

ROUTES OF ENTRY AND MODES OF ACTION

Herbert E. Stokinger, Ph.D.

Routes of Entry

There are at least three routes by which industrial substances can gain entry into the worker's body. In order of importance they are inhalation, skin contact, and ingestion. It is obvious that some substances may have multiple routes of entry. These will be noted in the appropriate section.

INHALATION

The adult human lung has an enormous gas-tissue interface (90 square meters total surface, 70 square meters alveolar surface). This large surface, together with the blood capillary network surface of 140 square meters, with its continuous blood flow, makes possible an extremely rapid rate of absorption of many substances from the air in the alveolar portion of the lungs into the blood stream.

Some highly water soluble substances, such as the soluble halogen salts (but not their acids) and soluble chromates (but not chromic acid), may pass through the lung so fast that none can be detected in this organ directly after cessation of their inhalation. On the other hand, there are many industrially important substances which, by reason of their extreme insolubility in body fluids, or their rapid reactivity with lung constituents, remain for extended periods in the lung. They resist complete clearance by phagocytic or other forms of clearance action and may result in irritation, inflammation, edema, emphysema, granulomatosis, fibrosis, malignancy, or allergic sensitization.

Some of the highly reactive industrial gases and vapors of low solubility can produce an immediate irritation and inflammation of the respiratory tract and pulmonary edema. Prolonged or continued exposure to these gases and vapors may lead to chronic inflammatory or neoplastic changes or to fibrosis of the lung. Fibrosis, as well as granulomatosis and malignancy, also may be produced by certain insoluble and relatively inert fibrous and nonfibrous solid particulates found in industry. Indeed, it is now thought that one of the prerequisites for particulate-induced bronchogenic carcinoma may be the insolubility of the particulate in the fluids and tissues of the respiratory tract, which thereby allows requisite residence time in the lung for tumor induction.

GASES, FUMES, VAPORS

The irritant acid gases as a group (the halogen acid gases and the basic oxide fume particulates, vanadium pentoxide fume and copper fume) are examples of direct, fast acting substances in the upper airway passages. Irritation of these passages occurs from these substances at

concentrations only slightly above the industrial air standard. Bis(chloromethyl) ether, on the other hand, is an example of a slow-acting irritant gas. It produces an esthioneuroepithelioma of the olfactory epithelium of the rat at extremely low concentrations (ca. 100 ppb) after several months of exposure. Many of the metal oxides of submicron particle size (fume) produce both immediate and long term effects; the latter may occur in organs and tissues remote from the site of entry. For example, cadmium oxide fume inhaled at concentrations well above the industrial air standard may produce immediate pulmonary edema that can be fatal; in addition, inhalation over many years of the fume at concentrations of a few multiples of the standard can result in eventual renal injury and pulmonary emphysema.

Very soluble gases, such as sulfur dioxide and ammonia at maximum tolerated concentrations (about 20 ppm for sulfur dioxide, up to 500 ppm for ammonia), seldom proceed much farther down the respiratory tract than the bifurcation of the trachea. Indeed these two gases are so soluble that for all practical purposes they are usually completely absorbed in the nasal passages, whereas the highly irritant but less soluble gases, such as nitrogen dioxide, phosgene, and ozone, reach the deeper recesses of the respiratory tract, affecting mainly the bronchioles and the adjacent alveolar spaces, where they may produce pulmonary edema within a few hours. If exposure is of sufficient concentration and duration, emphysema and fibrosis may ultimately develop.

Gases and vapors of low water solubility but high fat solubility, on the other hand, pass through the lung into the blood to be distributed to organ sites for which they have special affinity, provided they do not combine with blood components. Typical of those gases and vapors that exert their principal effects after absorption from the lung are such volatile liquids as carbon disulfide, volatile aliphatic hydrocarbons (the methane series), volatile aromatic hydrocarbons (the benzene series), the volatile halogenated hydrocarbons, and the aliphatic saturated ketones, such as methyl ethyl ketone, alcohols, and glycols.

It should be recognized and taken as a general rule, that each industrial chemical can affect a variety of bodily reactions, depending upon the nature and degree of exposure. For example, single exposures to carbon disulfide at levels several fold above the threshold limit value (TLV), can lead to narcosis and its sequelae; repeated daily exposures for many years at levels a few fold above the TLV can result in effects on the central nervous system (polyneuritis and psychosis) as well as on the cardiovascular system, liver, and kidney. Similarly, certain halogenated hydrocarbon solvents produce narcosis after brief exposures above the TLV; after long repeated daily exposures a few fold above the TLV, they may injure the liver or the kidney. Single, massive exposures to some of these substances can produce pulmonary edema.

