3,5-disulfonate), Fe-carbamate (sodium diethyldithiocarbamate)

Reference: A. Minato, H. Fukuzawa, S. Hirose, and Y. Matsunaga, Radioisotopic studies on percutaneous absorption. I. Absorption of water-soluble substances from hydrophilic and absorption ointments through mouse skin, *Chem. Pharm. Bull. (Tokyo) 15*:1470–1477 (1967).
Species: mouse in vivo ✓ exposure
in vitro
Area: 5 cm^2, abdomen
Vehicle: ointments (≈50 mg/animal): 1 hydrophilic (o/w), 1 ''absorption'' (w/o), and 4 variations of these
Duration of exposure: 20 hr
Analytical method: γ-ray counting of the excised treated area of skin
Notes:

Ointment type	Form of Fe	Number of animals	Radioactivity of ointment[a] (cpm/50 mg)	Absorption of Fe in 20 hr (% ± SE)
w/o[b]	Fe-EDTA	4	7072	80 ± 4
w/o[b]	Fe-cupferron	4	6178	14.6 ± 0.3
o/w[b]	Fe-EDTA	4	5588	66 ± 5
o/w[b]	Fe-cupferron	4	6373	13 ± 2
w/o	Fe-EDTA	4	5752	58 ± 4
w/o	Fe-tiron	4	5404	16 ± 2
o/w	Fe-EDTA	4	6871	55 ± 2
o/w	Fe-tiron	4	6118	35 ± 2
w/o	Fe-cupferron	4	3843	10.4 ± 2.2
w/o	Fe-carbamate	4	3318	6.1 ± 0.5
White petrolatum	Fe-cupferron	3	10045	2.7 ± 1
PEG[c]	Fe-EDTA	4	3886	5.4 ± 1.7
PEG-POE[c]	Fe-EDTA	4	3873	21 ± 2
PEG-POE-petrolatum[c]	Fe-EDTA	4	3418	26 ± 1

[a] Amount of ointment applied ≈ 50 mg/animal; 50 nmoles Fe per 1–1.6 g ointment; the $^{59}FeCl_3$ has apparently been diluted with nonradioactive $FeCl_3$.
[b] 100 μL chloroform was added to each gram of ointment.
[c] PEG, polyethylene glycol 1500; POE, polyoxyethylene oleyl ether.

Fe-EDTA was only 5% but was increased 4–5 times by the addition of the surfactant polyoxyethylene oleyl ether.

For Fe-cupferron, one of two tested lipid-soluble chelates, absorption from the oil-in-water and water-in-oil ointments averaged 10–15% during 20 hr; from petrolatum, absorption was only 3%. Absorption of the other lipid-soluble che-

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late, Fe-carbamate, was reported only from the water-in-oil preparation and also exhibited relatively low absorption (about 6%).

D. IMMUNOLOGY

Certain immune defense mechanisms appear dependent on iron status. The influence of iron deficiency anemia on lymphocyte concentration, on humoral and cell-mediated immunity, as well as on phagocytosis has been studied in humans and animals. A reduced allergic response to skin test antigens was noted coincidental with iron deficiency anemia (5,13).

Few cases of allergic contact sensitization to iron have been recorded, but iron oxides as well as both ferrous and ferric salts confirmed sensitivity upon patch testing (40–46). Granulomatous dermatitis due to tattooing with iron oxide is also recorded (47,48). The clinical relevance of positive reactions to iron has not been established, however, since in a number of cases described reactivity was associated with sensitivity to nickel and cobalt, closely related elements of the same group of the periodic table, and the possibility of cross-reactivity has not been addressed. Normally occurring tolerance to iron is presumed to be due to genetic adaptation since it is a ubiquitous element, readily combining with protein to form a complete antigen. The same may also be true for magnesium and zinc tolerance. Since divalent iron has been shown to cross the placenta and accumulate in the fetus, such tolerance may develop during gestation (49). Iron failed to induce lymphocyte proliferation in the local lymph node assay (50,51).

E. SUMMARY

Homeostasis in humans regulates iron levels, ensuring that this critical essential element remains within the limits required for proper functioning of various important physiological processes, such as oxygen transport. Iron status plays a key role in infection and immunity; overabundance as well as deficiency causes deleterious effects. Recent studies have associated excessive iron accumulation in the body with coronary heart disease and colorectal cancer. Appropriate iron levels are critical for the functioning, health, and appearance of the skin and its appendages. Allergic contact sensitization to iron compounds is extremely rare. Quantitative data on the rate of skin absorption are available only for chelated forms of iron.

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17
Mercury

A. GENERAL COMMENTS

Mercury is widespread in the environment, occurring in its elemental state (mainly as a vapor), as inorganic mercurous or mercuric salts, and as methylmercury. All three states are interconverted with relative ease in the environment. Biological and environmental conversion mechanisms include reduction to metallic mercury, oxidation to mercuric or mercurous ion, and methylation (1). The latter is a natural process occurring microbially in aquatic sediments. This bacterial biotransformation of inorganic mercury compounds probably accounts for the significant mercury levels seen in fish and leads to significant body burdens at the higher end of the food chain (1,2). In the mammalian organism, all forms of mercury are preferentially converted to bivalent mercury, which attaches to sulfhydryl ligands. Of the organomercurials, the alkyl derivatives are the more environmentally persistent. In the elemental state, mercury has a relatively high vapor pressure, 0.67 Pa at 37°C, and it is rapidly taken up via the lungs. In North America, pre-industrial annual atmospheric mercury deposition has been estimated at 3.7 $\mu g/m^2$ annually (3). However, atmospheric concentrations are steadily increasing; by some estimates modern levels are now four times higher (4). The major sources of the estimated 159 tons of mercury emitted annually into the atmosphere in the United States are coal-fired electric utilities, municipal waste incinerators, boilers, and chloralkali plants. Further, mercury levels in soil can attain significant values, due to degassing of unstable amalgams and volatile mercury salts such as mercuric chloride ($HgCl_2$) of natural occurrence. According to the U.S. Department of Energy's Oak Ridge National Laboratory, plants and soils discharge gaseous elemental mercury at rates significant enough to have a measurable impact on environmental levels. Gas phase emissions can be as high as 100 $ng/m^2/hr$ in a heavily forested area, and up to 7.5 ng from contaminated soil. Plants absorb mercury and then release the metal through transpiration in amounts inversely related to ambient concentrations (5).

Mercury is toxic to humans in all its forms and its presence in the body

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serves no known useful purpose. The toxicity of mercury to different organs depends on the route of exposure and the form in which the metal reaches its target; biodistribution is, to a large extent, a function of polarity and lipid solubility. For example, in animals the lipid-soluble methylmercury (MeHg) and the relatively lipophilic elemental Hg in vapor form readily cross the blood–brain barrier, whereas inorganic mercury does not enter the central nervous system (CNS) (6). In mammals, inorganic mercury accumulates predominantly in the kidneys where it is concentrated in the proximal tubules (7). Such accumulation is thought to proceed through glomerular filtration and subsequent reabsorption at the distal part of the proximal tubules (8). Chronic exposure to mercuric ion has also been found to result in renal tumors (9). In the human organism, mercuric ion is known to accumulate in the thyroid gland also, potentially leading to the formation of premalignant and malignant thyroid nodules (10–12). Carcinogenic activity of mercury is not altogether clear but appears to result from ingestion or inhalation of the metal, its oxidation to the mercuric state, and translocation to the thyroid. Results from earlier studies suggest that cytotoxicity and mutagenicity of mercuric ion, potentially resulting in malignant tumors, may be due to interference with DNA replication, DNA repair, RNA transcription, and protein synthesis (9,13–15). A recent in vitro study involving the intact multiprotein DNA replication apparatus of human cells demonstrated that DNA replication, DNA polymerase activity, as well as DNA replication fidelity are inhibited in the presence of mercuric ion, confirming such suspected carcinogenicity of the element (16). Such interference with the DNA replication process, mediating mutagenic effects, seems to be involved in the process leading to the formation of malignancy in the thyroid (15). The association between mercury exposure through the diet (MeHg in seafood) and thyroid cancer appears confirmed by a Norwegian investigation into the incidence of cancer among wives of men employed in the fishing industry; in comparison with the general female Norwegian population, the risk of thyroid cancer among those women was significantly elevated, with a standardized incidence ratio of 1.91 (CI 1.65–2.21; $n = 40{,}839$), supporting the suggested role of seafood as an etiological factor (17).

Mercury exposure of pregnant women leads to severe brain damage in the newborn and is associated with spontaneous abortions. Male occupational exposure has been associated with altered libido, and the reproductive toxicity of mercury causes altered sperm production and decreased fertility (18).

Ingestion, and contact by skin and mucosa, of all types of mercury (metallic, volatilized from amalgams, salts, and organic mercurials) and inhalation of mercury vapors can result in systemic toxicity. In humans, a number of reactions occur in response to environmental or occupational exposure to mercury, leading to a variety of hypersensitivity reactions and to suppressed immunoreactivity (19).

Widely occurring MeHg poses a serious health hazard to the general popu-

lation. Because it is a natural metabolite, is persistent, and bioaccumulates in the aquatic environment, it is recognized as one of the most significant environmental toxicants, particularly to the development of the CNS in the developing embryo; its biochemical activity in the organism also induces oxidative stress, affects calcium homeostasis and protein phosphorylation, and interferes with mitochondrial function (20). Studies also implicate MeHg in changes observed in cell cycle kinetics in developing and mature organisms (21).

The recurring Minamata disease in Japan was the consequence of large-scale consumption of seafood contaminated with mercury from industrial discharges into the sea; in Iraq, MeHg poisonings repeatedly occurred from the consumption of wheat that had been treated with the chemical in the form of a preservative (22). A readily absorbed mercury compound, MeHg is also capable of crossing biological membranes other than the skin, such as the placental barrier, from which it is transported to the developing nervous system. Its hazard is dependent on its accumulation in the organism with repeated exposure; thus, knowledge of its pharmacokinetic behavior is important for appropriate risk analysis. The Minamata and Iraqi experiences have shown that fetuses are much more susceptible to the toxic action of methylmercury. The effects of in utero exposure can be gauged using hair mercury levels: for example, New Zealand children born to mothers with levels higher than 6 ppm in hair exhibited abnormal development (22).

Following MeHg absorption, the only significant metabolic product is inorganic Hg and, over time, this form represents an increasing portion of the body burden (23). The main route of inorganic mercury elimination is urinary excretion. In blood, MeHg partitions primarily to the erythrocytes (24). Since the total mercury level in the organism is a composite of the parent organomercurial and its biotransformation product, models describing mercury pharmacokinetics in humans include both the prevalent parent compound (MeHg) and its metabolite (inorganic mercury). Such models predict a MeHg half-life of 44 days (25).

Besides naturally occurring MeHg, the synthetic chemical dimethylmercury, used in research among other things as the mercury nuclear magnetic resonance (NMR) and mass spectroscopy (MS) standard, has proven extremely damaging to the CNS. Minimal skin exposure has repeatedly resulted in fatal poisoning. Also, inhalation poses grave health risks due to the compound's high volatility. Exposure to severely toxic or even fatal doses goes unnoticed and when signs and symptoms appear, irreversible brain damage has already occurred (26).

Other potentially toxic organomercurials, that are also easily absorbed through the skin are the industrially important biocides ethylmercuric chloride and diethylmercury.

The toxicity of mercury is reduced in the presence of selenium in the bloodstream, as a complex is formed between a specific plasma protein and the two elements at an equimolar ratio (27). This process of mercury detoxification oper-

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ates in both humans and marine mammals (28,29). In animals, formation of the complex leads to a marked decrease in renal toxicity (30,31).

For the general population, the major route of mercury exposure is dietary intake, primarily through seafood in which the metal occurs mainly as MeHg. Upon consumption, Hg is preferentially absorbed and stored in red blood cells. Blood concentrations are therefore a reliable measure of the body burden. Individuals who never consume fish have levels of less than 20 ppb in blood cells, well within WHO safe limits, whereas regular fish consumption can result in values of over 200 ppb (32). In the United States, 46 states have fish consumption advisories for commercial seafood due to potential MeHg in excess of FDA recommendations and safety limits (33). The FDA's MeHg reference dose is 0.5 μg/kg body weight every day for a lifetime as the first indicator of an adverse effect.

Mercury, like other transitional or heavy metals, can form protein complexes, which result in immunotoxicity. Such activity becomes manifest in symptoms of hypersensitivity, of both the cell-mediated (34) and humoral type (35,36). The ability of mercuric chloride to enhance specific and total IgE has been confirmed in animal studies (37). While this is a frequent cause of allergic contact dermatitis (ACD) due to multiple systemic and dermal exposure, both type I and IV reactions have been noted in the same patient after topical application of mercurials (36,38). This phenomenon was confirmed by prick and patch tests (39). Also observed was the gradual transition from urticaria to eczema on the same test site (40). Following parenteral administration of mercurials for diuretic purposes, severe and sometimes fatal cases of anaphylactic shock have been recorded (41).

In certain animal strains, mercury modulates the immune response and may also play a role in causing autoimmune disease (42).

Like other electropositive, potentially protein-denaturing metals such as arsenic, antimony, and silver, mercury compounds have long been used in the treatment of bacterial infections. More recently, they are also used in cosmetics (bleaching creams), in a large number of over-the-counter drugs (eye, ear, nose and throat preparations, contraceptives, antiseptics, laxatives, diuretics, fungicides, topical drugs and acne preparations) (43), in agricultural pesticides, and in paints. With such multiple sources of exposure, there exists the potential for accumulation of toxic levels.

B. MERCURY IN DENTAL MATERIALS

Amalgam, an alloy consisting of mercury and silver, copper, tin, or zinc, is the compound most often used in dental restorative materials, and the rate of allergic reactions to these and other metals used in filling materials (e.g., gold, palladium) seen in routinely patch-tested patients raises concerns over possible sensitization

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through the oral mucosa. Of 118 patients with oral lichenoid lesions, 68% were found positive to a variety of metal compounds included in their dental appliances upon patch testing, 64% to one or more mercury preparations included in the test series. When dental fillings were replaced, 45% of patch test–positive patients showed complete healing (44). Because evaporation of mercury from amalgam fillings in the oral cavity contributes to the total mercury body burden [levels in urine (45) and serum (46) correlating positively with the number of such fillings], its use in dentistry has given rise to concern. There exists no evidence, however, that such mercury release is toxicologically significant, nor do the levels observed due to this source attain those observed in individuals whose regular diet includes seafood. The amount of mercury measured in saliva during a typical amalgam filling or removal operation is 15–20 μg Hg. Manipulation of amalgam by dental professionals does not result in exposures that approach the maximum time-weighted average work environment value of 0.05 mg/m^3 air (as elemental mercury) mandated by the ACGIH (see Appendix for comments on skin notation for mercury) (47) and thus is no reason for concern as a professional hazard.

When dental patients have a significant number of amalgam fillings removed, their urine and plasma mercury levels at first increase significantly, before reverting toward preoperative values after 30 days. One year later, the plasma levels had decreased by 50%, urinary excretion to 25% of the preoperative measurements (48). The American Dental Association (49,50), the US. Department of Health and Human Services (51), and the German Toxicological Society (48), among others, have concluded that concern over the safety of amalgam fillings is not warranted and that its use as a dental restorative material can continue. Replacement of fillings on a major scale, and mobilization of the metal in the oral cavity may, however, provoke significant release of mercury vapor in the organism. Thus, removal of amalgam fillings is avoided unless medically indicated. Sometimes removal of dental fillings in patients with oral lichen planus attributed to the presence of metals in dental filling materials is carried out without any histological or immunological examinations (52); since amalgam replacement is time consuming and expensive, prior diagnosis is recommended as a prerequisite. Patch test results and localization of the oral lesions in relation to dental fillings appear as the best diagnostic markers prior to removal of fillings (44).

C. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

All forms of mercury are absorbed through biological membranes including mucosa and skin (53). The ACGIH occupational exposure guidelines warn that cutaneous penetration contributes to overall exposure [see Appendix for comments on skin notation (47)]. Skin penetration rates vary as a function of the particular

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form of the metal. The organomercurials, Hg vapor, and certain oil soluble salts (e.g., $HgCl_2$) can penetrate the skin and other biological membranes to a significant degree (including the blood-brain barrier) (54–56). The penetration route of mercuric chloride across human skin in vitro involves intercellular pathways (57,58). Uptake into apical corneocytes was also observed. By contrast, autoradiographic studies in humans following dermal application of $HgCl_2$ under occlusion showed permeation through the epidermis, via both the hair follicles and sweat ducts (55,59).

Chronic dermal application of mercurials used as skin bleaches (mercurous chloride, ammoniated mercury, mercurous oxide) can lead to tissue accumulation of metallic mercury. Such deposits are observed in the stratum corneum and epidermis, and are characterized by slate-gray pigmentation (ironically the opposite of the desired action). Histopathology reveals disperse granules of mercury concentrated in the upper dermis (60–63).

Absorbed through the lungs, the GI tract, and the skin, mercury accumulates in the body and reacts with protein sulfhydryl groups, affecting both the activity of enzymes and the structure and function of membranes. For instance, the bleaching action of mercury ions in the skin is due to the inactivation of tyrosinase, the key melanin-forming enzyme (64). The equilibrium affinity constant between mercury and the sulfur of a thiol group is orders of magnitude greater than that for the more readily reversible binding between mercury and other electronegative functional groups (54,65). Mercury accumulates in the body and is primarily retained in the kidneys (bound to cysteine groups of metallothionein) (66,67).

Electron microscopy shows that application of aqueous $HgCl_2$ (0.1% w/v) to normal human skin rapidly results in mercury deposits below the stratum corneum. Later, mercury can be found in keratinocytes, melanocytes, Langerhans cells, and mononuclear cells of the dermis. Lower doses of $HgCl_2$ (0.025% w/v), however, do not result in significant deposition (68).

In subjects allergic to mercury or on application of irritant $HgCl_2$ concentrations on the skin (1–5% w/v), mercury deposits were seen intra- and extracellularly in the stratum corneum, but not in the deepest layers. In analogy to Cd and Zn, in clinically normal skin Hg is deposited as a metallothionein complex. Bound to that protein apparently designed to shield this tissue from heavy-metal damage, this can result in significant accumulation of the metal. In clinically compromised skin, on the other hand (e.g., skin affected by contact allergy or nonspecific irritation), this metallothionein defense mechanism can be overwhelmed and becomes ineffective (69). A case study of an occupationally exposed patient revealed such high mercury concentrations (exceeding normal values by a factor of 1000) in a skin biopsy analyzed by atomic absorption spectroscopy (70). No unusual levels were present in blood or urine in this case. A number of skin conditions, neurological changes, and systemic autoimmune disease accompanied such elevated levels

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of the metal, corresponding to symptoms reported in experimental animals with similar systemic concentrations (71–73).

Severe, and in some cases fatal, mercury poisonings have been documented in patients undergoing prolonged topical treatment with various alkylmercurial compounds to treat fungal skin infections (74,75).

In the general population not exposed to elevated levels of industrially generated, exogenous airborne mercury, scalp hair analysis is related to blood mercury levels and is a useful indicator therefore of environmental exposure, occupational exposure, and abnormal dietary intake (e.g., consumption of contaminated fish) (76).

D. QUANTITATIVE ABSORPTION DATA

Most investigations of the percutaneous absorption of mercury considered either inorganic forms of the metal, such as mercuric chloride ($HgCl_2$), or elemental mercury itself. Mercury compounds with a more organic character, such as mercuric oleate and phenylmercury compounds including phenylmercuric acetate and merbromin, have been given some experimental attention.

Sadly, an organomercury compound, dimethylmercury, for which there were no absorption data, was apparently absorbed transdermally with fatal consequences (26). Evidently, in this case, a single exposure through disposable latex gloves to 0.1–0.5 mL of pure dimethylmercury raised the concentration of mercury in whole blood to 4000 μg/L 5 months after exposure, far above both the normal range (<10 μg/L) and the usual toxic threshold (50 μg/L). With a density of 3 g/mL, just 40 μL of dimethylmercury would constitute a severely toxic dose.

The history of quantitative investigations of mercury's percutaneous absorption began in the 1920s because of its use in medicine, but the early methods were neither precise nor sensitive. For example, by applying various mercury-containing ointments to 20 in^2. and, after a period of absorption, weighing and analyzing the recoverable ointment, Wild and Roberts reported that ''after ten minutes inunction an absorption not exceeding 0.17 gram [of mercury]''; this is a flux equivalent to 7.9 mg $Hg/cm^2/hr$ (77).

In 1947, with the objective of delivering mercury more efficiently ''for cutaneous prophylaxis against venereal disease,'' Laug et al. reported a more rigorous measure of total mercury absorption (78). Rats were exposed to two unspecified calomel (Hg_2Cl_2) ointments for 24 and 48 hr. As determined by chemical analyses of the excreta and entire carcasses (excepting only the skin at the application site), mercury was absorbed at 0.059–0.129 $\mu g/cm^2/hr$ (Table 1). The kidneys accumulated the highest concentration of mercury.

A follow-up report used chemical analyses of rats kidneys to compare the percutaneous absorption of various mercury compounds from various vehicles

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Table 1 Mercury, as Hg_2Cl_2

Reference: E. P. Laug, E. A. Vos, E. J. Umberger, and F. M. Kunze, A method for the determination of cutaneous penetration of mercury, *J. Pharmacol. Exp. Ther. 89*:42–51 (1947).

Species: rat, rabbit in vivo ✓ exposure
in vitro

Area: clipped area of back, 29 cm^2 (1.5 × 3 in.) in rats and 155 cm^2 (4 × 6 in.) in rabbits

Vehicle: ointments containing 30% calomel (Hg_2Cl_2); the vehicles/ointments are not specified in this paper

Duration of exposure: 24 and 48 hr

Analytical method: chemical analysis of kidneys, livers, and, in one experiment, whole carcasses

Notes:

1. The concentrations of mercury were 10 or more times higher in the kidneys than in the livers.
2. The analysis of the whole body excluded only the skin at the site of application.

Percutaneous absorption by rats of mercury from calomel

Exposure (hr)	Average Hg flux[a], μg/cm^2/hr	
	Ointment A	Ointment B
24	0.059	0.160
48	0.079	0.129

[a] Flux = μg Hg (in carcass and excreta)/(29 cm^2 × hr).

3. Since the ointments are unspecified, the concentration of dissolved mercury is unknown (solubility in water at 25°C, 2 μg/cm^3; at 43°C, 10 μg/cm^3) and the permeability coefficients cannot be determined.
4. Decreasing the exposed area by one-half decreased the mercury concentration in kidneys by about one-third in both rats and rabbits.
5. Washing the skin with soap and water before treatment had no effect on mercury concentration in rat kidney.
6. Wiping off the excess ointment with tissue paper immediately after inunction reduced the mercury concentration in rat kidney by an average of 37%.
7. Not occluding the application site decreased the mercury concentration in rabbit kidney by an average of 67% for six ointments each tested in duplicate.

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Table 2 Mercury, as Hg_2Cl_2 (calomel), ammoniated mercury, metallic mercury, yellow oxide mercury (HgO), and mercuric oleate

Reference: E. P. Laug, E. A. Vos, F. M. Kunze, and E. J. Umberger, A study of certain factors governing the penetration of mercury through the skin of the rat and the rabbit, *J. Pharmacol. Exp. Ther. 89*:52–63 (1947).
Species: rat, rabbit in vivo ✓ exposure
in vitro
Area: clipped area of back, 29 cm^2 (1.5 × 3 in.) in rats and 155 cm^2 (4 × 6 in.) in rabbits
Vehicles: water; oleic acid; one or more of the following as major components: propylene glycol, corn oil, mineral oil, anhydrous lanolin, hydrous lanolin, petrolatum, lard; in smaller proportions: sulfathiazole, wetting agents (Triton NE, Aerosol OT, sodium lauryl sulfate) and other materials
Duration of exposure: 24 hr
Analytical method: chemical analysis of kidneys and sometimes also of livers
Notes:
1. The total absorption of mercury was not determined in these experiments.
2. Among single-ingredient vehicles, absorption of mercury from Hg_2Cl_2 by rats was 2–5 times higher from propylene glycol, corn oil, lard, and oleic acid than from water, petrolatum, mineral oil, and lanolin.
3. In two of the three tested calomel ointments 2% Triton NE significantly increased mercury absorption. In a comparison of surfactants with a single ointment base, the other two wetting agents had about the same effect as Triton NE.
4. The two experiments with the highest absorption of mercury in rats compared Hg_2Cl_2 in oleic acid with mercuric oleate in oleic acid; the kidney concentrations produced by these two systems were the same.
5. Absorption of Hg_2Cl_2 by rabbits and rats was higher from smaller particles (1–10 μm; 26–35 μg Hg/g wet kidney) than from larger particles (10–100 μm; 11–18 μg Hg/g wet kidney).
6. In comparisons with four mercury species, namely, Hg_2Cl_2, metallic mercury/mercuric oleate, ammoniated mercury, and yellow oxide mercury, the concentration of metallic mercury (0.93–50%) had little effect on the accumulation of mercury in the kidneys.

(Table 2) (79). By that test, mercuric oleate in oleic acid and Hg_2Cl_2 in oleic acid produced higher absorption than other mercury compounds from other ointment bases. Only ''micronized'' calomel (1–10 μM particle size) in a separate experiment led to similarly high mercury concentrations in the kidneys.

In the 1960s Wahlberg and his associates, in a series of at least nine reports (80–89), measured the absorption of mercury through guinea pig skin and, in one case, also through human skin. All of these investigations included mercuric chloride; some also involved methylmercury dicyandiamide (a seed fungicide)

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and potassium mercuric iodide. For various vehicles, concentrations, chemicals, etc., in diverse experiments, most of the estimated permeability coefficients for mercury in the first five hours after application were in the range 10×10^{-4} to 30×10^{-4} cm/hr (Tables 3–11).

It is important to note that in all of those experiments the absorption of radioactive mercuric chloride and of methylmercury dicyandiamide by live guinea pigs was demonstrated by a decrease in counting rate from the site of application, but in one case ''when the experiment was made with an animal that had previously been killed, the counting rate remained constant throughout'' (80). It is conceivable that metal ions, whether mercury or other, bind to components of the stratum corneum. In Wahlberg's experiments, the sample chambers contain 1 mL of liquid which, given the typical area of application, would form a cylinder of water about 3 mm high. A transfer of radioactivity from the bulk solution to the stratum corneum would slightly increase the effective shielding between the Geiger counter and the decaying isotope. The observable result would be a slight decrease of the measured radiation by a degree dependent on the energy of the emitted γ ray. A lower energy γ ray would be more affected by increased shielding than a higher energy gamma ray. The difference between results for dead and live guinea pigs supports the interpretation of the data as the result of percutaneous absorption of the metal ions followed by translocation of the radioactive ions by the circulatory system in vivo.

In the first of these papers (80), mercuric chloride and methylmercury dicyandiamide were applied at concentrations from 1 to 48 mg Hg/mL to the abdomen of guinea pigs. There was little difference in apparent permeability coefficients between the two chemicals (Table 3). There also was little variability of K_p with concentration, although K_p was lowest at the highest concentration. Pretreating the skin with either 1% soap, 1% detergent, or 1:1 acetone-water affected K_p significantly in only 1 of 12 experiments.

In a follow-up study, which yielded similar results, the same compounds were applied instead to back skin of guinea pigs (82). In addition, the soap and detergent solutions were not pretreatments but were the vehicles for the mercury compounds (Table 4). Compared to absorption from water without surfactant, the average absorption of mercury from 1% soap or detergent solutions was significantly different in only 1 of 8 cases; absorption of mercury from methyl mercury dicyandiamide (at 8 mg Hg/mL but not at 16 mg Hg/mL) was about 50% greater from 1% detergent than from water without detergent.

Additional data (Table 5) for other mercury concentrations in soap and detergent were published later (85). Absorption by stripped skin was included. Differences from the controls were modest and generally insignificant.

The absorption of $HgCl_2$ from plain aqueous solutions by guinea pigs was the same whether it was applied to the back (81) or to the abdomen (80).

Wahlberg and Skog compared absorption of $HgCl_2$, which is a skin irritant

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and a protein precipitant, with K_2HgI_4 (formed by the addition of KI to $HgCl_2$); in the latter compound, mercury is complexed with iodide and is a lesser irritant and protein precipitant (81). At various concentrations (1–48 mg Hg/mL) applied to the back of guinea pigs, the only noteworthy differences were at the higher concentrations. From mercuric chloride applied at concentrations greater than 16 mg Hg/mL there was no increase in absorption; on the other hand, mercury absorption from K_2HgI_4 increased proportionally with concentration up to 48 mg Hg/mL (Table 6).

Additional data from the same laboratory on the absorption of $HgCl_2$, K_2HgI_4 and methylmercury dicyandiamide concurred with the earlier results (Table 7) (83). Skog and Wahlberg remarked that the relative absorption (expressed as the absorbed percentage of the applied dose or as a permeability coefficient) of these compounds and of some other metal compounds was smaller at the lowest and highest applied concentrations than at intermediate concentrations. However, no explanation for this observation was offered.

In vitro, the effects of application time, kinetics postapplication, temperature, species (human, guinea pig), penetrant concentration, and receptor composition have been investigated (86). The time postapplication was the most influential variable for absorption rate. The percutaneous absorption of mercury from mercuric chloride solutions in Wahlberg's experiments was greatest in the first measurement period (0–5 hr) and decreased in each successive period to the lowest rate usually in the final period (36–48 hr) or sometimes in the next to last period (24–36 hr) (Table 8). By the end of the experiments, the absorption rate was not more than 40% of the initial rate, often 25–35%, and sometimes as little as 1%. This trend was seen with both guinea pig skin and human skin.

Absorption rates at 34°C were higher than those at 24°C. The in vitro absorption was the same whether the receptor chamber contained distilled water, saline, or heparinized blood.

The in vitro comparison with human skin gives some perspective to the guinea pig data for mercury. For three $HgCl_2$ concentrations and various intervals up to 48 hr, guinea pig skin was about 2–4 times more permeable than human abdominal skin. The permeability of human mammary skin was greater than abdominal skin but still less than that of guinea pig skin.

To influence the absorption of mercury, three procedures—removal of the stratum corneum, pretreatment for 5 hr with distilled water, and pretreatment for 5 hr with 0.239 M $HgCl_2$—resulted in scattered statistically significant changes. Water pretreatment decreased absorption; $HgCl_2$ pretreatment increased absorption; stripping increased absorption slightly at only one of the three mercury concentrations. The results for mercury absorption, when considered with those for sodium absorption in similar circumstances, do not shed any light on these disparate, and not easily explained, observations.

The use of neat dimethylsulfoxide (DMSO) as the vehicle for 0.239 M

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Table 3 Mercury, as $^{203}HgCl_2$ and $CH_3 \cdot {}^{203}Hg \cdot NHC(=NH)(NHCN)$ (methylmercury dicyandiamide)

Reference: L. Friberg, E. Skog, and J. E. Wahlberg, Resorption of mercuric chloride and methyl mercury dicyandiamide in guinea-pigs through normal skin and through skin pre-treated with acetone, alkylaryl-sulphonate and soap, *Acta Derm. Venereol.* (*Stockh.*) *41*: 40–52 (1961).

Species: guinea pig, M & F in vivo ✓
in vitro

Area: 3.1 cm^2 on the belly (later publications dealt with back skin).

Vehicle: water

Duration of exposure: 5 hr

Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter

Notes:

1. This paper, the earliest in a series of related publications, has the most detail about Wahlberg's procedure including an example of the output from the counting ratemeter used.
2. Small quantities of ^{203}Hg were detected in animal organs after exposure.
3. ''When the experiment was made with an animal that had previously been killed, the counting rate remained constant throughout, thus indicating that Hg^{203} is not resorbed by dead skin.''
4. To determine whether or not covering the skin with water for 5 hr would increase mercury absorption, a controlled experiment was done. When 1 mL of distilled water was in the donor chamber for 5 hr before being replaced by a $^{203}HgCl_2$ solution (16 mg/mL), the absorption of Hg was determined to have a disappearance constant of 15×10^5 min^{-1} compared to 5×10^5 min^{-1} without the water pretreatment. ''Thus no increase in resorption could be shown.''

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Percutaneous absorption of mercury (The $HgCl_2$ data appeared again in a later paper (81). The 8- and 16-mg/mL values are also in the following pretreatment table.)

Conc. (mg Hg/mL)	$HgCl_2$		Methyl mercury dicyandiamide[a]	
	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)
1	8	15	—	—
8	11	21	8	15
16	14	29	21	40
24	8	15	6	12
32	8	15	6	12
40	6	12	5	10
48	<3	<6	<3	<6

[a] Methyl mercury dicyandiamide's solubility in water is only about 16 mg Hg/mL, so that some of these solutions are ''supersaturated.'' Its oleyl alcohol–water distribution coefficient is 8.

Percutaneous absorption of $HgCl_2$ and methylmercury dicyandiamide after pretreatment

		$HgCl_2$				Methyl mercury dicyandiamide			
		16 mg Hg/mL		8 mg Hg/mL		16 mg Hg/mL		8 mg Hg/mL	
Pretreatment[a]	N	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)
No pretreatment	9	15 ± 1	29	11 ± 1	21	21 ± 1	40	8 ± 1	15
Soap, 1%	3	12	23	13	25	13	25	11	21
Acetone-water, 1:1	3	10	19	7	14	12	23	12	23
Alkylarylsulfonate 1%	3	18	35	14	27	20	38	22[b]	42[b]

[a] Pretreatment was accomplished by painting the animals' abdomens for 2 min twice daily for the 6 days before the day of the experiment.
[b] Only this group is significantly different among the comparisons of pretreated with untreated groups.

5. ''Our results showed that there is no significant difference in penetration between the organic and inorganic mercury compounds.''

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Table 4 Mercury, as $^{203}HgCl_2$ and $CH_3 \cdot {}^{203}Hg \cdot C_2H_3N_4$ (methylmercury dicyandiamide)

Reference: E. Skog and J. E. Wahlberg, The effect of alkylaryl-sulphonate and soap on the percutaneous resorption in guinea-pigs of mercuric chloride and methyl mercury dicyandiamide, *Acta Derm. Venereol.* (*Stockh.*) *42*:17–20 (1962).
Species: guinea pig (back) in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: water, 1% soap in water (pH 10), 1% alkylarylsulfonate in water (pH 5)
Concentrations: 8 mg Hg/mL (0.04 M), 16 mg Hg/mL (0.08 M)
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:

Average percutaneous absorption of mercury[a]

	$HgCl_2$ 16 mg Hg/mL		$HgCl_2$ 8 mg Hg/mL		Methyl · Hg · DCD 16 mg Hg/mL		Methyl · Hg · DCD 8 mg Hg/mL	
Vehicle	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (cm/hr)	$10^5 \cdot k$ (min^{-1})	$10^4 \cdot K_p$ (min^{-1})	$10^5 \cdot k$ (cm/hr)	$10^4 \cdot K_p$ (min^{-1})
Water	12	23	10	19	15	28	11	21
Soap, 1%	8	15	7	13	11	21	11	21
Alkylaryl-sulphonate 1%	11	21	11	21	19	36	17[b]	32[b]

[a] For each combination of compound, concentration and vehicle there were six or eight experiments.
[b] Only this case differed significantly ($0.01 < p < 0.001$) from the corresponding value with water only.

Table 5 Mercury, as $^{203}HgCl_2$

Reference: J. E. Wahlberg, Some attempts to influence the percutaneous absorption rate of sodium (^{22}Na) and mercuric (^{203}Hg) chlorides in the guinea pig, *Acta Derm. Venereol.* (*Stockh.*) *45*:335–343 (1965).
Species: guinea pig (back) in vivo ✓
in vitro
Area: 3.1 cm^2, 2 cm^2 for some stripped skin experiments
Vehicle: water, 1% alkyl aryl sulphonate, 1% soap
Concentrations: 0.000078, 0.00013, 0.04, 0.08, 0.239 M
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter

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Table 5 Continued

Notes:

1. The factors tested for their influence on the absorption of mercury were (1) inclusion of 1% soap or 1% detergent in the applied mercury solutions, (2) pretreatment of the exposed area of skin with distilled water or 0.239 M $HgCl_2$ for 5 hr, and (3) removal of the stratum corneum by tape stripping 24 hr before the application of $HgCl_2$ solution.
2. Results are reported as the number of animals in various absorption ranges and, where all individual values are above the limit of detection ($k > 3.4 \times 10^{-5}$ min^{-1}; % absorbed/5 hr > 1), as an average absorption rate. Only the experimental groups with calculated averages are included below, but there are animals/absorption range data for other similar groups exposed to mercury in various vehicles (including water alone) and with pretreatments. (See Chap. 3).
3. The statistical significance (by analysis of variance or, where some individual values were below the limit of detection, by the χ^2 test) of the differences observed with these variations of method was generally low except in the following instances.
4. From alkylarylsulfonate solution only at 0.00013 M and 0.239 M $HgCl_2$ ''was a distinct rise in the absorption rate observed.''
5. From soap solution ''only in the highest concentration (0.239 M) of $HgCl_2$ was an increase obtained.''
6. Only at 0.239 M $HgCl_2$ did stripping, i.e., removal of the stratum corneum to produce a red glistening surface, lead to a ''distinct increase'' in absorption when, 24 hr later, radioactive mercury was applied.
7. Pretreatment with distilled water for 5 hr ''resulted in a decrease in the absorption rate of the 0.080 M $HgCl_2$ solution,'' the only $HgCl_2$ solution so tested.

Average absorption of mercury from $HgCl_2$ under various conditions

Conc. (M)	Vehicle/pretreatment	Disappearance constant $10^5 \cdot k$ min^{-1}	Permeability coefficient $10^4 \cdot K_p$ cm/hr^{-1}
0.00013	1% alkylarylsulfonate	18.4 ± 2.5[a]	35
0.040[b]	Distilled water	8.3 ± 0.3	16
0.040	1% soap	11.4 ± 1.5	22
0.040	1% alkylarylsulfonate	11.8 ± 1.8	23
0.080[b]	Distilled water	10.8 ± 0.8	21
0.080	1% soap	10.5 ± 1.4	20
0.080	1% alkylarylsulfonate	11.2 ± 1.6	21
0.080	Pretreatment: 0.239 M $HgCl_2$ 5 hr	14.4 ± 2.2	27
0.239	1% soap	10.1 ± 1.7	19
0.239	1% alkylarylsulfonate	16.0 ± 2.0	30

[a] Standard error.

[b] The data for these groups are found also in an earlier publication (83).

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Table 6 Mercury, as $^{203}HgCl_2$, $K_2{}^{203}HgI_4$

Reference: J. E. Wahlberg and E. Skog, Percutaneous absorption of mercuric chloride in guinea-pigs. Effect of potassium iodide and the pretreatment of the skin with irritant concentrations of mercury, *Acta Derm. Venereol.* (*Stockh.*) *42*:418–425 (1962).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: water
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:
1. Mercuric chloride precipitates protein.
2. $4KI + HgCl_2 \rightarrow K_2HgI_4$, which is water-soluble and causes slight or no protein precipitation.
3. ''The actual ionization of $HgCl_2$ is not high.''

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Percutaneous absorption of mercury with and without added potassium iodide.

	Back skin								
	$HgCl_2$				K_2HgI_4				
Hg conc. (mg/mL)	No. of expts.	$10^5 \cdot k$ (min^{-1})	%/5 hr	$10^4 \cdot K_p$ (cm/hr)	KI (mg/mL)	No. of expts.	$10^5 \cdot k$ (min^{-1})	%/5 hr	$10^4 \cdot K_p$ (cm/hr)
1	6	10	3.2	19	4	6	6	1.9	11
1	—	—	—	—	159	6	9	2.7	17
8	6	10	3.0	19	27	6	11	3.2	21
16	15	13	3.7	25	53	6	12	3.7	23
24	6	9	2.7	17	80	6	20[a]	5.7	38
32	6	8	2.5	15	106	6	17[a]	5.1	32
40	6	9	2.7	17	133	6	14[a]	4.2	27
48	6	<3	<1.0	<6	159	6	20[a]	5.9	38

[a] These values are significantly higher than those for $HgCl_2$ without KCl.

4. From 16 to 48 mg Hg/mL as $HgCl_2$, mercury flux was quite constant at 35–50 μg/cm^2/hr. From 24 to 40 mg Hg/mL the fluxes of Hg from K_2HgI_4 were significantly greater than from $HgCl_2$.
5. Results of previous experiments using abdominal skin without pretreatment were included in this paper though they were reported earlier elsewhere (see Table 3) (80). Results from the abdomen were not significantly different from absorption on the back.
6. Pretreatment with an irritant concentration of $HgCl_2$ (48 mg Hg/mL) for 5 hr produced only a statistically insignificant increase in absorption from $^{203}HgCl_2$ (16 mg/mL); the range of values was greater but most were in good agreement with the untreated experiments.

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Table 7 Mercury, as $^{203}HgCl_2$, $K_2{}^{203}HgI_4$, $CH_3 \cdot {}^{203}Hg \cdot C_2H_3N_4$ (methylmercury dicyandiamide)

Reference: E. Skog and J. E. Wahlberg, A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110m}Ag, ^{115m}Cd, ^{203}Hg, *J. Invest. Dermatol. 43*: 187–192 (1964).
Species: guinea pig, M & F in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: water
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:

Average disappearance constants, percutaneous absorption and permeability coefficients

Conc. (M)	Compound	Disappearance constant $10^5 \cdot k$ min^{-1}	Absorption % /5 hr	Permeability coefficient $10^4 \cdot K_p$ cm/hr
0.000078	$HgCl_2$	[a]	[a]	
0.00048	$HgCl_2$	[a]	[a]	
0.005	$HgCl_2$	[a]	[a]	
0.04	$HgCl_2$	8.3	2.5	16
0.08	$HgCl_2$	10.8	3.2	21
0.12	$HgCl_2$	7.8	2.3	15
0.16	$HgCl_2$	6.6	2.0	13
0.199	$HgCl_2$	[a]	[a]	
0.239	$HgCl_2$	[a]	[a]	
0.005	K_2HgI_4	[a]	[a]	
0.04	K_2HgI_4	8.3	2.5	16
0.08	K_2HgI_4	11.8	3.5	22
0.12	K_2HgI_4	13.9	4.1	26
0.16	K_2HgI_4	13.6	4.0	26
0.199	K_2HgI_4	13.1	3.8	25
0.239	K_2HgI_4	10.3	3.0	20
0.398	K_2HgI_4	5.9	1.7	11
0.753	K_2HgI_4	[a]	[a]	
0.04	MMDCD[b]	11.6	3.4	22
0.08	MMDCD[b]	15.3	4.5	29

[a] Some individual values were below the limit of sensitivity; no mean was calculated. (See Chap. 3).
[b] Methylmercury dicyandiamide.

1. Some of the mercury results were published earlier elsewhere.
2. Although Hg flux reaches a plateau at the higher concentrations, the authors were unable to explain this observation.

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Table 8 Mercury, as $^{203}HgCl_2$

Reference: J. E. Wahlberg, Percutaneous absorption of sodium chromate (^{51}Cr), cobaltous (^{58}Co), and mercuric (^{203}Hg) chlorides through excised human and guinea pig skin, *Acta Derm. Venereol.* (*Stockh.*) *45*:415–426 (1965).
Species: guinea pig, human in vivo
in vitro ✓
Area: 3.1 cm^2
Vehicle: 1 mL distilled water
Duration of exposure: 48 hr
Concentration: 0.005 M, 0.08 M, 0.239 M $HgCl_2$
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter and/or analysis of receptor solution or HgS precipitated from receptor solution
Notes:

Percutaneous absorption of mercury from 0.005 M $HgCl_2$

Interval (hr)	Disappearance constant, $10^5 \cdot k$ min^{-1} (mean ± SE)		Permeability coefficient $10^4 \cdot K_p$ cm/hr	
	Guinea pig, fresh	Human abdominal stored[a]	Guinea pig	Human
0–5	13.3 ± 1.4	4.6 ± 0.7	25.3	8.8
0–12	7.2 ± 0.6	2.2 ± 0.4	13.7	4.2
12–24	0.5 ± 0.2	0.2 ± 0.1	0.96	0.38
24–36	0.1 ± 0.1	0.1 ± 0.1	0.19	0.19
36–48	0.2 ± 0.1	0.2 ± 0.1	0.38	0.38
0–24	3.8 ± 0.4	1.1 ± 0.2	7.3	2.1
24–48	0.3 ± 0.1	0.1 ± 0.0	0.57	0.19
0–48	1.8 ± 0.1	0.5 ± 0.1	3.4	0.96

[a] $K_p = (1 - e^{-60k})/3.14$ (See Chap. 3).
[b] Experiments with abdominal skin began about 42 hours after death.

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Table 8 Continued

Percutaneous absorption of mercury from 0.239 M $HgCl_2$

Interval (hr)	Disappearance constant, $10^5 \cdot k\ min^{-1}$ (Mean ± SE)		Permeability coefficient $10^4 \cdot K_p$ cm/hr	
	Guinea pig, fresh	Human abdominal stored[a]	Guinea pig	Human
0–5	8.7 ± 0.6	3.2 ± 0.5	17	6.1
0–12	5.7 ± 0.3	2.1 ± 0.3	11	4.0
12–24	1.1 ± 0.3	1.1 ± 0.3	2.1	2.1
24–36	1.1 ± 0.2	0.7 ± 0.2	2.1	1.3
36–48	0.7 ± 0.3	1.3 ± 0.4	1.3	2.5
0–24	3.3 ± 0.2	1.6 ± 0.2	6.3	3.1
24–48	0.9 ± 0.2	1.0 ± 0.3	1.7	1.9
0–48	2.1 ± 0.2	1.3 ± 0.2	4.0	2.5

Percutaneous absorption of mercury from 0.080 M $HgCl_2$

Interval (hr)	Disappearance constant, $10^5 \cdot k\ min^{-1}$ (Mean ± SE)				Permeability coefficient $10^4 \cdot K_p$ cm/hr	
	Guinea pig	Human abdomen	Human mammary		Guinea pig, fresh	Human abdomen stored[a]
	Fresh	Stored[a]	Fresh	Stored		
0–5	7.7 ± 1.1	2.3 ±0.5	4.9 ± 0.6	4.7 ± 0.8	14.7	4.4
0–12	6.1 ± 0.6	1.7 ± 0.3	3.3 ± 0.2	3.0 ± 0.4	11.6	3.2
12–24	2.4 ± 0.4	0.5 ± 0.2	0.7 ± 0.2	1.1 ± 0.3	4.6	0.96
24–36	1.3 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	1.0 ± 0.3	2.5	1.5
36–48	1.1 ± 0.4	0.8 ± 0.3	1.2 ± 0.4	1.1 ± 0.4	2.1	1.5
0–24	4.2 ± 0.4	1.1 ± 0.2	2.0 ± 0.1	2.0 ± 0.3	8.0	2.1
24–48	1.1 ± 0.3	0.7 ± 0.2	0.9 ± 0.3	1.0 ± 0.3	2.1	1.3
0–48	2.6 ± 0.3	0.9 ± 0.1	1.4 ± 0.2	1.5 ± 0.2	5.0	1.7

[a] Experiments with abdominal skin began about 42 hr after death. Stored mammary skin was refrigerated 48 hr before an experiment.

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Mercury absorption through guinea pig skin from 0.08 M $HgCl_2$ with various receptor solutions, receptor flow rates and temperatures

Bathing solution	Distilled water	Saline 0.9%	Saline 0.9%	Saline 0.9%	Saline 0.9%	Heparinized blood
Flow rate, mL/hr	15	15	0	16.6 ± 0.4	167 ± 14.3	0
Receptor, °C	24	24	34	34	34	34
Conductivity at 1 volt	2.0–5.0	0.5–5.0	1.0–3.0	0.5–5.0	0.5–3.0	0.7–4.0
$10^5 \cdot k$ min^{-1} 0–5 hr	5.2 ± 1.0	5.4 ± 0.8	5.7 ± 0.8	7.7 ± 1.1	9.5 ± 1.1	5.7 ± 0.8
$10^4 \cdot K_p$ cm/hr	9.9	10	11	15	18	11
$10^5 \cdot k$ 0–12 hr	3.5 ± 0.8	3.5 ± 0.4	4.1 ± 0.5	6.1 ± 0.6	5.1 ± 0.6	4.3 ± 0.6
$10^4 \cdot K_p$	6.7	6.7	7.8	12	9.7	8.2
$10^5 \cdot k$ 12–24 hr	1.3 ± 0.6	1.1 ± 0.2	3.0 ± 0.8	2.4 ± 0.4	2.1 ± 0.5	1.3 ± 0.4
$10^4 \cdot K_p$	2.5	2.1	5.7	4.6	4.0	2.5
$10^5 \cdot k$ 24–36 hr	1.5 ± 0.5	0.9 ± 0.2	2.2 ± 0.4	1.3 ± 0.3	2.2 ± 0.3	1.6 ± 0.6
$10^4 \cdot K_p$	2.9	1.7	4.2	2.5	4.2	3.1
$10^5 \cdot k$ 36–48 hr	1.2 ± 0.4	1.4 ± 0.5	1.6 ± 0.4	1.1 ± 0.4	1.6 ± 0.4	1.8 ± 0.6
$10^4 \cdot K_p$	2.3	2.7	3.1	2.1	3.1	3.4
$10^5 \cdot k$ 0–24 hr	2.4 ± 0.6	2.3 ± 0.2	3.7 ± 0.5	4.2 ± 0.4	3.7 ± 0.4	2.8 ± 0.4
$10^4 \cdot K_p$	4.6	4.4	7.1	8.0	7.1	5.3
$10^5 \cdot k$ 24–48 hr	1.3 ± 0.4	1.1 ± 0.3	1.8 ± 0.4	1.1 ± 0.3	1.9 ± 0.3	1.7 ± 0.5
$10^4 \cdot K_p$	2.5	2.1	3.4	2.1	3.6	3.2
$10^5 \cdot k$ 0–48 hr	1.8 ± 0.5	1.7 ± 0.2	2.8 ± 0.4	2.6 ± 0.3	2.8 ± 0.3	2.2 ± 0.5
$10^4 \cdot K_p$	3.4	3.2	5.3	5.0	5.3	4.2

1. ''Variations in the type of bathing solution (distilled water, 0.9% NaCl or heparinized blood) did not have any influence [on the absorption rate].''
2. Over the course of 48 hr the disappearance constant was highest in the 0–5 hr time period, lower in the 0–12 hr period and still lower in each subsequent 12-hr interval.
3. ''Absorption was greater at 34°C than at 24°C.''
4. There was a discrepancy between the two methods of determining absorption. Based on the precipitation and recovery of HgS from the receptor solution, absorption was much lower than that determined from the disappearance measurements. Since the filtrate was still radioactive, some ^{203}Hg was not precipitated, and some mercury may have been volatilized. However, Wahlberg does not accept these reasons as complete explanations of the discrepancy.
5. For example, whereas in the first 12 hr the disappearance measurements (in vitro guinea pig) implied an average absorption of 52 μg of Hg from 1 mL of 0.005 M $HgCl_2$, the mercury recovered by precipitation was not more than 0.003 μg following application of 0.02 M $HgCl_2$ (1.5–2.0 mCi) with continuously flowing receptor fluid. (It would have been more appropriate to compare the results with disappearance measurements also from a 0.02 M solution.)

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$HgCl_2$ increased significantly the percutaneous toxicity of the compound in guinea pigs relative to a water vehicle (87). Mortality at 3 weeks post-treatment increased from 20% with water to 80% with DMSO.

There was a less dramatic effect of DMSO on the absorption of ^{203}Hg from topical $HgCl_2$ solutions. The uptake of mercury from 100% DMSO solutions in 5 hr into guinea pig skin in vivo increased relative to the aqueous controls at three out of five concentrations with the largest increase for 0.239 M $HgCl_2$. Absorption from 50% DMSO increased at one concentration of $HgCl_2$; at the other mercury concentration the absorption was decreased (Table 9). Permeability coefficients for mercury for four out of five concentrations in DMSO averaged 27×10^{-4} to 40×10^{-4} cm/hr the first 5 hr, among the highest obtained for mercury under any circumstances by Wahlberg and his associates. Pretreatment with DMSO increased the absorption of 0.239 M $HgCl_2$.

A second factor in the higher toxicity of $HgCl_2$ was evident in longer experiments; absorption from DMSO decreased less with time than from water. Over a period of 20 hr from 0.08 M $HgCl_2$, the absorption rate of mercury decreased by only about 20% from DMSO compared to 50% from water.

Experimental evidence supporting the importance of the follicles and sebaceous glands in guinea pig skin as routes of entry to the body is ambiguous (88). In vivo and in vitro absorption of mercury (0.239 M $HgCl_2$) from 1% detergent was the same through guinea pigs' hairy skin as through the nonhairy skin behind the ears (Table 10). Thus, in this case, follicles and sebaceous glands did not

Table 9 Mercury, as $^{203}HgCl_2$

Reference: J. E. Wahlberg and E. Skog, The effect of dimethyl sulfoxide on the percutaneous absorption of mercuric chloride in the guinea pig, *Acta Derm. Venereol.* (*Stockh.*) *47*:209–215 (1967).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: water, water-DMSO, DMSO
Duration of exposure: 5, 20 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:
1. 0.239 M $HgCl_2$ in 100% DMSO was much more toxic than in aqueous solution: 12 deaths in 1 week compared to 0 deaths, and 16 deaths in 3 weeks compared to 4 deaths. There were no deaths in a group of 5 control guinea pigs exposed only to DMSO without $HgCl_2$.
2. DMSO caused a moderate increase in absorption but only in 3 of 5 $HgCl_2$ concentrations studied; at 0.04 M in 50% DMSO the absorption decreased compared to 100% aqueous solutions.

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Table 9 Continued

Percutaneous absorption of $HgCl_2$

Conc. of $HgCl_2$ (M)	Vehicle	Disappearance constant $10^5 \cdot k$ min^{-1}	Permeability coefficient $10^4 \cdot K_p$ cm/hr
0.00013	Distilled water	(2.3–4.4)[a]	(4.4–8.4)
0.00013	100% DMSO	17.1 ± 2.4[b]	33
0.005	Distilled water	(7.5–8.2)[a]	(14–16)
0.005	100% DMSO	(7.6–8.3)[a]	(14.7–16.0)
0.04	Distilled water	8.3 ± 0.3[b]	16.0
0.04	100% DMSO	14.0 ± 1.2[b]	27
0.04	50% DMSO	(3.6–6.0)[a]	(7.0–11.6)
0.08	Distilled water	10.8 ± 0.8[b]	21
0.08	100% DMSO	14.8 ± 3.7[b]	28
0.239	Distilled water	(1.3–4.2)[a]	(2.5–8.0)
0.239	100% DMSO	20.6 ± 2.3[b]	40
0.239	50% DMSO	(8.0–8.3)[a]	(15.4–16.0)[a]

[a] See Chap. 3.
[b] Standard error.

1. Over 20 hr, whether from water or DMSO, the disappearance rate of $HgCl_2$ decreased gradually by 54% and 21% for water and DMSO, respectively, from the first 5 hr to the last 5-hr period.
2. Pretreatment for 5 hr by 100% DMSO for 0.08 M and 0.239 M $HgCl_2$ resulted in similar disappearance constants for both groups of pretreated animals. Since the control values were so different, 10.8×10^{-5} min^{-1} and $1.3–4.2 \times 10^{-5}$ min^{-1} for 0.08 M and 0.239 M $HgCl_2$, respectively, the difference between pretreated and not pretreated was significant only for the higher concentration.

Permeability coefficients of guinea pig skin for mercury from 0.08 M $HgCl_2$

Time period (hr)	$K_p \cdot 10^4$, cm · hr^{-1}	
	Distilled water	DMSO
0–5	15	28
5–10	12	25
10–15	9.9	24
15–20	6.9	22

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Table 10 Mercury, as $^{203}HgCl_2$

Reference: J. E. Wahlberg, Transepidermal or transfollicular absorption? In vivo and in vitro studies in hairy and non-hairy guinea pig skin with sodium (^{22}Na) and mercuric (^{203}Hg) chlorides, *Acta Derm. Venereol.* (*Stockh.*) *48*:336–344, (1968).

Species: guinea pig, hairy and nonhairy skin in vivo ✓
in vitro ✓

Area: 3.1 cm^2 in vitro; 1.57 cm^2 in vivo

Vehicle: water; 1% aqueous alkylarylsulfonate

Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter

Notes:

1. Behind the ears of guinea pigs there is an area of skin with few or no hair follicles, sebaceous glands, or sweat glands. This area was the ''nonhairy'' skin in these experiments. The hairy skin was from the back.

Absorption of mercury from 0.239 M $HgCl_2$ through guinea pig skin

Vehicle	Skin	N	Disappearance constant, $10^5 \cdot k \pm$ SE min^{-1}	Permeability coefficient $10^4 \cdot K_p$ cm/hr
Distilled water	In vivo, hairy	15	(1.3–4.2)[a]	(2.5–8.0)
Distilled water	In vivo, nonhairy	10	(3.9–5.2)[a]	(7.4–9.9)
Distilled water	In vitro, hairy	10	8.7 ± 0.6	17
Distilled water	In vitro, nonhairy	10	6.1 ± 0.8	12
1% alkylaryl-sulfonate	In vivo, hairy	10	16.0 ± 2.0	30
1% alkylaryl-sulfonate	In vivo, nonhairy	10	15.9 ± 1.8	30
1% alkylaryl-sulfonate	In vitro, hairy	10	6.1 ± 1.0	12
1% alkylaryl-sulfonate	In vitro, nonhairy	10	5.1 ± 0.6	9.7

[a] $K_p = (1 - e^{-60k})/3.14$. See Chap. 3.

2. ''It was found that absorption in vivo occurs principally via the transepidermal route.'' Hairy skin did not have a lower resistance to absorption.
3. Comparisons between vehicles, between concentrations, between in vivo and in vitro experiments, and between metals were not consistent. No one variable consistently dominated the relative absorption rates of metal.
4. In vitro absorption of $HgCl_2$ from distilled water was higher through hairy than through nonhairy skin according to a χ^2 test, but in vivo the relative ranking was reversed.
5. From 1% alkylarylsulfonate there was no significant difference between absorption through hairy and nonhairy skin either in vitro or in vivo.
6. Absorption of $HgCl_2$ at three concentrations in water through hairy skin was slower in vivo than in vitro at the lowest concentration (0.005 M), faster in vivo than in vitro at the intermediate concentration (0.08 M), and slower in vivo than in vitro at the highest concentration.
7. In vivo absorption was higher from alkylarylsulfonate than from water, but in vitro absorption was higher from distilled water than from alkylarylsulfonate although the difference was small and statistically insignificant with nonhairy skin.

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Table 11 Mercury, as $^{203}HgCl_2$

Reference: J. E. Wahlberg, Vehicle role of petrolatum. Absorption studies with metallic test compounds in guinea pigs, *Acta Derm. Venereol.* (*Stockh.*) *51*:129–134 (1971).
Species: guinea pig, M & F in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: petrolatum, distilled water
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter
Notes:
1. To test petrolatum, it was mixed with $HgCl_2$ crystals smaller than 0.3 mm.

Percutaneous absorption of $HgCl_2$ by guinea pigs

Conc. of $HgCl_2$ (%)	Time (hr)	N	Disappearance constant $10^5 \cdot k$ min^{-1}	Flux (μg Hg/cm^2/hr)	Permeability coefficient $10^4 \cdot K_p$[a] cm/hr	Vehicle
0.14	≥5	10	(7.5–8.2)[b]	(1.5–1.6)[b]	(14.3–15.6)	1 mL distilled water
2.17	≥5	20	10.8 ± 0.8 (SE)	33.4	20.6	1 mL distilled water
6.49	≥5	15	(1.3–4.2)[b]	(12.1–39.0)[b]	(2.5–8.0)	1 mL distilled water
0.14	≥5	10	(4.8–5.8)[b]	(0.5–0.6)[b]	(9.2–11.1)	0.5 mL petrolatum
0.14	≥5	10	(6.5–7.2)[b]	(1.3–1.4)[b]	(12.4–13.7)	1 mL petrolatum
2.17	≥5	10	(5.1–6.8)[b]	(15.8–21.1)[b]	(9.7–13.0)	1 mL petrolatum
6.49	≥5	10	(4.8–6.2)[b]	(44.6–57.6)[b]	(9.2–11.8)	1 mL petrolatum

[a] $K_p = (1 - e^{-60k})/3.14$. See Chap. 3.
[b] Values from some guinea pigs were below the limit of sensitivity. See Chap. 3.

2. The only statistically significant difference between vehicles was at 2.17% $HgCl_2$ where absorption was higher from water ($P < 0.001$).

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Table 12 Mercury, as $^{203}HgCl_2$

Reference: T. Dutkiewicz and M. Oginski, The absorption of mercuric chloride through the skin of the rats, *Zes. Nauk. Bromat. Chem. Toksykol. 1*:131–136 (1968).
Species: rat, F; tail in vivo ✓
in vitro
Area: about 10 cm^2
Vehicle: water
Duration of exposure: 1 hr
Analytical method: radioactivity in tissues, organs, and excreta
Notes:
1. Exposure to mercury was accomplished by immersing the tail in mercuric chloride solution.
2. The tail was rinsed of excess solution after the exposure period.

Absorption by rats

$HgCl_2$, applied conc. (M)	Total $HgCl_2$ absorbed[a] average (mg)	Average uptake of Hg ($\mu g/cm^2/hr$)	Apparent permeability coefficient $10^4 \cdot K_p$ cm/hr
0.01	1.5	143	710
0.1	7.4	540	270
0.2	11.1	1180	290

[a] Includes the mercury in all organs, tissues, and excreta.

3. Fourteen days after the exposure about 40% of the mercury was still at the site of application on the tail.
4. At least 54% of the mercury was recovered with the feces and urine.

serve as major routes of entry for mercury. Absorption of mercury from water was less than from detergent, but there was an in vivo–in vitro disagreement on the hairy versus nonhairy question. Hairy skin was statistically less permeable in vivo and more permeable in vitro than nonhairy skin, although the differences were not great. On balance, for mercury absorption, hairy and nonhairy skin were about equally permeable with permeability coefficients of about $5–15 \times 10^{-4}$ cm/hr from distilled water and $10–30 \times 10^{-4}$ cm/hr from detergent. Results for sodium were similarly mixed (Chap. 24, p. 275).

For two out of three tested concentrations (0.14%, 2.17%, and 6.49% $HgCl_2$), the absorption rate for mercury was about the same from small crystals of $HgCl_2$ in petrolatum as for aqueous solutions (Table 11) (89). At 2.17% $HgCl_2$ the mercury flux was greater from solution than from crystals in petrolatum. (Chromium and cobalt had slightly higher, but not statistically significant, absorption from aqueous solution compared to petrolatum mixtures.)

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There may be another lesson to be learned in these petrolatum experiments. Although in Wahlberg's disappearance experiments some of the radioactive substance could ''disappear'' under a thicker layer of shielding material (water) by binding and accumulating at the stratum corneum surface, it seems less likely that this would occur with crystals in petrolatum than in aqueous solution. The disappearance of radioisotope from petrolatum would then be more reliably attributed to absorption through the skin followed by relocation to deeper or more distant sites from which the radiation is less likely to be counted. The disappearance measured from petrolatum is similar to that from many aqueous solutions.

When rats tails were immersed for an hour in aqueous solutions of $HgCl_2$ (90), uptake of mercury was very rapid, corresponding to apparent permeability coefficients of 270–710 $\times$ 10^{-4} cm/hr (Table 12). Fourteen days after the exposure, about 40% of the mercury recovered from the animals was still in the skin of the tail.

Estimates of permeability coefficients of mercury through isolated human stratum corneum as two cosmetic ingredients, phenylmercuric acetate (a preservative) and ammoniated mercury (a bleaching agent), were extremely small, in the range 0.001–0.05 $\times$ 10^{-4} cm/hr (Table 13) (91).

The absorption of merbromin (0.5% mercury) through pig skin has been measured (92). For a 6-hr exposure, the permeability coefficient is about 0.05 $\times$ 10^{-4} cm/hr (Table 14). Mercury was absorbed more quickly from phenylmercury acetate (PMA) and phenylmercury borate (PMB) preparations. Even though the mercury concentration in both instances was lower at only 0.1% and the time of

Table 13 Mercury, as ammoniated ^{203}mercury (AMM) and phenyl ^{203}mercuric acetate (PMA)

Reference: F. N. Marzulli and D. W. C. Brown, Potential systemic hazards of topically applied mercurials, *J. Soc. Cosmet. Chem. 23*:875–886 (1972).
Species: human, forearm stratum corneum in vivo
in vitro ✓
Area: unspecified but 0.2 cm^2 in cited references
Vehicle: 95% alcohol (PMA), commercial bleach cream (AMM)
Duration of exposure: 24 and 72 hr
Analytical method: determination of radioactivity in samples collected from the receptor chamber of the diffusion cell
Notes:
1. Stratum corneum (stratum corneum conjunctum) was removed from cadavers with tape and removed from the tape with solvent (128).
2. PMA is a preservative used at concentrations up to 0.05% in cosmetics.
3. The lag time for PMA penetration was about 1 hr.
4. Although the quantified substance was ^{203}Hg, the penetration rates were reported in terms of PMA.

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Table 13 Continued

Maximum steady flux of alcoholic PMA through stratum corneum[a]

	Mean flux[b] ng PMA/cm^2/hr		Permeability coefficient $10^4 \cdot K_p$ cm/hr	
Conc. (% PMA)	0–24 hr	24–72 hr	0–24 hr	24–72 hr
0.06	0.2	0.1	0.003	0.002
0.13	0.3	0.1	0.002	0.0008
0.13	4	0.2	0.03	0.002
0.25	2	0.3	0.008	0.001
0.50	3	1	0.006	0.002
1.00	2	1	0.002	0.001

[a] Stratum corneum was from different sources and experiments were carried out at different times.
[b] Despite the range of values for the first 24 hr they were not significantly different. The decrease from 0–24 hr to 24–72 hr was significant only for the three lower concentrations.

Maximum steady flux of PMA through stratum corneum (parallel tests)[a]

Conc. (% PMA)	Mean flux ng PMA/cm^2/hr 0–24 hr	Permeability coefficient $10^4 \cdot K_p$ cm/hr 0–24 hr
0.001 parallel group 1	0.03[b]	0.03
0.063 parallel group 1	2[b]	0.03
0.063 parallel group 2	1	0.02
0.125 parallel group 2	2	0.02
0.250 parallel group 2	5	0.02
0.500 parallel group 2	2	0.004

[a] ''Parallel tests'' were done at the same time and used stratum corneum from the same source.
[b] Significantly different ($p < 0.02$).

5. 1–5% concentrations: ''[AMM] is practically insoluble in all useful solvent systems.'' Its solubility in isotonic saline, used as the receptor fluid, was about 0.6 μg/mL.

Maximum steady flux of ^{203}Hg-AMM through stratum corneum

	Mean flux ng AAM/cm^2/hr		Apparent permeability coefficient $10^4 \cdot K_p$ cm/hr	
Conc. (% PMA)	0–24 hr	0–24 hr	0–24 hr	24–72 hr
3.9	200	100	0.05	0.03
5.4	100	50	0.02	0.01
9.9	50	20	0.005	0.002

Table 14 Mercury, as ^{203}Hg-merbromin (mercurochrome), ^{203}Hg-phenylmercury borate (PMB), and ^{203}Hg-phenylmercuric acetate (PMA)

Reference: H. Pratzel, Untersuchen zur perkutanen Aufnahme—Quecksilber aus Desinfektionsmitteln (Studies on percutaneous uptake—mercury from disinfectants), *Klinikarzt 16*(*Suppl.*):3–10 (1987).
Species: pig in vivo ✓
in vitro
Area: 300 cm^2
Vehicle: water, isopropanol
Duration of exposure: for merbromin, 6 hr; for PMB and PMA, 10 min for propanol solutions and 1 hr for aqueous solutions
Analytical method: counting of γ radiation in excreta, blood, tissues, and organs; correction factors for estimating the total percutaneous absorption were derived from subcutaneous injections
Notes:
1. Percutaneous absorption of mercury during 6 hr contact with merbromin (0.5% Hg) totaled 0.1 μg Hg/cm^2.
2. Percutaneous absorption of mercury during 1 hr of contact with aqueous PMA (0.1% Hg) or PMB (0.1% Hg) led to at least a 50-fold increase in urinary excretion of Hg compared to the merbromin experiment.
3. Percutaneous absorption of Hg during 10 min contact with PMA or PMB in propanol (0.1% Hg in both solutions) caused about a 10-fold increase in the urinary excretion of Hg compared to the merbromin experiment.

Compound	Method of administration	Urinary excretion ng/cm^{-2} exposed area	$10^4 \cdot K_p$ cm/hr^{-1}
Merbromin, 6 h	Dermal	3.1 ng/cm^2 in 5 d	0.05[a]
PMA/water	Dermal	36 ng/cm^2 in 5 d	—[c]
PMB/water	Dermal	103 and 273 ng/cm^2 in 5 d	—[c]
PMA/propanol	Dermal	9.7 and 10.1 ng/cm^2 in 5 d	
PMB/propanol	Dermal	12 ng/cm^2 in 5 d	
Merbromin	Subcutaneous injection	4.4, 6.0, and 5.4 % in 5 d[b]	Not applicable
PMA	Subcutaneous injection	Not done	—
PMB	Subcutaneous injection	0.73 % in 5 hr[b]	Not applicable

[a] Assumes that the applied preparation was covered and that its concentration was constant at the concentration of the solution.
[b] not significantly different during comparable time periods.
[c] If the Kp for merbromin is used as a basis for estimating K_ps for PMA and PMB (by incorporating factors for contact time, concentration and excretion), then these values would be 300 and 1000 times larger, respectively.

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Table 15 Mercury, as ^{203}Hg

Reference: J. B. Hursh, T. W. Clarkson, E. F. Miles, and L. A. Goldsmith, Percutaneous absorption of mercury vapor by man, *Arch. Environ. Health 44*:120–127 (1989).

Species: human, forearm, M in vivo ✓
in vitro

Area: 357–427 cm^2

Vehicle: vapor (concentrations 18–43 times the threshold limit value of the U.S. National Institute for Occupational Safety and Health)

Duration of exposure: 27–43 min

Analytical method: γ ray counting of the whole body, body regions (counting efficiency for forearm = 21.4%), epidermal tape strips, and excreta

Notes:

1. The forearm was exposed to Hg vapor in a 12-L Saran bag; the hand was protected by a smaller Saran bag so that the hand would not be exposed. On average, 6.8% (range 3.0–10.6%) of the Hg vapor originally in the exposure chamber was absorbed by the arm.
2. Up to half of the mercury initially in the forearm was shed by desquamation of epidermal cells during several weeks. The remainder diffused into the general circulation and could be measured as systemic mercury.
3. For two subjects on the day following exposure, stratum corneum was collected from a 35-cm^2 area of the forearm by repeatedly applying and removing pieces of an adhesive cellophane tape. The ^{203}Hg measured on the tape normalized for the total exposed area corresponded to only 0.3% and 1.3% of the Hg in the arm at that time. When the process was repeated for one subject 14 days and again 23 days after exposure, the additional recoveries normalized to the entire exposed area were 24% and 10.7%.

Uptake of mercury vapor by skin

Skin area (cm^2)	Exposure duration (min)	^{203}Hg vapor concentration (ng/cm^3)	Hg in skin at the end of exposure (ng)	Permeability coefficient[a] $10^4 \cdot K_p$ cm/hr
427	43	1.21	546	14760
396	43	0.88	278	8100
357	34	1.76	216	6060
363	27	2.73	844	24120
369	27	2.06	287	8280

[a] The reader's attention is drawn to the very high numerical value of these K_ps.

4. Hursh et al. conclude that ''absorption of mercury vapor by the skin poses a very minor occupational hazard compared to inhalation.''

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exposure was shorter than for merbromin, the urinary excretion was higher. Applying these factors to the merbromin permeability coefficient puts the PMA and PMB permeability coefficients at about $15–50 \times 10^{-4}$ cm/hr.

Inhalation of mercury vapor has long been considered a hazard, but in 1989 Hursh et al. measured for the first time the uptake of mercury vapor by the skin (56). The rate determined corresponded to a very high permeability coefficient, about 1 cm/hr (Table 15). As much as half of the absorbed mercury was lost by desquamation in the following weeks. Despite the seemingly high rate of extraction from the air, Hursh et al. concluded that, unless the mercury concentration in air is much above the threshold limit value or unless mercury is trapped in clothing, inhalation was a much greater hazard than the dermal absorption of mercury.

E. IMMUNOLOGY

1. An Overview

Mercury compounds have been administered for medical purposes since the earliest times, applied directly to the skin, or given by mouth, and more recently by intramuscular or intravenous injection. Hypersensitivity (i.e., contact sensitivity) to mercury as a consequence of medicinal administration was first described in 1895 (93). Like other transition group elements and highly electropositive heavy metals, such as lead and cadmium, mercury can act as a coordination center for electronegative groups (SH groups and disulfide bridges, cysteinyl and histidyl residues) present in proteins, purines, and pteridines. Such complexation can cause conformational changes and hence immunogenicity (94). Hypersensitivity reactions to host proteins haptenized by mercury following skin contact or systemic exposure can be (a) of type I or anaphylactic, mediated by immunoglobulin E (IgE) (presenting clinically as urticaria); (b) of type III or Arthus reactions involving antigen–antibody complexes with the kidneys as main target organ; and (c) type IV or delayed hypersensitivity mediated by macrophages and sensitized T lymphocytes, resulting in contact dermatitis.

2. Autoimmunity

Exposure to mercury or mercurials induces autoimmune disease described as type III or Arthus reaction in several species including humans. Such reactions, characterized by lymphoproliferation, are mediated mainly by IgG and involve generation of antigen–antibody complexes. A genetic predisposition appears to be an important etiological factor for such metal-induced autoimmunity. For mercury, immune complexes are preferentially deposited in the vascular endothelium of the kidneys, leading to destructive inflammatory reactions and glomerulonephritis (70–72,95). Workers chronically exposed to mercury vapor in the indus-

trial environment develop proteinuria due to glomerulonephritis secondary to deposition of immune complexes in the basement membrane of renal epithelial cells (19). In experimental animals, exposure to mercury salts, particularly $HgCl_2$, has been shown to trigger a T-dependent polyclonal activation of B cells and to result in the formation of autoantibodies (96).

3. Contact Dermatitis and Urticaria

In the human repeat-insult patch test, mercuric chloride registered 92% positive reactions, making the salt a class 5 (extreme) sensitizer on the Magnusson-Kligman scale (97). In both children and adults, mercury (as amalgam) is a moderate topical sensitizer. Hypersensitivity, both of the cell-mediated (type IV or contact dermatitis) and humoral (type I or urticarial) type, is induced by mercury compounds. Among the various cutaneous manifestations of sensitization, the most characteristic is mercury exanthem, a generalized eruption that can result from exposure to either mercury or a variety of its compounds (34,40,98,99).

While mercury is a frequent cause of ACD due to multiple systemic and dermal exposure, immediate-type hypersensitivity as well as ACD has been noted to occur concurrently in a few patients (39).

4. Prevalence of Sensitization

Mercury allergy is relatively common due to multiple and widespread use of mercury-containing products, such as (a) medicaments and disinfectants based on mercury salts; (b) organic mercury compounds (e.g., mercurochrome or merbromin, thiomersol, also known as thimerosal or merthiolate, a common topical antifungal and antibacterial, also used as preservative in contact lens solutions, which cross-reacts with phenylmercuric acetate, a common industrial preservative and agricultural pesticide) (34,35,100,101); (c) mercury contamination of foods; and (d) metallic mercury, which is still found in antiparasitic powders, thermometers, and other instruments (34). Contact allergy to the metal, and to its organic and inorganic compounds, is well documented (102). Rate of sensitization ranges from 3% in France to 13% in Japan, and 21% (specifically to thimerosal), in Austria (103). The prevalence in the United States is estimated to be 5% (104,105).

5. Cross-Sensitization

Many cross-reactions between metallic mercury and organic or inorganic mercurials have been reported (100,106,107). An unusual type of cross-reactivity to different mercury species was noted in patients sensitized to gold; when patients exhibiting gold dermatitis were patch-tested with aqueous. mercuric chloride

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($HgCl_2$), two-thirds showed positive reactions to mercury; but of seven subjects sensitized to gold, only one tested positive with elemental mercury (in petrolatum). Finally, when individuals with systemic contact dermatitis to mercury were tested with $HgCl_2$ and Hg, all reacted to both the elemental and the ionic form of the metal (108,109).

6. Systemic Sensitization

Mercury-containing drugs can cause allergic contact dermatitis when used topically and also upon inhalation of the metal vapor (34,110). Asthma, urticaria, and anaphylactic shock can result from ingestion, injection or dermal application (36,111). Organic mercurials for diuresis were administered by many routes and were rapidly eliminated by the kidney, particularly when given intravenously; however, these compounds caused flushing, pruritus, and urticaria (112).

Both delayed and immediate reactions have been noted in the same patient after topical application of mercurials (38). The transition from urticaria to eczema has also been seen on the same test site (40). Following topical application, mercury is selectively taken up by the Langerhans cells of viable human epidermis (113). As for other heavy-metal-based pigments used in tattoos, red cinnabar (mercuric sulfide) is a common cause of cutaneous allergic and photo allergic reactions of the delayed type (114). Tattoo removal using lasers can provoke sensitization; the pigment-containing cells are fractured, thus releasing the compound into the extracellular milieu (115).

Inhalation of mercury vapor, e.g., from broken thermometers, can cause allergic reactions in previously sensitized individuals (34,99,116,117).

Allergic reactions to mercury attributed to dental amalgam restorations become manifest through signs and symptoms of contact allergy. Orally, reactions range from edema or ulcers, to gingivostomatitis (oral lichenoid lesion, OLL) (118–122); however, the skin is the site most commonly affected, with the occurrence of generalized urticarial erythema. In a significant measure, such reactions may be attributed to a systemic etiopathology, as antigenic material can penetrate the oral mucosa affected by chronic irritation caused by rough fillings, increased antigen penetration through diseased mucosa, or retention of antigen by the mucosa due to continuous exposure (44). Occupational respiratory exposure to dust, mist, and fumes of various metals, and particularly to measurable amounts of mercury vapor (such as may occur in amalgam synthesis in dental laboratories), can elicit contact dermatitis (123).

7. Diagnostic Tests

Because patch testing for mercury hypersensitivity is associated with false-positive reactions due to irritant nature of some of the mercury salts used, to establish

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the true nature of sensitization simultaneous patch testing with several mercury compounds are recommended: mercuric chloride, metallic mercury, ammoniated mercury, phenylmercuric acetate, thimerosal and amalgam powder, and preferably the combination of mercuric chloride (0.1%), mercury (0.5%), and mercury ammonium chloride (1.0%), all in petrolatum (44,124). In addition, the use of MELISA (memory lymphocyte immunostimulation assay) has also been proposed as an alternative. This method reveals lymphocyte reactivity to both mercury and a number of other metals (Au; Pd) in vitro (125). This technique has also confirmed the dependence of metal-specific responses on genetic predisposition (126).

8. Treatment of Hypersensitivity

When patients with mercury hypersensitivity have been treated with chelation therapy, their symptoms have improved (127). Selenium seems to play an antagonistic role with respect to mercury allergy. Applied epicutaneously or when ingested, low selenium concentrations eliminate the delayed hypersensitivity reaction to repeated dermal contact with Hg. Dietary selenium supplementation can also alleviate immediate hypersensitivity response to mercury vapor (98).

F. SUMMARY

Mercury, present throughout the environment, poses both a cutaneous and systemic toxicity hazard in all its forms: as salt, complexed with other elements, in organometallic compounds, and as the elemental vapor. Target organs in humans are the thyroid and the kidney, where accumulation of mercury can have carcinogenic effects. Hg is a developmental toxicant; methylmercury, naturally occurring in seafood, is a particular environmental health threat to the nervous system of the developing fetus in fish-eating populations. Cutaneous absorption of the metal vapor, (Skin notation by the ACGIH) of its alkyl and aryl derivatives and of inorganic mercury compounds has been characterized by the ACGIH as presenting a significant risk of systemic toxicity, and particular care is recommended to avoid direct skin contact with dimethylmercury in the work environment. In the general population, MeHg accumulated from the ingestion of seafood is the major source of exposure, associated with elevated risk of thyroid cancer; the presence of amalgam in dental restoration work does not give reason for concern, and in only the most hypersensitive is mercury replacement indicated. Mercury compounds are also immunotoxic. In humans, mercury and its compounds are moderate topical and systemic sensitizers, apparently affecting genetically predisposed individuals in particular, causing immediate- and delayed-type hypersensitivity,

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as well as generating immune complexes. Salts of Hg^{2+} in particular have such IgG-mediated autoimmune effects and stimulate IgE production.

In human and guinea pig skin in vitro, the permeability coefficients of Hg salts are about 10^{-5} cm/hr; but in vivo data from guinea pigs indicate values closer to 10^{-3} cm/hr. Mercury reacts strongly with skin proteins, particularly with sulfhydryl group; as a result, penetration does not increase commensurably with increasing exposure concentrations but rather approaches a plateau value.

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18
Indium

A. GENERAL COMMENTS

Indium is a rare metal, frequently used in plating jewelry, in semiconductors, and in dentistry. It poses no notable toxicological risk because at physiological pH its salts hydrolyze to the highly insoluble $In(OH)_3$. There is no record of health problems to the general population from any type of exposure to indium or its compounds.

B. SKIN REACTIVITY

Human skin maintained in direct contact with indium metal or various indium salts for up to 30 days showed no untoward effects (1).

C. IMMUNOLOGY

The open epicutaneous test (OET) on albino guinea pigs showed that indium trichloride is a strong contact allergen. When put in relation to the compounds palladium dichloride and vanadium sulfate, the latter two were found to be even more severe (2).

D. SUMMARY

There are no reports of toxicity to indium or its salts in humans following either oral ingestion or dermal contact, although its salts were shown to have sensitizing properties in animals. No significant skin penetration would be expected due to precipitation of insoluble metal hydroxide at physiological pH. No data were located regarding the skin penetration of any indium compound.

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19
Potassium

A. GENERAL COMMENTS

Potassium is an essential macroelement, vital in the maintenance of normal water balance, neurotransmission, and muscle and enzymatic activity. Present in cells at high concentrations, potassium is the principal intracellular metal ion. The extracellular concentration of K^+ is maintained at 5 mM, the intracellular at 140 mM (1). The normal homeostatic mechanism maintains steady levels of the metal ion in the body, despite wide fluctuations in intake. Absorption of excessive amounts of potassium may have toxic consequences (2), particularly if an imbalance in normal Na^+ levels is induced. Extracellular (serum) potassium acts as an indicator of total potassium status; it rises and falls only once intracellular levels exceed or fall below capacity. Those intracellular values can be subject to major fluctuations while serum levels remain constant. It is the extracellular concentration of potassium that produces major untoward effects; a 3% increase of serum levels will cause fatal hyperkalemia, usually observed in cases of severe renal failure. The fatal dose of potassium (as K_2CO_3) is 15 g in the average adult. Potassium deficiency or hypokalemia (serum concentrations below 3 mEq/L) becomes manifest by muscular weakness, increased nervous irritability, disorientation, cardiac irregularities, and, ultimately paralysis and respiratory failure.

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Measurements of potassium (and sodium) ion transport across human skin indicate that absorption increases with increased applied concentrations, following Fick's law of diffusion. There is some evidence that penetration from natural mineral waters is more efficient than that from a simple solution, though the precise reason for this observation has not been established (3,4).

Penetration rates of a number of metal ions through human skin in vivo have been placed on a relative scale. Based on the changes induced in the direct

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current resistance of the skin by their diffusion, potassium ion was the most mobile over Na, Li, Ca, Mg, and Al ions, in that order (5). For that group of metal ions, diffusivity seems to be determined by the size of the hydration sphere.

The nature of the counterion is important to absorption characteristics of potassium, e.g., potassium chloride is better absorbed than potassium iodide (6,7). Potassium is excreted in significant amounts in sweat. Values fluctuate as a function of perspiration rate and other variables, such as diet and hormonal control (8). K^+ losses through arm skin of male volunteers during maximum sweating amounted to 30% of the total daily excretion (0.125 g/hr) (9). Measured over a period of 6 hours on the human forearm at rest, the average potassium concentration in transudate was 0.9 mmol/L (10).