ADSORPTION

The toxicologic action of some gases and vapors may be consider-

ably enhanced by adsorption on solid particles. The physicochemical theory for this is that those adsorbed gases which would normally never reach the deeper, more sensitive portion of the lung are carried there in very high concentrations when adsorbed on particles of small size.

There are now a number of instances that appear to confirm the above theory. A 27-fold increase in pulmonary airway resistance occurred from the inhalation of a mixture of the irritant gas, sulfur dioxide, and the inert particulate, sodium chloride, over that produced by sulfur dioxide alone at a concentration of 2.6 ppm. Other irritant gases in a sodium chloride admixture behave similarly. The serious health effects at Donora, Pa., are now attributed to the adsorption of sulfur dioxide on zinc ammonium sulfate. The Los Angeles eye irritation resulted from some unidentified vapors (aldehydes? peroxyacetyl nitrates?) adsorbed on smog particulates. Perhaps the most striking example of enhancement of toxicity from gaseous adsorption is that of radon; essentially no body retention of radon occurs if the inhaled air is dust-free.

BREATH ANALYSIS

If the inhaled gases and vapors are body-fat soluble and are not metabolized, they may be cleared from the body primarily via the respiratory system. Examples of these are some of the well-known industrial organic solvents; the volatile halogenated hydrocarbons; the volatile aliphatic, olefinic, and aromatic hydrocarbons (the methane and benzene series and certain olefinic homologues); some volatile aliphatic saturated ketones and ethers; aliphatic esters of low molecular weight; and certain other organic solvents such as carbon disulfide.

For those industrial solvents that continue clearing from the body in the exhaled breath for several hours following exposure, analysis of the rate of excretion in the breath of the exposed worker offers a laboratory test that may be very helpful in showing not only the nature of the substances to which the worker was exposed, but also the magnitude of the exposure and probable blood levels. By the use of gas chromatographic or infrared analysis of the breath samples, the identification of the substance is established, permitting comparison of the exposed workers' breath decay rate with published excretion curves (1). The physician can then estimate the magnitude of the original exposure. There is, however, considerable individual variation and it is not easy to set standard values.

PARTICULATES

The factors governing the sites of deposition, retention, distribution, and ultimate health effects of solid and liquid particulates obviously differ in most respects from those just mentioned for gases, fumes, and vapors. There are two exceptions, submicron particles $\leq 0.05 \mu\text{m}$ in diameter, which may act as gases adsorbed on particulates, and liquids adsorbed on particulates.

DEPOSITION

There are four major factors that influence the site of the ultimate toxicologic response to inhaled particulates: 1) the anatomic arrangement and physical dimensions of the respiratory system, 2) the physiologic character of breathing rate and depth, 3) the physical nature of the particle-size, surface area, "solubility," and hygroscopicity, and 4) the biochemical reactivity of the soluble components of the particle.

The knotty problem of handling particles of all sizes, shapes, and densities has been resolved by relating all particles to a median aerodynamic diameter. This is the diameter of a unit-density sphere with the same settling velocity as the particle of concern. The cut-off point for respirable size is conventionally taken as 5 μm expressed as an aerodynamic diameter.

The aerodynamic diameter of particulates determines which particles will or will not present exposure to the respiratory system and gives some indication of the degree of impaction in the various compartments of the respiratory system, and hence the site of particle deposition. Thus, a particle such as uranium dioxide with a high density of 10.9 and diameter of 0.5 μm will behave as a unit-density spherical particle of 1.65 μm , and can be expected to settle out of undisturbed air at the rate of a larger diameter particle, and impact more in the upper respiratory passages than its measured size would indicate. The sedimentation rate of fibers depends on their diameter and is independent of length. Fiber geometry is also important in relation to certain toxicologic properties, for example, in the induction of mesotheliomas.