Determined by electron probe analysis and electron microscopy, the potassium concentration profile in human skin cells shows fairly uniform distribution throughout the dermis but drops dramatically at the inner stratum corneum; a large gradient exists between dermis and epidermis. This gradient, similar to that observed for the other diffusible elements sodium and chlorine, could be due to an elaborate nutrient recycling mechanism causing inward diffusion of potassium from the skin surface after sweating (11).

In living organisms the free flow of potassium ions in and out of cells through designated channels is critical for normal physiological function and is interdependent with Ca ion flow. A defect in such channeling has been detected in the brain cells of patients with Alzheimer's disease (AD), and subsequently observed to occur also in their skin. Early diagnosis of the disease may thus become possible through a simple skin test, i.e., skin fibroblast cultures are tested for the presence of potassium channels, visualized by patch-clamp technique and fluorescence imaging of calcium flow. Since K^+ channel dysfunction is AD-specific, the method will allow adequate distinction between AD patients and other conditions due to normal aging or disease states not associated with defective potassium channels such as Parkinson's, Huntington's, and Wernicke-Korsakoff diseases (12).

Potassium, like calcium and magnesium, plays a role in epidermal barrier homeostasis, acting synergistically with calcium. In experiments with mice in vivo, the reestablishment of skin barrier properties following delipidization was blocked by either increased exogenous potassium or increased calcium. This indicates that lipid barrier repair is initiated by both lowered Ca and K concentrations in the upper epidermis, occurring as a consequence of passive loss through increased liquid flux (transepidermal water loss) (13).

Cross-sectional and longitudinal localization of the element in human scalp hair by micro-PIXE analysis reveals that K is mainly present in the hair bulb and in the zone of keratinization (14). Elemental analysis by plasma atomic emission spectroscopy (15) of samples of human cerumen, a compound consisting of secre-

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tions from sebaceous and ceruminous glands, similar to apocrine glands, and of exfoliated epidermal cells, showed the presence of potassium in the range of 5.7–19 mg/g dry weight (16).

Potassium hydroxide, used widely in industry, poses a severe occupational hazard; in concentrated form it is capable of rapidly destroying skin and eye tissue on contact.

C. QUANTITATIVE ABSORPTION DATA

The in vitro permeabilities of rabbit and pig skin to potassium from aqueous 0.155 M potassium chloride have been determined with the use of radioactive ^{42}K (Table 1) (17). The ion flux reached a steady value after 1–3 hr and indicated K_p values of 22×10^{-4} cm/hr for rabbit skin ($N = 6$) and 1.9×10^{-4}cm/hr for pig skin ($N = 3$). Through rabbit skin the flux of potassium was not significantly different from the flux of sodium or bromide.

In experiments that measured the reverse of percutaneous absorption, the potassium flux from the inside of normal skin to water on the stratum corneum was $3.5 (\pm 3.6) \times 10^{-9}$ mol/cm^2/sec (Table 2) (18). Removing the stratum corneum doubled the potassium flux. The brief duration of these experiments made it impossible to determine a reasonable permeability coefficient.

Table 1 Potassium, as ^{42}KCl

Reference: R. T. Tregear, The permeability of mammalian skin to ions, *J. Invest. Dermatol. 46:*16–23 (1966).
Species: pig (flank) and rabbit (flank) in vivo
in vitro ✓
Area: 4 cm^2
Vehicle: not specified, probably water
Concentration: 155 mEq/L (11.5 mg KCl/mL)
Analytical method: radioisotope content of receptor fluid (0.9% NaCl)
Notes: The flux reached a steady value after 1–3 hr; the total length of the experiments was not reported.

Species	Number of experiments	Flux ± SE (nEq K^+ cm^2/min)	$10^4 \cdot K_p$ (cm/hr)
Rabbit	6	5.6 ± 3.4	22
Pig	3	0.5 ± 0.2	1.9

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Table 2 Potassium, as physiological salts

Reference: J. S. Lo, H. A. Oriba, H. I. Maibach, and P. L. Bailin, Transepidermal potassium ion, chloride ion, and water flux across delipidized and cellophane tape-stripped skin, *Dermatologica 180:*66–68 (1990).
Species: human in vivo ✓
in vitro
Area: 0.8 cm^2
Vehicle: distilled water
Concentration: not applicable
Duration of experiment: 20 minutes
Analytical method: potassium-specific electrode
Notes:
1. These experiments determined the outward flux of ions from the dermal side of the skin through the epidermis into 0.5 mL distilled water on the skin's stratum corneum side; they did not measure percutaneous absorption.
2. The observed value of potassium flux was 3.5 ($\pm$ 3.6) $\times$ 10^{-9} mol/cm^2/sec from normal skin in seven individuals.
3. Tape stripping of the stratum corneum until the skin glistened, but not delipidization with 1:1 acetone-ether, significantly ($p < 0.05$) increased the potassium flux to 7.5 ($\pm$ 4.2) $\times$ 10^{-9} mol/cm^2/sec.

D. SUMMARY

Potassium is an essential element, primarily as an intracellular cation, and only toxic at excessively nonphysiological levels. In the metallic form it is highly reactive, and in contact with water it yields the hydroxide, which is corrosive to live tissues. Its permeation through animal skin appears to fall in the same range as that of most other metal salts, with a K_p on the order of 10^{-4} to 10^{-3} cm/hr.

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20
Lithium

A. GENERAL COMMENTS

The question of whether lithium is an essential trace element is not settled (1). Possibly the most orally toxic among alkali metal salts, it is found uniformly distributed throughout the animal and human organism in extremely small quantities. In healthy individuals the levels in plasma and in the erythrocytes are 90 and 95 nEq/L, respectively (1). The element has important psychotropic, hematopoietic, immunomodulating, and antiviral properties. In medicine, different lithium salts have various uses, e.g., the bromide is a hypnotic and sedative. Lithium chloride once was used as a condiment in place of table salt; however, since it became apparent that this resulted in significant accumulation and corresponding toxic effects, it is no longer used for this purpose.

Given as the carbonate, lithium was found effective in preventing or reducing frequency and severity of depression and mania in bipolar affective disorder, as well as unipolar depression. Li salts have also been recognized as effective in treating aggressive behavior abnormalities. Immunopotentiating and antiviral properties have been reported for Li salts as well (2).

While lithium has been found to be safe and effective in the treatment of affective disorders if blood levels are kept under periodic surveillance, the mechanism of Li^+ action has not yet been clarified. This is in part due to differential levels of Li between normal and dosed individuals being so small as to defy quantitative analysis. Because of the difficulty that Li^+ has in traversing biological membranes, a large load of the ion is necessary to reach effective intracellular levels in the treatment of manic depressive psychosis. Serum concentrations must be elevated by more than four orders of magnitude over normal levels to yield the desired prophylactic effects. This is accomplished by administration of 600–900 mg/day of the carbonate. Such levels come close to the upper end of the narrow therapeutic window (serum Li^+ concentrations from 0.5 to 0.8 mmol/L), beyond which lithium becomes cytotoxic. Chronic application of Li^+

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in the treatment of bipolar illness therefore requires close monitoring of serum levels. The upper tolerance limit is 1.5 mEq/L; levels beyond that possibly lead to impaired renal concentrating ability (3) and a number of other unwanted side effects, involving the skin, neuromuscular and CNS changes, and the endocrine, cardiovascular and immune systems (1,4). A certain risk of adverse side effects from chronic lithium therapy exists even within the accepted therapeutic range (5).

Based on physical and chemical properties, there exists a close biological interrelationship between lithium and the alkali and the earth alkali metals, particularly sodium, which may hold the answer to toxic effects of lithium (6). The two metal ions compete for reabsorption by the renal tubules (7). The sodium plasma concentration tends to change inversely with that of lithium, as the sum of their concentrations appears to remain constant. Thus a decreasing sodium intake will accelerate lithium retention and magnify its toxic effects (8). Through its capacity to replace sodium in the body, lithium perturbs intracellular potassium-dependent metabolic processes, causing untoward effects on the nervous and circulatory systems, resulting in the following manifestations, respectively: blurred vision, convulsions and stupor, pulse irregularities, circulatory failure, and circulatory collapse (9). Thanks to similarities in membrane transport, in salt formation and solubility, and in ionic radius with Mg^{2+} and hydrated Ca^{2+}, lithium tends to interfere with Mg- and Ca-dependent processes also (4).

In metallic form lithium is highly reactive, and in contact with water violently forms the hydroxide, corrosive to any living tissue with which it comes in contact.

B. SKIN REACTIVITY

Observations made in conjunction with the treatment of affective disorders showed that lithium taken orally causes side effects involving various systems of the body, including the skin; it can both aggravate preexisting dermatological conditions, and have positive dermatological effects in cases of eczema (contact and atopic) and herpes (oral and genital) (10,11). A study describing the preferential accumulation of lithium in epidermal, dermal and adipose tissues may explain the numerous dermatological effects occurring in conjunction with lithium therapy (12). Listed among the principal cutaneous side effects of lithium therapy are psoriasis, acneform and lichenoid eruptions, alopecia, contact and lichenoid stomatitis (13).

Applied topically, lithium salts show antiinflammatory activity and benefi-

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cial effects on herpes simplex infections and seborrheic dermatitis, indicating that lithium may boost the immune defenses of the skin (14).

C. QUANTITATIVE ABSORPTION DATA

The passive percutaneous flux of lithium ions was measured in vitro in conjunction with iontophoretic delivery of lithium and other model ions (15). In three species (humans, pigs, and rabbits), the passive permeability to lithium (Table 1) spanned more than a 10-fold range with dermatomed human skin ($K_p = 0.3 \times 10^{-4}$cm/hr) being the most resistant to lithium absorption and rabbit skin offering the weakest barrier ($K_p = 19 \times 10^{-4}$ cm/hr). The permeability of pig skin ($K_p = 9.9 \times 10^{-4}$ cm/hr) was more similar to that of the rabbit than to the human value.

The flux of lithium through rat and human skin exposed to 45%, i.e., saturated, LiCl has been reported (16) to decrease over a period of 4 hr such that in

Table 1 Lithium, as LiCl

Reference: J. B. Phipps, R. V. Padmanabhan and G. A. Lattin, Iontophoretic delivery of model inorganic and drug ions, *J. Pharm. Sci. 78,* 365–369 (1989).
Species: human, pig, rabbit in vivo
in vitro ✓
Area: 8 cm^2
Vehicle: aqueous
Concentration: 1.0 M
Duration: 24 hr
Analytical method: atomic absorption spectrophotometric analysis of the receptor fluid
Notes:
1. Rabbit skin was full thickness. Pig skin was dermatomed to a thickness of about 600 μm. Human cadaver skin was dermatomed at 350 μm.
2. The receptor fluid (0.1 M NaCl) in contact with the dermis was maintained at 37°C.

Passive percutaneous absorption of lithium

Skin source	Flux (μg/h/cm^2)	Permeability coefficient $10^4 \cdot K_p$ cm/hr
Human	0.2 ± 0.2[a]	0.3
Pig	6.9 ± 0.8	9.9
Rabbit	13 ± 1	19

[a] Standard deviation.

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the fourth hour the flux is 12% of the flux in the first 15 min. Decreases in flux during percutaneous absorption experiments have been observed for other metals, e.g., mercuric and cobaltous chlorides with both guinea pig and human skin (17).

D. IMMUNOLOGY

Observations made in patients treated with lithium for affective disorders indicated certain effects on components of the immune system and the development of certain autoimmune diseases and psoriasis. Investigations in humans and animals, in vivo and in vitro, reveal that Li^+ has an influence on levels and activity of polymorphonuclear leukocytes, monocyte/macrophages, lymphocytes, and thymocytes, among other immunologically relevant cells (18).

Several cases described in the medical literature attribute the various cutaneous conditions observed in patients during treatment with lithium to an immunological pathogenesis; contact stomatitis in particular has been associated with therapeutic doses of lithium carbonate (11,12,19,20).

E. SUMMARY

The antiinflammatory activity observed with topical application of certain lithium salts indicates significant skin barrier penetration. Through human skin in vitro, the K_p of lithium chloride was less than 10^{-4} cm/hr; across rabbit and pig skin, values on the order of 10×10^{-4} cm/hr have been measured. Although the body will accumulate lithium under conditions of sodium deficiency, no specific homeostatic mechanism is recognized for the regulation of lithium in the mammalian system. Acute toxicity of lithium salts becomes manifest neurologically. In long term therapeutic use of lithium for the treatment of bipolar affective disorders chronic elevated serum levels can involve the gastrointestinal, renal, neuromuscular, central nervous, endocrine, and immune systems.

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21
Magnesium

A. GENERAL COMMENTS

Magnesium is a biologically essential nutrient, found to be critical for phosphorylation reactions, protein synthesis, energy transfer, and lipid and carbohydrate metabolism (1). Magnesium generally performs biological functions under circumstances where there is a need for a small, electropositive, divalent cation that will coordinate to strongly nucleophilic ligands, primarily by ionic bonding. Like Ca^{2+}, Mg^{2+} shows strong affinity for negatively charged oxygen, which leads to crosslinking and conformational changes in proteins. Unlike Ca^{2+}, however, Mg^{2+} does not have appreciable affinity for covalently bound oxygen, which puts it at a competitive disadvantage in spite of significantly higher intracellular concentrations. Magnesium is one of the four substantial metals in the human body, and the second most abundant metal within cells, exceeded only by potassium. Magnesium stabilizes nucleic acids, occurs in over 300 enzymes as an activator and cofactor, and like Ca is subject to similar endocrine controls. In healthy mammalian organisms homeostasis maintains plasma levels at a constant 0.9 mM (2).

Approximately 50% of the adult maintenance requirement of magnesium is derived from foodstuff of plant origin; the balance comes from animal products. The richest sources of this metal are nuts, whole grains, green leafy vegetables, and seafood. It has been noted that the per capita magnesium intake in the industrialized world has been steadily decreasing, due to decreased consumption of whole grain products and increased consumption of processed foods (3). A significant segment of the U.S. population now has an intake below the RDA (420 mg/day for men and 300 mg/day for women) according to the National Academy of Sciences/National Research Council (4). Magnesium deficiency is also noted in plants due to overfertilization of soil leading to displacement of magnesium by potassium, as well as to acid rain that leaches magnesium from soil (1).

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Routine monitoring of magnesium status in the living organism is difficult with current technology. The metal exists in three different states: (a) bound to protein, in differing levels depending on the tissue; (b) complexed to anions, and (c) as free intra- and extracellular Mg^{2+}. Only the latter has biological activity, and no known correlation exists between free levels and those of bound metal. While prolonged fasting will lead to magnesium depletion in muscle tissue, the concentration in plasma remains unchanged. Tissue distribution studies indicate that the majority of the approximately 24-g total body burden of Mg exists intracellularly in bone and muscle. Magnesium and calcium concentrations are interrelated, and under conditions of calcium deficiency, bone concentrations of magnesium will increase. Magnesium reabsorption is high when blood concentration falls below species-specific threshold values, but the homeostatic mechanism for maintaining the Mg concentration in serum is poorly understood. As many as five hormones have been postulated for the regulation of Mg homeostasis (4). The normal intracellular concentration of this closely controlled cation ranges from 0.1 to 1.0 mM in mammalian tissues, and this concentration is well maintained even during transient Mg deficiency (5).

In ruminants, Mg deficiency was long known to result in ''grass tetany.'' Recently, evidence of neuromuscular, cardiovascular, and psychiatric disorders in humans due to hypomagnesemia has been revealed. Severe magnesium depletion has been identified as a stressor in controlled studies involving humans and animals. The clinical syndrome associated with magnesium deficiency includes spasmophilia, gross muscular tremor, ataxia, tetany, hallucinations, confusion, delirium, depression, vertigo, and muscular weakness. Also, a strong correlation was established between suboptimal serum Mg and stress-induced elevated blood pressure, hyperirritability, neuromuscular hyperexcitability (spontaneous cramps and muscle spasms), and cardiovascular manifestations (6). While overt toxicity due to magnesium deficit is rarely observed in humans, epidemiology now causally links nutritional magnesium deficit to mortality due to ischemic heart disease, myocardial infarction, cardiovascular disease, and hypertension (3).

Certain agents in diet and drugs can contribute to Mg deficiency by sequestering the metal and increasing diuresis. These include vitamin D in milk, phosphates present in milk and in carbonated beverages, acute and chronic alcohol consumption through inhibition of antidiuretic hormone, cyclosporin, tetracyclines, and digitalis (3).

Hypermagnesemia in healthy individuals is rare, as overloads of Mg are easily eliminated through normal renal clearance. Signs and symptoms of magnesium intoxication have been noted when blood Mg concentrations exceed 6 mEq/L (7) and are described as hypotension, narcosis, and respiratory paralysis.

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B. SKIN ELIMINATION AND REACTIVITY

Under conditions of deficiency, magnesium losses are minimized through strict renal conservation. In healthy volunteers kept on a magnesium-free diet for 18 days, the renal retention was efficient enough to conserve all but 1 mEq of Mg per day (8). Normally, most magnesium is excreted through the gut and kidneys, whereas elimination through skin plays a minor role. Because magnesium is incorporated into hair and skin tissue, elimination of this metal occurs through hair loss and desquamation. Loss through sweat can become significant during muscular work and at elevated temperatures (9). In test subjects exposed to high temperatures over extended periods, the loss of magnesium in sweat was estimated at 25% of the total excreted and reached 2.3 mg/hr (10). Elemental analysis by plasma atomic emission spectroscopy (11) of human cerumen showed the presence of magnesium in the range of 0.54–1.1 mg/g dry weight (12).

Magnesium is among the 30-odd metals detected in human hair to date. Scalp hair of young children up to 12–14 years of age shows an age-dependent, gradual increase in magnesium levels. Such age-dependence in the excretion of this structurally important metal is explained by the changing requirements during skeletal growth and development in children and adolescents (13).

In general, the divalent cations magnesium and calcium are necessary for intercellular adhesion. In the skin they maintain structural integrity between dermis and epidermis, and within the epidermis itself. The role of magnesium in maintaining cellular adhesion and preventing epidermolysis was demonstrated in mouse skin, when an EDTA solution, routinely used to separate various layers of the skin, failed to produce the desired epidermolysis in the presence of Mg^{2+}, and further, when loss of adhesion induced by EDTA could be reversed by addition of magnesium to the system. The fact that under physiological conditions EGTA (ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid), a strong calcium chelator but weak magnesium chelator, failed to produce epidermolysis the way EDTA did is presented as evidence that magnesium, rather than calcium, is needed for intraepidermal and dermal-epidermal integrity (14).

C. IMMUNOLOGY

Magnesium appears to play a significant role in modifying the immune response to antigens. Observations based on experimental and clinical data suggest that Mg deficiency may be a cofactor in atopic and anaphylactic reactions, and that it may decrease delayed hypersensitivity reactions in the skin, as observed in humans and animals. Patients suffering from allergic urticaria improved after magnesium therapy. In hairless rats sensitized to dinitrochlorobenzene, Mg depletion reduced allergic contact dermatitis challenge reactions (15).

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D. SUMMARY

An essential metal in the mammalian organism, magnesium plays an important role in maintaining the structural integrity of the skin. It also appears to have a positive immunoregulatory function in both humans and animals. Magnesium is virtually nontoxic orally due to effective homeostatic regulation.

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22
Manganese

A. GENERAL COMMENTS

Manganese is a trace element that is essential for both plants and animals. Present at low levels in all human tissues but at generally higher concentrations in tissues rich in mitochondria, it is a cofactor in a number of enzymatic reactions, notably in oxidative metabolism as well as in vitamin K, DNA, RNA, and protein syntheses (1). In response to excessive and toxic superoxide radicals, which can occur during aerobic metabolism, the mitochondria form Mn superoxide dismutase (MnSOD), representing a major class of SOD metalloenzymes that act to transform superoxide radicals to oxygen and hydrogen peroxide (2). When induced by ionizing radiation and hyperoxia, MnSOD is more effective than the corresponding CuZnSOD by virtue of the fact that its half-life in serum is on the order of 5–6 hr (compared to the 6–19 min found for the latter) (3,4).

Manganese is without systemic toxicity of note, except in industrial manufacture and mining, where heavy exposure to dust of its mineral forms (e.g., manganese dioxide) is likely. Chronic inhalation of such dust can cause a severe, crippling neurological syndrome called manganism, characterized by languor and spastic gait typical of Parkinson's disease (5). Manganese tricarbonyl compounds, used as fuel additives for inhibiting exhaust smoke formation and as antiknock agents, are recognized as industrial hazards, and the American Conference of Governmental Industrial Hygienists (ACGIH) recommends particular care in controlling human exposure in their manufacture. Both oral and dermal acute toxicity fall below 1 g/kg in the various animal species tested (6). Under normal conditions, manganese levels in the mammalian organism are regulated by a homeostatic system, and turnover is rapid (7).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Even under sustained, heavy exposure in the mining industry, intimate skin contact with manganese dusts was not seen to result in notable skin absorption (5).

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Occupational exposure to certain manganese tricarbonyls is highlighted by the ACGIH as a particular skin hazard due to their ease of permeation [see Appendix for comments on ''skin'' notation (8)]. On the abdominal skin of rats, the LD_{50} of (2-methylcyclopentadienyl)manganese tricarbonyl, a novel octane-enhancing gasoline additive also referred to as MMT, applied as a 10% solution in peanut oil is 665 mg/kg (6). Low-level excretion of manganese occurs in human sweat and hair (9). Typical concentrations of the metal in sweat are 21 mg/L (10). A mean level of 0.58 ppm Mn was found in human hair (11).

Manganese deficiency can have untoward effects on the skin, including dermatitis, discoloration of hair, and retardation hair growth (12).

Conversely, relatively high concentrations of manganese have been associated with normal pigmented tissues, such as dark hair and dark skin (6). Levels of manganese occurring in healthy human skin determined by neutron activation analysis varied according to anatomical site, ranging from 0.1 to 1 mg/g dry weight. There is a possible correlation between levels of Mn and the occurrence of dendritic cells in the region (13).

Permanganate anion, a powerful oxidizing agent readily soluble in water, has long been used externally as an antiseptic as dilute aqueous solution. At high concentrations, however, it can damage and then penetrate human skin in significant amounts. For example, repeated applications of a concentrated solution, have resulted in intravascular hemolysis (14).

C. SUMMARY

Manganese is an essential trace element whose deficiency or excess has undesirable effects (discoloration) on skin and hair. Chronic inhalation, occurring particularly in the mining industry, poses a risk of overexposure, resulting in respiratory and central nervous system toxicity. Cutaneous absorption of cyclopentadienylmanganese tricarbonyl and (2-methylcyclopentadienyl)manganese tricarbonyl has been recognized by the ACGIH as presenting a significant risk of systemic toxicity, and particular care is recommended to avoid direct skin contact in the work environment. In animals, manganese has been found to be carcinogenic. No data were located regarding the rate of skin absorption of any manganese compounds.

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23
Molybdenum

A. GENERAL COMMENTS

Molybdenum is an essential trace element that is ubiquitous in the environment and vital in plant and animal biochemistry. In human nutrition Mo is assimilated primarily from vegetables. It is closely associated with copper and iron metabolism and enzymatic redox processes involving an equilibrium between the Mo^{6+}, Mo^{5+}, and Mo^{4+} forms, e.g., in the oxidation of aldehydes, sulfites and nitrates, and of molecular nitrogen (1). In plants, the so-called MoFe protein, a component of nitrogenase, is essential for nitrogen fixation (2). Toxic levels of molybdenum are rarely attained, as the metal is rapidly eliminated renally from mammalian organisms. In humans, gout, characterized by high levels of uric acid excretion, is more prevalent in regions with high Mo concentrations in agricultural soil and plants (3). High levels of exposure to Mo as the mineral dust occur in the foundry industry and in molybdenum ore mining.

B. SKIN REACTIVITY

Frequent occurrence of occupational skin diseases (such as dermatitis and eczema) has been noted in molybdenum production workers where high levels of airborne metal dust were present. Studies in rats with systemic administration of ammonium molybdate show accumulation in skin, with Mo mainly bound to collagen. Molybdenum apparently alters the mechanical and chemical properties of collagen, with an inhibitory action on crosslinking in particular (4,5).

C. IMMUNOLOGY

Molybdenum as $MoCl_5$ has been found to be a potent contact sensitizer in the guinea pig maximization test (13).

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Significant incidence of molybdenum sensitivity in humans was noted in context with molybdenum refining, where exposure to metal dust in the work environment can result in IgE-dependent sensitization, as well as delayed-type hypersensitivity [verified by a positive patch test to aqueous ammonium molybdate(VI)] (6). In rare instances, systemic sensitization reactions have been ascribed to trace concentrations of molybdenum dissolved from orthopedic implants fabricated from stainless steel or Vitallium, all containing varying percentages of molybdenum, besides cobalt, nickel, and chromium (7). Systemic sensitization resulting in generalized urticaria was also ascribed to molybdenum leaching from denture material (8). Such leaching of metal into the tissues has been identified as a risk factor for sensitization, especially in patients fitted with stainless steel prostheses, and is described by the term orthopedic dermatitis (9–11). Risk of sensitization to molybdenum and other heavy metals used in artificial joints appeared heightened in the early days of total joint replacement practice, prior to the development of improved materials and safer alloys, which now minimize mechanical failure and abrasion through metal-to-metal contact (12).

D. SUMMARY

For the general population, molybdenum toxicity becomes an issue only in those few regions where high natural levels in the soil result in elevated dietary intake. In the metalworking industry, exposure can result in skin diseases, involving both cellular and humoral immunity. No data exist on the rate of skin absorption of any molybdenum compound.

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24
Sodium

A. GENERAL COMMENTS

The principal extracellular metal ion in humans, sodium is essential for life. As the most abundant cation in the extracellular fluid, its salts account for over 90% of the osmotically active solute in the plasma and interstitial fluid. In mammals, the intracellular sodium ion concentration is 5–15 mM, whereas in the interstitial fluid a steady concentration of 145 mM Na^+ is present. The normal body fluid molality is maintained by multiple regulatory systems. A principal regulator is renal sodium excretion, in turn controlled by a number of variables, principally glomerular filtration rate and elaboration of antidiuretic hormone(1). Together with other minerals, particularly potassium, sodium is vital to the maintenance of normal water balance, enzymatic activity, transmission of nerve impulses, and, hence, muscle contraction. Regardless of widely fluctuating dietary intake, in the healthy organism the kidneys maintain a steady level of sodium in the body.

In metallic form sodium is highly reactive; in combination with water it yields the hydroxide, which is corrosive to tissue on contact.

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Steady-state fluxes of sodium ions through human skin are essentially the same in vivo and in vitro. Human skin permeability to Na^+ ions is 10–100 times less than that of animal skin (pig, rabbit), suggesting that Na^+ transport is sensitive to appendageal density (2). This relatively low permeability also explains why earlier studies failed to measure detectable Na^+ penetration across human skin. In mouse skin studies, regardless of whether NaCl was delivered in a hydrophilic or a lipophilic emulsion, absorption of the sodium ion remained similar (3).

Na^+ is the main cation excreted in sweat. However, sweat concentrations are variable, depending on local skin temperature, humidity, sweat gland density, anatomical site, acclimatization, gender, etc (4). For example, with increasing

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sweat rate, the Na^+ concentration in human forehead sweat increased from 10 to >100 mEq/L (5). On the forearm, Na^+ loss averaged 26 mEq/hr during maximum sweating (6). At rest, however, mean Na^+ concentration in epidermal transudate was 7.2 mmol/L over a period of 6 hr (7).

The Na^+ concentration profile of human skin cells was delineated by electron probe analysis and electron microscopy, displaying fairly uniform distribution throughout the viable strata, a sharp decrease at the inner stratum corneum, followed by a large concentration gradient approaching the molar range toward the outer stratum corneum layers. This dramatic increase of Na^+ in the outer half of the stratum corneum may be a concentration effect due to solvent loss toward the very skin surface (8).

Elemental analysis by plasma atomic emission spectroscopy (9) of human cerumen samples showed sodium concentrations in the range of 3.6–8.8 mg/g dry weight (10).

Widely used in industry, NaOH in concentrated form comports a severe occupational hazard as it rapidly destroys skin and eye tissue on contact.

C. QUANTITATIVE ABSORPTION DATA

The first reported demonstration of sodium's percutaneous absorption was in 1943 from ointments containing aqueous $^{24}NaCl$ (11). During the next decade, it was further shown that chemically depilated rabbit and guinea pig skins were permeable to sodium, although in 8 hrs there was no detectable penetration of ^{22}Na that had been deposited on a human arm (Table 1) (2). Radioactivity in the serum of the rabbits and guinea pigs 24 hours after exposure indicated that an estimated 20–50% of the sodium had been absorbed. From one rabbit's skin about half the sodium from 10 μL of 0.9% saline disappeared every 5 min. The effect of the depilatory used on the barrier function of the skin in these experiments is unknown.

Research in the 1960s placed the permeability coefficients for sodium in the range of about $0.1–10 \times 10^{-4}$ cm/hr for several species. Tregear (12) found an in vivo sodium flux through the forearm that corresponded to a permeability coefficient of 0.62×10^{-4} cm/hr (Table 2). In vitro skin from the thigh was perhaps slightly less permeable ($K_p = 0.35 \times 10^{-4}$ cm/hr).

Compared to humans, rabbits and pigs allowed a greater flux of sodium in vivo, corresponding to K_p values of 30×10^{-4} cm/hr and 20×10^{-4} cm/hr, respectively. For rabbits, as the concentration of sodium was increased from 1.5 to 1500 mM, the in vitro permeability coefficients for sodium were constant; sodium ion transport followed Fick's law. Coincidentally, the bromide ion exhibited the same behavior with concentration, and its molar flux was about the same as that of sodium.

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Table 1 Sodium, as sodium chloride

Reference: O. Nørgaard, Investigations with radioactive nickel, cobalt and sodium on the resorption through the skin in rabbits, guinea-pigs and man, *Acta Derm. Venereol. (Stockh.) 37:*440–445 (1957).
Species: guinea pigs, rabbit, human — in vivo ✓ exposure; in vitro
Area: from a 10-μL drop
Vehicle: distilled water and Ringer's solution for human experiments; not specified for the other animals; evaporated right after application—with a hair dryer for rabbits and guinea pigs
Analytical method: Geiger-Müller counter
Notes:

Animal	Exposure time, (hr)	^{22}Na in 50 μL serum (cpm)	Max. possible ^{22}Na in 50 μL serum (cpm)[a]
Rabbit	1	1.6	1.25
Guinea pig	24	7	5
Guinea pig	24	4	5

[a] On the basis of absorption being complete, distribution being uniform, and animals being 100% serum. These conditions are, of course, not correct. The maximum possible ^{22}Na concentration could be higher but the observed ^{22}Na in 50 μL serum would be a substantial fraction of the applied ^{22}Na even if the volume of distribution were considerably reduced.

1. An experiment with 1 rabbit yielded a $T_{1/2}$ of about 5 minutes for ^{22}NaCl absorption from 10 μL of 0.9% saline (35 μg Na^+). The Geiger-Müller tube was held continuously against the skin and counts were recorded after every minute.
2. On a human forearm, however, ''no resorption took place during the 8 hours of the experiment'' wherein ''10 μL of Na^{22} in distilled water and in Ringer's solution . . . {was} applied to the skin'' and then dried (cpm ≈ 215).
3. The large difference between the observations in rabbits and guinea pigs and the observations in humans may be influenced by the calcium thioglucolate depilatory used on the furry quadrupeds.
4. No values for K_p or for flux are obtainable from this data.

Wahlberg's data with guinea pigs lead to permeability coefficients similar to those from the earlier animal experiments (13). This was true both in vivo and in vitro, and it was also true for both the hairy skin over most of the guinea pig's body and the nonhairy skin from behind the guinea pig's ear. For the guinea pig overall, the permeability coefficients averaged 8×10^{-4} to 16×10^{-4} cm/hr (Table 3).

Experiments with mice exposed to NaCl-containing ointments (3) led to

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Table 2 Sodium, as $^{24}NaCl$

Reference: R. T. Tregear, The permeability of mammalian skin to ions, *J. Invest. Dermatol. 46:*16–23 (1966).
Species: pig and rabbit (flank); human (dorsal forearm in vivo; thigh half-thickness in vitro) in vivo ✓ in vitro ✓
Area: 4 cm^2
Vehicle: water (not stated explicitly in the paper); 0.1 mL/4 cm^2 in vivo; approximately 200 μL/cm^2 in vitro
Concentration: 1.5, 15, 155, 1500 mEq/L
Analytical method: decrease in β emission measured by Geiger counter from the site of application; radioactivity in blood samples from contralateral arm; radioisotope content of in vitro receptor fluid (0.9% NaCl)
Notes:
1. The applied solutions were covered to prevent evaporation.
2. Penetration rates were based on the last 2–3 hr of the experiments, but the length of the experiments is unspecified. Reported data spans 5- to 8-hr periods. With pig and rabbit skin, the rate of sodium ion penetration was steady for the duration of the experiment, although it was also reported that ion penetration in vitro reached a steady rate after 1–3 hours.

	Percutaneous $^{24}Na^+$ flux, nEq/cm^2/min Mean ± standard error (*N*) {Permeability coefficient, $10^4 \cdot K_p$, cm/hr}		
Conditions	Rabbit	Pig	Human
In vivo	7.8 ± 1.5 (6)[a]	5.1 + 1.5 (5)	0.16 ± 0.05 (4)
155 mM NaCl	{30.2}	{19.7}	{0.62}
In vivo, stripped	—	61 ± 26 (3)	—
155 mM NaCl		{236}	
In vitro	6.1 ± 2.8 (3)	1.9 ± 1.4 (6)	0.09 ± 0.03 (3)
155 mM NaCl	{23.6}	{7.4}	{0.35}
In vitro	2.9 ± 0.9[c]	—	—
155 mM NaCl	{11.2}		
In vitro, upside down[b]	2.0 ± 0.5[c]	—	—
155 mM NaCl	{7.7}		

[a] Number of experiments.
[b] Upside down means that the skin was inverted and the radioactive solution was applied to the dermal surface.
[c] For 8 experiments comparing penetration rates for skin from the same animal, $Flux_{in}/Flux_{out} = 0.9$.

3. Fick's law was followed for absorption of sodium from sodium chloride through excised rabbit skin.

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Table 2 Continued

$^{24}Na^{+}$ concentration (mEq/L[a])	Percutaneous $^{24}Na^{+}$ flux in excised rabbit skin[a] (nEq/cm^2/min)	Permeability coefficient $10^4 \cdot K_p$, cm/hr
1.5	0.079, 0.042, 0.042	21.7
15	0.40, 0.37, 0.29	14.1
150	5.4, 4.2, 3.6	17.6
1500	106, 51, 28	24.7

[a] Estimated from Tregear's graph. In addition to NaCl, these donor solutions also contained 155 mM KCl.

Table 3 Sodium, as $^{22}NaCl$

Reference: J. E. Wahlberg, Transepidermal or transfollicular absorption? In vivo and in vitro studies in hairy and non-hairy guinea pig skin with sodium (^{22}Na) and mercuric (^{203}Hg) chlorides, *Acta Derm. Venereol. (Stockh.) 48:*336–344 (1968).

Species: guinea pig in vivo ✓
in vitro ✓

Area: 3.1 cm^2 in vitro; 1.57 cm^2 in vivo

Vehicles: water, 1% alkylarylsulfonate; 1 mL in vitro, 0.5 mL in vivo

Analytical method: disappearance of γ radioactivity from donor chamber; the procedure is not detailed in this paper but the reader is referred to previous papers for specific details

Notes:

1. Hairy skin was from the back; non-hairy skin was from behind the ear.
2. The receptor solution in the in vitro cells was distilled water instead of saline.

Sodium absorption from 0.239 M NaCl through guinea pig skin

Vehicle	Skin	N	Disappearance constant $k \cdot 10^5$ min^{-1} ± SE	Permeability coefficient for Na^{+}, $10^4 \cdot K_p$ (cm/hr)[a]
Distilled water	in vivo, hairy	12	8.2 ± 0.7	15.6
″	in vivo, non-hairy	10	6.9 ± 0.4	13.2
″	in vitro, hairy	10	5.5 ± 1.1	10.5
″	in vitro, non-hairy	10	4.1 ± 0.5	7.8
1% alkylaryl sulfonate	in vivo, hairy	10	(2.1–4.5)	(4.0–8.6)
″	in vivo, non-hairy	10	11.2 ± 1.4	21.3
″	in vitro, hairy	10	8.1 ± 0.8	15.4
″	in vitro, non-hairy	10	5.7 ± 1.2	10.9

[a] $(1 - e^{-60k}) \cdot$ (volume/area).

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Table 3 Continued

Sodium absorption in vitro (34°C) from various concentrations of aqueous sodium chloride

	Disappearance constants[a] ($k \cdot 10^5$ min^{-1})					
	0.005 M	0.08 M		0.239 M		0.398 M
Interval (hr)	Hairy $N = 10$	Hairy $N = 14$	Nonhairy $N = 10$	Hairy $N = 10$	Nonhairy $N = 10$	Hairy $N = 10$
0–5	6.5 ± 0.9	4.9 ± 0.8	6.5 ± 1.1	5.5 ± 1.1	4.1 ± 0.5	4.3 ± 0.4
0–12	4.0 ± 0.5	3.7 ± 0.7	4.3 ± 0.5	4.0 ± 0.5	3.0 ± 0.2	3.4 ± 0.3
12–24	1.2 ± 0.9	1.5 ± 0.6	1.1 ± 0.3	1.7 ± 0.5	1.1 ± 0.2	1.3 ± 0.2
0–24	2.6 ± 0.7	2.6 ± 0.6	2.7 ± 0.4	2.9 ± 0.4	2.1 ± 0.2	2.3 ± 0.2

[a] Mean ± SE.

Average apparent permeability coefficients for sodium absorption in vitro (34°C) from various concentrations of aqueous sodium chloride

	$10^4 \cdot K_p$ (cm/hr)[a]					
	0.005 M	0.08 M		0.239 M		0.398 M
Interval (hr)	Hairy $N = 10$	Hairy $N = 14$	Nonhairy $N = 10$	Hairy $N = 10$	Nonhairy $N = 10$	Hairy $N = 10$
0–5	12.4	9.3	12.4	10.5	7.8	8.2
0–12	7.6	7.1	8.2	7.6	5.7	6.5
0–24	5.0	5.0	5.2	5.5	4.0	4.4
12–24	2.3	2.9	2.1	3.2	2.1	2.5

[a] $K_p = (1 - e^{-60k}) \cdot$ (volume/area) where k is the disappearance constant.

estimates of permeability coefficients for sodium that are 2×10^{-4} to 6×10^{-4} cm/hr (Table 4). These values, smaller than for rabbits, pigs, and guinea pigs, may be underestimates of K_p since half or more of the applied sodium was absorbed during the experiment.

More recently, investigations of iontophoresis as a potential drug delivery technique have included determinations of skin's passive permeability to ions. Sodium is one such ion. In order to study the role of sodium in iontophoresis and to measure the effect of iontophoresis, the passive flux of sodium, i.e., the sodium flux in the absence of an electrical current, has also been measured. The earliest report of this type (14) allows only an estimate of the upper limit of the permeability to sodium for dermatomed human skin: $K_p < 9 \times 10^{-4}$ cm/hr (Table 5). In an early group of such in vitro experiments with nude mouse skin (15),

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Table 4 Sodium, as $^{22}NaCl$

Reference: A. Minato, H. Fukuzawa, S. Hirose, and Y. Matsunaga, Radioisotopic studies on percutaneous absorption. I. Absorption of water-soluble substances from hydrophilic and absorption ointments through mouse skin, *Chem. Pharm. Bull. (Tokyo) 15:*1470–1477 (1967).
Species: mouse in vivo ✓ exposure
in vitro
Area: 5 cm^2
Vehicle: ointments, one hydrophilic (o/w) and one ''absorption'' (w/o); 50 μL of 0.001 M $^{22}NaCl$ or 50 μL of 0.1 M $^{22}NaCl$ per gram of ointment base
Analytical method: γ-ray counting of the excised dosed area of skin
Notes:

Duration (hr)	Ointment type	Carrier {NaCl} (M)	*N*	Radioactivity of ointment[a] (cpm/50 mg)	Absorption of ^{22}Na, (% ± SE)
10	o/w	0.001	4	9863	50.8 ± 4.1
10	o/w	0.1	4	9202	48.4 ± 3.5
5	w/o	0.001	3	10129	68.4 ± 4.4
5	w/o	0.1	4	9199	73.5 ± 2.1

[a] Amount of ointment applied ≈ 52 mg/animal.

Calculation of approximate K_p values:

1. For Na^+ from o/w ointment:

Assume that the $^{22}NaCl$ is diluted by the water in the ointment base and that the concentration in contact with skin is that of the diluted solution.

Na^+ per application =
0.05 mL Na solution/g ointment × 0.1 mol Na/L × 0.001 × L/mL × 0.052 g/animal = 2.6×10^{-7} moles ^{22}Na/animal

Average flux = $2.6 \times 10^{-7} \times 50\% \div (10\ hr \times 5\ cm^2) = 2.6 \times 10^{-9}$ moles $Na/cm^2/hr$

Na concentration in ointment = 0.1 M × 0.05 ÷ (0.05 + 0.37)
= 0.0119 M = 0.0000119 mol/mL

$K_p = 2.6 \times 10^{-9}$ moles $Na/cm^2/hr \div 0.0000119$ mol/$cm^3 = 2.2 \times 10^{-4}$ cm/hr

Because % absorbed was similar for both concentrations of carrier, the estimated K_p values are also similar. Because the % absorbed is large, the sodium-22 concentration changed significantly during this experiment and the conditions for determining an accurate permeability coefficient were not met.

2. For Na^+ from w/o ointment:

If it is assumed that the $^{22}NaCl$ is diluted by the water in the ointment base, then the sodium concentration in this ointment's aqueous phase differs by less than 5% from the concentration in the o/w ointment.

The same amount of Na^+ was applied in this case as was applied with the o/w ointment.

Average flux = $2.6 \times 10^{-7} \times 70\% \div (5\ hr \times 5\ cm^2) = 7.3 \times 10^{-9}$ moles $Na/cm^2/hr$

Na concentration in ointment = 0.1 M × .05 ÷ (0.05 + 0.35)
= 0.0125 M = 0.0000125 mol/mL

$K_p = 7.3 \times 10^{-9}$ moles $Na/cm^2/hr \div 0.0000125$ mol/$cm^3 = 5.8 \times 10^{-4}$ cm/hr

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Table 5 Sodium, as $^{22}NaCl$

Reference: R. R. Burnette and B. Ongpipattanakul, Characterization of the permselective properties of excised human skin during electrophoresis, *J. Pharm. Sci. 76:*765–773 (1987).
Species: human (thigh) in vivo
in vitro ✓
Area: 0.64 cm^2
Vehicle: Hepes-buffered saline with mannitol (25 mM Hepes-133 mM NaCl–1 mM mannitol with 12 mL 1 M NaOH per L of buffer)
Concentration: 147 mM sodium ion
Duration: 9 hr
Analytical method: determination by γ-ray counter of radioactivity in the receptor solution
Notes:
1. Human cadaver skin was dermatomed to 0.8 mm thickness.
2. ''Because of the large counting errors associated with the passive flux values, no statements concerning them will be made other than they were at least an order of magnitude lower than the smallest of the observed iontophoretic fluxes.''
3. The lowest iontophoretic Na^+ flux occurred with the lowest current density, i.e., about 1.3 μmol/hr/cm^2 at 0.078 mA/cm^2. The lowest passive flux would have been no larger than about 0.13 μmol/hr/cm^2.
4. The permeability coefficient would be no larger than (0.13 μmol · hr/cm^2) ÷ (147 μmol/cm^3), i.e., $\leq 9 \times 10^{-4}$ cm/hr.

the passive sodium fluxes were approximately 0.01–0.16 μmol/hr/cm^2 after 8 hrs (Table 6). The resulting permeability coefficients are 0.7×10^{-4} to 11×10^{-4} cm/hr.

In a later report, the same authors determined that the average passive sodium flux, also through nude mouse skin, from buffered saline after about 8 hr was 0.246 ± 0.216 (S.D.) μmol/hr/cm^2 (Table 7) (16), corresponding to a permeability coefficient of 18×10^{-4} cm/hr. This value is similar to earlier results in other species.

In similar iontophoresis-based experiments, dermatomed human skin showed much lower passive sodium permeability than mouse skin (17, 18). In one report (17), dermatomed cadaver skin (0.25 mm) that had been stored frozen was used for determinations of electrical resistance and 19 values of the passive permeability coefficient for sodium. Fifteen values were 0.06×10^{-4} to 0.65×10^{-4} cm/hr, and the others were scattered higher to 11.9×10^{-4} cm/hr (Table 8).

A comparison with fresh skin in a follow-up report (18) produced a median permeability for sodium that was 45% lower for fresh skin than frozen skin,

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Table 6 Sodium, as $^{22}NaCl$

Reference: R. R. Burnette and T. M. Bagniefski, Influence of constant current iontophoresis on the impedance and passive Na^+ permeability of excised nude mouse skin, *J. Pharm. Sci. 77:*492-497 (1988).
Species: nude mouse in vivo
in vitro ✓
Area: 0.64 cm^2
Vehicle: Hepes-buffered saline (25 mM Hepes–133 mM NaCl with 12 mL 1 M NaOH per L of buffer to adjust to pH 7.4)
Concentration:147 mM sodium ion on both sides of the skin
Duration: 8.5 hr
Analytical method: determination by γ-ray counter of radioactivity in the receptor solution
Notes:
1. $^{22}NaCl$ was introduced 2.5 hr after the beginning of exposure to nonradioactive buffered saline.
2. Passive flux was based on a 1-hr period beginning 5 hr after the epidermal side of the skin was first exposed to $^{22}NaCl$. Control experiments showed that at that stage Na^+ flux had reached steady state.
3. Reported Na^+ fluxes ($N = 5$) were about 0.01–0.16 μmol/hr/cm^2 (median ≈ 0.05 μmol/hr/cm^2).
4. Corresponding K_p values = (0.01–0.16) μmol/hr/cm^2 ÷ 147 μmol/cm^3
$= (0.7–11) \times 10^{-4}$ cm/hr.

Table 7 Sodium, as $^{22}NaCl$

Reference: T. Bagniefski and R. R. Burnette, A comparison of pulsed and continuous current iontophoresis, *J. Controlled Release 11:*113–122 (1990).
Species: mouse, nude in vivo
in vitro ✓
Area: 1.6 cm^2
Vehicle: aqueous buffered saline
Concentration: 0.133 M
Duration: 8.25 hr
Analytical method: determination of radioactivity in the receptor solution
Notes:
1. The average passive flux of Na^+ during the last 1.5 hr was 0.246 ± 0.216 (SD) μmol/hr/cm^2 (number of experiments = 34); no electric current was involved in determining these values.
2. K_p = 0.246 μmol/hr/cm^2 ÷ (0.133 mol/L × 0.001 L/cm^3 × 10^6 μmol/mol)
$= 18 \times 10^{-4}$ cm/hr.

Table 8 Sodium, as $^{22}NaCl$

Reference: G. B. Kasting and L. A. Bowman, DC electrical properties of frozen, excised human skin, *Pharm. Res. 7:*134–143 (1990).
Species: human in vivo
in vitro ✓
Area: 0.7 cm^2
Vehicles: A: Dulbecco's phosphate-buffered saline with 0.02% sodium azide (pH 7.4); B, C, D: 0.025 M Tris-HCl (pH 7.4) with 0.02% sodium azide and three concentrations of NaCl
Concentration: 0.015, 0.147, 0.156, and 1.59 M sodium ion
Duration: 17 and 23 hr, but the permeability coefficients for sodium were based only on the sodium ion which penetrated 2–17 or 6–23 hr, respectively, after application of the $^{22}Na^+$
Analytical method: determination by γ-ray counter of radioactivity in the entire receptor solution
Notes:
1. Skin from cadavers was dermatomed at 0.25 mm, frozen in 10% glycerol to liquid nitrogen temperature, and stored at −80°C for up to 2 months.
2. Electrical resistance of the skin was measured before the passive sodium flux was determined. In fact, these measurements constituted brief periods of iontophoresis. For each sample of skin exposed to vehicle A there were three sequences of direct currents applied in both directions (e.g., 0, +2, −2, +4, −4 μA, etc.) and increased in five steps from 0 to 10 μA, 0 to 50 μA, and 0 to 250 μA. Each sequence lasted about 10 min. With vehicles B, C, and D, only the ''ascending'' portions of the current sequences were applied.
3. Passive diffusion of sodium began the day after the skin was thawed and mounted in the diffusion cells.

Passive percutaneous absorption of sodium

Vehicle	Sodium con. (M)	Permeability coefficients for sodium $10^4 \cdot K_p$ cm/hr
A	0.015	0.25, 0.38, 1.28, 11.9
B	0.147	0.19, 0.55, 3.6
C	0.156	0.10, 0.27, 0.50, 0.54, 0.61, 0.62, 0.65, 8.1
D	1.59	0.06, 0.12, 0.33, 0.46

4. Median $K_p = 0.50 \times 10^{-4}$ cm/hr.
5. Excepting three samples, ''there was a strong inverse correlation between K_p and initial resistance.''

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Table 9 Sodium, as $^{22}NaCl$

Reference: G. B. Kasting and L. A. Bowman, Electrical analysis of fresh, excised human skin: a comparison with frozen skin, *Pharm. Res. 7:*1141–1146 (1990).
Species: human in vivo
in vitro ✓
Area: 0.7 cm^2
Vehicle: Dulbecco's phosphate-buffered saline with 0.02% sodium azide
Concentration: 156 mM sodium ion
Duration: 71 hr
Analytical method: determination by γ-ray counter of radioactivity in the receptor solution
Notes:
1. Skin was dermatomed to 0.25 mm.
2. Flux was linear between 6.5 and 71 hr.

Skin condition	Permeability coefficient for sodium $10^4 \cdot K_p$ (cm/hr), median ± MAD[a]
Fresh	0.28 ± 0.23
Frozen[b]	0.50 ± 0.23

[a] Median absolute deviation.
[b] Frozen slowly in 10% glycerol to −150°C, stored for up to 2 months and thawed prior to use.

0.28×10^{-4} cm/hr and 0.50×10^{-4} cm/hr, respectively, but the difference was not statistically significant (Table 9).

These results of Kasting and Bowman agree well with the permeability coefficients for human skin determined nearly 30 years earlier by Tregear.

D. SUMMARY

Sodium is an essential mineral and the principal cation in the body of animals. The permeability coefficient of sodium ion through human skin is on the order of 0.5×10^{-4} cm/hr, a value 10–100 times smaller than that in other mammalian species investigated. The skin is an important excretory organ for sodium, with concentrations varying widely as a function of environmental and physiological factors.

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Nickel

A. GENERAL COMMENTS

Nickel is generally considered to be an essential trace element. It is subject to homeostatic regulation and mobilization in response to disease and physiological stress, and its absence or lack in the mammalian organism produces well-defined deficiency symptoms (1). A number of critical physiological functions are clearly dependent on the metal—notably the metabolism of carbohydrates, as nickel titers were seen to rise in parallel with insulin following glucose intake in humans. Conversely, the liver could not build glycogen stores in a state of nickel deficiency (2,3).

Forming coordination complexes with organic molecules, Ni shows a special affinity for sulfur in peptides and proteins (4–7). The outstanding biological characteristic of nickel, from a public health perspective, is its immunotoxicity, as allergen and immunosuppressant. None of the naturally occurring nickel compounds are known to be immunogenic. Rather, the many anthropogenic salts and alloys, as well as the metallic form of nickel itself, have proven a health hazard with the advent of the industrial age. What started out as a prime occupational hazard reported from the metalworking and refining industry in the late nineteenth century and the first part of the twentieth century due to nickel allergenicity (8,9), since the Second World War nickel dermatitis has become a consumers' affliction as well, when the metal found entry into most alloys used in the manufacture of common materials and articles of daily contact. At long-term occupational exposure levels, it is also characterized as genotoxic and identified as a carcinogen, known to cause malignancy in the respiratory organs, the GI tract, and the kidneys (10). On a relative scale of carcinogenicity of metals based on human and animal studies and short-term bioassays, nickel compounds actually were ranked highest in activity, on par with hexavalent chromium (11). The principal cancer-causing forms are suspected to be various nickel sulfides, encountered in the metal fabrication and refining industries (12,13). Various cell types react with the Ni^{2+} ion; epidermal tissue in particular binds to it reversibly (14). Levels acquired through

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the major exposure routes (dermal, GI, and respiratory) have an additive effect. Inhalation augments GI exposure, for example, and oral intake exacerbates a cutaneous sensitization response. Upon skin contact, the metal is easily dissolved by sweat, especially the relatively abundant sweat of individuals classified as ''rusters'' (15,16).

A significant elevation of nickel concentration was seen in the serum of psoriasis patients, as determined by electrothermal atomic absorption spectrophotometry (17). While the pathogenesis of this condition remains unknown, this finding points to abnormal nickel homeostasis as one biochemical defect in the psoriatic process.

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Nickel shows a special affinity for keratin that appears to have a retarding effect on skin penetration rates (18). When applied on the skin, certain nickel salts thus require considerable induction times before any measurable penetration is observed (24–90 hr) (19–21). Such binding to epidermal and dermal tissue is also responsible for establishing depots of the metal in the skin, which appears to function as a local reservoir for a number of xenobiotics (14,20,22). In addition to stratum corneum penetration, nickel is also absorbed through the skin appendages, where it appears to be sequestered by chelation, primarily by urocanic acid and histidine occurring in human sweat. Proposed as one of the functions of such components of sweat is the removal of nickel and other potentially toxic metal ions from the body, as well as prevention of their absorption (23). Absorption studies of nickel on powdered stratum corneum showed that the metal binds preferentially to carboxyl groups rather than amino groups (14). Sustained exposure to nickel metal dust in the industrial setting can induce various forms of dermatitis collectively described as ''nickel rash'': papules, erythema, and vesicles progressing to weeping eczema. These effects seen in the epidermal tissues all confirm the ability of the metal to penetrate beyond the stratum corneum.

Divalent nickel, topically applied to excised hairless mouse skin, apparently penetrates the stratum corneum via the intercellular lipids, where it was visualized in the intercellular space by x-ray microanalysis (24). Localization of nickel in the shunt pathways was not attempted in that investigation. The concentration of nickel in human skin following exposure to a Ni solution appears highest on the skin surface and within the stratum corneum. This contrasts with the results obtained for chromium, another prevalent allergen, which was found predominantly at a depth of 30 μm (25,26).

Autoradiography of human skin exposed to $^{63}NiC1_2$ revealed that nickel accumulated within 1 hr in the hair shafts besides depositing in the stratum corneum (27). After 4 hr the basal and suprabasal epidermal cells were also labeled.

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Incubation of homogenized human epidermis with nickel chloride solutions indicated establishment of an equilibrium depending on the nickel concentration in the incubation medium (28). Such reversible binding to constituents of the skin is consistent with the observation in vivo that the epidermis can function as a dynamic nickel reservoir (14).

The role of the counterion, of concentration, and of occlusion on the percutaneous absorption rate and irritation potential of nickel salts has been demonstrated in human skin in vivo and in vitro. The skin irritation potential of different salts was determined by application under occlusion on healthy volunteers and evaluation of irritancy by objective measurement with the laser Doppler technique. Comparison of equimolal concentrations showed a clear dose-response relationship, whereby the chloride and the nitrate were more irritating than the sulfate (29). Applied as a chloride, nickel permeates at 50 times the rate of the sulfate; occlusion increases skin penetration 10-fold. Under all experimental conditions, induction times observed are substantial (approximately 50 hr) for either sulfate or chloride (20).

The standard protocol of skin tape stripping was implemented to examine the penetration of nickel salts through human stratum corneum in vivo following single open application at levels of 0.001–1% of the metal as the chloride, sulfate, nitrate, and acetate salts over 30 min to 24 hr. Application sites were stripped 20 times and analyzed for metal content by inductively coupled plasma-atomic emission spectroscopy (30). The concentration gradients across the stratum corneum were seen to converge towards nondetectable levels ($<$7 ppb) beyond the fifteenth strip, regardless of concentration applied. This again seems to confirm the observation that in-depth diffusion of nickel through the stratum corneum occurs to a minimal degree and only after considerable lag times (19–21). Material recovery calculation points to the fact that, particularly at higher concentrations (1%), up to 50% of the applied dose remains unaccounted for. This seems to indicate that nickel chooses the alternate, shunt pathway for diffusion and deposition to a significant degree, as was observed by other investigators using autoradiography or micro-PIXE analysis, and helps explain the ease of eliciting skin reactions in those sensitized (27,31,32).

Analysis of human nails showed a nickel concentration in fingernails that is significantly higher than that found in toenails, indicating that most of the levels found in the former originate from environmental exposure (33). This finding gave rise to the concept of analyzing fingernail for nickel as an indicator of significant occupational exposure: a nail nickel content of <1 μg/g can be considered normal, whereas values of >8 mg/g point to chronic occupational exposure (34). Women sensitized to nickel had significantly higher levels of the metal in their toenails (but not fingernails), hair, and plasma than the nonsensitized controls. Toenails thus appear to be more indicative of endogenous nickel levels prevailing at the time of their formation than do fingernails (33). Hair analysis

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has also been found to be a suitable method for biological monitoring of exposure to the metal in the work environment, as atomic absorption analysis revealed geometrical mean values for Ni in exposed workers to be significantly higher than those seen in nonexposed individuals matched by age (216.75 μg/g versus 3.31 μg/g) (35).

Sweat contains significantly higher nickel levels than does normal blood serum, pointing to active nickel secretion by the sweat glands. This makes perspiration an important excretory pathway for the metal, surpassing urinary elimination, particularly under conditions of profuse sweating. Values measured range from 7 to 270 mg excreted per day, compared to urinary levels of 1 to 10 mg/day (36,37).

C. QUANTITATIVE ABSORPTION DATA

Despite its prominent role as an allergen, quantitative reports about nickel's percutaneous absorption are scarce and involve few subjects. In the first such report (38), Nørgaard in 1955, with an eye toward comparing normal and hypersensitive individuals, first demonstrated the absorption of nickel through normal skin. In 24 hr, the radiation from protected, dried aqueous deposits of $^{57}NiSO_4$ (12–100 μg Ni) on the skin at several body sites decreased by about two-thirds (Table 1) with the most rapid reduction occurring at the beginning of the experiment. Since the half-value thickness of skin for ^{57}Ni is 0.3 mm and the epidermis is about 0.1 mm, measured radiation will not be reduced to the degree observed in these experiments until the ^{57}Ni has passed through the epidermis and entered the dermis, where it can be transported by blood or lymph. In other words, the decrease in measured radiation corresponds to absorption of nickel. Results with nickel-sensitive individuals were the same as with normal individuals. In similar experiments radiation from $^{57}NiCl_2$ in lanolin decreased by 61% in 41 hr.

In a subsequent demonstration that nickel can be absorbed through the skin (Table 2). Nørgaard applied ^{57}Ni (unspecified compound) to two rabbits and two guinea pigs (39). After 24 hr, the kidneys, liver, and blood of all the animals were radioactive. Rabbit urine also contained ^{57}Ni. Since the sites of application had been treated with a calcium thioglucolate depilatory, however, these experiments are not completely satisfactory for demonstrating absorption by normal untreated skin.

Other similar in vivo guinea pig experiments (27) established that measurable nickel, 0.005% and 0.009% of 40 μCi of $NiCl_2$, reached the plasma and urine, respectively, within four hours (Table 3). After 12 and 24 hr the amounts rose to 0.05–0.07% in plasma and 0.21–0.51% in urine. The washed skin at the site of application retained much more, 5.3%, after 24 hr, but neither the amount of nickel in the washes nor that in the whole body was reported.

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Table 1 Nickel, as $^{57}NiSO_4$ and $^{57}NiCl_2$

Reference: O. Nørgaard, Investigations with radioactive Ni 57 into the resorption of nickel through the skin in normal and in nickel-hypersensitive persons, *Acta Derm. Venereol. (Stockh.) 35:*111–117 (1955).

Species: human (forearm, thigh, thorax) in vivo ✓
in vitro

Area: approximately 0.2 cm^2

Vehicle: 10 µL water (allowed to dry); lanolin ointment

Time: 24 hr

Analytical method: Geiger-Müller counter to determine radioactivity at site of application

Notes:

1. In skin the half-value thickness for ^{57}Ni radiation is 0.3 mm.
2. After the applied solutions had evaporated to dryness, the sites were occluded.

Disappearance of $^{57}NiSO_4$ from the skin in 23 hr[a]

Conc. of $NiSO_4 \cdot 7H_20$	5% (178 mM)[c]	2.5% (89 mM)	1.25% (45 mM)	0.68% (22 mM)
Av. % disappeared[b]	76 ± 2	60 ± 5	77 ± 3	55 ± 4

[a] For one subject to whom all four concentrations were applied on the extensor surface of the forearm. The results were the same for the flexor surface of that subject and of two other subjects as well as for a second site on the latter subjects (anterior thigh, anterior thorax).
[b] $N = 4$ counting intervals; ± SD. [c] 10 µL contains 101 µg Ni.

3. The radioactivity (corrected for decay) from $NiCl_2$ in a lanolin ointment decreased by 61% in 41 hr.
4. In experiments where the radioactivity was measured several times in 24 hr, the absolute decrease in radioactivity was greatest at the beginning of the experiment.
5. In another experiment $^{57}NiSO_4 \cdot 7H_20$ was applied for 24 hr to nine dermatological patients who had all responded to nickel patch tests:

	% Decrease in ^{57}Ni radioactivity/% left after wiping			
Applied concentration	5% (178 mM)	2.5% (89 mM)	1.25% (45 mM)	0.68% (22 mM)
Case 1	80/8	60/40	44/12	39/4
Case 2	57/5	65/4	46/2	50/4
Case 3	84/8	72/9	62/19	60/6
Case 4	58/4	92/1	81/5	74/5
Case 5	88/5	71/3	76/9	56/7
Case 6	62/10	32/10	61/1	65/1
Case 7	54/	55/	68/	72/
Cases 8 and 9		45–75% resorbed in 24 hr		
Average	69	64	63	59

6. There was no apparent difference in absorption of nickel between normal and hypersensitive individuals.
7. Although average fluxes are estimable in these experiments, the concentration is unknown after evaporation and occlusion; thus, the permeability coefficient cannot be determined.

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Table 2 Nickel, as ^{57}Ni

Reference: O. Nørgaard, Investigations with radioactive nickel, cobalt and sodium on the resorption through the skin in rabbits, guinea-pigs and man, Acta Derm. Venereol. (Stockh.) 37:440–445 (1957).
Species: rabbits, guinea pigs in vivo ✓
in vitro
Area: approximately 0.2 cm^2 on the basis of a preceding paper by Nørgaard
Vehicle: 10 μL water (dried with hair dryer); covered with ''plastic foil and an adhesive plaster''
Time: 24 hr
Analytical method: radioactivity in organs, blood, and urine
Notes:
1. The hair was first clipped (5 × 5 cm) and then removed with a calcium thioglucolate depilatory, which caused ''a pronounced keratolysis of the uppermost layers of the epidermis.''
2. ''The organs examined [liver and kidney] were distinctly radioactive in all cases.''
3. The urine and blood were also radioactive.
4. There is no mention of the amount of Ni applied or which salt was applied or the percentage absorbed.
5. Neither flux nor K_p can be calculated.

Diffusion of $NiSO_4$ (applied at concentrations of 0.001, 0.01, and 0.1 M) through heat-separated epidermis was very slow (14). After 17 hr of diffusion only one of six cells indicated any measurable diffused nickel, and after 90 hr that spanned two more sampling times, two of the six cells at no time presented measurable nickel in the receptor chamber (Table 4). Estimated permeability coefficients (diffusion area is not reported) corresponding to measured nickel were 0.03×10^{-4} to 0.1×10^{-4} cm/hr. Five nonionic detergents as 2% solutions carrying the ^{63}Ni ''do little to enhance the diffusion of nickel.''

The in vitro percutaneous fluxes of $NiCl_2$ and $NiSO_4$ through full-thickness human skin were compared in 1986 by Fullerton et al (20). After lag times of about 50 hr, in experiments lasting 144–239 hr, occluded $NiCl_2$ entered the receptor fluid about 5–40 times more rapidly than (a) $NiSO_4$, (b) $NiCl_2$ with added Na_2SO_4, or (c) $NiSO_4$ with added NaCl (Table 5). Solutions (b) and (c) had identical ionic activities, and the Ni^{2+} and Cl^- concentrations were the same as for $NiCl_2$ solution. Without occlusion, the permeation of nickel was reduced by more than 90%. Nickel in the skin tissue at the end of the experiments (calculated as the difference between the applied nickel and sum of the nickel in the receptor solution plus the nickel recovered by washing the skin) was also greater after exposure to pure $NiCl_2$ than to sulfate-containing solutions. These results are in

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Table 3 Nickel, as $^{63}NiCl_2$

Reference: G. K. Lloyd, Dermal absorption and conjugation of nickel in relation to the induction of allergic contact dermatitis—preliminary results, Nickel Toxicology (S. S. Brown and F. W. Sunderman, Jr., eds.), Academic Press, London, 1980 pp. 145–148.
Species: guinea pigs (shaven) in vivo ✓
in vitro
Area: not reported (flanks, occluded)
Vehicle: water (assumed; it is not specified)
Time: 4, 12, and 24 hr
Analytical method: radioactivity of urine, plasma, and skin (homogenate and nonhomogenizable debris)
Notes:
1. ''A small percentage of dermally applied soluble nickel [dose = 40 μCi ^{63}Ni, i.e., 0.6 μg since no carrier is mentioned] is absorbed across the skin and appears in the plasma after 4 hours exposure and increases after longer exposure periods.''

Absorption of $^{63}NiCl_2$

	^{63}Nickel content, % of applied dose (ng Ni)		
	Exposure time (hr)		
Location	4	12	24
Plasma	0.005 (0.03)	0.07 (0.42)	0.05 (0.3)
Urine	0.009 (0.05)	0.21 (1.3)	0.51 (3.1)
Excised, washed skin[a]	1.94 (11.6)	7.30 (44)	5.33 (32)

[a] Excised to a depth of 0.3 mm over the entire application site

1. Just over half (53–59%) of the ^{63}Ni in the excised skin was in the ''nonhomogenizable skin.''
2. ''Zonal centrifugation of homogenized epidermis . . . showed that a major portion of nickel within the skin was associated with the soluble portion of the homogenate.''
3. No K_p can be calculated because the treated area is not given and there was no measure of total absorption.

agreement with cited patch test results wherein occlusion and the use of nickel chloride rather than nickel sulfate are more likely to produce positive reactions in patients. Estimated permeability coefficients after the lag time were 0.5×10^{-4} to 15×10^{-4} cm/hr for pure $NiCl_2$ and 0.03×10^{-4} to 0.2×10^{-4} cm/hr in the presence of sulfate.

Subsequently, additional, similar in vitro experiments with human skin ex-

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Table 4 Nickel, as $^{63}NiSO_4$

Reference: M. H. Samitz and S. A. Katz, Nickel—epidermal interactions: diffusion and binding, *Environ. Res. 11:*34–39 (1976).
Species: human epidermis (autopsy) in vivo
in vitro ✓
Area: <5.1 cm^2 (The exact area was not reported, but the epidermis was approximately 25.4 × 25.4 mm and, therefore, diameter < 2.54 cm). [It is estimated that the radius of the exposed epidermis was not more than (1.27 – 0.4) = 0.87 cm, and it follows that the exposed area would have been less than or equal to 2.4 cm^2.]
Vehicle: physiological saline solution, sweat, or 2% surfactant in saline (5 separate surfactants)
Time: 1 hr to 8 days exposure
Analytical method: liquid scintillation counting of the ^{63}Ni in 1-mL samples
Notes:
1. Epidermis was separated ''by gentle scraping after a brief immersion in boiling water.''
2. The volume of the receptor compartment was 5 mL.

Percutaneous absorption of Ni from nickel sulfate

Donor $[Ni^{2+}]$ (mM)	% of donor ^{63}Ni[a] in receptor after			Permeability coefficient for Ni^{2+}[b] $10^4 \cdot K_p$ (cm/hr)		
	17 hr	24 hr	90 hr	17 hr	24 hr	90 hr
100	0.000	0.024	0.000	[c]	0.1	[c]
100	0.018	0.026	0.031	0.1	0.1	0.03
10	0.000	0.000	0.000	[c]	[c]	[c]
10	0.000	0.007	0.025	[c]	0.03	0.03
1	0.000	0.009	0.066	[c]	0.04	0.07
1	0.000	0.000	0.000	[c]	[c]	[c]

[a] 2 μCi/diffusion cell; 2 mL/cell.
[b] It is assumed that area = 2 cm^2 and that reported amounts are totals from 0 hr.
[c] The limit of detection is not reported. If 0.001% of the donor were in the receptor chamber, then 1 mL taken from the receptor would contain 0.4×10^{-5} μCi, i.e., perhaps 5–8 cpm. For that amount of percutaneous penetration the K_p would be 0.006×10^{-4}, 0.004×10^{-4}, and 0.001×10^{-4} cm/hr for 17, 24, and 90 hr, respectively.

Table 4 Continued

Percutaneous absorption of Ni from 2 mM $NiSO_4$[a] in various vehicles[b]

Duration (hr)	Ni in the receptor solution, ng (average of 5 experiments)						
	Saline	X-100	EOB	915	LF-6	LR-74	Sweat
1	n.d.[c]	8	n.d.	50	8	2	8
2	n.d.	n.d.	n.d.	n.d.	n.d.	2	8
3	n.d.	50	n.d.	100	200	2	8
4	n.d.	—	n.d.	20	—	2	2
5	n.d.	n.d.	—	80	—	2	2
48	—	50	—	130	160	—	—
192	—	180	—	7000	1800	—	—

[a] 0.2 μCi ^{63}Ni/diffusion cell; 2 mL/cell. The concentration was ambiguously reported both as 2×10^{-3} M and as 2×10^{-3} M that was diluted by an equal volume of the various vehicles, i.e., 1×10^{-3} M. The concentration used here is 2 mM.

[b] Saline, physiological saline; X-100, Triton X-100, alkylphenoxypolyethoxyethanol; EOB, KYRO EOB, ethoxylated ethanol; 915, Swift No. # 915, *N*,*N*-hydroxyalkyl-(*n*-alkyl)amide; LF-6, aliphatic alcohol/ethylene oxide adduct; LR-74, *n*-alkoxypolyethoxyethanols; sweat, human sweat collected from volunteers.

[c] n.d., none detected.

Percutaneous absorption of Ni from 2 mM $NiSO_4$ in various vehicles[a]

Duration (hr)	Permeability coefficient,[b] $10^4 \cdot K_p$ (cm/hr)						
	Saline	X-100	EOB	915	LF-6	LR-74	Sweat
1	[c]	0.3	[c]	2	0.3	0.1	0.3
2	[c]	[c]	[c]	[c]	[c]	0.05	0.15
3	[c]	0.7	[c]	1	2.7	0.03	0.1
4	[c]	—	[c]	0.2	—	0.025	0.025
5	[c]	[c]	—	0.6	—	0.02	0.02
48	—	0.04	—	0.1	0.12	—	—
192	—	0.04	—	1.5	0.36	—	—

[a] Saline, physiological saline; X-100, Triton X-100, alkylphenoxypolyethoxyethanol; EOB, KYRO EOB, ethoxylated ethanol 915, Swift No. # 915, *N*,*N*-hydroxyalkyl-(*n*-alkyl)amide; LF-6, aliphatic alcohol/ethylene oxide adduct; LR-74, *n*-alkoxypolyethoxyethanols; sweat, human sweat collected from volunteers.

[b] It is assumed that area = 2 cm^2 and that reported amounts are totals from 0 hr.

[c] Below the limit of detection.

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Table 5 Nickel, as $NiSO_4$ and $NiCl_2$

Reference: A. Fullerton, J. R. Andersen, A. Hoelgaard, and T. Menné, Permeation of nickel salts through human skin in vitro, *Contact Dermatitis 15:*173–177 (1986).
Species: human (surgical, stored frozen) in vivo
in vitro ✓
Area: 1.8 cm^2
Vehicle: water, 1.32 mg Ni^{2+}/mL; 0.25 mL/cell
Time: 144–239 hr
Analytical method: adsorption differential pulse voltammetry
Notes:
1. On the dermal side of the skin, the receptor chamber was filled with deionized water.

Permeation of nickel ion in various conditions

Donor (tissue)	Solution	Ni permeated after 144 hr (%)	Estimated permeability coefficient,[a] $10^4 \cdot K_p$ (cm/hr)
A (breast)	$NiCl_2$, not occluded	0.23	0.034
A (breast)	$NiCl_2$, occluded	3.6	0.53
B (breast)	$NiCl_2$, occluded	15.5	2.3
B (breast)	$NiSO_4$, occluded	0.2	0.03
C (breast)	$NiCl_2$, occluded	12.2	1.8
C (breast)	$NiSO_4$ + NaCl, occluded	0.62	0.09
D (leg)	$NiCl_2$, occluded	3.7	0.55
D (leg)	$NiCl_2$ + $NiSO_4$, occluded	1.1	0.16

[a] On the basis of 144 hr exposure less an approximate lag time of 50 hr for each case.

1. The donor C and donor D experiments were continued to 239 and 187 hr, respectively, when the skin was washed five times to remove all nickel still on the skin surface; nickel found in neither the recipient phase nor the washes was considered to be in the epidermis and dermis. The results were as follows:

Nickel permeation and distribution

Donor	Solution applied	Ni permeated (%)		$10^4 \cdot K_p$[a] (cm/hr)		Ni in washes (%)		Ni in skin[b] (%)	
		Cell 1	Cell 2	Cell 1	Cell 2	Cell 1	Cell 2	Cell 1	Cell 2
C	$NiSO_4$ + NaCl	1	1	0.073	0.073	96	88	3	11
C	$NiCl_2$	22	18	1.6	1.3	40	33	38	49
D	$NiCl_2$ + Na_2SO_4	2	2	0.20	0.20	97	92	1	6
D	$NiCl_2$	5	5	0.51	0.51	80	85	15	10

[a] Following a lag time estimated at 50 hr; based only on the permeated nickel.
[b] By difference, 100 − (% permeated + % in washes).

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plored the effect of six hydrogels as vehicles for $NiCl_2$ and of petrolatum for both $NiCl_2$ and $NiSO_4$ (21). Petrolatum was the poorest vehicle for $NiCl_2$; less than 1 μg Ni/cm^2 reached the receptor in 70–98 hr (Table 6). For $NiSO_4$ in petrolatum, again a poorer penetrant than $NiCl_2$, no detectable nickel reached the receptor phase after 96 hr.

Compared to a simple solution of $NiCl_2$ in water (1.32 mg Ni/mL), methylcellulose and Methocel gels containing 5% $NiCl_2 \cdot 6H_2O$ (12.3 mg Ni/mL) delivered 25–100% as much nickel to the receptor from a similar dose (0.34 mg Ni/cell), but it should be noted that the nickel concentration was 10 times higher. The aqueous gels were less efficient vehicles than water alone. Three other gels—poly(oxyethylene)-poly(oxypropylene), polyvinyl alcohol, and polyvinyl alcohol with triethanolamine—delivered little or no nickel through the skin to the receptor.

The accumulation of nickel in the receptor over 48 and 96 hr increased with increasing nickel concentrations in Methocel E 4M gels for $NiCl_2 \cdot 6H_2O$ concentrations from 0.62% to 5%. The relative increase in nickel accumulation was greater than the increase in concentration. This is reflected by apparent permeability coefficients over 96 hr that are not constant but rise from 0.045×10^{-4} cm/hr for 0.62% nickel chloride to 0.29×10^{-4} cm/hr for 5%. Since the results after 48 hr were similar, these experiments clearly do not support the same lag times, which were about 50 hr, reported earlier (20). Such a long lag time would have led to much smaller apparent permeability coefficients for the first 48 hr than for 96 hr.

Other measures of nickel absorption, namely, nickel content of the dermis, of the epidermis, and of the fourth and fifth tape strips of the stratum corneum, demonstrated a more linear dependence on the concentration of nickel applied.

Less nickel appeared in the receptor phase after 96 hr than was found in layers of the skin. Because the skin was not washed before tape stripping, a large amount of nickel was in the tape strips (% of the dose in the first two strips), but even the tenth strip contained more nickel than the receptor phase. Despite its lower weight the epidermis contained far more nickel than the dermis.

The permeability of human breast skin to $NiCl_2$ in vitro was not significantly different at two moderate temperatures (22°C, and 40°C), but at 60°C the nickel flux into the receptor chamber was significantly greater than at 22°C (Table 7) (40). Based on the median nickel flux over 18 hr, estimated permeability coefficients were 0.46×10^{-4} cm/hr, 0.49×10^{-4} cm/hr, and 0.82×10^{-4} cm/hr at 22, 40 and 60°C, respectively. Minimum individual values were 0.3–0.4 $\times 10^{-4}$ cm/hr, and maximum values were 1.6×10^{-4} cm/hr at 22°C and about 6×10^{-4} cm/hr at 40°C and 60°C.

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Table 6 Nickel, as $NiCl_2$ $6H_2O$ and $NiSO_4$ (hydrate?)

Reference: A. Fullerton, J. R. Andersen and A. Hoelgaard, Permeation of nickel through human skin in vitro—effect of vehicles, *Br. J. Dermatol. 118:*509–516 (1988).

Species: human (surgical, stored frozen) in vivo
in vitro ✓

Area: 1.8 cm^2

Vehicles: for $NiCl_2$: water (0.25 mL/cell) with 1.32 mg Ni^{2+}/mL, 6 aqueous hydrogels {approximately 28 mg/cell; methylcellulose 1500, hydroxypropylmethylcellulose (three types, Methocel E 4M, F 4M, and K 4M), poly(oxyethylene)poly(oxypropylene) copolymer (Pluronic F-127), polyvinyl alcohol (Polyviol W 25/140)} with 5% nickel chloride hexahydrate, and petrolatum; for $NiSO_4$: petrolatum.

Time: 24–163 hr

Analytical method: adsorption differential pulse voltammetry

Notes:

1. The skin was full thickness from either breast or abdomen.
2. The cells were occluded except for the last 48 hr of one experiment.
3. ''All experiments were carried out at least in duplicate.''
4. The amount of nickel delivered in 0.25 mL of aqueous solution was approximately the same as that in 28 mg of ointment with 5% $NiCl_2 \cdot 6H_2O$, i.e., approximately 0.34 mg Ni/cell.
5. From Methocel E 4M the flux of Ni was similar to flux from simple aqueous solution.
6. From methylcellulose, Methocel F 4M and Methocel K 4M the fluxes were approximately 25–60% of flux from water.
7. From polyvinyl alcohol with triethanolamine, from polyvinyl alcohol and from Pluronic F-127 fluxes were 0–10% of the simple aqueous flux.
8. In the first 24 hr the flux of Ni was less than 1 mg/cm^2 for all gels and for aqueous solution. The fluxes increased in each of the next three 24-hr periods above the flux in the previous period.

Permeation of Ni into receptor phase

	Ni in receptor phase (mg/cm^2)[b]				
Vehicle[a]	26 hr	48 hr	70 hr	92–98 hr	163 hr
Water, 1.32 mg Ni^{2+}/mL	0.1	1.2	5.5	12.6	
Petrolatum/$NiCl_2$			0.5	0.7	
Petrolatum/$NiSO_4$	No permeation was detected				
Methylcellulose gel (gel A)		0.5	2.2	7.2	
Methocel F 4M gel (gel B)	0.4	0.9	2.1	3.4	
Methocel K 4M gel (gel C)		1.1	1.9	5.7	
Methocel E 4M gel (gel D)	0.5	1.8	5.1	9.6	
Methocel E 4M gel (gel D)		0.5	1.8	2.9	11.6

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Table 6 Continued

Permeation of Ni into receptor phase

Vehicle[a]	Ni in receptor phase (mg/cm^2)[b] 26 hr	48 hr	70 hr	92–98 hr	163 hr
poly(oxyethylene)-polyoxypropylene gel (gel E)	No permeation was detected				
Polyvinyl alcohol gel (gel F)				0.5	
Polyvinyl alcohol + ethanol + triethanolamine (gel G)				<0.5	

[a] All gels contained 5% $NiCl_2 \cdot 6H_2O$.
[b] By interpolation on published graphs.

Dependence of nickel permeation on its concentration in Methocel E 4M gel

Conc. of $NiCl_2 \cdot 6H_2O$ in gel D (%)	Ni in the receptor phase (mg/1.8 cm^2) 48 hr	96 hr	Permeability coefficient, $10^4 \cdot K_p$ (cm/hr) 48 hr	96 hr
0.62	<0.6	1.2	<0.045	0.045
1.25	3.5	4.7	0.13	0.088
2.5	8.6	14.1	0.16	0.13
5	39.6	61	0.37	0.29

1. The amount of nickel in the skin layers after 96 hr was far higher than the amount of nickel in the recipient phase.

Nickel content after 96 hr exposure to 5% $NiCl_2 \cdot 6H_2O$ in Methocel E 4M gel

Layer[a]	Ni content, µg Ni/1.8 cm^2	% of applied dose	Permeability coefficient, $10^4 \cdot K_p$ (cm/hr)
Tape strippings 1 & 2	143	42.2	—
Tape strippings 3–10	29.7	8.8	—
Epidermis	35.8	10.6	(1.5)
Dermis	5.2	1.6	(0.23)
Receptor phase	1.2	0.4	0.06
Total Ni recovered	214.9	63.8	—

[a] The skin was not washed before stripping.

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Table 7 Nickel, as $^{63}NiCl_2$

Reference: A. Emilson, M. Lindberg, and B. Forslind, The temperature effect on in vitro penetration of sodium lauryl sulfate and nickel chloride through human skin, *Acta Derm. Venereol. (Stockh.) 73:*203–207 (1993).
Species: human in vivo
in vitro ✓
Area: 1.33 cm^2
Vehicle: distilled water
Concentration: 5% $NiCl_2$ (w/v)
Duration: 18 hr
Analytical method: liquid scintillation counting of ^{63}Ni in donor and receptor chambers
Notes:
1. The barrier tissue was full-thickness breast skin from surgery.
2. Experiments were done concurrently at 22°C (ambient temperature), 40°C and 60°C.
3. Results were reported in units of counts per minute (cpm); the counting efficiency is not reported.
4. A reasonable value for ^{63}Ni counting efficiency is approximately 65%. On the basis of that assumption, the median Ni^{2+} flux ($N = 14$) at 22°C = 30 samples/chamber $\times$ 150 cpm/sample $\div$ 0.65 cpm/dpm $\div$ 18 h $\div$ 1.33 cm^2 = 289 $dpm/cm^2/h^1$.

Radioactivity of the donor solution = 0.17 mCi $\times$ 2.22×10^9 dpm/mCi $\div$ 60 mL = 6.3×10^6 dpm/mL.

The corresponding permeability coefficient, K_p = 289 $dpm/cm^2/h^1 \div 6.3 \times 10^6$ $dpm/cm^3 = 0.46 \times 10^{-4}$ cm/hr.

5. Differences between results at 22°C and 40°C were not statistically significant.
6. At 60°C the flux was significantly increased ($p < 0.05$) such that the median $K_p = 0.82 \times 10^{-4}$ cm/hr.

Summarized in tabular form, the data obtained were:

	Permeability coefficient for Ni^{2+} (estimated)[a] $10^4 \cdot K_p$ (cm/hr)		
	22°C	40°C	60°C
Mean	0.55	1.2	1.4
Median	0.46	0.49	0.82
Minimum	0.29	0.33	0.37
Maximum	1.6	5.5	6.1
Comparison to 22°C[b]		Not significant	$p < 0.05$

[a] $N = 14$ at each temperature.
[b] Nonparametric Wilcoxon's rank sum test.