To simplify calculations of the deposition pattern of the aerosol of concern, the manifold compartments of the respiratory system are reduced to three: the nasopharyngeal, tracheobronchial, and pulmonary (2). If the particulates are assumed to be present as log-normal distributions and three tidal volumes are used, a table can be developed showing the amount of particles deposited in each of the three compartments according to unit-density sizes, ranging from 0.01 to 10 μm . As might be expected, no particles less than 0.6 μm were deposited in the nasopharynx at any of the three breathing rates, whereas practically all of the particles greater than 6 μm were deposited at this site at all breathing rates. Thus, the major site of deposition of the smaller particles is the lung; deposition in the tracheobronchial compartment never exceeded 25 to 30% of the total particles inspired even at the smallest sizes (around 0.01 μm) and the slowest breathing rate (3). Although various degrees of mouth-breathing would upset the calculations of deposition in the upper respiratory tract, it is not believed to affect seriously pulmonary deposition.

Hygroscopicity, however, seriously affects deposition of smaller, highly water-soluble particles by increasing their size as they travel down the respiratory tract in its 95% humidity. Thus, in some instances, it is possible for a small size particle in an atmosphere of low humidity to

so increase its size in the respiratory tract as to alter considerably the deposition pattern characteristic of the entering particle size.

As is true with all generalizations, differences in deposition patterns can be expected, and indeed have been demonstrated, particularly for the lung where ventilation among the five lobes is normally variable.

Another factor which alters deposition patterns is electric charge, commonly associated with particle sizes less than $0.1 \mu\text{m}$ in diameter, i.e., newly generated fume. Such particles have enhanced nasopharyngeal deposition.

CLEARANCE AND RETENTION

In evaluating the health hazards from inhaled aerosols, four combined physio-chemical actions must be considered: 1) ciliary movement, 2) phagocytosis and lymphatic drainage, 3) direct intercellular penetration, and 4) solubilization or leaching.

Respiratory tract cilia do not extend beyond the terminal bronchioles. Particle clearance by upward ciliary movement takes place from the terminal bronchioles upward to the throat where the particles are transferred to the gastrointestinal tract by swallowing. Thus, exposure of the respiratory tract to particles may also involve exposure of the intestinal tract. With the exception of soluble particles impacting in the nasal passages and being absorbed there, clearance by solubilization from the tracheobronchial passages is not important.

Phagocytosis represents the major mechanism for clearing most particulates from the lung. Moreover, the presence of dusts stimulates the appearance of phagocytes at the site, so that repetitive exposures increase the rate of phagocytosis and hence the rate of clearance of dust from the lungs. Lymph drainage of the dust-filled phagocytes to the lymph nodes represents 2 to 10% of the clearance of the total pulmonary dust burden for certain insoluble oxide dusts.

Direct intercellular penetration offers another clearance mechanism of variable magnitude depending on the solubility, shape, and biologic activity of the dust. Thus, a particle that is not readily coated with serous protein, or other lung constituent, would penetrate the cell and then be cleared by this mechanism more readily than one that is coated.

Obviously solubilization represents the dominant clearance factor for particles readily soluble in respiratory tract fluids. Highly soluble dusts, such as the chromates of the alkali metals, pass through the lungs in a matter of minutes, and even grossly insoluble mineral dusts, such as certain types of asbestos, are subject to leaching of their metals and consequent clearance of these elements from the lung. A partial listing of inorganic compounds according to three pulmonary clearance classifications has been attempted for those compounds cleared in less than one day to 10 days, those requiring more than 10 days to 100 days for clearance, and those greater than 100 days, clearance time being expressed as biologic half-life (2).

While the site and degree of particle deposition is altered by a variety of factors and the magnitude of these factors is largely known, much less is known about the factors governing clearance. Mucociliary action, one of the major factors, varies with any factor or agent that affects ciliary beat or mucus production. They include temperature, humidity, industrial respiratory irritants, and cigarette smoking (4). The amount, site, and frequency of deposition also affect clearance rates indirectly, presumably by stimulating phagocytosis to varying degrees. Clearance rates differ appreciably among individuals (5).

SKIN CONTACT

Upon contact of a substance with the skin, four actions are possible: 1) the skin and its associated film of lipid can act as an effective barrier against penetration, injury, or other forms of disturbance; 2) the substance can react with the skin surface and cause primary irritation (dermatitis); 3) the substance can penetrate the skin and conjugate with tissue protein, resulting in skin sensitization; and 4) the substance can penetrate the skin, enter the blood stream, and act as a potential systemic poison.

Although one of the skin's principal physiologic functions is to serve as a protective barrier against entry of foreign substances into the body (6, 7), serious and even fatal poisonings have occurred from brief exposures of confined areas of the skin to highly toxic substances such as parathion and related organic phosphates, the organometallics, the alkyl leads and tins, and aniline, phenol, and hydrocyanic acid. Abrasions, lacerations, and cuts may greatly enhance the penetration of the skin.