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D. IMMUNOLOGY

1. An Overview

While nickel is a transition metal with an ionic radius too small to be antigenic itself, it has a tendency to complex with electronegative molecular sites, e.g., by forming Ni-complexed protein, and thus cause immunogenic conformational changes in proteins that are recognized by hapten-specific T cells (41). In model experiments with amino acids, the nature of the protein-hapten conjugates that can result in nickel hypersensitivity were investigated by means of radiochromatographic analysis. They showed the metal preferentially binding to glycine, histidine, and lysine and, to a lesser extent, to aspartic acid and serine groups. Quantitative assay by liquid scintillation counting revealed lysine to be the major nickel-binding amino acid (42). The presence of other electropositive metals that have the ability to form complexes was seen to significantly modify such nickel reactivity with skin proteins in vivo in humans, presumably by interfering with the complexing action of nickel ions. Magnesium, for example, is known to form complexes but does not induce contact sensitization. When nickel-sensitive patients were patch-tested with equimolar (0.1 M) nickel sulfate and magnesium sulfate solution as well as zinc sulfate and manganese sulfate, the positive reaction to nickel sulfate decreased in frequency and severity with statistical significance (43,44). The fact that allergic reactions to $NiSO_4$ can be inhibited or reduced by sulfates of other metals with physicochemical similarities means that nickel can replace other divalent ions in some biomolecular structures, and thus lose part of its antigenic activity.

Nickel compounds can induce multiple allergies: (a) contact dermatitis of the delayed type, (b) antibody-mediated, immediate-type urticaria and asthma, and, in some cases, both types (a) and (b) from exposure through the skin, diet, inhalation, or orthopedic implants. Exposure through inhalation presents a particular occupational hazard, as 30–50% of the nickel inhaled is absorbed by the organism (37,45–47). While, on the one hand, the common occurrence of nickel in virtually all metal objects with which man comes into constant, sometimes intimate contact help explain the frequency of nickel hypersensitivity, on the other hand, the metal was determined to be an allergen of moderate potency only. In a classification of allergens, based on the human maximization test of metal compounds among 90 diverse chemicals by the repeated insult patch test protocol, the score for nickel sulfate was 48% positives, making the salt a class III (moderate) sensitizer on the Magnusson-Kligman scale (48,49). Inducing nickel allergy in animals has proven difficult (50). Animal models commonly used in predictive screening for allergenicity of materials do not appear to react significantly to nickel exposure, and only most recently have guinea pigs been sensitized to nickel in a consistent manner (51).

It has been demonstrated histochemically that nickel, applied as a nickel

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chloride solution, is selectively taken up by Langerhans cells in whole viable human epidermis (52). In agreement with its classification as a moderate immunogen, $NiCl_2$ in the murine local lymph node assay (LLNA) only causes moderate to weak lymph node cell (LNC) proliferation (53). Under modified conditions, however, using a more polar solvent (dimethylsulfoxide, or DMSO) chosen to facilitate skin penetration by the metal ion, and the addition of an irritant (sodium lauryl sulfate, or SLS) to the vehicle, cutaneous application of nickel sulfate significantly increased LNC proliferation in the mouse, as corrected for exposure to DMSO-SLS alone (54,55).

Frequent exposure to nickel results in a buildup in the stratum corneum, resulting in formation of a reservoir (14), and this may serve to explain the significant prevalence of nickel hypersensitivity and often a lifetime persistence of that condition. Nonspecific irritation and damage to keratinocytes by nickel ions may be the key event leading to sensitization. Nickel sulfate in nontoxic doses, for example, was shown to cause a dose- and time-dependent inhibition of human keratinocyte growth and viability in culture, with a concomitant increase in inflammatory cytokine release such as interleukin-1, and activation of lipoxygenase in leukocytes (56,57).

The immunogenic form of nickel is its divalent ion (58) and the ease of its formation through oxidation of the metal with exudates on contact with the skin is another important factor in the etiology of nickel allergy. The sodium chloride and fatty acid contents of sweat alone will leach the metal from coins in significant amounts (59), sufficient to give a positive reaction when a nickel alloy coin is taped to the skin of a sensitized individual (60,61). Highly sensitive individuals can react to coined money by developing eczematous lesions to a degree that will preclude their occupation involving routine handling of change (62).

2. Systemic Sensitization and Dental Problems

Immunologically mediated skin reactions of the immediate or delayed type can occur as consequence of systemic induction of sensitivity or challenge by an allergen. Nickel can serve as a model hapten for systemic sensitization since, together with chromium and cobalt, it is a ubiquitous and most common cause of various types of such immune reactions (37,63). Induction as well as elicitation reactions are seen following various types of systemic exposure: oral, gastrointestinal, respiratory, parenteral, or iatrogenic. Conversely, there is increasing evidence that low-level, long-time oral exposure to nickel has a desensitizing effect.

Nickel-induced hand eczema is a common problem in systemically sensitized women; this periodically recurring, vesicular, palmar eczema is known as pompholyx and is the most common clinical form of systemic nickel hypersensi-

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tivity. Pompholyx is never elicited by external contact (64). When nickel-sensitive individuals are exposed intravenously to incidental nickel, as can be present in medical devices, microgram quantities can be sufficient to produce contact dermatitis (36,65) or even anaphylaxis (66).

Dental health care workers and their patients are at risk of developing contact sensitivity or urticaria to nickel; only the clinical presentations differ. Dental workers may develop ACD and cheilitis of the hands from handling nickel-plated instruments, whereas patients risk the development of allergic contact stomatitis, gingivitis, or cheilitis [confirmed histologically (67)], or generalized systemic ACD without buccal involvement (68,69). Also asthma (70–73) and chronic urticaria (74) have been observed in dental patients.

The incidence of nickel hypersensitivity among dental patients was found to be significantly higher than that seen among the average dermatological patients; in an analytical study of 403 patients, 31.9% of the women and 20.7% of the men showed positive reactions to patch tests with $NiSO_4$ (75).

With recent advances in orthodontic materials, stainless steel, which customarily contained 8% nickel, has been replaced with alloys claiming titanium for improved performance, but which proved to contain as much as 78% nickel. What used to be occasional elicitation of buccal or generalized allergic reactions, de novo sensitization from routine orthodontic therapy has also been reported recently (76,77). The causal relationship between allergic reactions and nickel-containing appliances was demonstrated in patients in whom local and generalized dermatitis cleared completely upon removal of the appliances (69,78).

The amount of nickel dissolved from orthodontic appliances has been determined in a model experiment. Immersed in a 0.05% sodium chloride solution, up to 40 μg Ni per day was released, an amount capable of sensitizing a naïve organism, and certainly adequate to elicit a reaction in presensitized individuals (79). However, sensitization or allergic reactions due to the metal liberated from denture materials is not recognized as a risk factor of comparable import as that from total joint replacement or pacemakers. This is probably due to significant differences in the microenvironment surrounding the contact surfaces. Thus, hypersensitivity does not necessarily result in adverse oral reactions on exposure to nickel-containing dental alloys.

Higher concentrations of the allergen appear to be required for elicitation of reactions in the oral mucosa than those leading to involvement of the skin (80,81). In a controlled study ($n = 10$) involving subjects with skin reactions to nickel, prosthetic appliances containing as much as 66% Ni caused no adverse general or oral clinical or histological reactions over exposure times of up to 40 months (82). Also, patients with fully banded or bonded orthodontic appliances did not show signs of increased nickel blood levels stemming from such exposure (83).

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Subcutaneous implantation in mice of nickel alloys used in nonprecious dental castings showed that, due to corrosion, nickel was released and then accumulated in various tissues and organs (84). A number of systemic sensitization reactions have been ascribed to trace concentrations of nickel dissolving in contact with tissues. The implanted sources of nickel include joint prostheses, plates, and screws for fractured bones fabricated from stainless steel or Vitallium, all containing varying percentages of nickel, cobalt chromium, and molybdenum, or from nickel-cadmium battery pacemakers that contain up to 35% nickel (37). The use of such alloys comports the risk of nickel hypersensitivity developing de novo, as well as the risk of allergic reactions in patients already sensitive to nickel. Model solubilization studies show that ppm levels of nickel are released from stainless steel prosthesis materials on immersion in physiological saline, sweat, or blood plasma for 1 week at room temperature (85). Such leaching of metal into the tissues is called orthopedic dermatitis (86–88). In the majority of earlier cases, metal sensitization due to implanted joint replacements was correlated with loosening or mechanical failure of the prostheses leading to increased solubilization of the alloy components (89). The risk of sensitization to nickel and other heavy metals used in artificial joints appears to be negligible now, thanks to the development of improved materials and safer alloys, which minimize mechanical failure and the incidence of abrasive metal-to-metal contact (90).

The systemic sensitization to nickel via articles of personal adornment such as earrings appears to be more subtle. Even though earrings are often gold-plated, the nickel interliner beneath the gold plating can become exposed with use, inducing nickel hypersensitivity and contact dermatitis. Scanning electron microscopy and x-ray microanalysis of the surface of both used or unused gold-plated jewelry, for instance, reveals that the gold surface can be defective, allowing the underlying nickel to be corroded and released on skin contact (91).

In the occupational setting, worker exposure to dust and aerosols high in nickel content does not seem to cause inordinate helath problems. It is not clear, however, whether the workers are nickel-sensitized but show no symptoms, whether they are not sensitized, or if they have become immunotolerant through chronic exposure.

3. Release of Nickel from Alloys

The increase of nickel allergic hypersensitivity (NAH) noted among the general population and particularly in dermatology patients is becoming a significant public health problem. The incidence has more than doubled in some countries over the past 10–15 years (92–97). This is believed to be due to facile release of the metal from alloys upon tissue contact, e.g., with mucous membranes or intact

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skin. Two types of exposure are the principal causes of primary or secondary NAH: (a) sustained and intimate contact with costume jewelry, particularly when the skin is pierced and subsequently embellished with metal articles, and (b) contact with orthodontic devices that remain in prolonged contact with the buccal mucosa. NAH traced to surgical implants, e.g., joint replacement, is much less prevalent these days. Leaching or release of nickel from metal objects in contact with biological substrates is a continuous variable that defeats prediction. Aside from immediate, external environmental factors, the microenvironment within the particular alloy is a principal determinant, due to the action of electromotive forces generated by the presence of a multitude of possible accompanying metals (98). Denture and orthodontic materials, for example, may contain as many as 5–20 different metals. Furthermore, published corrosion data on corrosion show that release rates are not necessarily correlated with Ni content. Other metals in immediate proximity can provoke electrochemical corrosion of the alloy, either accelerating or diminishing the release of nickel (98,99).

The amount of nickel liberated from various metal objects, including metal fashion accessories and orthodontic appliances, has been determined in several model experiments. When immersed in a 0.05% sodium chloride solution, a level typical for saliva, such appliances released 125 μg ± 22 over 12 days at 37°C. While this is a minor quantity compared to average dietary intake of 300–500 μg/day, it is still an amount capable of sensitizing a naïve organism, and certainly enough to elicit a reaction in presensitized individuals (79).

Model solubilization studies show that ppm levels of nickel are released from stainless steel prostheses (2–5 cm^2 surface area) on immersion in physiological saline, sweat, or blood plasma for 1 week at room temperature (85).

Nickel release from orthodontic appliances was also investigated over 14 days. The amounts liberated ranged from essentially zero from archwires, to 10.4 μg/day from face bows.

Analysis of used and unused studs/clasps and earrings showed that they all released nickel to some degree upon 1-week storage in synthetic sweat and that neither gold nor silver plating prevented this. Release ranged between 0.005 and 442 μg nickel (100).

Nickel is also released in appreciable amounts from stainless steel cooking utensils at the low-pH characteristic of certain foods. In an experiment simulating different foodstuffs, Ni release correlated inversely with pH, with an average 6.0 μg released in 80 min at 100°C and pH 3.2 (101).

In cooking experiments with various foods, oxalic acid is most aggressive in provoking the release of Ni from steel cooking utensils, with up to 9.5 mg Ni/L liberated in 1 hr (102). This is much more than the level of 0.6 μg Ni/L that is considered to be the triggering threshold in highly sensitive individuals with NAH (103). Such amounts leached in the process of cooking contribute to

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the oral intake of allergen and help explain, at least in part, the permanent state of NAH often observed.

4. Orally Induced Tolerance to Nickel

The tolerogenic effect of oral exposure to diverse types of antigen seems to affect all aspects of the systemic immune response that have been studied: IgM, IgG, and IgE antibody responses are suppressed, as is cell-mediated immunity as measured by lymphocyte proliferation in vitro or ACD in vivo (104).

Observations following Ni exposure in animals and in humans (105,106) led to the hypothesis that systemic delayed hypersensitivity reactions can be suppressed following prior oral exposure to haptens (also known as the Chase-Sulzberger phenomenon) under certain specific conditions, i.e., if prolonged antigenic contacts occurred during childhood and adolescence of naive individuals.

Thus, since the late 1980s, dermatologists and immunologists focused increasingly on corroborating such a correlation, motivated particularly by a steady increase in the prevalence of NAH in the young (a phenomenon correctly attributed to the pervasive practice of skin perforation with base metal objects for the sake of embellishment).

In a prospective study in 1986 of 303 hairdressers and nurses, representative of a population at heightened risk for developing NAH, an effect contrary to sensitization was noted when contact with nickel occurred at an early stage in life (and particularly if such exposure occurred prior to ear piercing). Nurses who had worn dental prostheses in childhood showed a markedly lower incidence of NAH (5.4%) than those without orthodontic treatment (18.4%; $p < 0.05$) (106). Subsequently, several further studies confirmed the tolerizing effect of slow oral release of nickel present in dental prostheses. A European multicenter study by van Hoogstraten, for example, showed that orthodontic treatment did not contribute to NAH (108). Of 431 patients with pierced ears, 168 (39%) were patch-positive, whereas of the 86 who had been wearing braces before piercing, only 24 (27.9%) were positive ($p < 0.05$). In a survey of 294 patients in a patch test clinic in Ulster, the incidence of nickel allergy was reduced from 36% to 25% in those subjects whose orthodontic treatment had preceded ear piercing (107). In an investigation involving seven western European clinics, it was demonstrated that when the wearing of dental braces had preceded ear piercing, NAH was reduced from 39.3% (without braces) to 25.0% ($p < 0.006$) in female patients. In the Netherlands, specifically, the incidence was 37.5% versus 15.6% ($p < 0.025$), and corresponding figures in Denmark were 38% versus 21.7% ($p < 0.05$) (108,109). Finally, a Finnish study of 700 adolescents from 14 to 18 years of age showed that while orthodontic treatment did not increase the risk for NAH, when such treatment had preceded ear piercing it prevented NAH in girls (110).

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5. Allergic Contact Dermatitis

Immunochemically, allergic contact dermatitis (cell-mediated or type IV hypersensitivity) to nickel was first demonstrated by the lymphocyte transformation test and the macrophage migration test on peripheral blood samples obtained from nickel-sensitive patients (111).

Nickel is present in most base metal alloys used in utensils and commonplace consumer commodities, including tools, coins, garment accessories, jewelry, wristwatches, etc., and casual skin contact is virtually unavoidable. Thus, the different types of stainless steel incorporate varying amounts of nickel for improved corrosion resistance. Depending on nickel content, they will release nickel on skin contact, resulting in hypersensitivity reactions. The American Iron and Steel Institute (AISI) austenitic steel grades 303, 304, 316, and 430 contain 8.45, 8.65, 11.29, and 0.11% Ni, respectively. In a leaching study conducted with various austenitic steel grades, with high-sulfur-containing (resulfurized) stainless steel (AISI 303) and nickel-plated steel, nickel release in synthetic sweat was correlated with clinical provocation of contact allergy in already sensitized patients by skin patch testing. The potential for sensitization was found to correlate inversely with corrosion resistance in chloride media. Austenitic grades releasing less than 0.5 $\mu g/cm^2$/week were found to be safe when used in prolonged contact with skin, as they caused no allergic reactions. Resulfurized grade releasing 1.4 $\mu g/cm^2$/week provoked allergic reactions in 14% of the volunteers, and Ni-plated steel in 96%; the latter two types therefore should be avoided for occupational safety purposes (112,113). Nickel release was also increased due to galvanic corrosion when the metal was combined with precious metals (98).

While nickel is statistically recognized as the premier cause of ACD overall, it is likely that it plays this role as the eliciting, secondary agent evoking a reaction in those already sensitized systemically, e.g., by ear piercing. This was corroborated in studies that more closely investigated the etiology of ACD. First, it is recognized that all types of studs and earrings release nickel in synthetic sweat, ranging from 0.005 to 442 μg/week, and neither gold nor silver plating prevents this (100). Second, the incidence of nickel allergy is much more likely in individuals whose ears are pierced (114–116) and over 50% of nickel-sensitive women suspect that they were sensitized before age 20 (117). It would appear, then, that primary nickel sensitization is due primarily to metal contact when the epidermis is damaged, and that later contact with the reepithelized tissue represents a further risk. Thus ACD reactions upon casual or occasional contact with nickel are likely due to elicitation rather than induction reactions to relatively low levels of allergen released. The prognosis for nickel dermatitis appears more favorable when nickel-allergic patients avoid all further contact with white metal objects, particularly in clothing and jewelry (118).

The incidence of nickel hypersensitivity is increasing in most industrialized

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countries where epidemiological surveys have been conducted. This holds true for ACD, in particular, from occupational and environmental exposure. In these cases, nickel is consistently found to be the most common contact allergen, also causing the most persistent form of ACD due to the cumulative effect of multiple exposure (96).

In response to this trend, dermatologists have proposed a regulatory standard that would limit nickel released from commonplace metal items to less than 0.5 μg/cm^2/week (119,120). Subsequent to verification that such a maximum threshold is appropriate, a standard analytical methodology that includes a modification of the dimethylglyoxime test to ascertain observance of these limits was adopted by the European Union (EU) in the European Nickel Directive in 1994 (94/27/EC) (117). This is intended to reduce the prevalence of sensitization (primary prevention) and recurring dermatitis in the sensitized population (secondary prevention). In Denmark, the Nickel Directive became law in 1989 (121), followed by the entire EU in 1996. The legislation states that nickel may not be used (a) in earring post assemblies used during epithelialization unless they are homogeneous and in which the concentration of nickel is less than 0.05%; (b) in products intended to come into direct and prolonged contact with the skin, such as earrings, necklaces, watch straps, or zippers, if the nickel release is greater than 0.5 mg/cm^2/week; and (c) in coated products under (b), unless the coating is sufficient to ensure that nickel release will not exceed 0.5 μg/cm^2/week after 2 years of normal use (122). This threshold of 0.5 mg/cm^2/week will avoid ACD in most sensitized subjects, albeit some nickel-allergic individuals may still be expected to react even to levels of 0.05 mg/cm^2/week (100,123,124).

The potential threshold for inducing nickel sensitivity due to contact with irritated skin, also the major cause of so-called ''housewife dermatitis,'' was investigated by a hand immersion experiment. However, upon exposure twice daily for 23 days to a surfactant solution, there was no evidence of nickel sensitization up to concentrations of 1 ppm (125).

Finally, it should be noted that in an analysis of patch test responses to nickel in subjects of varying age, the incidence of positive reactions to nickel decreased with increasing age (126).

6. Induction and Elicitation Thresholds

Consensus for a safe exposure limit to avoid sensitization by nickel has not yet been found. The ''best'' number so far is 10 mM or 0.6 ppm (127); however, some dermatologists see the safety limit concentration at one-tenth this value (102,128). The levels of incidental nickel encountered in articles of everyday use normally lie below 5 ppm and were not found to represent a hazard, even to sensitized individuals, as such exposure is brief (129). In a single open application on the forearm, of 51 subjects none reacted to 100 ppm, indicating a threshold

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above that level (130). In the application of nickel disks, the release rate of metal determined in synthetic sweat at 0.5 mg Ni/cm^2/week appeared to represent a threshold, having induced weak reactions only (119). No reaction was seen upon repeated open nickel application at 1 ppm for 23 days on the skin of four sensitized volunteers following application of sodium dodecyl sulfate (131). Twelve subjects sensitive to nickel were patch tested with serial dilutions of $NiSO_4$ in petrolatum. The lowest amount of nickel producing reactions ranged from 5.2 to 47 mg (132).

Patch test under occlusion (True Test) was conducted on the back of 72 test subjects and left for 48 hr; reaction was read at 96 hr. The results showed that 38 subjects responded to 3–0.3 mg Ni/cm^2 (threshold). This range in response, even in this homogeneous cohort, indicates the individual variation in susceptibility to sensitization and in reactivity (133).

A number of patients testing positive to various patch concentrations of nickel sulfate were checked for reactivity to nickel in coins. All patients positive to 1.25% or lower patch concentrations reacted to contact with coinage as well (134).

7. Prevalence of Allergic Contact Dermatitis

Consistently, nickel ranks as the most common cause of ACD in the countries where statistics on occurrence of sensitization are recorded. The female population is particularly affected due to its frequent use in jewelry and accessories (135). The incidence of contact allergy to nickel is seen to be increasing; in patch-tested women it rose from 12% in 1967 to 21% in 1976, and in men from 1% to 4% over the same interval (136). Presently it is seen to reach 22% of the female and 4.7% of the male dermatology patients in the United States and Europe (137–139). A random Danish population showed 11.1% and 2.2% Ni sensitivity for females and males, respectively (116). In both cohorts nickel ranks as the number one contact allergen (Table 8). This incidence among the general population rose to 19.6% in the group aged 15–34 years, and such an elevated rate of sensitization in the young finds confirmation in a Spanish study involving children up to the age of 14. Patch testing of 272 children in a dermatology clinic resulted in 101 cases with a positive ACD reaction to at least one allergen, of which 57 (54.4%) were considered relevant. Among these, 21 tested positive to nickel. In Japan, where ear piercing is also becoming increasingly popular, a study involving 377 dermatology patients compared the occurrence of dermatitis due to diverse metal allergies and identified that practice as a definite risk factor; the rate of hypersensitivity to nickel (tested with 5% nickel sulfate) was 38.3% in patients with pierced ears, versus 25.6% of patients without (140). The second highest rate was recorded for gold: 34.6% positive reactions to 0.2% gold chloride.

The reason for this prevalence of nickel contact dermatitis was again seen

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Table 8 Prevalence of Contact Allergens in German and Danish Populations

Compound* (metal allergens are in boldface)	Positive reactions (%)					
	Total		Women		Men	
	n = 3389†	n = 567‡	n = 2123†	n = 288‡	n = 1266†	n = 279‡
$NiSO_4$	**15.5**	**6.7**	**22.0**	**11.1**	**4.7**	**2.2**
Fragrance mix	6.6	1.1	6.8	1.0	6.4	1.1
Balsam of Peru	5.4	1.1	6.6	1.4	5.3	0.7
Cobalt	**5.4**[a]	**1.1**[b]	**6.1**[a]	**1.4**[a]	**4.4**[a]	**0.7**[b]
Kathon CG	5.2	0.7	5.6	1.0	4.7	0.4
p-phenylenediamine	4.6	0.0	5.0	0.0	3.8	0.0
K_2CrO_4	**3.6**	**0.5**	**3.3**	**0.3**	**4.3**	**0.7**
Neomycin sulfate	3.5	0.0	3.7	0.0	3.3	0.0
Colophony (rosin)	3.3	0.7	3.3	1.0	3.3	0.4
Mercury	**3.2**[c]	**3.4**[d]	**3.3**[c]	**3.1**[d]	**3.2**[c]	**3.6**[d]

* Metal allergens are in boldface.

† Frequency of positive reactions to standard battery patch test in dermatology patinets form 12 German clinics (142).

‡ Frequency of positive reactions to dermatological tests in an unselected Danish population (116).

[a] Sulfate; [b] chloride; [c] amide chloride; [d] thimerosal.

in the increasing use of imitation jewelry, often involving perforation of the skin on various parts of the body, and the intimate contact with buttons, rivets, and other metallic fasteners on clothes (141).

Nickel has been described as the ubiquitous allergen (143), and it is the consensus among dermatologists that nickel allergy prognosis is very poor due to constant reexposure through ingestion, inhalation, and skin contact (144). This unfortunate conclusion can be appreciated when one realizes that particulate nickel suspended in atmospheric aerosols has been measured to reach levels of 180 ng/m^3 in urban air, and goes as high as 3.3 mg/m^3 near nickel smelters (19,145) versus maximum levels of 0.8 pg Ni/m^3 air measured as natural background in remote areas. Typical house dust contains 40 mg nickel/kg (146). The biological half-life of inhaled particulate nickel is >330 days (147); that of pulmonarily deposited nickel oxide aerosol was calculated in function of particle size, expressed as the mass median aerodynamic diameter (MMAD): 11.5 months for NiO with MMAD = 1.2 mm and 21 months for particles with MMAD = 4 mm (148). Among patients with industrial nickel dermatitis, none among the female patients (n = 29) completely cleared up, and only 27% of men (n = 11) healed over a 3 year period, even after a change in occupation or discontinuation of work. Atopic patients were excluded from that longitudinal study (149).

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8. Blocking Cutaneous Contact

Given the high incidence of hypersensitivity to nickel effected by skin contact with that antigenic metal or exacerbation of allergy due to systemic (nutritional) intake, considerable efforts have gone to prevention of such disease or alleviating its severity, especially to benefit those occupationally exposed to nickel. On the one hand, the therapeutic approach using antiinflammatory topical steroids or, in cases of extremely hypersensitive patients, the systemic administration of chelating agents such as diethyldithiocarbamate or triethylenetetramine only yields limited success, as the dermatitis is not completely suppressed and the drugs produce toxic side effects (150,151). On the other hand, preventive application of barrier ointments to reduce or avoid skin contact and penetration by the allergen has proven successful. Nickel can be immobilized by appropriate formulation of various barrier systems, thus avoiding elicitation of allergic reactions. Such auxiliary barrier materials shielding the stratum corneum carry either a chelating agent (21,152,153) (5-chloro-7-iodo-8-hydroxyquinoline or Clioquinol, L-histidine, D-penicillamine, or ethylenediaminetetraacetate) or an antioxidant, either of which would reduce the oxygen-dependent dissolution of nickel on skin contact (16). The protective effect of barrier gels could be measured in vitro by determining the degree of nickel penetration by means of permeation cells, or in vivo by noting the incidence of patch test reactions in hypersensitive patients. Of the antioxidants and chelators studied, Clioquinol was shown to be the most effective inhibitor of nickel-induced hypersensitivity reactions, completely abrogating the allergic reaction in all subjects tested (29,154). Application of a carboxyvinyl polymer gel (Carbopol) containing 10% $CaNa_2$-EDTA on the skin beneath a nickel disk also prevented the allergic contact response in all hypersensitive patients tested (21,153).

9. Cross-Reactivity and Multiple Sensitivities

Nickel cross-sensitivity to cobalt was previously assumed due to the prevalence of concurrent sensitivity to both metals but has been refuted by careful experimentation. In human volunteers presensitized to nickel, no cross-sensitivity to cobalt could be demonstrated in vivo (155). Nickel and cobalt were shown to occupy different binding sites in epidermal tissue (28), also confirming the view that nickel does not cross-sensitize with cobalt. A synergistic relationship between nickel and cobalt sensitivity, presumed to exist in humans, has been demonstrated in a guinea pig model (156). The presumed cross-sensitivity to nickel and cobalt could be either the result of multiple primary sensitizations (concomitant sensitization) or a reaction to contaminated challenge materials. Nickel compounds are normally contaminated with cobalt, as the two metals are naturally associated and difficult to separate quantitatively. Even when patch testing for

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nickel or cobalt dermatitis, it is difficult to obtain reagents in which one metal compound is totally devoid of the other (157).

On the other hand, hypersensitivity to copper and palladium, occasionally noted in nickel-allergic patients, may well be due to cross-reactivities at the T-cell clonal level rather than to concomitant sensitization. Nickel-reactive T-lymphocyte clones from different human donors sensitized to $NiSO_4$ were analyzed for their ability to cross-react with other metals neighboring nickel in the periodic system of elements: 10 of 20 Ni-reactive T-lymphocyte clones cross-reacted with Cu, and 6 of 20 with Pd. The finding that Ni-reactive clones only exceptionally cross-reacted with Co (1 of 20) supports the view that in vivo Ni and Co hypersensitivities are separate and independent entities (158), and the concomitant positive reactions of nickel-sensitive patients to copper and palladium may signify true cross-reactivity at the T-cell clonal level.

A different pattern of sensitization to transition metals was observed upon testing with specific metal salts only; the pattern of concomitant positive patch test reactions leads to the conclusion that they only can occur if they form the same shaped complexes with similar ligands inside the skin, an immune response toward antigenic complexes with similar geometry, i.e., reactions provoked by inclusion of palladium in the patch test program, but without clinical relevance (159).

10. Diagnostic Methods and Models for Nickel Dermatitis

Cutaneous testing for nickel sensitivity has a limited reliability. False-negative results are seen, which can be due to the extremely long induction or lag times demonstrated in skin penetration experiments, or to the irritant effect of the patch test concentration used (14,20,29), and intradermal testing has been proposed as an alternative to bypass the stratum corneum barrier (160). Cases with a clinical history of nickel sensitivity have occasionally shown negative test results when patched with the standard 5% (0.19m) nickel sulfate in petrolatum, probably due to the considerable lag time involved in the salt's penetration through the stratum corneum. Upon pretreatment of nickel test areas with sodium lauryl sulfate, however, such asymptomatic sensitivity will nevertheless become manifest, with the inflammatory reaction elicited by the pre-treatment facilitating the penetration of the immunogen (161).

The minimum challenge concentration that will still elicit a positive patch test reaction to $NiSO_4$ in 10% of highly nickel-sensitized individuals, at 48 hr, under occlusion and on normal forearm skin compromised by pretreatment with sodium dodecyl sulfate, was 0.5 ppm (3/12) (162).

Allergic patch test reactions are remarkably longlasting. A median duration of response to nickel sulfate was 9 days, and 15% of responses extended to 17 days or longer. Nickel quantification in involved versus noninvolved tissue

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showed no significant qualitative or quantitative differences in the immunocytochemistry of the tissues, however (difference in surface receptors on activated T lymphocytes or Langerhans cells). This is seen as an indication that the elevated local nickel concentration occurring at the challenge sites does not play a significant role in the time course of the immune reaction (163).

Since nickel is such an outstanding cause of ACD in humans, the search for a reliable in vitro Ni allergy test has been pursued for several years, focusing mainly on the lymphocyte transformation test which affords a measure of increased DNA synthesis upon stimulation (164–166). False-positive results have limited the value of this test because T-cell proliferation is sometimes observed in otherwise clinically healthy, patch-negative individuals (167). However, the combination of the lymphocyte transformation test with the macrophage migration inhibition test for nickel-induced release of migration inhibition factor in peripheral blood lymphocytes can significantly improve the reliability of in vitro diagnosis for nickel allergy (168).

11. Immunological Contact Urticaria

Occupational exposure to nickel salts or volatile nickel carbonyl dispersed in the breathing air can lead to asthma and pulmonary infiltrates with eosinophilia (45,169,170). Such antibody-mediated, immediate-type hypersensitivity or immunological urticaria was repeatedly demonstrated by positive reaction to inhalation challenge and skin prick test with nickel sulfate (171). Nickel-reactive IgE antibodies as well as elevated levels of the IgG, IgA, and IgM types have been identified in the sera of asthmatics exposed to metal particles suspended in the air (71,72,172–174). A distinct pattern of changes in serum immunoglobulin levels was seen in response to nickel-exposed workers: IgG, IgA, and IgM levels were elevated, with a concurrent drop in the level of IgE. These changes in serum protein concentrations may have an impact on the susceptibility of the organism to infection (35).

Rhinitis (albeit with negative chamber and prick tests) can develop upon inhalation of nickel present in soldering fumes; the immunological nature of such a reaction could be demonstrated by a positive challenge reaction to nickel sulfate, resulting in impaired nasal air flow. The dual nature of such sensitization became obvious with positive skin patch reactions (type IV) and inhalation reactions (type I) when patients were tested with the same nickel compound (175).

12. The Dietary Factor

Induction of nickel hypersensitivity by the oral route is unlikely. However, the elicitation of a systemic dermal reaction in those already sensitized may occur when they are exposed to soluble nickel species in certain foods. Chronic sensi-

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tivity can thus be maintained by nickel that is ingested and may elicit both immediate- and delayed-type reactions. This effect has been demonstrated by the controlled exposure of eczematous patients to a soluble nickel salt in their diet (176,177). The cutaneous reaction following ingestion shows a dose-dependent response; only a few react to a dose below 1.25 mg, but nearly all react to amounts over 5.5 mg. Such exposure elevates serum levels and increases urinary excretion. The latter is also the most practical indicator of chronic exposure to the metal. In controlled experiments the converse was also found to hold true; a nickel-poor diet resulted in reduced urinary excretion of the metal and alleviated symptoms of dermatitis in allergic subjects (178), commensurate with the degree of sensitization (179). Nickel intake is almost unavoidable through the normal human diet, as Ni occurs in most plants, being especially concentrated in plant seeds (180). Nuts and cocoa beans, for example, are reported to contain as much as 5–10 mg Ni/kg (181), which may explain the frequently voiced complaint of ''allergy to nuts.'' When a group of nickel-sensitive subjects was given controlled, limited amounts of nickel sulfate in their diet, the majority were able to adapt and finally showed no clinical reaction to continuing oral administration of the allergen. Challenge by patch test or incidental dermal exposure, however, produced an unchanged allergic reaction as observed before. That was taken as an indication of intestinal adaptivity rather than a buildup of tolerance (182), which is contrary to observations made in animals (58). The role and impact of dietary nickel in nickel-sensitive patients has been investigated in dietary depletion studies, as it is possible to reduce nickel intake by half by adhering to a low-nickel diet. Such restriction generally improves, but does not cure, clinical manifestations of hypersensitivity; only approximately 50% of patients with eczema involved in that program showed marked improvement of the hypersensitivity state (183).

13. Immunotoxicity

The immunotoxicity of nickel has been demonstrated in a number of animal models (184–188). Regardless of type of exposure, nickel will significantly alter the functioning of host defense mechanisms: Alveolar macrophages are reduced in number and lose activity, primary antibody production is reduced, lysozyme levels and activity are significantly decreased. All effects were reflected in enhanced mortality upon challenge of the animal model with infectious microorganisms.

E. SUMMARY

Nickel, ubiquitous in the environment and a common respirable-sized particulate pollutant, is an essential trace element, but also a carcinogen that is of major concern in industrial medicine. From the point of lethality, pulmonary absorption

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is the most hazardous route of exposure in humans, and in particular poses the risk of cancer in the industrial environment. The dermal toxicity aspect of greatest importance for the general population is the allergenicity of nickel; while a continuing, significant frequency of sensitization to the metal is still noted in the occupational setting, prevalence in nonoccupational exposure is on a rapid increase. The principal way in which sensitization can be induced appears to be systemic exposure or skin contact with a high concentration of sweat-soluble nickel. Casual contact with the metal in articles such as coinage, tools, instruments, and equipment does not appear to be a factor in the induction of ACD for the population in general. Once an individual is sensitized, elicitation of a dermal reaction can occur by contact with much lower concentrations of the metal.

Success was seen in preventive application of barrier gels featuring metal-chelating materials, blocking the elicitation of contact hypersensitivity reactions in those already sensitized. Clinically relevant are contact dermatitis of the delayed type, as well as immediate urticaria and asthma. Induced either by skin contact or systemic exposure such as occurs in ear piercing, they are the most obvious signs of induced hypersensitivity. Conversely, chronic oral (dental materials) and inhalation (occupational) exposures to low concentrations appear also to be important routes in the development of immunotolerance to nickel, primarily in previously unexposed individuals.

Although nickel allergy is often diagnosed concurrently with cobalt allergy, this is best explained by the close association of the two metals in nature and in alloys, and not by cross-sensitization.

Percutaneous absorption rates of nickel salts are low; following a characteristically long lag time, the observed permeability coefficients are on the order of 10^{-6}–10^{-5} cm/hr.

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26
Osmium

A. GENERAL COMMENTS

Metallic osmium is relatively inert. However, when powdered osmium is heated from room temperature to approximately 100°C, the metal oxidizes to volatile osmium tetroxide. No adverse effects have been noted from casual contact with this rare element in its metallic state. But the industrially important catalyst osmium tetroxide is a strong, pyrophoric oxidant that is rapidly reduced to the inert dioxide or to metallic osmium upon contact with organic materials.

B. SKIN REACTIVITY

Osmium tetroxide is a primary irritant that has caustic action on the skin. Contact with this compound results in eczema and severe, nonimmunological contact dermatitis (1). Similar to other metal complexes traditionally used as antimicrobials, chelated complexes of osmium with phenanthroline and bipyridyl ligands are effective against gram-positive bacteria. Systemically, however, they exhibit curariform action (paralysis of skeletal muscle) and thus are limited to topical use (2,3).

C. SUMMARY

Exposure to osmium as it occurs in the environment poses no toxicological threat. Only occupational exposure to the tetroxide in the chemical and metalworking industries can result in pulmonary and skin damage due to its severe irritancy. No data were located regarding the rate of skin absorption of any osmium compound.

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27
Lead

A. GENERAL COMMENTS

Lead is a nonessential element, a ubiquitous environmental contaminant of considerable general and cumulative toxicity, and a suspected human carcinogen. Having similar physicochemical properties, Pb^{2+} competes with Ca^{2+} inhibiting the release of neurotransmitters, interfering with the regulation of cell metabolism by binding to calcium receptors, and blocking calcium transport to protein binding sites and mitochondria (1). It effectively and functionally displaces or substitutes for Ca^{2+} in calmodulin and other receptor proteins, with such interactions possibly representing a fundamental mechanism of Pb toxicity (2,3). Lead forms coordination complexes with sulfur, nitrogen, and oxygen, and it exerts its toxic action through inhibition of sulfhydryl dependent enzymes (4–6). It produces adverse effects in most tissues and organs of the body, particularly on the central nervous system (CNS), the kidneys, involving damage to proximal tubules, and on the hematopoietic system, with parallel effects on metabolic processes. The identification of causal relationships is complicated by the delay between the time of lead exposure and the onset of toxic manifestations.

Children are the principal target population for the subtle adverse effects of chronic low-dose lead exposure, particularly in the early developmental stages of the nervous system. Pregnant women and their fetuses thus are also at high risk (7). Heightened sensitivity of the fetus's CNS to the toxic effects of lead present in the maternal organism has been ascribed to the poorly developed blood-brain barrier. Different mechanisms for Pb^{2+} transport in different cell types, e.g., the adrenal medullary cells (8) or erythrocytes (9), have been reported, but the mechanism by which it crosses the capillary endothelial cell membrane, an important component of the blood–brain barrier, is not known. Two mechanisms of lead's neurotoxic action on cognition and other measures of brain function have been postulated: (a) irreversible impact on the neurodevelopment resulting in changes in the architecture of the nervous system and (b) reversible interference with signal transduction associated with neurotransmitter function (10). In 1991,

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the U.S. Centers for Disease Control and Prevention (CDC) established a maximum safe blood concentration level of 10 μg/dL, the so-called intervention level. This term indicates that, beyond this level, steps must be taken to reduce exposure to prevent impairment of CNS development, ultimately manifest as a variety of memory, attention, and learning deficits (11). In older children and adults lead also affects the cardiovascular system, heme synthesis, and erythropoiesis (12). Diminished hemoglobin synthesis eventually leads to anemia; threshold levels in the blood for this effect in children are 20-25 μg/dL. Impairment of erythropoiesis occurs at approx. 40 μg/dL lead in blood (13). Epidemiological studies, however, show that a true no-effect threshold for toxicity cannot be defined for this metal.

Prenatal exposure to lead correlates with maternal exposure and body burden. Lead is also held responsible for raising blood pressure in pregnant women (14). There is apparently no impediment to lead transfer across the placenta, as measured maternal and fetal blood levels are similar. In the nonhuman primate lead concentrations measured in fetal bone can be higher than in maternal bone (15). Excretion of lead into the milk of lactating mothers has also been reported, further exposing the neonate to the toxicant during a critical and vulnerable period of development (16). Lead is now characterized as a reproductive toxicant. Lead exposure of pregnant women increases rates of spontaneous abortion. In nonpregnant females, lead exposure has been associated with menstrual disorders and infertility. In men, there are effects on sperm that correlate with lead exposure (17).

Lead levels (μg/dL) in whole blood are empirically categorized as: normal $<$40; acceptable 40–80; excessive 80–120, and dangerous $>$120 (18). Lead blood levels that cause the onset of overt toxicity are difficult to assign, however. While 20 μg Pb/dL is considered the no-effect ceiling, i.e., the concentration at which no toxicity, such as neurological impairment, occurs in adults, some individuals can tolerate 120 μg/dL or higher. Blood levels of intoxicated individuals undergoing detoxification can fluctuate substantially without reexposure to the metal, a sign that lead stores in the organism can be mobilized by factors not all of which are presently understood. Deposition and mobilization of bone lead is in dynamic equilibrium and is related to the rate of bone turnover. Such turnover is heightened during pregnancy, and a corresponding increase of lead concentration was measured in the maternal blood of pregnant monkeys (19). The capacity of human erythrocytes to bind lead is limited (20,21), whereas lead partitioning between plasma and red cells depends on the plasma concentration. As plasma lead increases as a function of whole-body lead burden, the concentration of lead in the red cells eventually plateaus. Plasma and whole-blood lead concentrations are not directly correlated; therefore, the situation must be considered carefully when assessing acute lead exposure (22, 23). The correct determination of plasma lead levels is essential to an understanding of the metal's pharmacoki-

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netics. Fortunately, the cynomolgus monkey provides a useful and relevant model for such studies (24).

Induction of renal tumors in rodents by lead acetate or subacetate has satisfied EPA's criteria for carcinogenicity (25). Lead compounds have also been shown to be tumor promoters and cocarcinogens, such that lead now is classified as a probable human carcinogen (26,27).

A low molecular weight lead-binding protein that has a protective function characteristic of metallothionein has been identified in erythrocytes of workers exposed to high lead levels. The higher the erythrocyte concentration of this protein, the more resistant the individual is to overt poisoning (3,28–30) The protein is inducible at a threshold of about 38 μg/dL or less; typical of inducible proteins, the amount present is related to the intensity of exposure.