How large a role the skin plays as a route of entry in occupational exposure can be seen by consideration of those substances in the American Conference of Industrial Hygienists' Threshold Limits list bearing the notation "Skin." Of the 579 items (*) in the 1976 Threshold Limit Value (8) booklet, 138 are listed under skin, indicating about one in four industrial substances presents an appreciable exposure via the skin. It should be noted, however, that appreciable exposure via the skin occurs generally from direct contact with undiluted substances, and for the most part exposure is not appreciable at or around the TLV. How important appreciable skin contact can be in industrial exposures is shown by substances, such as benzidine, which have negligible vapor pressure, but are readily absorbed through the skin. For such substances, the skin provides the major route of entry, and it is for this reason that no air standard has been set for these substances. Exposure is controlled by biologic monitoring or engineering and personal protective procedures.

(*) Items, not substances, for two reasons. Some listings refer to groups of substances, e.g., metals and insoluble compounds; others represent typical examples, e.g., substances in Appendices E and F.

ABSORPTION PATHWAYS

Although technically the two main routes of skin absorption are through epidermal cells (transepidermal) and through hair follicles and sebaceous glands (pilosebaceous), the transepidermal is the principal route because of the relatively small absorbing surface of the pilosebaceous units, even though these units offer greater permeability than the epidermal cell layers. Transepidermal absorption is influenced by the superficial barrier lying between the stratum corneum and the uppermost layer of living epidermis.

In general, percutaneous absorption of inorganic substances (electrolytes), including water, is negligible. Only those inorganic substances that are nonionic or ionize very slightly, such as boric acid, certain salts of mercury, and the halides of beryllium, are absorbed to any degree. On the other hand, fat-soluble substances (mainly organic compounds) are absorbed fairly rapidly.

Most substances that are both water- and fat-soluble, e.g., amines and nitriles, penetrate so rapidly that the rate of absorption is comparable to that of gastrointestinal or even pulmonary absorption. The numerous factors that affect absorption of hazardous substances through the skin can be grouped into physicochemical and physiologic.

PHYSICOCHEMICAL FACTORS

Chemical structure and its associated physical property, lipid/water solubility, are major determinants of absorption. Concentration and tissue reactivity, however, can take precedence over other physicochemical factors.

As a general rule, any substance having a caustic effect (such as phenol or cresols) or having a protein-coagulation effect (such as heavy or polyvalent metals), when contacted in high concentration, will be absorbed in relatively smaller amounts than when contacted at lower concentrations that do not cause protein coagulation. Moreover, prior contact of the skin with caustics or astringents decreases the absorption of other absorbable substances. Gases and vapors usually show increasing penetration with increasing concentration. However, skin absorption of gases, at or below the TLV contributes negligibly to the overall toxicity from air exposure. There appears to be a barrier, though relative, to even lipid and water-soluble substances; e.g., pentanediol and diethyleneglycol.

The reasons for the relative barrier effect are not known, but it is possible that chemical combination with skin constituents may fix such substances *in situ*. Some substantiation for this view is the finding of mustard gas fixed in the epidermis and corium of human skin 24 hours after application (9).

Changes in pH have been shown to aid penetration of some of the few substances that have been measured; certain surfactants are absorbed most readily from buffered solutions with pH values greater than 10.5. On the other hand, certain polyvalent metals are absorbed more readily from solutions of low pH.

Prior application of any solvent that removes cellular lipid, such as benzene, alcohol, or chloroform, increases barrier-cell permeability. An increased penetration of iodine and dyes follows the use of saponin, a cholesterol precipitant. Conditions leading to minimal ionization at skin pH probably lead to greater absorption.

Vehicles, despite common impression otherwise, play a negligible role in increasing percutaneous absorption; substances incapable of penetrating the barrier are not "carried through" by a vehicle. Vehicles do, however, enhance absorption by transappendageal route (7). This is accomplished by diminishing the surface tension between the liquid and the follicular pore and by bringing the substance into more intimate contact with the follicular pore and the hair canal.

Temperature elevation may be expected to increase skin absorption by increasing vasodilatation and thus increasing the rate of transport away from the skin, and by increasing the rate of diffusion.

PHYSIOLOGIC FACTORS

The barrier effect on percutaneous absorption of both the lipid surface film on the skin and the horny layer has been overestimated. The horny layer has large pores that can be penetrated even by large molecular aggregates; the waxy lipid film is miscible with water because it contains cholesterol, its esters and waxes, all of which are emulsifying agents. Hence, the major barrier to absorption lies between the stratum corneum and the uppermost layer of living epidermis.

Human skin shows great differences in absorption at different anatomic regions (10). If the skin of the forearm is used as a frame of reference, the palm of the hand shows approximately the same penetration as the forearm for certain organic phosphates and carbamate insecticides. The dorsum of the hand and the skin of the abdomen have twice the penetration potential of that of the forearm, whereas follicle-rich sites, such as the scalp, forehead, angle of the jaw, and postauricular area have 4-fold greater penetration. The intertriginous axilla has a 4- to 7-fold increase; the skin of the scrotum allows almost total absorption.

The physiologic factors promoting absorption are chiefly due to elevated temperature effects on the skin resulting in hyperemia and sweating (hydration of skin). Hyperemia, which can be caused by some factors besides temperature elevation, promotes skin absorption by increasing the rate of removal of the penetrated substance from the corium by providing greater concentration gradients between skin surface and deeper tissues. Gases and vapors are the substances most affected when in aqueous solution. Hair, an excellent collector of fine dusts, materially increases transappendageal absorption.

SWEATING AND HYDRATION

It has been recognized clinically and observed in industry (11) that absorption of toxic substances is more likely to occur when the clothing that is worn keeps the skin wet than when it keeps the skin dry. Sweat-

ing may increase the skin lipids, suggesting that the absorption of lipid-soluble substances should increase. Sweat is also instrumental in eliciting certain allergic cutaneous hypersensitivities (e.g., from chromium or nickel). As these apply to the metals themselves, it is the acidity of the sweat that leaches small amounts from the metals in contact with the skin to induce the allergic hypersensitivity. Data on the relative magnitude of the increase in absorption from a wet skin are very limited; absorption of ethyl nicotinate was increased by a factor of 6 for a cold-soaked arm, 12 for a hot-soaked arm (10).

ABRADED SKIN

It is evident from skin-stripping experiments that abrasion of the skin dramatically increases percutaneous absorption as far as the substances histamine and Privity are concerned. Differences between intact and abraded skin ranging from 10,000 to 100,000 times were found for both substances (10).

INGESTION

Health hazards to the worker from ingestion of industrial substances in comparison with those from the inhalation and skin contact routes are generally of such a low order as to warrant only limited discussion.

First, the number of substances that can be ingested are fewer as it is virtually impossible to ingest a vapor or gas.* Second, the frequency and degree of contact are very limited; mouth contact with substances on hands, in food, in drink, and on cigarettes is far less frequent, of shorter duration, and lesser in amount during the work shift than that with other routes of entry.

Third, and most important, toxicity by mouth is generally of a lower order than that by inhalation. Reasons for this include: 1) poor absorption into the blood stream; 2) subjection to relatively high acidity (pH 1 to 2) in passing through the stomach; 3) subjection to the alkaline medium of the pancreatic juice on passing through the small intestine. Both these latter may act to reduce toxic organic substances through hydrolysis to less toxic substances. Moreover, the pancreatic enzymes begin to convert (metabolize) some substances to less toxic moieties well before the parent substance is absorbed.

Favoring low absorption also are the following: 1) Food and liquid mixed with the toxic substance not only provide detoxifying dilution, but also frequently reduce absorption because of the formation of less soluble substances resulting from complex interaction with ingested substances in the gastrointestinal tract. 2) There is a certain selectivity in absorption through the intestine that tends to limit absorption of "unnatural" substances. 3) After absorption into the blood stream, the

*Exceptions—mouth-breathing, gum and tobacco chewers can absorb appreciable amounts of gaseous substances during an 8-hour workshift.

toxic material goes directly to the liver which further metabolically alters, degrades, and detoxifies most substances.

It is worth noting, however, that the ingestion route contributes secondarily to the intake of particulates by inhalation. That portion of inhaled material that lodges in the upper parts of the respiratory tract is swept up the tract by ciliary action and is subsequently swallowed.

Although the foregoing statements on reduced toxicity by ingestion in comparison with that by inhalation are true in general, there are obviously striking exceptions. Notable among the exceptions are those highly toxic elements with slowly cumulative action such as arsenic, cadmium, lead, and mercury. Recognition of the potential of such elements to add to the body burden through ingestion has led to prohibiting eating, drinking, and smoking in areas where there are such exposures.