Historically, lead levels in the environment correlate with human use (31). Analysis of the Greenland ice core showed that Greek and Roman smelting activities in 500 B.C. to 300 A.D. produced lead fallout levels four times the natural background level (0.55 pg/g) (32). This amount is equivalent to approximately 15% of that measured during peak lead emissions due to the use of leaded gasoline.

Lead poisoning in children was first recognized in Australia before the turn of the century (33), and lead-based paints were banned there in 1920; the United States followed suit half a century later. Since the elimination of lead-based fuel additives, termination of the use of lead-based paint, strict regulation of lead in water conduits, and severe controls imposed on permissible levels in food, mean blood levels dropped 78% from 12.8 to 2.8 μg/dL during the periods 1976–1980 to 1988–1991 (34). Contaminated soil, drinking water, and leaded paints in housing predating 1978 are the major remaining sources of Pb that pose a health threat. For occupational exposure, major lead sources are smelting and metal refining, Pb battery recycling, plumbing and pipe fitting, and alkyl lead manufacture. Environmental lead contamination results mostly from combustion of fossil fuels, and from mining and manufacturing which leads to lead release in aerosolized form; lead is also found in surface water and groundwater. The maximum contaminant level for lead in municipal water distribution systems was set at 0.05 mg/L by the EPA in 1985 (35). The current federal action level is 15 ppb, or 0.015 mg/L for air or water, at which point the EPA intervenes to investigate the source of excess pollution.

B. EXPOSURE, ABSORPTION, AND DISPOSITION

Lead absorption in humans was originally thought to occur predominantly through the GI and the respiratory tract; skin absorption measured conventionally

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in vivo and in vitro was extremely low, i.e., the statum corneum appeared to be a very efficient barrier to lead penetration. Now, however, lead absorption is perceived to occur (a) by rapid delivery via respiration or ingestion (and subsequent incorporation in erythrocytes) and (b) by transport across the skin to the extracellular circulation from which the metal is circulated throughout the organism and rapidly excreted. In the latter case, slow, low-level incorporation in erythrocytes occurs.

Lead deposits in the upper portion of the respiratory tract are removed by ciliary clearance, then swallowed and absorbed from the intestine. Particles less than 1 μm in diameter can be absorbed through the lung. About 30–50% of inhaled lead is thus retained by the lungs, almost all of which is absorbed rapidly (36). For nonoccupationally exposed individuals, lead uptake from the GI tract is the main route of absorption: via foods and water in adults; from soil and paints in children. Oral bioavailability is estimated to be 10–15% in adults, 50% in children. Such resorption can be as high as 400 mg/day (37,38)

Lead is first taken up into plasma and then is completely redistributed to the erythrocytes, soft tissues, and bone, where long-term storage occurs. Skeletal stores are a significant source of endogenous lead; approximately 90% of the body burden in adult humans is in bone, continually remobilized by normal turnover (39). Accumulation continues until the sixth or seventh decade of a human life. Lead is released from bone in adults in response to significant reduction in exposure, e.g., a change in environment or job, metabolic stress, dietary changes, or bone-mineral homeostatic changes that occur in old age.

Measurement of blood lead is the only way to unambiguously establish acute or subacute exposure (12) and is the biological marker of choice. However, blood lead concentration as a measure of exposure is less accurate for subjects whose skeletal lead contributes substantially to total blood count. Thus, estimating total body lead burden from blood analysis is complex and requires that all potential sources be considered, as total blood lead is made up of fractional contributions of recent and cumulative exposure. Thus, mobilized bone lead can be an important contributor to the steady-state blood concentration and a skeletal burden can become the dominant source after active exposure ceases.

C. SKIN ABSORPTION AND ELIMINATION

The ability of Pb to form complexes with anionic ligands in proteins appears to prevent trans- or intercellular skin penetration of inorganic lead in significant amounts. Lipid-soluble organolead compounds, however, are readily absorbed, particularly the lead alkyls (fuel additives), lead naphthenates and lead oleates (lubricants). These compounds pose significant hazards in the industrial environ-

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ment. Earlier investigations of skin penetration by lead in vivo and in vitro pursued conventional protocols and relied on blood level data for occupational exposure monitoring. More recent studies, principally conducted in Australia, now point to appendageal uptake through sweat glands and hair follicles, which have been suggested to act as potential diffusion shunts for rapid absorption of polar compounds (40). Thus, the stratum corneum barrier is bypassed and the resulting penetration rates of the heavy metal may represent a substantial toxicological risk that was previously unrecognized (22,23,41). Lead absorbed through the skin appears principally in the extracellular fluid; total lead in urine does not increase and only insignificant amounts are detectable in blood. It is postulated that skin-absorbed lead rapidly transfers from plasma to extracellular fluid, and is found in sweat and saliva within 1–3 hr of exposure. As blood lead is the main biomarker for standard exposure monitoring, skin-absorbed lead may be difficult to detect through blood analysis. Saliva was confirmed as a useful alternative marker for short-term environmental exposure to certain environmental pollutants, lead among them (42). Skin is usually disregarded as a port of entry, and this is a particularly serious risk in the work environment, where exposure and prevention still focuses primarily on inhalation and ingestion.

An established, intentional exposure of human skin to lead is the use of hair-coloring agents based on lead acetate, with Pb levels ranging from 2300 to 6000 μg/g (43). The darkening effect is due to a reaction between lead and sulfur in the scalp hair shafts producing lead sulfide which is black. The percutaneous absorption of the metal from products based on lead acetate (e.g., Grecian Formula, Lady Grecian Formula) has been investigated in humans. When commercial preparations, spiked with ^{203}Pb acetate, were applied to the volunteers' foreheads once a month for 6 months, the conclusion reached was that the quantity absorbed appears insignificant when compared to the average lead absorption estimated from all cumulative sources in the course of a normal lifestyle (44). A separate review of all lead acetate skin penetration data concluded that exposure to the metal from hair-coloring preparations represents approximately 0.5% of the total amount absorbed from the environment and could therefore be deemed toxicologically insignificant (45). It should be said, however, that these assessments, which were based uniquely on blood level data, may (for the reasons stated above) underestimate true exposure and should perhaps be reconsidered, particularly since use of the relatively short-lived ^{203}Pb isotope also renders the validity of these results questionable. A certain urgency in clarifying this issue appears warranted by the fact that lead acetate hair-coloring products are designed for use by the female population, and thus for women of childbearing age.

The depth and pathway of lead acetate penetration were also investigated qualitatively in human skin in vitro, and in rat skin in vivo and in vitro. The presence of lead was determined by microscopic visualization after precipitation of the

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sulfide. Penetration was slow, and exposures longer than 6 hrs were necessary to detect lead beyond the outermost stratum corneum.

The standard protocol of skin tape stripping has been used to examine the penetration of lead salts through human stratum corneum in vivo following single open applications at 0.1% and 1% of the metal as the acetate and perchlorate salts (46). The lead concentration gradient across the stratum corneum decayed toward nondetectable levels (<20 ppb) beyond the fifteenth strip, confirming earlier observations that diffusion of lead through the stratum corneum occurs minimally and only after considerable lag times (44,45,47). However, mass balance indicates, particularly at higher concentrations (1%), that up to 50% of the applied dose is ''missing.'' Such unaccountability has been discussed elsewhere (22).

Abrasion of the skin does not cause deeper penetration, presumably due to the formation of lead–protein complexes. But after 24 hr, lead can be observed in the intercellular spaces, inside keratinocytes, in the mitochondria and nuclei of the epidermis, and on the surface of collagen fibrils. Skin appendages contribute significantly to the penetration of lead, with accumulation seen around the openings of follicles and sweat glands (47).

Application of lead nitrate solution or finely powdered lead metal (100 mesh) to the forearm skin of human volunteers resulted in elevated lead levels in the contralateral arm within 2 hr. These values reached 10 times background after 2 days, even though the lead source had been removed at 24 hr. Similar, rapid increases in lead concentration were measured in serum and saliva, but not in blood or urine, where lead levels remained essentially unchanged throughout the study. Applied in inorganic form, lead can apparently penetrate through the sweat ducts, concentrating in the extracellular fluids through which it is then rapidly eliminated without significant uptake by erythrocytes (22).

Lipid-soluble organolead compounds show relatively rapid absorption compared to lead salts. Lead naphthenate and lead oleate, for example, are well absorbed through human skin in vivo (48,49). The absorption rate of tetraethyllead through rat skin is much greater than that of the acetate, orthoarsenate, or oleate salts. After exposure to lead salts, the amounts of the metal stored in the rat kidney were extremely small; in the case of tetraethyllead, on the other hand, the amount was significant (50) (see Sec. D).

Sweat, hair, nail loss, and desquamation have all been characterized as important modes of lead detoxification. However, the relative contributions from these routes, in relation to total lead elimination, has not been resolved (22,51–55).

Lead levels measured in hair reflect in part systemic absorption from dietary intake and environmental exposure. Lead is incorporated into the hair matrix during growth and is only lost through mechanical removal (56,57). Lead has

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been found in the hair root sheath (having been delivered via radial transcellular transport) and in increasing amounts along the hair shaft, indicating exogenous origin (58). Deposition of lead in bone and hair has been investigated in rats dosed orally with drinking water containing lead acetate. Bone and hair levels increased in a dose-dependent manner during continuous exposure. At 4–10 weeks post cessation of exposure, hair levels had returned to normal, whereas the amount in bone remained significantly elevated (59).

D. QUANTITATIVE ABSORPTION DATA

The subject of scientific investigation since 1922 (60), quantitative data for the percutaneous absorption of lead are thinly spread over three species (humans, rats, and guinea pigs); about 10 chemical forms of lead, including elemental lead, inorganic salts, salts of organic acids and alkyllead compounds, have been studied. While the measures of absorption are often not readily compared, it seems fair to say that the most readily absorbed class, i.e., alkyllead, is absorbed 100–1000 times faster than compounds that are more inorganic in nature.

In vivo exposure of rats to four lead compounds in different vehicles (Table 1) was assessed from the amounts subsequently found in the kidneys (50). The relative accumulation of lead during 24 hr of exposure was lead arsenate $<$ lead oleate $\leq$ lead acetate $\ll$ tetraethyllead. After tetraethyllead exposure, the kidneys contained 60- to 210-fold more lead per gram than that following exposure to the other chemicals; the carcass contained 6.4% of the applied 106 mg lead, and the exposed skin retained 1.6% of the dose (the remainder probably evaporated).

More than 40 years later (61), five similarly diverse lead compounds showed a similar ranking of percutaneous absorption through human skin in vitro: lead oxide $<$ lead acetate $<$ lead naphthenate $<$ lead nuolate (a mixture of oleate and linoleate) $<$ tetrabutyllead (Table 2). Across guinea pig skin, on the other hand, lead nuolate flux was less than that of lead naphthenate and less than one-fifth the flux of the nuolate through the human tissue. In vivo, the same ranking of absorption was also found in guinea pigs.

In humans and other mammals, the percutaneous absorption of lead naphthenate, lead salts of a mixture of cycloalkane carboxylic acids with various alkyl side chains and a lead-based additive in lubricating oil, has been determined (48,62,63) Skin contact with the lubricating oil for an hour caused the lead concentration in the blood to increase rapidly. A crude estimate of the permeability coefficient under these circumstances is of the order 23×10^{-4} cm/hr (Table 3).

In comparing lead naphthenate and lead acetate absorption from different

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Table 1 Lead, as lead acetate, lead orthoarsenate ($Pb_3(AsO_4)_2$), lead oleate, and tetraethyllead

Reference: E. P. Laug and F. M. Kunze, The penetration of lead through the skin, *J. Ind. Hyg. Toxicol. 30:*256–259 (1948).

Species: rat in vivo ✓
in vitro

Area: 29 cm^2

Time: 24 and 48 hr

Vehicle: lead acetate in water (77 mg Pb per rat; no concentration given); lead orthoarsenate as aqueous paste (102 mg Pb per rat); lead oleate in petrolatum (0.8 g ointment containing 148 mg Pb per rat); lead oleate in oleic acid; tetraethyllead was pure (0.1 mL containing 106 mg Pb)

Analytical method: chemical analysis (dithizone method) of kidneys and sometimes other tissues

Notes:

1. Much more lead was absorbed from tetraethyllead than from the other compounds even though analysis of the whole carcass revealed the loss of most, perhaps 90–95%, of the compound by evaporation.
2. For tetraethyllead after 24 hr exposure 6.4% of the dose was recovered in the carcass excluding the exposed area of skin. This allows the calculation of a minimum permeability coefficient as follows:

$$K_p > 6.4\% \times 106 \text{ mg Pb} \div 24 \text{ hr} \div 29 \text{ cm}^2$$
$$\div 106 \text{ mg Pb} \times 0.1 \text{ cm}^3 = 0.09 \times 10^{-4} \text{ cm/hr}.$$

3. The lead content of the carcass was not determined for any of the lead salts; in other words, the total absorption of lead was not determined for those lead sources.
4. On the basis of increased lead in the kidneys, the relative absorption of lead from the four substances as applied, i.e., with different amounts of lead from different vehicles, was as follows: lead arsenate $<$ lead oleate $\leq$ lead acetate $\ll$ tetraethyllead.
5. For lead oleate in petrolatum, increasing the exposure time from 24 hr to 48 hr did not result in a corresponding increase in lead concentration in the analyzed organs.
6. Judged by the content of lead in the kidneys, absorption of lead from lead oleate or lead acetate was about three times greater through scarified skin than through uninjured skin.

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Table 2 Lead, as lead ''naphthanate'' [sic], lead nuolate (mixture of oleate and linoleate), tetrabutyllead, lead acetate, and lead oxide

Reference: W. C. Bress and J. H. Bidanset, Percutaneous in vivo and in vitro absorption of lead, *Vet. Hum. Toxicol. 33:*212-214 (1991).
Species: guinea pig in vivo ✓ human in vivo
in vitro ✓ in vitro ✓
Area: 1.3 cm^2 in vitro; 2 cm^2 in vivo
Time: 24 hr exposure in vitro; 7 days exposure in vivo
Vehicle: in vitro, not specified; in vivo, water for lead acetate and lead oxide, petroleum ether for nuolate, naphthanate, and tetrabutyllead
Analytical method: in vitro—dithizone extraction of saline from the diffusion tube followed by plasma spectroscopy; in vivo—dithizone analysis of blood and plasma spectroscopy of brain, liver, and kidney samples
Notes:

In vitro percutaneous absorption of lead in 24 hr[a]

Applied chemical (10 mg Pb)	Human, 37°C µg	Guinea pig, 37°C µg	Guinea pig, 23°C µg
Tetrabutyllead[b]	632 ± 56	107 ± 5	124 ± 14
Lead nuolate	130 ± 15	26 ± 4	17 ± 1
Lead naphthanate	30 ± 3	42 ± 3	47 ± 2
Lead acetate	5.0 ± 0.9	3 ± 0.2	5.0 ± 0.5
Lead oxide	<1.0	<1.0	<1.0
Control	<1.0	<1.0	<1.0

[a] average of 10 observations ± SEM
[b] Reservoir was covered to prevent evaporation of tetrabutyllead.

1. Ranking of absorption through human skin:

   tetrabutyllead > nuolate > naphthanate > acetate > oxide (≈0).

2. For human skin, average in vitro fluxes were 20, 4.2, 1.0, 0.16, <0.03 µg/cm^2/hr, respectively. However, because concentration/vehicle was not reported, K_p values cannot be calculated.
3. The major differences between human and guinea pig skin permeability were seen as higher fluxes of tetrabutyllead and lead nuolate through human skin.
4. In vivo in guinea pigs, daily occluded applications for 7 days totaled 300 mg Pb/kg. The organ and blood lead concentrations after dermal exposure had the same ranking as in vitro fluxes of the five lead sources; the total absorption was not determined.

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Table 3 Lead, as lead naphthenate

Reference: L. Rasetti, F. Cappellaro, and P. Gaido, Contributo allo studio del saturnismo da olii lubrificanti addittivati [Contribution to the study of saturnism by inhibited oils], *Rass. Med. Ind. Ig. Lav. 30:*71–75 (1961).
Species: human in vivo ✓
in vitro
Area: 64 cm^2 (4 × 8 cm on each forearm)
Time: 1 hr exposure
Vehicle: lubricating oil (6 mL) containing 10% lead naphthenate (192 μg Pb/6 mL)
Analytical method: analysis of blood and urine; the specific method was not reported
Notes:
1. Blood lead concentration rose from 36 mg Pb/100 mL (SD = 15, 3 subjects) to 59 mg Pb/100 mL (SD = 15), i.e., to 176% of the initial value, within 10 minutes after application. The maximum increase in any subject 30–60 min after application was 338%. From that point the concentration fell quickly and returned to the pretreatment level by 8 hr.
2. On the basis of the increase in blood Pb concentration in one subject, 2.5% of the applied Pb was absorbed in an hour. For that result, flux and permeability coefficient can be estimated:

$$\text{Flux} = (2.5\% \times 192\ \mu g) \div (64\ cm^2 \times 1\ hr) = 0.075\ \mu g/cm^2/hr$$

$$K_p = 0.075\ \mu g/cm^2/hr \times 6\ cm^3 \div 192\ \mu g = 23 \times 10^{-4}\ cm/hr.$$

3. The urine analyses were not used to measure absorption but rather to investigate the body chemistry of occupationally exposed subjects.

solvents, rats exposed to five topical applications (Table 4) over 10 days developed increased lead levels in muscle, brain, kidney, and liver but not in the spleen (62). Lead naphthenate absorption led to a higher muscle concentration than lead acetate, whereas uptake of the acetate was greater in the kidney and liver. No measurement of the total absorption was made.

Percutaneous absorption of lead acetate has come under special examination due to its use in hair dyes (44,45,64). Two commercial hair products were spiked with radioactive lead and applied in four different ways to the forehead of eight human subjects. Twelve hours after each exposure, the treated area was washed. Lead absorption in two cases was 0.057% and 0.058% (Table 5). The calculated apparent permeability coefficient was small: $\approx 0.005 \times 10^{-4}$ cm/hr. The lead remaining at the site of application, even after washing, was generally about 1% of the applied dose, much more than that absorbed; the final disposition of this surface associated material is unknown.

The in vivo (in rats) absorption of three lead acetate preparations, one a

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Table 4 Lead, as lead naphthenate and lead acetate

Reference: S. C. Rastogi and J. Clausen, Absorption of lead through the skin, *Toxicology 6:*371–376 (1976).
Species: rats, M & F in vivo ✓
in vitro
Area: 4 cm^2
Time: 10 days
Vehicle: water for lead acetate (0.24 M Pb, 0.5 mL every 2 days); ether/ethanol (1:3) for naphthenate (0.24 M Pb, 0.5 mL every 2 days)
Analytical method: atomic absorption spectrophotometry of brain, liver, kidney, spleen and muscle samples
Notes:
1. Precautions to prevent the possible oral ingestion of topically applied lead were not reported.
2. All rats (six animals per group) were killed 2 days after the fifth dose was administered.
3. Toxicity, aminolevulinic acid dehydratase activity and tissue lead concentrations after percutaneous and subcutaneous doses of both lead sources were compared.
4. No measurement of total absorption was made.
5. Cutaneous doses caused decreases in enzyme activity and also increases in most tissue Pb concentrations, but the changes were not so great as those following subcutaneous administration.
6. As much or more lead was absorbed from the acetate compared to the naphthenate when the absorption is judged by decreased enzyme activity and increased lead in organs. The appearance of the lead exposed area, however, was more affected by naphthenate than by acetate.
7. Two subcutaneous doses produced effects that were not present even after five topical doses. Stratum corneum lead naphthenate caused two deaths, retarded growth, and, in fact, caused a 10% decrease in weight; topical doses had no such effect.

commercial hair dye, each containing about 5 mg of the compound, was in the range 1–6% (Table 6) (64). But even in animals treated three times per week for 4 weeks, the absorption was only 3–5% of the total quantity applied. Assuming a median absorption of 3% of the dose for each application, then 15 μg lead/cm^2 was taken up per application.

Unrecoverable lead from a 24-hr exposure of a human subject to 0.5 M lead nitrate implied that absorption and adsorption together over 4.9 cm^2 totaled 1.3 mg of lead (Table 7) (23). If all of this material is considered to be absorbed, then the corresponding apparent permeability coefficient is 1.3×10^{-4} cm/hr.

Table 5 Lead, as $^{203}Pb(CH_3COO)_2$

Reference: M. R. Moore, P. A. Meredith, W. S. Watson, D. J. Sumner, M. K. Taylor and A. Goldberg, The percutaneous absorption of lead-203 in humans from cosmetic preparations containing lead acetate, as assessed by whole-body counting and other techniques, *Food Cosmet. Toxicol. 18:*399–405 (1980).

Species: human (forehead) in vivo ✓ exposure
in vitro

Area: 8 and 10 cm^2

Time: 12 hr exposure

Vehicle: Commercially available hair-darkening preparations: (a) hydroalcoholic solution (alcoholic strength 6%) of lead acetate containing colloidal sulfur (6 mmol lead acetate/L); (b) a cream containing 9 mmol lead/kg

Analytical method: Gamma counting of ^{203}Pb in whole blood, urine, and ''whole body'' (actually the calves); an intravenous correction factor was used to correct calf count rate to whole-body activity and a second iv correction factor was used to correct blood sample count rates to whole body activity. A correction factor for urinary excretion was also used but the resulting whole-body estimate was judged by the authors as less reliable.

Notes:

1. ''It was found that absorption of lead through the skin was essentially zero, with results ranging between 0 and 0.3% of the dose applied to whole skin.''

	^{203}Pb content at 24 hr (± SD; $N = 8$) % of dose[a] on basis of measurements of following	
Treatment	''Whole body''	Urine
Wet	0.177 ± 0.146	0.0144 ± 0.0120
Cream	0.037 ± 0.026	0.0026 ± 0.0013[b]
Dry	0.142 ± 0.173	0.020 ± 0.023
Dry + scratch	0.257 ± 0.464	0.007 ± 0.004[c]

[a] 0.1 mL of solution or 0.1 g of cream.
[b] $N = 7$.
[c] $N = 4$.

1. There were four treatments: wet (occluded), cream, dry (dried and protected with a permeable cover), and dry + scratch. Scratched skin allowed the greatest percutaneous uptake. The results in all the other treatments were averaged together for the following estimates of absorption.

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Table 5 Continued

2. Each treatment was done in eight subjects; each subject was assigned randomly to the different treatment modes.
3. Applied lead from solution = 6 mmol/L $\times$ 10^{-4} L = 6 $\times$ 10^{-4} mmol = 0.12 mg of Pb.
4. Applied from cream = 9 mmol/kg $\times$ 10^{-4} kg = 9 $\times$ 10^{-4} mmol = 0.19 mg of Pb.
5. After the exposed skin was washed at the end of the treatment, the amount of radioactivity remaining at the site of application was ''generally low—in the region of 1% of the applied dose—but in some cases it rose to as high as 7%.''
6. By measuring the radiation in pooled blood samples taken at several times during the first 12 hr after the application of ^{203}Pb, the investigators claimed that at the midpoint of the period an average of 0.023% of the dose was in the blood. In addition, they asserted that ''in the initial stages of absorption, 50% of the absorbed lead lies in the blood.'' By extrapolation, they estimated from blood samples that the total lead absorbed during 12 hr was the equivalent of 0.057% of the applied dose.
7. Based on a second parameter, i.e., the radioactivity measured in the calf of the leg (representing the absorbed lead content of the whole body) 12 hr after beginning the exposure, the total absorbed lead was calculated to be 0.058% of the applied dose.

Source of data	Absorbed lead (% of dose)	Permeability coefficient, $10^4 \cdot K_p$ (cm/hr)
Blood	0.057	0.005
Whole body (calf)	0.058	0.005

Quantitation of lead absorption has been difficult. For example, absorption of lead does not always produce an increased concentration of lead in blood throughout the absorption period. Use of the stable lead isotope, ^{204}Pb, has allowed another demonstration that inorganic lead salts are absorbed through the skin, although resulting in little change in the lead concentration in blood (23). Lead nitrate (0.4 M) enriched in ^{204}Pb was applied to the arm and occluded for 48 hr. The concentration in whole blood was subsequently unchanged, but the relative abundance of ^{204}Pb was elevated after 6.5 hr and continued to increase through 47 hr. Exposure to enriched lead acetate produced similar trends.

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Table 6 Lead, as $^{210}Pb(NO_3)_2$ (source of ^{210}Pb) with $Pb(CH_3CO_2)_2$

Reference: J. G. Pounds, *Percutaneous Absorption of Lead,* NCTR Technical Report, Experiment No. 199, National Center for Toxicological Research, Jefferson, Arkansas, 1979, pp. 1–44.
Species: rat, M in vivo ✓
in vitro
Area: 10 cm^2, dorsal
Time: 1, 2, 4, and 8 weeks
Vehicle: water, ethanol, Grecian Formula 16 [a commercial product labeled as <1% lead acetate and reported elsewhere to be 0.57% $Pb(CH_3CO_2)_2 \cdot 3H_2O$]
Analytical method: β and γ scintillation counting of tissues, organs, urine, feces, whole carcass
Notes:
1. Each application was 0.5 mL of solution containing 5 mg (or perhaps less in Grecian Formula 16) of lead acetate. Each group contained four animals.
2. After the vehicles had evaporated, the sites of application were protected with an occlusive polyethylene-lined wrap.

Absorption of lead following a single application

Exposure time (weeks)	Absorption[a] % of administered dose		
	Grecian Formula 16	70% Ethanol	Water
1	1.69 ± 0.83	1.51 ± 0.82	2.99 ± 1.87
2	2.75 ± 1.52	3.58 ± 2.38	5.60 ± 2.91

[a] Calculated as (sum of amounts recovered in urine, feces and carcass excluding skin at the application site) × 100 ÷ administered dose.

1. Differences in relative absorption between vehicles were not significant.

Absorption of lead following multiple applications[a]

Exposure time (weeks)	Absorption[b] % of administered doses		
	Grecian Formula 16	70% Ethanol	Water
4	3.28 ± 0.63	3.91 ± 0.84	3.58 ± 0.20
8	4.44 ± 0.56	4.14 ± 0.65	4.27 ± 0.43

[a] Three applications per week for 4 weeks. Sites of application were not washed between treatments.
[b] Calculated as (sum of amounts recovered in urine, feces, and carcass excluding skin at the application site) × 100 ÷ administered dose.

4. Total absorption was also estimated from urinary excretion; the radioactivity found in the urine was divided by the fraction of lead excreted in urine following an intravenous dose. This method gave results similar to the summation of lead in urine, feces, and carcass.
5. Because the solutions evaporated in both sets of experiments, there is no defined concentration and no permeability coefficients can be calculated.

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Table 7 Lead, in some cases ^{204}Pb or ^{210}Pb, as $Pb(NO_3)_2$, $Pb(CH_3COO)_2$, $PbCO_3$, PbO, and elemental Pb

Reference: J. L. Stauber, T. M. Florence, B. L. Gulson and L. S. Dale, Percutaneous absorption of inorganic lead compounds, *Sci. Total Environ. 145:*55–70 (1994).

Species: human (forearm) in vivo ✓
in vitro

Area: 4.9 cm^2

Time: 24 hr

Vehicle: water

Analytical method: anodic stripping voltammetry at a hanging mercury drop electrode (sweat), thermal ionization–mass spectrometry (sweat, urine, serum, blood), inductively coupled plasma–mass spectrometry (sweat, urine), inductively coupled plasma–atomic emission spectrometry (unabsorbed lead recovered from the arm)

Notes:

1. Of 4.4 mg of lead as occluded 0.5 M $Pb(NO_3)_2$ that was applied to one subject, 3.1 mg was recovered from the site of exposure; the remaining 1.3 mg had apparently been absorbed and/or adsorbed during 24 hr. For this situation, an average permeability coefficient can be estimated as follows:

$$K_p = 1.3 \text{ mg} \div 207000 \text{ mg/mol} \div 24 \text{ hr} \div 4.9 \text{ cm}^2 \div 0.4 \text{ mol/L} \times 1000 \text{ cm}^3/\text{L} = 1.3 \times 10^{-4} \text{ cm/hr}.$$

2. ''Preliminary experiments showed that lead as $Pb(NO_3)_2$, $Pb(CH_3COO)_2$, PbO and elemental Pb were absorbed through the skin and detected in sweat on the opposite arm within a few hours. Only lead as $PbCO_3$ was not absorbed through the skin on the forearm.''
3. In experiments involving the application of ^{204}Pb nitrate (0.4 M) or ^{204}Pb acetate (0.4 M), little or no increase in total lead in blood or urine was observed after application of lead to the skin, but changes in the isotopic composition of lead in blood and in urine demonstrated that percutaneous absorption of lead had certainly occurred.
4. Percutaneously absorbed lead can be quickly detected at sites of the body distant from the application site. It is hypothesized that this is possible because the absorbed lead quickly enters the bloodstream and is carried around the body. In contrast to injection of lead, percutaneous lead did not increase the lead concentration in serum in the first 24 hr and increases in blood levels were small.

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E. IMMUNOLOGY

Allergic reactions to lead or its salts are rare (65) and have been noted only as the result of chronic lead intoxication (66). Lead acetate, widely used cosmetically by men to darken hair, is not considered an allergen (67), and only isolated cases of sensitization have been reported (68).

The metal is also immunotoxic, possessing the potential to both enhance and inhibit the immune system, as noted in experimental animals (69). It enhances humoral immunity by stimulating antibody production, and suppresses cell-mediated immunity by blocking formation of macrophages and T cells (the Th1 subset) (70). Thus, subtoxic oral doses of lead salts may either reduce or increase host resistance to bacterial and viral infections (71,72).

F. SUMMARY

Lead compounds are widely distributed throughout the environment and present a constant, potentially toxic exposure to humans through all routes of entry. Historically, environmental levels correlate directly with industrial activities. The lipophilic alkyllead compounds, tetramethyl- and tetraethyllead in particular, can penetrate skin in toxicologically significant amounts, and the ACGIH lists them as occupational hazards in the work environment. Inorganic lead salts, on the other hand, cross the skin much less readily and appear to follow predominantly a transappendageal, shunt pathway. The latter absorption can be manifest by a rapid increase in extracellular lead concentration of as yet undetermined toxicological relevance. Earlier investigations of the bioavailability of lead contained in hair coloring cosmetics appear to underestimate true exposure and should be revisited.

Lead is a toxin that accumulates in the bone tissue, from which it is remobilized in the process of bone turnover and by a number of xenobiotics. Lead compounds can induce genotoxicity, neurotoxicity and developmental toxicity in humans. The greatest health concern is the exposure of infants and young children, as well as fetal exposure to lead. Serious effects on cognitive and neurobehavioral developments have been documented.

Percutaneous absorption of organolead compounds through human skin has been measured, and permeability coefficients of approximately 10^{-3} cm/hr have been deduced. For inorganic lead compounds, permeability coefficients are approximately 10^{-4} cm/hr or less.

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28
Palladium

A. GENERAL COMMENTS AND IMMUNOLOGY

A rare and chemically inert metal similar to platinum, palladium occurs at such low concentrations in alloys (e.g., in jewelry or dental fillings) as to pose no notable toxicological hazard. No acute or systemic effects in humans have been reported, other than the observation of allergic sensitization, which can involve the respiratory system, skin, oral mucosa, and eyes, ascribed to contact with the metal itself. Acute contact dermatistis (ACD) and oral mucosal lesions, such as erosive lichen planus, are the principal clinical expression of palladium sensitivity seen in the general population, acquired mainly from the metal in alloys used in dental prostheses which can contain up to 79% of the metal (1,2). Rare cases of immediate type allergy have been reported (3–8). The prevalence of reactions to dental materials has been on the increase since the introduction of palladium-silver alloys in 1973 as a substitute for other materials containing metals suspected of toxicity or allergenicity, such as mercury and nickel, respectively (9). In Germany, dentists have even been advised to stop using palladium or its alloys because of this trend (10).

Striking increases in hypersensitivity to palladium are not limited to oral exposure and corresponding manifestations. In a cohort of 2300 dermatology patients in Italy, 171 (7.4%) had positive reactions to palladium chloride (1% in petrolatum); notably, 169 also had a concomitant reaction to nickel sulfate. Contamination of palladium patch test material with nickel was excluded by atomic absorption spectrophotometry analysis. None of the reactants had ever worn a metal dental prosthesis or had an occupational history of exposure to palladium. The prevalence of positive patch tests to nickel in that cohort was 22% (11). Confirming such an increase is the sensitization rate; in Austria, palladium allergy is now 8.3% in eczema patients, and is only exceeded by nickel (12).

Sensitization to palladium has also been attributed to long-term occupational exposure, where 24-hr skin reactions to patch testing and leukocyte migra-

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tion inhibition has been recorded (3,13–15). Immediate-type allergy to palladium salts was diagnosed in industry, based on short-term (20 min) reaction to intracutaneous test and passive intracutaneous anaphylaxis (4,5,8). Palladium salts have higher sensitization potential than elemental Pd; however, the high corrosion resistance of Pd reduces the likelihood of solubilizing trace amounts of metal on skin contact, and thus exposure to Pd salts.

In simultaneous tests, dermatology patients tested positive to both $PdCl_2$ and $NiSO_4$, but none to discs of palladium metal itself (16). The same authors reported three cases of positive reactions to metallic palladium discs, but negative to palladium chloride or nickel sulfate. Similar results were obtained in a prevalence study of allergic patch test reactions to palladium chloride compared to nickel sulfate. Among a nondermatological population of Finnish schoolchildren who had received orthodontic treatment involving metallic materials, most patients with an allergic patch test reaction to palladium also reacted to nickel. Here again, only 3 of the total 700 subjects reacted to palladium chloride only (17).

Because exposure to palladium salts is rare in the general population, the majority of $PdCl_2$-positive reactions may be due to either (a) cross-reaction between Pd and Ni in nickel-sensitive individuals due to the proximity of the two metals in the periodic system of elements, (b) false positives due to low-level contamination of the palladium test material with nickel [most likely based on results of Cd test material analyzed for nickel (18)], or (c) concomitant sensitization to both metals (16,19).

In a comparative open epicutaneous test for allergenic potential on albino guinea pigs, palladium chloride was a stronger allergen than indium or vanadium. All animals tested positive at maximal nonirritating concentrations of $PdCl_2$. Furthermore, at the height of the skin reaction, blood analysis showed a statistical increase in B-lymphocyte concentration, and a doubling in the size of the local lymph node (20). Also in the guinea pig, palladium chloride has been proven to be a more potent sensitizer than nickel sulfate (21). Guinea pigs sensitized to palladium by the maximization test showed cross reactivity to nickel (22), consistent with the concurrent sensitization to both metals observed in humans (11,17,23–26). This evidence supports the theory of cross-reactivity between related metals, in this case the group VIII metals in the periodic system of elements.

B. SUMMARY

No adverse systemic effects have been reported as a result of occupational or environmental exposure to palladium. However, increased use of the metal in industry as a catalyst, in jewelry, and in dental prostheses, has resulted in an increased incidence of palladium sensitization which, until recently, was rare. Animal tests confirm the contact-allergenic potential of palladium salts. Most

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recently, evidence has confirmed that Pd and Ni cross-react. Whether Pd can act as a primary sensitizer in humans, has not been established.

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Platinum

A. GENERAL COMMENTS

Platinum and the platinum group metals (Ru, Os, Rh, Ir, and Pd) have a strong binding capacity for the electron donor groups in amino acids and have the ability to form chelates. This behavior determines their inactivating effect on enzymes and cell division and, in turn, makes them potential antibacterial and chemotherapeutic agents, as well as immunosuppressants (1–3). Platinum and its complexes stand out especially among platinum group metals for their activity in biological systems. From the perspective of occupational medicine, platinum salts represent a serious hazard, causing asthma, rhinitis, urticaria, and dermatitis. Platinum coordination complexes, a relatively new class of drugs with significant antitumor activity, appear to selectively interact with cellular DNA. Specifically, such activity is found in the neutral platinum complexes, and in the cis, e.g., cisplatin, or *cis*-$Pt(NH_3)_2Cl_2$, rather than trans isomers. Cisplatin binds to DNA by forming intrastrand crosslinks in which the two chlorine atoms on cisplatin are replaced by nitrogen atoms of adjacent guanine residues on the same DNA strand. As are most other anticancer drugs, cisplatin has also shown to be mutagenic and moderately carcinogenic in animals (4). Also pronounced nephrotoxicity and neurotoxicity side effects in humans led to the development of second-generation drugs; when malonate derivatives (carboplatin) replaced the original chloride (cisplatin), toxic side effects were significantly reduced, while the antitumor activity was retained (5). A different spectrum of antitumor activity was recently observed with bis(platinum) complexes (homodinuclear Pt,Pt compounds), which proved effective in the treatment of cancer cells against which cisplatin is ineffective (6,7). This widening spectrum of antiproliferative effects has placed platinum compounds among the most active and widely used clinical agents for the treatment of advanced cancer.

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B. SKIN ABSORPTION AND REACTIVITY

Platinum chelates also find clinical use in bacterial infections. Because these complexes exhibit intense neuromuscular toxicity (curariform activity) when given orally, however, they can be used only topically. Platinum chelate complexes are active primarily against gram-positive microorganisms, and in clinical tests they proved effective in the treatment of various skin and nail afflictions caused by microorganisms (1).

Because the skin can act as a slow release depot for a number of platinum complexes, transdermal delivery of antitumor cisplatin complexes has been adopted in a number of conditions. When administered orally, cisplatin causes GI irritancy, kidney, stomach, and lymphoid/thymus toxicity; and weight loss. It is well tolerated on intact skin, however, and transdermally, higher doses can be administered without adverse effects (8).

Application of ammonium chloroplatinate, $(NH_4)_2PtCl_6$, on guinea pig skin in vivo (187 mg/cm^2 over 3 days) resulted in reduced weight gain and reduced oxygen consumption. On dissection, all internal organs and the blood showed the presence of platinum (9). On hairless mouse skin in vitro, carboplatin was shown to penetrate in pharmacologically significant amounts. Applied on mouse skin in vivo, platinum complexes exhibit an antimitotic effect and therefore have a therapeutic potential against hyperproliferative skin diseases such as psoriasis (10).

C. QUANTITATIVE ABSORPTION DATA

Carboplatin is the only platinum compound for which there is any published percutaneous absorption data (10). Applied to hairless mouse skin for 6 hr, carboplatin (530 μg Pt/mL) from three aqueous vehicles with surfactant led to total amounts of 0.5–1.4 mg Pt in the receptor solutions (Table 1). With no exposed area reported, the magnitude of the permeability coefficient can be estimated for an assigned area of 1 cm^2 to be about 10^{-4} cm/hr.

D. IMMUNOLOGY

In recent years, platinum salts have attracted great interest due to their antitumoral activity. At the same time, contact with these compounds was seen to provoke contact allergy as well as anaphylactoid reactions. Although allergic reactions to platinum jewelry are rare, long-term contact with the metal has occasionally re-

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Table 1 Platinum, as carboplatin (*cis*-diammine-{1,1-cyclobutane-dicarboxylato}platinum)

Reference: J. Featherstone, P. J. Dykes, and R. Marks, The effect of platinum complexes on human skin cells in vivo and in vitro, *Skin Pharmacol. 4:*169–174 (1991).
Species: hairless mouse in vivo
in vitro ✓
Area: not reported
Vehicle: A. saline/DMSO/Nonidet P40, 58:40:2
B. saline/propane-1,2-diol/Triton, 88:10:2
C. saline/Triton, 98:2
Concentration: 0.1% carboplatin (0.53 g Pt/L)
Duration of exposure: 6 hr
Analytical method: atomic absorption spectroscopy
Notes:

	Total platinum penetrated, μg[a]		
Time (hr)	Vehicle A	Vehicle B	Vehicle C
1	0.01	—	0.18
1.5	—	0.34	—
2	0.1	0.45	0.27
3	0.26	0.59	0.35
4	0.7	0.67	0.40
5	1.22	0.76	0.47
6	1.4	0.82	0.53

[a] Estimated from the published graph.

1. Although platinum concentration is reported and the platinum penetration is also reported, there is no mention of the area of skin exposed in the diffusion chamber. Therefore, neither flux nor K_p can, be calculated.
2. By assigning a value of 1 cm^2 to the exposed area, we can estimate the magnitudes of flux and K_p. Then, from vehicle B for the last 4 hr of the experiment, the estimated average penetration rate is 0.092 μg/hr. So then the average flux would be 0.092 μg Pt/hr/cm^2, and $K_p = 0.092\ \mu g/hr/cm^2/(0.53 \times 10^6\ \mu g/1000\ cm^3) = 1.7 \times 10^{-4}$ cm/hr. From vehicle C during the same period if the exposed area is 1 cm^2, then $K_p = 1.2 \times 10^{-4}$ cm/hr.

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sulted in contact hypersensitivity, probably due to dissolution of trace amounts of platinum by skin fatty acids (11). This potential of platinum salts to induce cell-mediated hypersensitivity was confirmed by the murine ear swelling test, and induction of contact sensitivity by Pt salts was demonstrated in the animal model also (12).

Occupational exposure to soluble Pt salts in metal refining, on the other hand, can cause type I respiratory as well as cutaneous hypersensitivity, which proves to be a relatively common and serious health problem, occurring in occupational exposure as well as in the therapeutic application of platinum salts. Sensitization expresses as immunological contact urticaria, rhinoconjunctivitis, bronchial asthma, and anaphylactic reactions. Specifically, ammonium tetrachloroplatinate and hexachloroplatinate salts were demonstrated as the haptens responsible; confirmation was also seen through identification of platinum-specific antibody in sensitized patients (13–18).

Platinum forms stable chelation complexes with many organic compounds, including proteins, and perhaps due to that property, certain water-soluble, complex salts of platinum, as they are manufactured and widely used in industry, can cause allergy in humans (19,20). Prolonged dermal or inhalation exposure to complex platinum salts, such as tetra- and hexachloroplatinates, can result in severe and disabling conditions termed collectively as ''platinosis'' or platinum salt sensitivity (PSS) (14,21–23). A definite association between PSS and smoking was documented in a study estimating risk as eight times greater in smokers than in nonsmokers (24). Allergic symptoms were seen to recur upon subsequent exposure to platinum, independent of the time interval between exposures (21). Diagnostic scratch or intradermal testing, standard procedures for confirmation of allergy, can be life-threatening to sensitized individuals; test concentrations of 1 mg/mL K_2PtCl_6 have caused anaphylactic reactions (13). Thus, for diagnosis of platinum hypersensitivity, the least invasive procedure, the skin prick test is recommended. With a typical delivery of 3×10^{-15} g of platinum compound, it is sufficient to elicit a positive reaction in a sensitized individual (25). A number of workers with type I hypersensitivity to platinum also reacted positively to palladium salts, although the converse was not observed. Nevertheless, this suggests that cross reactivity occurs to both platinum and palladium salts in some individuals (26).

Different valences of platinum elicit specific responses; thus, hexachloroplatinate sensitivity was demonstrated ot be different histochemically from tetrachloroplatinate sensitivity (22).

Not all platinum compounds are equally potent allergens. Neutral complexes, such as $(Pt(NH_3)_4)C1_2$ or $K_2(Pt(NO_2)_4)$, containing strongly bound ligands are immunologically inactive, presumably because little or no interaction occurs with endogenous proteins (15). However, ionic complexes containing reactive halogen ligands, are highly immunogenic.

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E. SUMMARY

Platinum coordination complexes are antibacterial and chemotherapeutic agents, as well as immunosuppressants. Whereas elemental platinum is among the least reactive of metals, ionic platinum compounds belong to the most immunogenic agents known, being capable of causing both type I and type IV sensitization upon all types of exposure, including dermal. The affinity of ionic platinum for electron-donating groups in amino acids favors the formation of chelation complexes that are potent complete antigens. Occupational sensitization to platinum, most often through inhalation, causes both anaphylactoid and delayed-type reactions of the skin and respiratory system. Sensitized individuals do not become asymptomatic again in a platinum-containing environment. Cisplatin, carboplatin and bis(platinum) complexes show antitumor activity and are rapidly becoming widely used chemotherapeutic agents, particularly in the treatment of solid tumors.

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30
Antimony

A. GENERAL COMMENTS

Antimony has no known function in living organisms. In its trivalent and pentavalent state it has long been used for therapeutic purposes as an antibacterial, emetic, and expectorant, as well as for cosmetic purposes. Antimony preparations are poorly absorbed from the GI tract; they cause irritation of the intestinal mucosa, however, and are preferentially given parenterally. In current medical practice, pentavalent antimony is still used to treat leishmaniasis (see also Sec. B). Trivalent antimony was given intramuscularly as the potassium tartrate or dimercaptosuccinate to treat schistosomiasis, a helminthic infection, but in most recent practice it is no longer recommended because of unacceptable toxicity (1,2). More reactive with sulfhydryl groups than the pentavalent Sb compounds, the trivalent antimonials have a high cellular affinity and can inhibit enzymes such as succinic oxidase and pyruvate oxidase, and thereby interfere with cellular respiratory mechanisms. Due to this reactivity with tissue components, the trivalent antimonials are retained in the organism significantly longer than the pentavalent antimonials (3).

In the industrial setting, a major hazard is the inhalation of antimony fumes and its volatile compounds, e.g., antimony oxide or the hydride stibine.

B. SKIN ABSORPTION AND REACTIVITY

Antimony compounds are irritating to the skin, with lesions occurring in the area surrounding the ostium (opening) of the sweat ducts, the site of antimony penetration sufficient to cause tissue reaction, as demonstrated by histology of affected skin. There was no evidence of eczematous reaction or immunological involvement of the skin tissue (4).

Irritant dermatitis with the formation of characteristic ''antimony spots'' may occur as a consequence of long-term occupational exposure, particularly to

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Sb_2O_3, under humid environmental conditions or where heavy sweating occurs. Pentavalent antimony preparations are used in modern medicine, given parenterally as the sodium antimony gluconate to treat leishmaniasis, a protozoan infection characterized by widespread skin lesions resembling those of lepromatous leprosy (5).

C. IMMUNOLOGY

The immunological nature of rare cases of contact dermatitis due to antimony compounds has not been confirmed (6,7). Patch testing of workers with dermatitis was negative when antimony trioxide was applied as a dry powder, as a suspension in water, or as a 50% mixture in paraffin (4). Injection of antimonials for therapeutic purposes, however, has resulted in anaphylactoid response, characterized by urticarial rash, and fatal hemolytic anemia has also been reported (8–10).

D. SUMMARY

Environmental exposure to antimony poses no significant toxicological risk. In modern medicine, preparations of pentavalent antimony are used in the treatment of parasitic infections. In industry, particularly in mining, the principal hazard posed by antimony and its compounds is accidental ingestion, inhalation, and skin absorption that can attain toxic levels, with the latter frequently resulting in irritant dermatitis. No quantitative data on skin absorption of any antimony compound is available.

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31
Selenium

A. GENERAL COMMENTS

Selenium is an essential trace element, chemically resembling sulfur, with metal and nonmetal characteristics (1). Toxicologically, high levels of selenium in the mammalian organism cause GI damage and loss of hair and nails, and become carcinogenic, embryotoxic, and teratogenic (2–5). On the other hand, selenium deficiency in the mammalian organism as a result of low levels present in the environment is associated with various diseases in animals and an increased risk of cancer in humans (6). An epidemiological study involving 27 countries indicated significant inverse correlations between relatively low-level Se dietary intake and the increased incidence of leukemia and other forms of cancer, notably of the stomach, pancreas, intestine, breast, prostate, skin, and lung (7–9). Selenium deficiency may also be associated with other deleterious effects, such as hemolytic anemia (10).

Anticarcinogenic properties of selenium in both animals and humans have been well documented through epidemiological studies and animal models (1,11,12). Particularly the regulation of oxidative damage by selenium is recognized to be of significant physiological importance. Glutathione peroxidase (GSH-Px) is a selenium-dependent enzyme that catalyzes the destruction of lipid peroxides (13) in a mechanism similar to that of vitamin E (14,15). This peroxidase keeps levels of hydrogen peroxide low and inhibits lipid hydroperoxides that are likely to form in the cell. SeGSH-Px, in association with superoxide dismutase and catalase, thus composes part of the cell enzymatic system preventing formation of free radicals. Other than the antioxidant function of selenium, little is known of its mechanism of beneficial action. L-Selenomethionine is now being evaluated as a potential topical cancer-preventing drug. Applied topically on the skin to attenuate potential actinic radiation effects, it has the potential advantage that it does not act simply as a sun block but as a free radical quencher in the skin due to its role as a cofactor in the SeGSH-PX enzyme system (16).

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When present in the organism along with mercury, selenium attenuates the toxicity of several mercury compounds. It appears that selenium diverts mercury from critical tissues to less critical ones through disruption of established mercury–protein bonds (17). This metalloid thus also appears to react with mercury in the bloodstream, forming a highly stable complex with a specific plasma protein. Selenium thus appears to play a vital role in the detoxification of certain heavy metals, as such equimolar complexes have been observed in human as well as animal tissues (e.g., kidney) (18–21).

In humans, the major intake of selenium is nutritional; highest concentrations are found in seafood, animal organs, and grains (4). Levels attained from normal exposure are homeostatically controlled, with excess amounts being rapidly excreted. Like arsenic, selenium accumulates preferentially in the hair. In the form of selenate, selenium competes with sulfur such that it can inhibit certain sulfhydryl enzymes. Adverse effects from selenium exposure have been sometimes traced to inhalation of flue gases from fossil fuel combustion. The chemical forms absorbed in this way are the water-soluble selenium oxide and selenious acid (H_2SeO_3), and the oil-soluble, elemental form of the metal itself (22).

Selenium is easily absorbed from soil by plants, and soil concentrations of more than 2 ppm are considered hazardous to grazing livestock (23). Ease of translocation of the element into specific plants characterized as hyperaccumulator phenotypes is increasingly used for phytoremediation of soils that are heavily contaminated by toxic metals: mercury, cadmium, and selenium. Particularly selenium is found in unnaturally high levels in low-lying agricultural areas due to irrigation drainage. Selenium can also be accumulated by plant roots and converted to a volatile species such as dimethylselenide (phytovolatilization) (24,25).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Selenious acid (H_2SeO_3) was absorbed transdermally in appreciable amounts when applied to rat skin in vivo: cutaneous absorption was 9–27% as measured over a 9-day period (26).

While selenium metal poses no hazardous threat via skin contact, the volatile SeO_2 can combine with water, sweat, or tears to form selenious acid, a severe irritant. Selenium oxychloride, used in chemical synthesis as a chlorinating agent, is a vesicant upon contact with human skin (27).

Accumulation and retention of selenium are regulated by homeostasis. The daily intake of selenium from a standard diet is calculated to be 62 mg; 50% of this amount is excreted in the sweat, and the balance is excreted in urine, feces, and expired air (4). Adverse cutaneous effects, due to elevated systemic levels of the element, have been noted in particular geographic areas, e.g., South Dakota

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(28), Venezuela (29), China (30), where chronically high levels of selenium in soil and water result in high dietary intake. The dermatotoxic pathologies attributed to selenium exposure observed in human skin include icteroid discoloration, chronic dermatitis, changes in nail structure, and partial or total loss of hair (31).

The apparent effective management of inflammatory skin diseases, such as psoriasis and atopic dermatitis, by balneotherapy using spa waters high in selenium prompted the investigation of possible modulatory effects of selenium on the production of cutaneous inflammatory cytokines (32). Effects on the induction in keratinocytes of three cytokines, IL-1α, IL-6 (IL = interleukin), and TNF-α (TNF = tumor necrosis factor), have been monitored in culture. The addition of selenium (or strontium) salts to both normal and inflammatory reconstituted human skin had a significant inhibitory effect, particularly on the induction of IL-6, reflected at both the intra- and extracellular levels. This confirms earlier observations made in animals showing that a deficit of selenium salts is associated with an increased inflammatory reaction. The latter disappears following selenium supplementation of the diet (33).

The role of selenium has been investigated in the occurrence or prevention of skin cancer. Patients with various forms of cancers were found to have a significantly lower mean plasma selenium concentration than controls (9). Also, serum selenium levels associated with two types of skin tumors were monitored longitudinally; for both melanomas and lymphomas, selenium concentrations in serum decreased with progression of the disease (34).

L-Selenomethionine was used effectively in humans to decrease the acute inflammation due to UV irradiation in a dose-dependent manner, using a solar UV simulator. While application of the methionine analog on rodent skin demonstrated percutaneous absorption of selenium, such application on the skin of human volunteers did not result in detectable increases of blood plasma levels of the metalloid (16).

C. QUANTITATIVE ABSORPTION DATA

Rats, during a 1-hr exposure to 0.1 M aqueous sodium selenite, absorbed selenium through the intact skin of the tail at a rate described by an average permeability coefficient of 8.8×10^{-4} cm/hr (Table 1) (35). The absorbed selenium left the body very slowly; 10 days after the exposure 53% remained. Much of the absorbed selenium was in the skin at the site of exposure for days after the exposure: 73% after 1 day and 37% after 10 days. Except for the tail skin, no analyzed single tissue ever contained more than 4% of the absorbed selenium; at least 10 times as much selenium was in that skin as in any other single tissue.

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Table 1 Selenium, as $Na_2{}^{75}SeO_3$

Reference: T. Dutkiewicz, B. Dutkiewicz, and I. Balcerska, [Dynamics of organ and tissue distribution of selenium after intragastric and dermal administration of sodium selenite], *Bromatol. Chem. Toksykol. 4*:475–481 (1971).
Species: rat, F, 150–180 g in vivo ✓
in vitro
Area: 10 cm^2, tail (T. Dutkiewicz, personal communication)
Vehicle: 0.1 M aqueous solution
Duration of exposure: 1 hr
Analytical method: scintillation counting of radioactivity in tissues, organs, and excreta
Notes:
1. Percutaneous absorption, i.e., ^{75}Se content, was measured in 18 animals (6 groups of 3) analyzed at various times after exposure.

Chemical form	Flux (μg Se/cm^2/hr)	Permeability coeff. $10^4 \cdot K_p$ (cm/hr)
Sodium selenite	7.0[a]	8.8

[a](T. Dutkiewicz, personal communication)
2. Ten days after exposure of the tail to selenite solution, 37% of the absorbed ^{75}Se was still in the skin of the tail and 15% of the absorbed amount was elsewhere in the body.

D. IMMUNOLOGY

In industrial processes where selenium is employed at elevated temperatures, this element readily oxidizes to form selenium dioxide, which is a confirmed airborne sensitizer. Thus, SeO_2 has been ranked comparably with nickel and chromium compounds for allergenic potency. In the industrial hygiene literature, selenium is reported to cause both a generalized urticarial-type body rash and delayed-type contact dermatitis, with the recommendation that workers thus affected be permanently removed from potential sources of reexposure (36–39).

Selenium can act as a prophylactic against dermal reaction to mercury. Dietary selenium supplements taken in tandem with cutaneous application of mercurials eliminated the allergic contact dermatitis reactions normally associated with mercury preparations (15). Taken during low-level exposure to mercury vapor, dietary selenium ameliorated immediate-type hypersensitivity. Selenium appears to compete with mercury for protein binding sites, thus altering the nature of that allergen.

Selenium disulfide is used extensively in medicated shampoos for the treatment of seborrheic dermatitis and tinea versicolor. Several investigations of these

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products and their efficacy have revealed no damaging effects (40–43) and allergic reactions to such preparations are rare (27).

E. SUMMARY

Selenium, an essential trace element, has an ambivalent nature. It protects against mercury and methylmercury toxicity by preventing free radical damage or by the formation of inactive selenium–mercury complexes. At low levels, selenium acts as an anticarcinogen, but selenium deficiency can also reesult in a number of disease states. Natural sources of elevated selenium salt concentrations in local water supplies have caused widespread adverse skin reactions. Beyond a threshold level, selenium is potentially carcinogenic, embryotoxic, and teratogenic. Due to ease of its translocation into plants, soils with high levels of selenium can be detoxified by phytoremediation. In the industrial environment, certain selenium compounds have caused generalized urticaria as well as delayed-type contact dermatitis. No data are available regarding the rate of skin absorption of any selenium compound in humans.

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32
Tin

A. GENERAL COMMENTS

Traditionally, tin was considered a relatively nontoxic element; the main route of exposure to the metal is through nutritional intake, although even by that route it is absorbed minimally (1). The levels found in vegetables and meats are less than 0.3 ppm and 3 ppm, respectively, and only in canned preserves do they reach several hundred ppm (2). Recently, however, evidence of toxic effects associated with elevated levels of tin have been discovered. Hypocalcemia, which affects hemoglobin metabolism and biosynthesis, can be induced by exposure to tin (3). By apparently interfering with iron and copper, elevated tin levels can also result in the development of anemia (2).

Organotin compounds, on the other hand, represent a serious toxicological hazard. They are of anthropogenic origin apart from methyltins, which may be produced by environmental biomethylation also (4). Organotins are inhibitors of energy production in cells, interfering with mitochondrial oxidative phosphorylation in a variety of cells. They also appear responsible for the apoptosis of thymocytes, resulting in immunotoxicity. The trisubstituted organotin molecule coordinates with cysteine and histidine, with corresponding impact on intracellular proteins (5). Evidence is also provided by recent studies that Tributyltin (TBT) interferes with normal steroid metabolism, i.e., the aromatization of androgens to estrogens. It thus leads to an increase in testosterone by inhibiting its conversion to 17β-estradiol (6–9).

Since the discovery of the biocidal properties of organotins in the 1930s, their industrial production and use have grown considerably, and their widespread dissipation throughout the environment due to their direct introduction into soil and water has become a hazard that all industrialized countries are attempting to contain through severe limitation or outright interdiction of use. Their agricultural and marine uses, the latter as biocides in paints, give rise to the largest proportion of free organotins in the environment. Tributyltin in particular gained widespread application as a marine antifouling paint biocide, due to its leaching

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it rapidly accumulated in aquatic organisms, becoming a general hazard to aquatic ecosystems (10). Since the U.S. government restricted the use of organotins in paints in 1988, average TBT concentration in coastal and harbor waters declined from an average 470 ng/L to 50 ng/L (5,11).

General toxicity of organotins increases with progressive alkylation, with a maximum for trialkylated compounds and decreasing toxicity with increasing length of the organic moiety (12,13).

B. SKIN ABSORPTION AND REACTIVITY

No adverse effects have been reported from skin contact with tin or inorganic salts of tin, and their skin absorption appears insignificant. However, certain therapeutic organotin compounds can readily penetrate the skin and cause irritation and systemic effects (14).

Application of a homologous series of organotin compounds—the dimethyl, diethyl, and diisopropyl dichlorides—to rat skin resulted in necrotic effects, decreasing in severity with molecular size of compound. The dipentyl homolog induced no lesions (15,16).

Tributyltin is one of several organotin compounds used as biocides in wood preservation, marine antifouling paints, and slime control in cooling towers and paper mills. Exposure of workers occurs mainly during the manufacture and formulation of TBT compounds, in application and removal of TBT-containing paints, and during its use in wood preservatives. Exposure can result in skin irritation and severe dermatitis (13,17).

Due to their potential for skin penetration, the ACGIH warns against dermal exposure to organotin compounds used as biocides in crop protection and antifouling agents, as they represent an obvious occupational risk; see Appendix for comments on ''skin'' notation (18). Triphenyltin derivatives are also severe irritants, but do not have any such effect when tested on rabbit skin, through which they appear to penetrate more slowly than their trialkyl counterparts (19).

Used topically to treat staphylococcal infection, acne, and other skin conditions, diethyltin diiodide has resulted in a number of fatalities when contaminated with triethyltin (20). Similarly, dermal application of triphenyltin and triethyltin for therapeutic purposes has resulted in adverse systemic effects (19).

C. IMMUNOLOGY

Although the incidence of tin hypersensitivity is rare, the metal or its salts can induce both immediate and delayed immune reactions, as seen in humans and demonstrated in animal experiments.

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The metal or its salts are weak contact sensitizers, as confirmed by human patch tests using highly purified metallic tin discs (21) and $SnCl_2$ (22).

Significant accidental exposure of the arms to triphenyltin acetate in the work place resulted in long-term elevated tin levels in plasma and equally protracted generalized urticaria accompanied by elevated circulating IgE levels (23). Several reports also document contact dermatitis due to occupational exposure, as well as due to the presence of the metal in prosthetic materials (21,22,24,25). In a cohort of 118 patients with oral lichenoid lesions patch-tested for possible allergy to metals used in dental filling materials, three patients reacted to stannic chloride (2.5% in petrolatum). The one patient who agreed to a total replacement of the fillings healed completely (26).

Attempts to sensitize guinea pigs to triphenyltin hydroxide, a representative organotin compound, were unsuccessful (14). Compounds such as di-*n*-octyl- and di-*n*-butyl-tin dichloride have marked modulating effects on cell-mediated and humoral immunity, as observed in vitro and in vivo in rats (27).

When used in the treatment of skin conditions such as staphylococcal infections, dialkyltin compounds can have immunotoxic effects. They seem to be related to the interaction of tin with sulfhydryl groups, resulting in a modification of the plasma membrane and, ultimately, a proliferation of lymphocytes (28).

D. SUMMARY

Environmental exposure to tin and tin salts poses no significant hazard to the general population, although a potential for human sensitization has been recognized. The accumulation of the metal or its salts in the organism is precluded by poor solubility and minimal absorption from the respiratory and GI tracts. Organotin compounds, however, appear to be absorbed through the skin of humans and animals, resulting in tissue irritation and systemic toxicity. Their widespread use as biocides and release into the environment represents a serious ecotoxicological hazard, particularly to aquatic organisms. Cutaneous absorption of organotin compounds has been recognized by the ACGIH as presenting a significant risk of systemic toxicity, and particular care is recommended to avoid direct skin contact in the work environment. No quantitative data are available on the rate of skin absorption of any tin compounds.

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Strontium

A. GENERAL COMMENTS

Strontium salts are virtually nontoxic orally, and no reports of adverse effects from industrial use of Sr are available. Acute inhalation toxicity for strontium has been observed in experimental animals, causing respiratory failure. In biological systems an interdependence between Sr^{2+} and Ca^{2+} exists because the two ions are similarly metabolized (1,2). In fact, strontium metabolism is not directly regulated by levels of the element in the body but is determined by homeostatically controlled calcium levels. There is evidence that Sr is essential for the growth of animals and especially for the calcification of bones and teeth (3). A functional association of strontium with calcium was observed to occur in cell differentiation processes: the presence of strontium-induced terminal differentiation of cultured mouse keratinocytes in low-calcium growth media; however, higher doses of calcium were required to produce the same effects when strontium was absent in the media (4). While chemically strontium is virtually nontoxic, it is nevertheless of major toxicological interest due to the abundance of its radionuclides, primarily of ^{90}Sr, introduced into the biosphere as a result of nuclear detonations and other uses of nuclear energy (5). Its radioactive isotopes released into the environment become incorporated into the food chain and hence accumulate in the body tissues of humans. Adding to the hazard is long-term retention in skeletal tissues of ^{90}Sr, where it is particularly resistant to removal by chelation. The nuclide has a biological half-life (the retention half-time corrected for physical decay) of 28 years, and lodged in bone it destroys nearby capillaries, decreasing local blood flow, and also can result in an increased incidence of neoplasia due to radiation. Among the most frequently observed neoplasms is epidermoid carcinoma (6). Metabolic studies have been conducted in great detail on a number of animal species to clarify the potential adverse effects of food chain contamination with strontium nuclides (7).

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B. SKIN REACTIVITY AND ELIMINATION

The apparent effective amelioration of inflammatory skin diseases such as psoriasis and atopic dermatitis by balneotherapy, using spa waters high in strontium (and selenium), prompted the investigation of possible modulatory effects of strontium on the production of cutaneous inflammatory cytokines (8). Effects on the induction of three cytokines in human keratinocytes—namely, IL-1α, IL-6, and TNF-α—were monitored in culture medium. The addition of strontium salts to both normal and inflammatory reconstituted skin in the medium had an inhibi-

Table 1 Strontium, as $^{89}SrCl_2$

Reference: J. E. Wahlberg, Percutaneous absorption of radioactive strontium chloride Sr 89 ($^{89}SrCl_2$), *Arch. Dermatol. 97:*336–339 (1968).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 1 mL distilled water
Duration of exposure: 5 hr
Concentration: 0.00013 M, 0.008 M, 0.08 M, 0.239 M, 0.398 M, 0.753 M
Analytical method: decrease of Bremsstrahlung radiation from the donor chamber detected by Geiger-Müller counter
Notes:
1. At the time of application, the pH values of the solutions were between 6.3 for the lowest concentration and 5.5 for the highest concentration.
2. The weight of strontium that was absorbed increased approximately in proportion to the increase in the applied concentration except perhaps at the two highest concentrations, 0.398 and 0.752 M. This proportionality was also true for sodium (NaCl) but not for some other metal compounds, e.g., $HgCl_2$ and $CrCl_3$.

Conc. (M)	N	Disappearance constant $k \cdot 10^5$ min^{-1} ± SE	Permeability coefficient for Sr^{2+}, $10^4 \cdot K_p$ (cm/hr)[a]
0.00013	10	(4.6 − 6.0)[b]	(9 − 11)
0.008	10	8.8 ± 0.9	17
0.08	10	10.4 ± 1.4	20
0.08	10	9.7 ± 1.0	18
0.239	10	8.4 ± 1.0	16
0.398	10	8.7 ± 0.7	17
0.753	10	(3.1 − 4.8)[b]	(6 − 9)

[a] See Chap. 3 for the method of calculation.
[b] Some values were below the limit of detection.

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tory effect on TNF-α production, but most pronounced was the decreased production of IL-6, both on the intra- and extracellular levels.

Strontium is among the 30 odd metals found to be incorporated in hair. Longitudinal monitoring of scalp hair of young children up to 12–14 years of age shows an age-dependent, gradual increase in Sr levels. Such age dependence in the excretion of this structurally important metal is explained by the changing requirements during skeletal growth and development in children and adolescents (9).

C. QUANTITATIVE ABSORPTION DATA

The in vivo absorption by guinea pigs of strontium from four concentrations (0.008–0.398 M) of aqueous strontium chloride coincides with permeability coefficients of 16×10^{-4} to 20×10^{-4} cm/hr (10). At higher and lower concentrations the permeability coefficients were reduced by about 50% (Table 1). Other authors reported that about 10% of ''minute'' quantities of radiostrontium was absorbed in just 10 min by intact rat skin (11).

D. SUMMARY

Through animal skin, the permeability coefficient of strontium chloride is about 10^{-3} cm/hr. Strontium salts are relatively nontoxic, and only the strontium radionuclides pose a serious hazard due to their accumulation in skeletal tissues.

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Thallium

A. GENERAL COMMENTS

Both metallic thallium and water-soluble thallium salts are highly toxic. Unlike the other group IIIA metals, thallium does not precipitate as an oxide at physiological pH. Of the two main valences, Tl^{1+} and Tl^{3+}, the monovalent (thallous) salts are the more toxic, yet for a period they were used therapeutically in human medicine. Following dermal, parenteral, or oral absorption, the metal is rapidly distributed throughout the body tissues, and over time accumulates due to its slow rate of elimination (1). Because of the severe toxicity of thallium salts, the use of these salts for medicinal purposes was halted in the early 1900s (2).

Thallium^{1+} is isomorphic with potassium and, based on its approximately 10 times higher affinity for enzymes, can compete successfully with that alkali metal in enzymatic reactions. To a great extent this explains the toxicity of thallium (3). Tl^+ is only slightly larger than K^+ and exhibits similar ability to cross cell membranes (4).

Thallium preparations were first used to reduce night sweats in tuberculosis and to treat scalp ringworm (a fungal infection) in children. At an oral dose approaching 8 mg/kg, thallous sulfate was seen to induce (temporary) alopecia in humans, without other signs of toxicity, and for a period of time was used for the removal of unwanted hair. The lethal dose of thallium for humans lies near to that value, at 12 mg/kg; due to the small therapeutic window, the internal use of thallium salts for therapeutic as well as esthetic purposes was discontinued (5,6).

Most recently, thallium sulfate has been used as an odorless, colorless, and tasteless adulterant in illegal drugs such as cocaine (7).

The populations at risk now from thallium poisoning are those living in the proximity of metallurgical industries and coal-burning power plants due to biomagnification, as significant airborne emissions of the metal can occur and become incorporated in the food chain (8,9).

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B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

The skin and its appendages seem to be one of the important target organs for thallium toxicity in mammals, and the appearance of abnormality in the skin is the earliest manifestation of elevated concentration in the human organism. To underscore the hazard of percutaneous absorption, the ACGIH has assigned a ''skin'' notation to thallium and its water-soluble salts; see Appendix for comments on ''skin'' notation (10). Cutaneous involvement becomes evident through acne, eczematous lesions and scaling, hyperkeratosis, and disintegrating hair shafts (7). The metal itself as well as its salts are rapidly absorbed through all the membranes of the body, including the mucous membranes and the skin. This affinity for skin appears confirmed by the cutaneous LD_{50} of 117 mg/kg determined for thallous carbonate in the rat (11).

Following skin exposure, traces of thallium rapidly appear in the urine, which is the main, albeit slow, route of elimination (1,12). The reported half-life of thallium in the human body is between 14 and 35 days (9).

Specific clinical symptoms of thallotoxicosis are hair loss and changes in nail structure (leuconychia striata or Mee's stripes) (5).

Also when applied topically, thallium acetate removes superfluous hair, and such preparations were at one time recommended for relatively large areas of application. When toxic symptoms became associated with such topical applications of thallium, its use as a depilatory was terminated (2,5). In the rat, autoradiography showed that thallium (applied as the nitrate) accumulates in the skin, primarily during the anagen phase or period of active hair growth. The resulting deposition of thallium in the hair follicles causes their atrophy, thereby explaining the occurrence of alopecia as a consequence of skin absorption (13–15). Thallium is excreted slowly by deposition in hair and nails, where it accumulates (2), and is also excreted to some degree in sweat (6). Presence of the element in toenail material can be a useful indicator of sublethal thallium poisoning because this metal accumulates in the toenails at higher and more accurately detectable concentrations than in other deposition sites (3).

C. SUMMARY

Thallium and thallium slats, which are now used primarily in the elctronic and semiconductor industries, belong in the class of most toxic metal compunds. Cutaneous absorption of thallium metal and its soluble salts (e.g., the carbonate and acetate) has been recognized by the ACGIH as presenting a significant risk of systemic toxicity, and particular care is recommended to avoid direct skin contact in the work environment. As a stable monovalent ion, thallium competes with potassium for membrane transport systems. Although several reviews mention

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thallium as being readily absorbed by human skin, no quantitative data were located in the literature.

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Vanadium

A. GENERAL COMMENTS

Vanadium occurs in biological systems primarily in the tetravalent and pentavalent forms. In body fluids at pH 4–8 the predominant forms of free vanadium are orthovanadates, HVO_4^{2-} and $H_2VO_4^-$, where its oxidation state is +5. Once inside a cell it is reduced to the +4 state or vanadyl, i.e., VO^{2+}. Both oxidation states have similar biochemical and physiological function. Vanadate competes with phosphate for active sites in phosphate transport proteins, inhibiting the action of phosphates, and also has a pronounced inhibitory action on Na^+/K^+ ATPase activity. As vanadyl, the metal competes with other transition metal ions for binding sites on metalloproteins (1). Vanadium is an essential nutrient for higher animals, including humans; it is present in mammalian tissues at concentrations below 1 mM, which was only recently determined with the advent of modern analytical techniques (2). A homeostatic mechanism maintains normal levels in the mammalian organism, and excess is rapidly excreted in the urine (3).

Vanadium-containing enzymes are found in plants and microorganisms (1). In higher animals vanadium is absorbed in moderate amounts from foods of both plant and animal sources. In both in vitro and whole-animal studies vanadyl and vanadate compounds display insulin mimetic effects and are being evaluated as orally active drugs that would replace insulin, which must be administered by injection (4). More recently, other vanadium species (the peroxovanadium complexes) have been characterized as even more potent mimetics; in addition, they are insulin potentiators, increasing the activity of injected insulin, and current research also focuses on their potential as orally active agents (5). It has been suggested that vanadium is a factor involved in the causation of manic-depressive illness. Evidence supporting that role is the finding that the mean plasma vanadium concentration in manic-depressive patients is higher than in normal controls (6). Vanadium deficiency induced in animals suggests that the metal has a func-

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tion in thyroid hormone metabolism and can mimic growth factors such as epidermal and fibroblast growth factors (2).

Significant exposure to vanadium occurs only in certain industrial operations: mining, petroleum refining, steel and utilities industries, as fossil fuels; in addition, certain ores contain this metal. Particularly in times of energy crisis when still bottoms from petroleum refining operations are increasingly used in oil-fired, electricity-generating plants for their Btu value, accumulated vanadium residues are volatilized as oxides or the carbonyl complex. Toxicity of vanadium is primarily associated with inhalation of vanadium pentoxide. Chronic exposure gives rise to symptoms of irritation, sometimes delayed, of the upper and lower respiratory tract, and inflammatory changes characterized by cough, wheezing, mucus production, chest tightness, bronchopneumonia, rhinitis, and sore throat; these symptoms, collectively described as ''boilermakers' bronchitis,'' can be disabling (7–9).

At the turn of the century, vanadium therapy was widespread in the treatment of tuberculosis, chlorosis, and diabetes. Evidence gathered from that medical use shows that, taken orally, it produces no signs of toxicity, due to poor absorption from the intestine (about 1% of the amount given in soluble form) and its relatively rapid excretion (about 60% in the urine within 24 hr (1,3). Clinical data indicate that a chronic oral dose of 24–80 mg daily vanadium is well tolerated (10).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

In rabbit skin, absorption of sodium metavanadate from a saturated solution (20%) produced an irritant effect (10). For purposes of occupational exposure, vanadium pentoxide is classified as a primary irritant, involving the skin, but most often affecting the respiratory system (11).

Given orally to rats as the pentoxide at 0.01% concentrations in food, vanadium produced structural changes in hair and depressed the cystine level, which indicates interference with metabolism of sulfur-containing amino acids. The observed effect could be counteracted by administration of methionine to the experimental animals (12).

Since vanadium has such a marked effect on cystine levels present in the integument, determination of cystine in chronically exposed metal workers can be useful in assessing vanadium absorption in these individuals. In particular, analysis of fingernail cystine provides a temporal record and an early detectable indication of exposure, which can be used diagnostically prior to the appearance of adverse clinical symptoms associated with vanadium excess.

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C. IMMUNOLOGY

Allergic responses of the immediate and delayed type to vanadium compounds have been recorded following industrial exposure to vanadium pentoxide (13,14). In patients fitted with stainless steel prostheses, leaching of metal into the tissues has been identified as a risk factor for sensitization, particularly in cases of prosthesis loosening, which results in enhanced mechanical abrasion. When patients ($N = 50$) who had received total joint replacements were patch-tested with various metal salts, a positive reaction was recorded to several of these metals, and one patient was positive to vanadium when tested with 0.1% ammonium metavanadate (15). The mouse ear swelling test confirmed the allergenic nature of vanadium sulfate, and the open epicutaneous test on albino guinea pigs with a 3% (w/v) solution of that same salt (a maximum nonirritating concentration) resulted in sensitization of all test animals ($N = 80$), thereby categorizing the metal as a strong allergen (14).

Vanadium was also shown to be immunotoxic by modulating immune responses in humans and experimental animals. Asthma, pneumonia, boilermakers' bronchitis and death from respiratory failure secondary to bacterial infections are recognized as consequences of acute and chronic exposure to high levels of vanadium dusts or fumes (9). A particular immunomodulating effect of vanadium is seen in vitro when murine macrophage-like cells are exposed to ammonium metavanadate (NH_4VO_3) or vanadium pentoxide (V_2O_5) in a standard immunocompetence test undertaken to elucidate the possible basis for altered host resistance. The capacity of the cells to interact with cytokines was reduced following exposure, and they become refractive to stimulation by host antigen or bacterial challenge (16). Due to the importance of the mature macrophage in initiating both cell- and humoral-mediated immune responses, impairment of such function may be the basis for immunomodulating effects that occur in the affected host.

D. SUMMARY

Environmental exposure to vanadium does not pose a significant toxicological risk to the general population. In the industrial setting, certain vanadium compounds have been found to be inhalatory and skin irritants, and strong contact allergens. On chronic inhalatory exposure, vanadium toxicity affects the respiratory system and the results can be disabling. Peroxovanadium complexes are currently under investigation as orally active alternatives to insulin. No quantitative data were located regarding the rate of skin absorption of any vanadium compound.

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Zinc

A. GENERAL COMMENTS

Zinc is an essential trace element on which many biochemical functions depend (1), because it is a constituent and cofactor in over 200 human enzymes that regulate lipid, protein, and nucleic acid synthesis and degradation (2). Zinc is one of the most abundant elements in the human body, present mainly in skeletal muscle (57%), bone (29%), teeth, hair, skin (6%), and testes. Only 0.1% is bound to plasma proteins (3). In the plasma, one-third is loosely attached to albumin and two-thirds are firmly bound to globulins. The metal protects against the formation of free radicals, by displacing iron ions from membrane proteins, and by inducing significant metallothionein (MT) levels which, with their high content of sulfhydryl residues, scavenge hydroxyl and superoxide radicals (4,5). ZnMT also appears to protect against cadmium-induced nephrotoxicity (6). A similar protective role is exhibited by preexposure to zinc sulfate (7). Parallel protective mechanisms are apparent with respect to the hepatotoxicity of cadmium, as pretreatment with zinc induces elevated MT levels in the liver of experimental animals (8–12).

In a recent review on the role of MTs in the brain (13), this regulatory action of free zinc in the CNS shed new light on the possible role of this metal in the pathology of Alzheimer's disease (AD). MTs are of critical importance in attenuating the cytotoxicity of free Zn in the brain. Indeed, MTs have a key function in the sequestration and dispersal of metal ions and in maintaining zinc homeostasis. Identification of different isoforms of MTs (MT I–IV) has revealed a particular abundance of MT-III in the brain and the possibility that it might have important neurophysiological functions in the hippocampus. Free zinc (as Zn^{2+}) is known to be neurotoxic (14,15) and various pathophysiological conditions can increase extracellular Zn released from the hippocampus (16). Since MT-III is preferentially expressed in the hippocampus, it would appear to act there as a local sink for the free metal. In the brain, as elsewhere, MTs are induced by a variety of xenobiotics such as lipopolysaccharide, dexamethasone, ethanol,

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or kainic acid (17). However, while MT-I mRNA is induced, MT-III mRNA expression is downregulated, particularly in the hippocampus, thereby increasing the local concentration of free zinc. This has the potential for neurotoxic effects, such as the formation of amyloid deposition in the brain, a pathological hallmark of AD (18).

Mechanisms that regulate zinc absorption from dietary intake or via the skin are not entirely understood; only 20–30% of ingested zinc is absorbed (19). Absorption does not take place by simple diffusion but seems to be regulated by homeostasis, being inversely related to the body's load of the metal. Also the regulating role of the various metalloenzymes, including Zn-binding MT in the skin, is not clear (20). Given systemically or topically, zinc leads to rapid clinical improvement of a number of abnormal conditions and diseases, particularly those stemming from zinc deficiency or depletion (21). Endogenous zinc is mobilized from body stores and utilized during protein synthesis in response to hemolysis, injury, and infection. During inflammation, particularly in arthritis, zinc is sequestered in both the injured tissues and the liver as MT, which results in hypozincemia (22). Interrelated homeostatic mechanisms regulate both zinc and copper distribution in the body. Corticosteroids are hormones which influence the homeostatic control of certain metals, including zinc and copper (23). The ratios of zinc and copper in the serum of patients with abnormal metabolism are inversely related (24). Levels and activity of zinc are also closely related to the tissue levels of the other essential elements magnesium, manganese, and selenium. Due to myriad biological mechanisms involving zinc, this metal is the most physiologically significant, and zinc deficiency can lead to a variety of diseases, including dermatological disorders. Plasma Zn levels below 70 mg/dL are generally indicative of deficiency, which can result from either inadequate nutritional intake, pathological malabsorption, defective metabolism, or increased mobilization in connection with stress; there are no significant body stores of this essential trace element. Serious conditions associated with chronic deficiency, congenital as well as acquired, are dwarfism, hypogonadism, infertility, and acrodermatitis enteropathica; the latter is an inherited disorder involving the skin, mediated by malabsorption, and the most serious and potentially lethal manifestation of zinc deficiency (25).

Animal protein is the main dietary source of zinc, some of which may be lost in normal food processing. Chelation of dietary zinc by high fiber and phytate content of certain grains may also lead to reduced absorption and symptoms of deficiency. Breast-fed infants, especially those born prematurely, are vulnerable to zinc deficiency (26). Human breast milk contains a special zinc-binding ligand that increases the bioavailability of zinc to the infant. The concentration of this dietary element, especially critical in early infant development, is highest in colostrum (5.59 mg/L), then decreases gradually in mature milk (1.18 mg/L)

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(27). Concentration of the element does not correlate with maternal serum zinc levels (28).

Inhalation of zinc chloride and zinc oxide fumes as they are generated at high temperatures in welding and soldering operations comports the risk of developing asthma, a condition also referred to as ''metal fume fever.'' For long-term occupational exposure, 8-hr threshold limit values (TLVs) have been set by the ACGIH to protect workers against adverse health effects from chronic exposure, at 1 mg/m^3 for zinc chloride and 5 mg/m^3 for zinc oxide (29). Adequacy of that TLV for zinc oxide fume has been questioned by a study when human volunteers were exposed at and below the TLV on an experimental basis. Of 12 subjects, 10 developed a fever and specific cytokines (plasma IL-6 levels) between 6 and 12 hr after exposure, and all but one complained of fatigue, muscle ache, and cough (30). Cases have also been described in the literature that showed no involvement of the immune system in connection with the fever condition (31,32).

B. SKIN ABSORPTION, ELIMINATION, AND REACTIVITY

Of the total amount of zinc in the body, 6% is present in the skin, with approximately three quarters of that amount found in the epidermis (33). Zinc levels in the skin of healthy subjects ($n = 40$) are highest in the epidermis (60 ± 14 mg/g), and they decrease progressively in the papillary (40 ± 10 mg/g) and reticular dermis (10 mg/g) (34). There appears to be no correlation between serum zinc levels and concentrations observed in the different skin strata. Other investigators have determined the concentration profile of zinc across normal human skin by micro-PIXE analysis, identifying a maximum level in the stratum germinativum ($90 \text{ mg} \pm 15$ ppm) (35,36). Histochemical studies of normal cutaneous tissue reveal that zinc accumulates primarily in hair shafts and follicles, and in the subcutaneous muscle layer (37).

Measurements of the rate of skin penetration of zinc compounds have been contradictory, due at least in part to the dynamics of zinc homeostasis, a rapid exchange between applied zinc and the large pool of endogenous zinc. Zinc reversibly bound to sulfhydryl storage site becomes available for immediate systemic absorption when deficiency develops (38). Chronic, intimate skin contact with a number of zinc organic compounds in the workplace can have an irritant effect, and exposure limits have been defined for a number of such compounds due to the potential for their cutaneous absorption (39). Zinc chloride has the greatest irritancy potential, causing parakeratosis, hyperkeratosis, inflammatory changes in the epidermis and superficial dermis, and acanthosis of the follicular epithelia. Irritancy decreased with the acetate and sulfate, in that order, and no irritation was observed following application of zinc oxide (40).

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Occlusive dermal application of a concentrated ZnO ointment to healthy human volunteers on a normal diet did not result in a significant increase in their serum zinc concentration. However, when a similar application was made to the skin of patients on total parenteral nutrition, a feeding routine that typically results in zinc deficiency, serum zinc levels were maintained at surprisingly normal and constant levels. In addition, in vivo in rats, dietary zinc deficiency can be adequately remedied by topical application of zinc salts (41). Both observations suggest the facile percutaneous absorption of zinc compounds.

Occlusive cutaneous application of various Zn compounds to normal human skin in vivo reveals both transepidermal and transfollicular penetration. Traces of the metal were seen in the stratum corneum within 2 hr of application, and in the dermis after 4 hr. Autoradiographic results correlated strongly with the histochemical data, indicating that the metal is bound to sulfhydryl groups (37,42). In vitro, histidine has been shown to be the preferred binding site for zinc (43).