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Modes of Action

Toxic substances exert their effects by physical, chemical, or physiologic (enzymatic) means, or by a combination of these. This classification is consistent with a definition of toxicity which includes irritation, narcosis, tissue injury and disease, sensitization, carcinogenicity, mutagenicity, and teratogenicity. Depending upon the state of development of toxicologic knowledge, the mode of action for a particular substance or group of substances may be described at the level of an organ or tissue, at the cellular level, or at the subcellular, molecular, or free-radical level. In what follows, the lowest level of effect consistent with available knowledge will be used. In some cases, this could lead to erroneous classification; i.e., those mechanisms now regarded as physical may later be shown to be chemical or physiologic.

PHYSICAL

Harmful substances that have a solvent or emulsifying action can produce, after prolonged or repeated contact, a dry, scaly, and fissured dermatitis. This effect is commonly attributed to the physical removal of surface lipid, but may also be caused by denaturation of the keratin or injury to the water barrier layer of the skin. Acid or alkaline soluble gases, vapors, and liquids may dissolve in the aqueous protective film of the eye and mucous membranes of the nose and throat, or in sweat, causing irritation at these sites. Moreover, such insults may erode teeth and produce changes in hair structure.

Irritants

On the inner surfaces of the body, the lungs and gastrointestinal tract, physical contact with unphysiologic amounts of substances causes irritation. This may lead to inflammation, or produce contraction, as in the reflex constriction of the respiratory passages upon inhalation of an irritant gas with resultant coughing or choking. In the upper gastrointestinal tract, the effect may include vomiting and, further down in the tract, the irritation may result in abnormal peristalsis and defecation.

Inert Gases

Inert gases can exert serious and often fatal effects simply by physical displacement of oxygen, leading to asphyxia. Under pressure, inert gases such as nitrogen can produce compressed air illness by dissolving in unphysiologic amounts in the blood, lymph, and intercellular fluids, or may rupture delicate membranes such as the eardrum. Sudden, or too rapid, decrease in pressure results in decompression sickness. Less inert gases such as carbon dioxide and oxygen under greater than atmospheric pressure can lead to narcosis and other more serious effects, such as pulmonary hemorrhage and nerve and brain damage.

Adsorption

Physical adsorption of gases or vapors on solid or liquid particulates (aerosols), may, upon inhalation, lead to physiologic effects out of proportion to that anticipated from their inhaled concentration prior to adsorption. The action is known as synergism when the effect of gas and particulate exceeds the sum of the effects expected from either alone, or antagonism when the effect is less than expected. A physical theory has been developed to explain these abnormal actions. It is based on the molecular properties of gases, and accounts for the synergism by postulating that "adsorbed" layers of the gas on inhaled particulates will carry to the sensitive lung tissue enormously increased concentrations of the gas that become localized, point sources of contact. Synergism or potentiation results when a rapid rate of desorption of the gas from the particulate to the tissue occurs; antagonism, when the desorption rate is very slow or nonexistent, or when chemical combination has occurred.

Radioactivity

Radioactive particles can cause dislocation and breaking of chromosomal linkages, apparently from local energy release, commonly referred to as the "oxygen effect."

CHEMICAL

Few body reactions progress by purely chemical processes. Among these few are the production of acids and bases (chlorides, phosphates, and sodium) and water and the liberation of bicarbonate into the urine.

There are other industrially important types of poisoning which proceed through mechanisms that do not involve the intervention of enzyme action but for which the energy may be supplied by chemical action.

Direct Combination

Among the best known and understood mechanisms of poisoning is that of direct chemical combination of the toxic substance and a body constituent, as illustrated by carbon monoxide poisoning. In this instance, the gas combines rapidly and rather firmly with hemoglobin, forming a new compound, carboxyhemoglobin, which cannot perform the usual function of hemoglobin, transporting oxygen to the tissues.

Hydrogen sulfide likewise unites with hemoglobin to convert it to sulfhemoglobin, a nonoxygen carrying pigment, although this mechanism is not important in hydrogen sulfide poisoning.

Indirect Combination

A less well understood mechanism of injury, but on which there is nevertheless an enormous amount of indirect evidence, is the release by

toxic substances of natural body constituents, such as histamine, in abnormal amounts that lead to injury and even death. Instances of this mechanism are numerous and involve the intake into the body of such common substances as "hay fever" allergens or other biologic allergenic materials, raw cotton dust and subtilisins, for example. Prominent industrial chemical allergens are the organic isocyanates which act as haptens with body proteins to become allergenic.