$ZnCl_2$ is used in chemosurgery to destroy remaining malignant cells following surgical excision of cutaneous carcinomas (44).

Zinc oxide is a widely used, so-called nonchemical sunscreen. It can be applied at high concentrations without risk of adverse effects (unlike the similar application of other, conventional sunscreens). No dermal irritation was observed after a 25% zinc oxide patch (2.9 mg/cm^2) was placed on human skin for 48 hours (45).

Repeated application of 5% $ZnCl_2$ to rat and guinea pig skin in vivo caused a dose-dependent induction of hepatic MT, indicating significant zinc absorption through skin into the systemic circulation (46).

Zinc excretion via skin desquamation is normally insignificant; however, in certain abnormal conditions such as dandruff and psoriasis, increased scaling can result in significant zinc losses, compounded by the elevated zinc levels present in keratinocytes in such conditions: 70–100 mg/g versus 50 mg/g for diseased and normal epidermis, respectively (25). Zinc levels in human skin vary as a function of anatomical site, ranging from 10 to 100 mg/g dry weight. A correlation has been stipulated between zinc levels and the occurrence of dendritic cells in the respective anatomical region (47).

Sweating is an important excretory pathway for zinc under extreme atmospheric conditions and stress, with significantly higher losses than urinary elimination, and higher losses in males than in females. The significant amount of zinc excreted during prolonged heavy exercise may be an important factor in the development of heat stroke (48). In temperate climates and with normal activities, however, zinc loss through sweat is estimated to be only approximately 1% of daily intake (49). In normal adults, daily loss of endogenous zinc via sweat approximates 0.5 mg (1).

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Cross-sectional and longitudinal localization of the element in human scalp hair by micro-PIXE analysis reveals that Zn is incorporated through the root sheath, resulting in homogeneous distribution throughout the hair shaft. Once the hair shaft has left the confines of the skin, becoming exposed to the environment, zinc is further absorbed from exogenous sources (50). Scalp hair of children up to 15 years of age shows an age-dependent, gradual increase in zinc levels. Such an age-dependent increase is explained by the changing requirements for zinc during growth and development (51). Regarding the relationship between hair and systemic levels of zinc, the results of early studies have been contradicted by later investigations. Early studies indicated a correlation between systemic zinc deficiency and low zinc levels in both sweat and hair (52). More recent studies of hair zinc levels in a large cohort of malnourished versus healthy subjects led to a different conclusion: While low hair zinc levels do reflect low serum levels of the metal, high or normal zinc levels in hair are not a good index of body zinc status or of environmental exposure to the metal. The case of zinc demonstrates the fairly general inadequacy of hair concentrations of essential elements as indices of nutritional status (51,53,54).

Vitally important for skin integrity, zinc is rapidly transported to the skin following intestinal absorption (25). A number of skin conditions and diseases other than acrodermatitis enteropathica result as a consequence of nutritional zinc deficiency, inadequate resorption or metabolic disorders; atrophic and eczematous skin, leg ulcers and bed sores, oral ulcers, acne, dandruff, skin lesions in leprosy, herpetic keratitis, and dermatitis both in the aged and in alcoholics (25). These manifestations are collectively described as zinc deficiency dermatoses; however, the mechanisms underlying such cutaneous involvement in zinc deficiency are unknown (55). These conditions respond promptly to treatment, and dramatic improvement occurs within days of initiating zinc therapy (56). Genetic insufficiency in intestinal zinc absorption is recognized to play the key role in the pathogenesis of acrodermatitis enteropathica. Its severity is commensurate with the zinc level present in the organism. If left untreated, this condition results in characteristic skin lesions, alopecia (including loss of eyebrows), nail dystrophy, recurring infections, delayed wound healing, irritability, GI distress, and even death (1,25).

Because of the interdependence of zinc and copper in human physiology, particularly with respect to skin metabolism, the concentration ratio of these two metals in serum can reveal abnormal metabolism. The Cu/Zn ratio is significantly different among three disease groups—skin cancer, inflammatory diseases and non-inflammatory diseases—indicating the usefulness of Cu/Zn ratios for differential diagnosis compared to that of the individual metals themselves (24). In most inflammatory reactions of the skin the keratinocytes are activated, producing a variety of cytokines, including tumor necrosis factor–α (TNF-α) (57). The

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antiinflammatory effects of zinc-based therapies involve the reduction of keratinocyte activation markers and may explain the inhibiting role in allergic contact dermatitis (58).

An adequate zinc level is necessary during maturation of the epidermis and for maintaining cutaneous integrity (collagen synthesis and fibroblast proliferation) (45). The beneficial effect of systemically or topically administered zinc compounds on tissue repair, in particular in the curing of skin lesions, has been established (21). Zinc derivatives applied topically in the treatment of acne produce an ameliorating effect that may be due to inhibition of sebum secretion. Zinc inhibits bacterial lipase activity, and, as a result, free fatty acid levels in sebum and on the skin surface are reduced (25). Zinc acetate appears to be the most effective zinc salt in topical therapy, presumably due to its favorable penetrating properties. Chronic leg ulcers, a difficult problem that often requires surgery in the aged, were successfully treated by zinc chloride applied to the wound bed (59). Zinc supplementation expedited healing of scarified skin in zinc-deficient animals. This effect does not occur in animals on a well-balanced diet (56).

That zinc plays an important role in epidermal metabolism and keratogenesis of the hair was demonstrated on a cellular level by histological and autoradiographic analysis of the follicles (60,61). Zinc deficiency was shown to lead to a drastic reduction in cystine incorporqtion into the skin (62).

C. QUANTITATIVE ABSORPTION DATA

Percutaneous absorption of zinc has been studied in some well-defined systems in which the compound is definitely dissolved (e.g., $ZnCl_2$ in water), but more often in situations where the compounds are not completely soluble in the vehicle (such as $ZnCl_2$ in petrolatum and zinc pyridinethione in aqueous suspension). Results for some situations cannot be reduced to apparent permeability coefficients. Data are divided among four species: humans, guinea pigs, rabbits, and rats.

The quantitative measurement of zinc absorption was first reported in guinea pigs. Application of 200–500 μCi of $^{65}ZnCl_2$ followed by the discovery of ^{65}Zn in various organs made it clear that zinc was absorbed transdermally. As a measure of zinc absorption through the skin, the in vivo decrease of ^{65}Zn radiation in 5 hr from a simple aqueous solution of $ZnCl_2$ was determined for six concentrations (0.005–4.87 M) (63). In these early experiments with 95 animals (Table 1) there were 62 absorption rates of zinc, some at each concentration, that were too small to be accurately determined, i.e., $K_p < 6.4 \times 10^{-4}$ cm/hr (see chap. 3). The remaining 33 absorbed 1.0–3.9% of the applied zinc ($K_p = 6.4 \times 10^{-4}$ to 25×10^{-4} cm/hr). For individual animals, the highest fractions of zinc were absorbed, corresponding to the largest apparent permeability coefficients,

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Table 1 Zinc, as $^{65}ZnCl_2$

Reference: E. Skog and J. E. Wahlberg, A comparative investigation of the percutaneous absorption of metal compounds in the guinea pig by means of the radioactive isotopes: ^{51}Cr, ^{58}Co, ^{65}Zn, ^{110m}Ag, ^{115m}Cd, ^{203}Hg, *J. Invest. Dermatol. 43:* 187–192 (1964).
Species: guinea pig, M & F in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: water
Duration of exposure: 5 hr
Analytical method: decrease of γ radiation from the donor chamber detected by Geiger-Müller counter; scintillation counting of isolated organs
Notes:
1. Liver, kidneys, intestines, and feces were the ''most suitable'' organs for measuring absorbed ^{65}Zn; no figures were reported.
2. No average values are given; no exact absorption values are reported.
3. The absorption was reported as <1% of the dose in 62 of the total of 95 experiments; in the remaining 33 cases the absorptions were reported as being in the range 1.0–1.9%, 2.0–2.9% or 3.0–3.9%.

		Absorption of zinc	
		% Zn/5 hr <1%	% Zn/5 hr = 1%
		Disappearance constant $k < 3.4 \times 10^{-5}$ (min^{-1})	Disappearance constant $k = (3.4 - 13.5) \times 10^{-5}$ (min^{-1})
Conc. (M)	pH	$K_p < 6.4 \times 10^{-4}$ (cm/hr)	$K_p = (6.4 - 26) \times 10^{-4}$ (cm/hr)
0.005	5.8	10/10	0/10
0.08	6.1	7/11	4/11
0.08	5.7[a]	10/11	1/11
0.08	1.8[a]	4/11	7/11
0.239	5.7[a]	5/10	5/10
0.398	5.6[a]	8/15	7/15
0.753	5.3[a]	10/15	5/15
4.87	3.7	8/12	4/12

[a] These solutions were acidified with HCl to dissolve precipitated $Zn(OH)_2$.

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for the intermediate applied concentrations. Conversely, the lower absorbed fractions were associated with the extremes of applied concentrations.

In related work, guinea pigs that were exposed for several weeks to 2 mL of 0.239 M $ZnCl_2$ did not gain weight after the first week, but all of the animals survived (Table 2) (64). In this report, Wahlberg presents the average absorption rate of 0.239 M $ZnCl_2$ as $<1\%/5$ hr; this is the equivalent of $K_p < 6.6 \times 10^{-4}$ cm/hr.

In humans, zinc oxide is used in the treatment of skin disorders and its percutaneous absorption has been quantitatively investigated. The zinc content of 4 cm × 5 cm 25% zinc dressings applied to forearms of 15 subjects was measured before and after 48 hr application (Table 3) (65). The average flux over 48 hr was 5 μg Zn/cm^2/hr including ''a large proportion of solubilized zinc oxide . . . in the horny layer.''

There are two reports considering the quantitative absorption of zinc in human skin in vitro. The in vitro fluxes of two soluble zinc salts, $ZnCl_2$ and $ZnSO_4$, in the presence of copper have been measured over 72 hr from three vehicles, two hydrogels and petrolatum, through human skin (66,67). Zinc fluxes into the receptor solution from zinc sulfate formulations peaked in the first 6 hr; from zinc chloride such early peaks of flux, if present at all, were not so distinct.

Permeability coefficients were based on the 24- to 72-hr or 48- to 72-hr periods. The permeability coefficients for 1.14% zinc from Carbopol-$ZnSO_4$-

Table 2 Zinc, as $ZnCl_2$

Reference: J. E. Wahlberg, Percutaneous toxicity of metal compounds, *Arch. Environ. Health 11:*201–204 (1965).
Species: guinea pig in vivo ✓
in vitro
Area: 3.1 cm^2
Vehicle: 2 mL water
Duration of exposure: 8 weeks
Concentration: 0.239 M
Analytical method: bioassay ($N = 20$)
Notes:
1. Applied amount = 31 mg zinc.
2. No animals died when exposed to zinc chloride.
3. Weight gain ceased by the first week in animals treated with zinc chloride; control animals and distilled water–treated animals gained weight for the 8 weeks of the experiment and grew from approximately 375 g to 500 g or more.
4. As cited in this paper, for 0.239 M $ZnCl_2$ the mean rate of zinc absorption is less than 157 nmol/hr/cm^2. This corresponds to $K_p < (157 \text{ nmol/hr/cm}^2)/(0.239 \times 10^9 \text{ nmol/1000 cm}^3) = K_p < 6.6 \times 10^{-4}$ cm/hr.

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Table 3 Zinc, as ZnO

Reference: M. S. Ågren, Percutaneous absorption of zinc oxide applied topically to intact skin in man, *Dermatologica 180:*36–39 (1990).
Species: humans in vivo ✓
in vitro
Area: 20 cm^2, flexor side of the lower arm
Duration: 48 hr
Vehicle: adhesive (natural rubber, gum rosin, and white mineral oil) with 25% ZnO on PVC-coated cotton fabric
Analytical method: analyzed zinc content of new and used dressings by flame atomic absorption spectrophotometry
Notes:
1. Initial Zn concentration of dressings: 2.66–3.21 mg/cm^2.
2. Zn concentration of used dressings: 2.52–2.99 mg/cm^2.
3. Difference between mean zinc content of new and used zinc dressings = 0.23 mg/cm^2 (10 subjects, $p < 0.02$), i.e., about 8% less after 48 hr.
4. Average flux = 5 μg/cm^2/hr (0.23 mg/cm^2/48 hr = 4.8 μg/cm^2/hr). ''But it was found that a large proportion of solubilized zinc oxide was retained in the horny layer. Therefore, the flux of 5 μg/cm^2/hr was an overestimation of the actual zinc quantities passing through the skin.''
5. Zinc-treated skin had a significantly higher pH after treatment (5.6 ± 0.3) than before (5.1 ± 0.5); there was a ''positive correlation ($r = 0.68$) between skin pH and the zinc content of the dressing at 48 hr.'' That is, at higher pH less zinc was absorbed by the skin.
6. In five other subjects treated similarly epidermis for zinc analysis was taken from suction blisters. The treatment increased the epidermal zinc by about 10 times and increased zinc in the blister fluid by a factor of about 3.

$CuSO_4$ and from petrolatum-$ZnSO_4$-$CuSO_4$ were not significantly different, 0.048 $\times 10^{-4}$ cm/hr and 0.060 $\times 10^{-4}$ cm/hr, respectively (Table 4). For 2.4% zinc from petrolatum-$ZnCl_2$-$CuCl_2$ the permeability coefficient was similar, i.e., 0.082 $\times 10^{-4}$ cm/hr. From Metolose 60 SH hydrogel containing the chloride salts, the zinc permeability coefficient, 0.29 $\times 10^{-4}$ cm/hr, was more than three times higher. All of these permeability coefficients are more than an order of magnitude below many values reported by Skog and Wahlberg from guinea pigs in vivo (63).

Even after removal of the zinc formulation there was still the potential for continued movement of zinc into the receptor or into the body as the case may be. The skin after 72 hr contained one to three times as much zinc as had reached the receptor except in the case of zinc sulfate in Carbopol. In the latter case, the zinc content of the skin was one-fifth of the amount in the receptor.

Percutaneous zinc absorption rates from some commercial emulsions and

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Table 4 Zinc, as zinc chloride and zinc sulfate

Reference: F. Pirot, F. Panisset, P. Agache, and P. Humbert, Simultaneous absorption of copper and zinc through human skin in vitro, *Skin Pharmacol. 9:*43–52 (1996).
Species: human, from surgery in vivo
in vitro ✓
Area: 3.14 cm^2
Duration: 72 hr
Vehicles: white petrolatum, carboxypolymethylene gel (Carbopol), and hydroxypropylmethylcellulose gel (Metolose 60 SH); 20 mg/cm^2. Each also contains an equal concentration of copper chloride or copper sulfate.
Concentration: 1.14% and 2.40% zinc
Analytical method: flame atomic absorption spectrometry of receptor fluid and skin layers
Notes:
1. Average thickness of the dermatomed skin = 410 μm.
2. Diffusion cells were Franz-type static cells. Receptor fluid was isotonic saline with antibiotics and 5% human albumin. Temperature = 33°C.
3. There were five cells for each formulation.
4. The ''transcutaneous fluxes always showed a peak between 1.5 h and 6 h, followed by immediate or delayed subsiding.''
5. When each salt was delivered from petrolatum, accumulation of zinc in the skin was greater in both cases than the accumulation in the receptor fluid. From Metolose 60 SH gel for zinc chloride, the skin and receptor fluid contained approximately equal amounts of zinc. Zinc sulfate in Carbopol 940 gel led to less than 10% as much zinc in the skin as from any of the other preparations and more zinc in the receptor than in the skin.

Salt	Formulation	Conc. (% Zn)	Apparent permeability coefficient $10^4 \cdot K_p$ (cm/hr)[a]
$ZnSO_4$ (& $CuSO_4$)	Petrolatum	1.14	0.048 ± 0.029
$ZnSO_4$ (& $CuSO_4$)	Carbopol 940 gel	1.14	0.060 ± 0.007
$ZnCl_2$ (& $CuCl_2$)	Petrolatum	2.40	0.082 ± 0.025
$ZnCl_2$ (& $CuCl_2$)	Metolose 60 SH gel	2.40	0.29 ± 0.12

[a] Based only on the zinc in the receptor fluid.

ointments were also measured in vitro with human skin by Pirot et al. (67). Three zinc compounds were used: zinc 2-pyrrolidone 5-carboxylate (ZnPC), ZnO, and $ZnSO_4$. Fluxes were highest in the first 2 hr after application and then decreased to less than a tenth of the initial rates (Table 5). For similar time periods the zinc permeability coefficients with one exception were 0.12×10^{-4} cm/hr to

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Table 5 Zinc, as zinc 2-pyrrolidone 5-carboxylate (ZnPC), ZnO, and $ZnSO_4$

Reference: F. Pirot, J. Millet, Y. N. Kalia, and P. Humbert, In vitro study of percutaneous absorption, cutaneous bioavailability and bioequivalence of zinc and copper from five topical formulations, *Skin Pharmacol. 9:*259–269 (1996).
Species: human, dermatomed abdominal, 400 μm in vivo
in vitro ✓
Area: 3.1 cm^2
Vehicle: three emulsions (two commercial products and a custom-made variant of one of the former) and two commercial ointments. All contained both zinc and copper compounds. Each formulation was applied at the rate of 16 mg/cm^2.
Emulsion A: Cu/Zn d'Uriage, water/oil, contains ZnO, ZnPC, and CuPC.
Emulsion B: same as A except that $CuSO_4$ and $ZnSO_4$ replace CuPC and ZnPC.
Emulsion C: Dermalibour, water/oil, contains $ZnSO_4$, ZnO, and $CuSO_4$.
Ointment D: Dalibour Monot, contains $ZnSO_4$, ZnO, and $CuSO_4$
Ointment E: Dermocuivre, contains ZnO and $CuSO_4$
Analytical method: atomic absorption spectrometry of receptor solution and of tissues
Duration of exposure: 72 hr
Notes:
1. The receptor fluid was 0.9% NaCl solution held at 33°C by a water bath.

Percutaneous absorption of zinc

Formulation	Zinc compound	Soluble zinc conc. (mg/cm^3)	Permeability coefficient[a] $10^4 \cdot K_p$ cm/hr 0–2 hr	25–48 hr	0–72 hr
		Experiment 1			
Emulsion A	ZnPC, ZnO	0.5 as PC	4.9	0.15	0.4
Emulsion B	$ZnSO_4$, ZnO	0.6 as sulfate	5.0	0.12	0.35
Emulsion C	$ZnSO_4$, ZnO	0.2 as sulfate	13	0.55	1.1
		Experiment 2			
Emulsion A	ZnPC, ZnO	0.5 as PC	17	0.4	1.1
Ointment D	$ZnSO_4$, ZnO	0.2 as sulfate	56	1.2	3.6
Ointment E	ZnO	(80.3 as ZnO)[b]	0.21[b]	0.002[b]	0.011[b]

[a] Based on concentration of Zn as $ZnSO_4$ or ZnPC, except for ointment E which contains only ZnO; for ointment E the Zn concentration used in calculating K_p values above was the total concentration of Zn from ZnO.

[b] The aqueous solubility of ZnO is only 4–6 μg/mL (66 p. 262), i.e., $<10^{-4}$ the ZnO concentration applied, and using that solubility figure in calculating K_p (flux ÷ concentration) would raise the value of K_p by more than a factor of 10,000. On the basis of the Zn^{2+} concentration being 5 μg/mL, the values of $10^4 \cdot K_p$ for the same time periods would be extremely large, e.g., 3380, 32, and 178 cm/hr, for 0–2, 25–48 and 0–72 hr, respectively.

Table 5 Continued

2. As the exposure time lengthens, apparent K_ps and fluxes decrease. The flux and the apparent K_p for the period 3–6 hr were smaller than for 0–2 hr but larger than for 7–24 hr.
3. Over 72 hr the average percutaneous absorptions ranged from 0.12% to 1.6% of the applied zinc, i.e., 1.5–6.4 μg/cm^2.
4. The percentage of zinc absorbed from emulsion C was significantly less than from emulsions A or B ($p < 0.05$). The percentage of zinc absorbed from ointments D and E was also significantly less than from emulsion A ($p < 0.01$).
5. The concentrations of zinc were significantly increased in the dermatomed skin and in its epidermal and dermal layers. Concentrations in the exposed epidermis (2.3–16 mg Zn/g dry tissue) were far higher than in controls (0.6 mg Zn/g dry tissue). Concentrations in the exposed dermal layers (0.02–0.36 mg Zn/g dry tissue) were also higher than in control tissue (0.01 mg Zn/g dry tissue), although the concentrations of zinc in the dermis were about two orders of magnitude below the epidermal concentrations.

1.2×10^{-4} cm/hr, somewhat larger than zinc K_p values from hydrogels and petrolatum. The exception was an ointment containing ZnO, a poorly soluble compound, as the only source of zinc. Depending on whether the zinc concentration is taken as the total concentration of zinc (80.3 mg/mL) or as the solubility of ZnO (4–6 μg/mL), the 25–48 hr permeability coefficient is either very small (0.002×10^{-4} cm/hr) or very large (32×10^{-4} cm/hr), respectively.

Percutaneously absorbed zinc delivered from 7500 ppm zinc chloride in corn oil can maintain the plasma concentration of zinc in rats being fed a zinc-deficient diet (Table 6) (41). In this case, the plasma concentration was determined for rats on a normal diet, on a zinc-deficient diet, and on a deficient diet supplemented with topically administered zinc. The actual amount of zinc absorbed was not ascertained, and so the flux of zinc is unknown.

At pH 1 more carrier-free zinc-65 chloride was absorbed by rats in 2 hr than at pH 4 (Table 7) (68). When the applied concentration at pH 1 was increased 100-fold to 125 μg Zn/mL by adding nonradioactive zinc chloride, there was no significant difference between groups in the total fraction of zinc absorbed. The absorbed fraction from a suspension of zinc oxide at pH 8 and 125 μg Zn/mL was about the same as from pH 4 zinc chloride. This was supported by autoradiography; no differences were observed between applications of zinc oxide and zinc chloride. In these experiments, the applied volume (50 μL) was so small that the zinc concentration in contact with the skin might not be constant enough to calculate a reliable permeability coefficient. However, setting aside that concern for these experiments with zinc chloride in rats, the range of permeability coefficients would be 0.6×10^{-4} to 2.5×10^{-4} cm/hr.

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Table 6 Zinc, as $ZnCl_2$

Reference: C. L. Keen and L. S. Hurley, Zinc absorption through skin: correction of zinc deficiency in the rat, *Am. J. Clin. Nutr. 30:*528–530 (1977).
Species: rats, F in vivo ✓
in vitro
Area: 3 cm × 4 cm = 12 cm^2; treated with depilatory
Duration: 8, 24 hr
Vehicle: 0.4 mL corn oil, 7500 ppm Zn (i.e., approximately 6 mg $ZnCl_2$ in 0.4 mL of oil; no information about preparation)
Analytical method: plasma zinc by atomic absorption spectrophotometry
Notes:
1. ''Plasma zinc levels in pregnant rats fed a zinc-deficient diet were found to drop within 24 hr of deficiency.'' In these experiments pregnant rats were fed the deficient diet for 24 hr and concurrently treated for either all 24 hr or the last 8 hr or not at all with zinc chloride/corn oil.

Diet/topical treatment[a]	Length of treatment (hr)	Plasma zinc (μg/100 mL)
Control diet	0	115 ± 5
Zinc-deficient diet:		
Corn oil	8	75 ± 2
Corn oil	24	63 ± 3
Corn oil + zinc	8	115 ± 4
Corn oil + zinc	24	182 ± 9

[a] Treated areas were covered and bandaged. Animals/group = 5–7.

2. It is clear that enough zinc was absorbed transdermally to prevent the decrease in plasma concentration of zinc.
3. There are no data for amount of zinc absorbed. Therefore, neither flux nor K_p can be calculated.

Table 7 Zinc, as ^{65}ZnO and $^{65}ZnCl_2$

Reference: G. Hallmans and S. Lidén, Penetration of ^{65}Zn through the skin of rats, *Acta Derm. Venereol. (Stockh.) 59:*105–112 (1979).
Species: rats, M in vivo ✓
in vitro
Area: ''approximately 3 cm^2 of the shaved areas on both sides of the animals'' for solution and for suspension, i.e., a total of 6 cm^2; 5 cm^2 ''on both sides'' for the tape formulation, i.e., a total of 10 cm^2
Duration: various, 10 min—4 days
Vehicle: aqueous $ZnCl_2$; ZnO in suspension; 17% ZnO in adhesive (gum and resin) on cotton gauze as tape

Table 7 Continued

Analytical method: γ radioactivity in blood, serum, coagulum from blood, tissue samples, whole body

Notes:

1. Controls with topical ^{3}H-thymidine produced no ''incorporation'' of radioactivity in lymph nodes; this indicated that during shaving there had been no major damage to the stratum corneum.
2. ^{65}ZnO was produced by irradiation of zinc oxide powder.
3. When the zinc tape was applied, the concentration of ^{65}Zn in blood but not the total absorption of Zn was determined.
4. Volume applied was ''25 mL of ^{65}Zn solution or suspension to 3 cm^2 . . . on both sides of the animals,'' i.e., 50 μL in total.
5. The test areas were covered with plastic film.
6. After application of $^{65}ZnCl_2$, ''substantial'' radioactivity was present in blood, liver and heart only 10 minutes later and the maximum amounts were reached in 1–4 hr.

Treatment[a]	pH	Total absorbed ^{65}Zn radioactivity[b] (%)
$ZnCl_2$, 1.3 μg Zn/mL	1	4.1 ± 0.6
$ZnCl_2$, 1.3 μg Zn/mL	4	1.6 ± 0.3
ZnO suspension, 125 μg Zn/mL	8	1.9 ± 0.2

[a] Rats were killed 2 hr after treatment.

[b] The GI tract, liver, and the contaminated skin were removed and measured separately from the remainder of the body. This total was absorbed by the whole body not including the contaminated area of skin.

Treatment[a]	pH	Total absorbed ^{65}Zn radioactivity[b]
$ZnCl_2$, 1.1 μg Zn/mL	1	6.1 ± 1.5
$ZnCl_2$, 125 μg Zn/mL	1	3.6 ± 0.9

[a] Rats were killed 2 hr after treatment.

[b] The treated area of skin is not included.

7. ''If the penetration of zinc through rat skin obeys Fick's law of diffusion then no difference is to be expected in the percentage ^{65}Zn penetrated per unit of time among solutions with different zinc concentrations.'' The difference in ^{65}Zn absorption between carrier-free (1.1 μg/mL) and supplemented (125 μg/mL) concentrations of zinc (as chloride) was not significantly different.
8. The small applied volume makes concentration of zinc very uncertain and so K_p is not reliably calculable.

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Table 8 Zinc, as [^{65}Zn]zinc oxide, omadine (pyridinethione), sulfate, and undecylenate

Reference: S. P. Kapur, B. R. Bhussry, S. Rao, and E. Harmuth-Hoene, Percutaneous uptake of zinc in rabbit skin, *Proc. Soc. Exp. Biol. Med. 145:*932–937 (1974).
Species: rabbit in vivo ✓ exposure
in vitro
Area: 1-in-diameter circles on the back (shaved), i.e., 5.1 cm^2
Duration: 6 and 24 hr after single dose; 6 and 24 hr after the second dose which was 24 hr after the first dose
Vehicle: 1:1 glycerin-propylene glycol; no volume mentioned
Analytical method: ^{65}Zn by γ counting
Notes:
1. The aim was ''to demonstrate, in situ, localization of zinc and sulfhydryl (-SH) groups in rabbit skin after topical application of various zinc compounds.''
2. Applied 2.5 mg of zinc compound per dose. There is no mention of whether or not unabsorbed zinc compounds on the surface were removed before counting ^{65}Zn. The treated skin was excised and fixed in cold alcoholic formalin apparently before counting ^{65}Zn.
3. The authors conclude: ''No significant differences were found in the amount and location of ^{65}Zn in skin treated with four different zinc compounds.''
4. ''It was observed in this study that ^{65}Zn almost completely disappears from skin 24 hr after the first and second application though its concentration is high at 6 hr.'' However, later, Hallmans and Lidén reported that formalin fixation removes most of the zinc from tissue (68).
5. There is no indication of whether or not treated areas were protected from loss of ^{65}Zn by rubbing or whether or not rubbing, etc., was a likely factor in loss of ^{65}Zn from the treated areas.

	Total applied dose		Time after last application: 6 hr	Time after last application: 24 hr
Compound	Compound (mg)	Zn (mg)	% retained	% retained
	Single dose			
Zinc oxide	2.5	2.0	20.6	23.6
Zinc omadine	2.5	0.51	4.1	29.0
Zinc sulfate	2.5	1.0	65.0	19.0
Zinc undecylenate	2.5	0.15	37.3	22.7
	Double dose			
Zinc oxide	5	4.0	21.2	25.0
Zinc omadine	5	1.0	7.2	8.3
Zinc sulfate	5	2.0	3.3	11.9
Zinc undecylenate	5	0.3	5.5	8.0

6. From a single dose of $ZnSO_4$ 35% disappears in 6 hr. Then, flux = 2.5 mg × 0.35 × 65 g Zn/mol ÷ (161 g $ZnSO_4$/mol × 6 hr × 5.1 cm^2) = 0.012 mg Zn/cm/hr.

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Table 9 Zinc, as [^{65}Zn]zinc [^{14}C]pyridinethione (ZPT)

Reference: C. D. Klaassen, Absorption, distribution and excretion of zinc pyridinethione in rabbits, *Toxicol. Appl. Pharmacol. 35:*581–587 (1976).
Species: rabbits, M, New Zealand white, 1.5–2.5 kg in vivo ✓ absorption
in vitro
Area: 7 cm × 12.5 cm on the back
Duration: 4 hr exposure
Vehicle: 1% aqueous suspension in 0.1% triethanolamine–alkyl sulfate
Analytical method: ^{65}Zn in blood, urine, and 13 tissues was measured in a γ ray spectrometer; ^{14}C was determined in a liquid scintillation counter
Notes:
1. IV dose = 1 mg ZPT/kg; dermal dose = 40 mg/kg.

Isotope content (% of dose) after zinc pyridinethione administration

	Dermal application[a]		Intravenous administration[b]	
Sample	^{14}C	^{65}Zn	^{14}C	^{65}Zn
Major organs	0.5	0.008	4	55
Urine	<0.5	0.02	75	0.5

[a] After 8 hr (4 hr exposure + 4 hr after removing ZPT).
[b] After 6 hr.

2. ''The [zinc pyridinethione] molecule does not remain intact in its distribution and excretion from the body.''
3. After intravenous administration the ^{14}C/^{65}Zn ratio in the major organs was about 1:14. Most of the ^{14}C had been excreted and most of the ^{65}Zn remained in the organs. After dermal application, in contrast, the ratio was reversed, i.e., about 62:1, because the zinc portion of ZPT apparently penetrates the skin less readily than the organic portion.
4. There is not enough information to determine precisely the percutaneous absorption of zinc.
5. An *estimate* of the permeability coefficient of zinc can be made as follows: If the major organs contain the same fraction of absorbed zinc after dermal absorption as after intravenous administration, then for a 2-kg rabbit, absorbed zinc = 40 mg/kg × 65 g Zn/mol ÷ 317 g ZPT/mol × 0.00008 ÷ 0.55 × 2 kg = 0.0024 mg Zn absorbed through the skin, and average flux = 0.0024 mg ÷ (4 hr × 87.5 cm^2) = 6.8 × 10^{-6} mg/cm^2/h. If the zinc in 1% ZPT were in solution, Zn concentration = 2.05 mg Zn/cm^{-3}, and K_p = (6.8 × 10^{-6} mg/cm^2/hr^1) ÷ 2.05 mg Zn/cm^3 = 0.033 × 10^{-4} cm/h.

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Four zinc compounds [zinc oxide, zinc omadine (pyridinethione), zinc sulfate and zinc undecylenate] with diverse properties were applied by Kapur et al. to shaved rabbit skin in order to determine the location and amount of zinc in the skin (Table 8) (37). Although there were no significant differences between compounds in the amount or location of zinc in the skin, Hallmans and Lidén (68) later reported (based on experiments using rat skin) that most of the zinc in the tissue would have been removed by the Kapur et al. fixation procedure. In both of these studies, autoradiography revealed relatively high concentrations of zinc in the hair follicles. Both also found that, after being fixed in alcoholic formalin, most of the zinc was gone from the surface of the skin. They differ in that when Hallmans and Lidén autoradiographed frozen, unfixed sections, high concentrations of ^{65}Zn were found on and near the epidermis; the dermis and panniculus carnosus also contained ^{65}Zn. After fixation most ^{65}Zn in the tissue had been removed. The lack of agreement between these two reports makes uncertain both the absolute and relative percutaneous zinc absorption from the applied compounds.

Percutaneous absorption of ^{65}zinc ^{14}C-pyridinethione was subsequently measured, again in rabbits (69). It was clear from the ^{65}Zn/^{14}C ratios following intravenous and topical administrations that the two parts of the compound, the zinc ion and the pyridinethione moiety, were not absorbed together (Table 9). With assumptions for the zinc concentration (the applied zinc pyridinethione was in a suspension) and for the total amount absorbed, an estimate for the permeability coefficient for zinc is 0.03×10^{-4} cm/hr.

D. IMMUNOLOGY

For the general population, neither zinc nor its salts are known to be significant allergens. Salts of the metal failed to induce lymphocyte proliferation in the murine local lymph node analysis (70). Organozinc compounds, such as zinc pyrithione (a widely used antidandruff agent) and zinc dimethyl- and diethyldithiocarbamate (chemicals used in the manufacture of rubber), occasionally cause allergy, with isolated cases of ACD and immunological contact urticaria having been described (71–73).

Occupational exposure to respirable particles of metallic zinc, vaporized at elevated temperatures in galvanization processes and in work with soldering fluxes containing zinc salts, was reported to result in isolated cases of asthma. Positive skin tests for immediate-type sensitization and bronchial provocation tests confirm hypersensitivity, although specific IgE could not be demonstrated (73–76).

Given systemically at high concentrations to animals, e.g., as the sulfate, zinc exhibited antitumor properties; this effect possibly results from a combina-

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tion of enzyme inhibition and a boosted immune response due to increased lymphocyte transformation (77). Since severe zinc deficiency is associated with increased susceptibility to infection, a number of investigations have focused on the role of zinc in the integrity of the immune system (21). Zinc deficiency depresses cell-mediated immunity, as revealed by delayed onset of cutaneous hypersensitivity. Such deficiency apparently causes lymphatic tissue atrophy and reduced T-cell concentration (78). This condition has been reported to improve after zinc supplementation therapy. When zinc-deficient children were treated on one arm with topical zinc sulfate ointment and then patch-tested on both arms with a battery of standard allergens, the delayed-type allergic reaction was significantly reduced on the untreated arm, which implies that local absorption of zinc was sufficient to restore the normal ACD response (79). Alopecia areata (hair loss) appears to be associated with zinc deficiency. Both the zinc status and immune competence were systematically investigated on a cohort (24) of alopetic patients. This study identified a close relationship between the immune system and zinc metabolism in alopecia areata. Most of the patients in this group were either deficient or marginally deficient in zinc and exhibited low B-lymphocyte counts ($p < 0.01$) (80), confirming the link established between Zn metabolism and immunity (81). Thymic atrophy, substantial reduction in T cell and impaired capacity of antigen presentation by Langerhans cells were associated with zinc deficiency in mice (82,83), and resulted in the failure to induce hypersensitivity to a potent contact allergen, dinitrochlorobenzene (84). Dietary zinc deficiency also led to significantly fewer Langerhans cells, even though the zinc-deficient and control animals showed equivalent zinc levels in the skin (85).

E. SUMMARY

Zinc is an essential trace metal as a critical component of membranes, and of the skin and its appendages in particular. Levels and activity of zinc in the animal organism are closely related to tissue levels of the other metals Cu, Mg, Mn, and Se. In the developing mammalian organism, deficiency of zinc results in loss of appetite, inability to gain weight, skeletal abnormalities, parakeratotic esophageal and skin lesions, hair abnormalities, and inhibition in sexual maturation. Zinc deficiency syndrome is rapidly manifest when the diet is deficient in this element, and severe deficiency can be fatal. Pathological conditions disappear just as rapidly, however, after oral zinc supplementation, and also when delivered in the skin. Such rapid improvement following therapeutic supplementation through cutaneous application of zinc salts indicates ease of skin penetration, although the measured absorption rates do not account for the rapid establishment of equilibrium observed. Quantitative penetration experiments in animals indicate a K_p of approximately 10^{-3} cm/hr or less. Only isolated cases of immediate or delayed

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type sensitivity have been attributed to this metal ion. In addition, proper immune function depends on zinc availability; deficiency leads to impaired cell-mediated immunity and decreased antibody production.

Inhalation of zinc chloride and zinc oxide fumes is an occupational hazard, potentially causing ''metal fume fever,'' and threshold limit values for 8-hr workroom exposure have been set by the ACGIH.

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Zirconium

A. GENERAL COMMENTS

Zirconium occurs in the environment mainly as a constituent of mineral deposits. These deposits are abundant and widespread, but they are not known to pose an environmental or human health threat because they are insoluble and hence essentially unavailable to living organisms. Furthermore, zirconium cannot form covalent bonds with carbon—an important condition for interaction with biological systems—and in fact the metal has no known biological significance. Water-soluble zirconium compounds that are discharged by industry are converted to highly insoluble hydrous zirconia (ZrO_2) or zirconium hydroxide [$Zr(OH)_4$] at pH 4–9.5 in wastewater and are deposited as environmentally inert sediments. The solubility product for precipitated zirconium oxide was computed as 1.1×10^{-54} (1).

B. SKIN ABSORPTION AND REACTIVITY

Formerly the widest commercial use of zirconium complexes was in deodorants and antiperspirants, often together with aluminum compounds. Most commonly used was zirconium oxychloride hydrate. Zirconium compounds are among the agents that cause closure of the sweat duct (emphraxis) due to protein precipitation or hydroxide gel formation, or both (2).

Once they penetrate the skin, such as through abrasions, or are inhaled as aerosols, zirconium salts are likely to form granulomas. Consequently, their use in cosmetic products has now ceased. The granulomatogenic agent in such cases may be either the soluble salt (e.g., as was used in antiperspirants) (3) or the insoluble hydroxide or acetate, (e.g., as were used in topical preparations to treat Rhus plant dermatitis) (4). Such granulomas are benign growths that envelop the irritating agent and thereby remove it from further tissue contact. Granulomas, such as induced by zirconium, are transitory if caused by soluble salts, but perma-

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nent if induced by insoluble particles that remain embedded indefinitely in tissue. In this latter case, they are refractory to therapy and require surgical removal.

C. IMMUNOLOGY

Following repeated use of zirconium-based stick deodorants or topical zirconium remedies for urushiol hypersensitivity, granulomatous hypersensitivity was induced in the skin, characterized as epithelioid cells that organize into characteristic tubercles and do not phagocytize zirconium. Due to such adverse reactions the original zirconium deodorant formulations had to be removed from the market upon order by the FDA. A zirconium oxychloride–aluminum chlorohydrate complex, however, also proved to be effective as a deodorant, without releasing zirconium into the skin, and can be used without the risk of granuloma formation even in persons with granulomatous hypersensitivity to zirconium (5).

In humans, allergic hypersensitivity to zirconium as a secondary reaction to granulomatous hypersensitivity is possible but is rarely seen (3-6). Certain zirconium compounds can also elicit sarcoidal granulomas when introduced into the skin (7).

Delayed-type hypersensitivity caused by sodium zirconium lactate has been induced experimentally in guinea pigs (8).

D. SUMMARY

Zirconium compounds as they occur in nature pose no toxicological threat. However, in the past, use of certain zirconium salts in personal care and medicinal products resulted in granulomatous hypersensitivity if they became embedded in skin tissue.

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Appendix

A limited number of compounds are considered by the occupational health authorities of a number of countries to be an occupational hazard upon cutaneous exposure and are identified as such by a ''skin'' notation in the listing of hazardous chemicals. The purpose of such labeling is to raise attention to the fact that cutaneous absorption can present a significant risk of systemic toxicity. These listings are among the standard references routinely consulted by occupational health professionals.

While the ''skin'' notation has been adopted by several countries in their lists of occupational exposure limits, the number of chemicals so labeled varies, and the criteria for labeling are not uniform. Often those criteria are not made public, nor is justification given in the relevant supporting documentation.

In the present review, mention of ''skin'' notation is made when used by the American Conference of Governmental Industrial Hygienists (ACGIH) in the most recent (1993–1994) listing of threshold limit values (TLV), developed as guidelines to assist in the control of health hazards in the industrial environment. The criterion most frequently used for listing is acute animal toxicity from skin absorption, i.e., a dermal LD_{50} below 1000 mg/kg. This may be an indication of rapid skin penetration or extreme toxicity, or both. Also important is the fact that no procedure for determining the dermal LD_{50} has been standardized to date. While some materials can cause irritation, dermatitis, or sensitization following direct skin contact, these properties are not considered relevant for the assignment of a ''skin'' notation by the ACGIH.

Metals and their compounds currently listed as a skin hazard in the United States are decaborane; tin organic compounds; tetraethyllead; tetramethyllead; manganese cyclopentadienyltricarbonyl; 2-methylcyclopentadienylmanganesetricarbonyl; *tert*-butyl chromate; mercury metal vapor; mercury alkyls, aryls, and inorganic compounds; and thallium metal and its soluble salts (e.g., carbonate, acetate).

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Abbreviations

ACD	allergic contact dermatitis
ACGIH	American Conference of Governmental Industrial Hygienists
CNS	Central nervous system
Da	Daltons
DMSO	dimethylsulfoxide
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
EU	European Union
FCAT	Freund's Complete Adjuvant Test
FDA	Food and Drug Administration
GI	gastrointestinal
GPMT	Guinea Pig Maximization Test
IL	interleukin
LLNA	local lymph node assay
LNC	Lymph node cell
MMAD	Mass median aerodynamic diameter
MT	metallothionein
NAS	National Academy of Sciences
NMR	nuclear magnetic resonance
NRC	National Research Council
pg	picogram
PIXE	proton induced x-ray emission
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
RAST	radioallergosorbent test
ROS	Reactive oxygen species
SC	stratum corneum
SLS	sodium laurylsulfate
WHO	World Health Organization

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