Intake of these substances results in release of histamine or histamine-like substances locally in large amounts with characteristic development of inflammation, edema, and other evidences of injury. Certain types of amines are capable of histamine release; in these instances the mechanism involved is believed to be one of displacement, whereby the tissue-bound histamine is displaced and liberated by the unnatural amine. Similarly, any type of simple cellular damage such as caused by respiratory irritants, e.g., ozone, results in the liberation of histamine-like substances.

There is accumulating evidence also that release of hormones from nerves may be the common mechanism by which a number of chemical substances exert their toxic action.

Chelation

A toxic mechanism that is increasingly being recognized to be one of the more common pathways of toxic action is chelation. Chelation is the term applied to the chemical combination of an organic substance and a metal whereby the metal is very firmly bound to the organic substance by both nonionic (organic) and ionic bonding. For example the therapeutic agent EDTA (ethylene-diamine tetraacetic acid) binds metals by chelation. Many drugs and antibiotics are now believed to act by chelation. By so acting, these substances exert their effects in a number of ways:

1) By removing biologically active metals that are normally bound in the cell or its components with resulting inactivation and cell damage. For example, treatment of lead poisoning with EDTA to remove lead may in addition remove other metals, such as zinc, that are required for important functions in certain kidney enzymes (e.g., carbonic anhydrase).

2) By reacting with fixed intracellular metals.

3) By chelating firmly with a fixed tissue constituent. This is believed to be the mechanism by which boron, as borate, exerts its toxic action. Borate is known to chelate with adjoining carbon atoms containing hydroxyl groups. If the structure prior to chelation happens to be a critical one in a metabolic chain, ordinary function ceases and injury occurs as a result of the altered chelated structure.

4) By increasing the absorption of a toxic agent. Instances are being recognized of toxicity resulting from abnormally increased amounts of absorption into the blood stream by a chelating compound. Iron, normally nontoxic when absorbed by the usual regulatory mechanism, may under unusual circumstances be absorbed in toxic amounts by the mechanism of chelation to form a soluble, easily absorbed substance.

TOXIC MECHANISMS

Of the three modes of action of toxic substances in the body—physical, chemical, and physiologic—the physiologic mode is most common.

Enzymes, the biologic catalysts of the body, estimated to total one million (1000 genes each governing an estimated 1000 enzymes), control the action of toxic substances. In what follows, an attempt is made to summarize the multitudinous ways that enzymes are involved in handling toxic substances following their entry into the body.

The toxicity concept can best and most simply be understood as the net result of the following two opposing reactions:

Reaction I — The toxic agent acts on the body.

Reaction II — The body acts on the toxic agent.

The net result of these two opposing reactions is the toxicity that is observed in any animal species. This is diagrammatically represented by Figure 1. The upward-pointing arrows are indicative of Reaction I; the downward-pointing arrows, Reaction II; arrows pointing in both directions are indicative of the known fact that in some instances, the homeostatic and adaptive Type II instead of providing beneficial reactions and reducing toxicity, actually result in harmful reactions and hence increased toxicity. In a typical example the “detoxication” of pyridine by methylation results in methyl pyridinium chloride which is eight times more acutely toxic than pyridine. See Figure 2.

Many toxic agents (insecticides, carcinogens, and drugs) stimulate liver enzyme activity, which in turn accelerates destruction of the toxic agent. Also, the production of immune bodies tends to counteract the action of a harmful substance (Reaction II) and hence reduces toxicity.

Chelation and combination represent a toxicity-reduction mechanism limited to metals. For example, the body’s methallothionein chelates firmly with cadmium (and zinc), removing it to less sensitive sites and reducing its toxicity. The limiting factor in such toxicity reduction is the body’s methallothionein reserves and capacity for methallothionein induction. Combinations of trace elements can be so effective a means of detoxication as to entirely antagonize the toxicity of highly toxic elements; e.g., an *in vivo* combination of mercury with selenium (and sulfur) can remove all traces of mercury toxicity.

The foregoing reactions result in an observed toxicity that is but a small fraction of the maximal toxic potential for those substances which react less than instantaneously. Obviously, Reaction II cannot occur with those substances that react on contact, e.g., unstable or highly reactive irritants such as ozone, nitrogen dioxide; or with high concentrations of those substances that immediately overwhelm these body defenses before they have time to come into play, such as high concentrations of the general asphyxiants, hydrogen sulfide, hydrogen cyanide, and carbon monoxide.

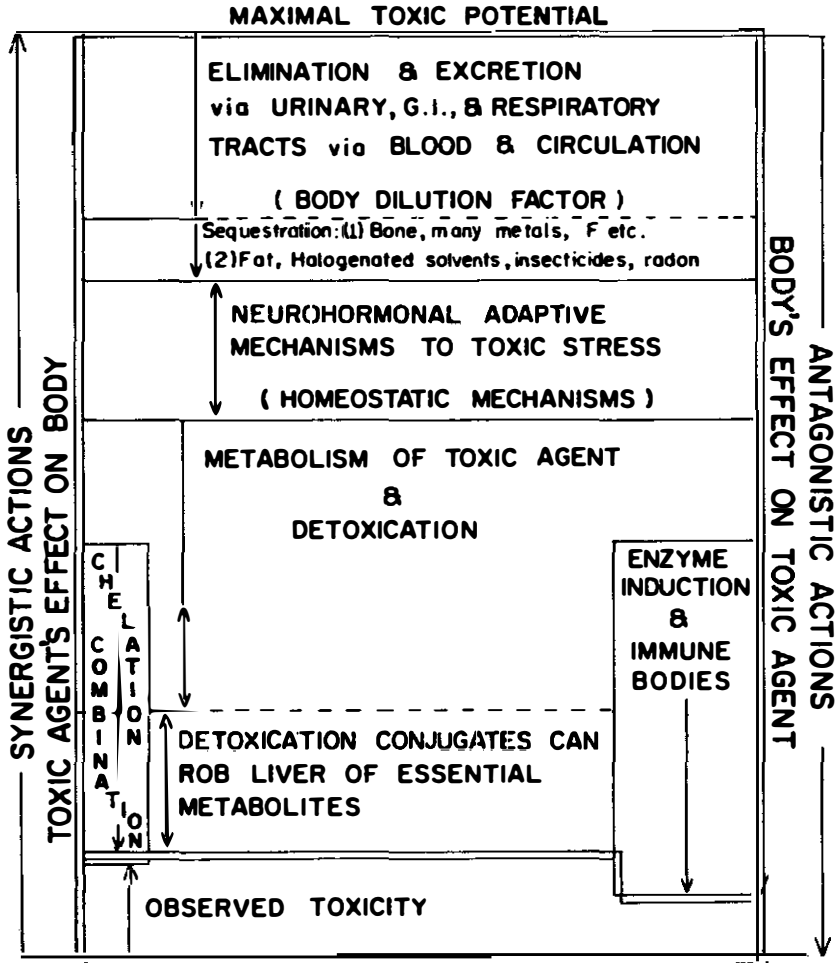


Figure 1. Diagrammatic concept of toxicity.





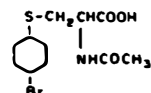

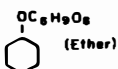
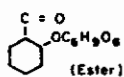
| Type | Toxic substance | Detoxication product examples |
|---|--|--|
| Methylation -CH ₃ | Inorg. compounds of As, Te, Se. Ring N compounds Certain complex aromatic phenols | (CH ₃) ₂ Se   |
| Acetylation CH ₃ CO- | Aromatic amines Amino acids (Known exceptions: aromatic amine carcinogens, also aliphatic amines.) |  RCHCOOH NHCOCH ₃ e.g., benzidine-hydroxylated aliphatic amines - aldehydes. |
| Ethereal sulfate -OSO ₃ H | Phenols (Cyclohexanol glucuronide) |  |
| Acetyl Mercapturic acid -SCH ₂ CHCOOH NHCOCH ₃ | Aromatic hydrocarbons Halogenated aromatic HC's Polycyclic HC's Sulfonated esters C ₂ H ₅ SO ₃ -CH ₃ Nitroparaffins (C ₄ H ₉ NO ₂) |  C ₂ H ₅ -acetyl cysteiy- C ₄ H ₉ -acetyl cysteiy- |
| Thiocyanate | Cyanide, inorganic Organic cyanides (Nitriles) | RCNS |
| Glycine -NHCH ₂ COOH | Aromatic acids Aromatic-aliphatic acids Furane carboxylic acids Thiophene " " Polycyclic " " (Bile acids) |  |
| Glucuronoside | Aliphatic (1°, 2°, 3°) and aromatic hydroxyl Aromatic carboxyl |  (Ether)  (Ester) |
| Glucose hydrazone | Hydrazine Hydrazine derivatives ? | NH ₂ N = CMC ₅ H ₉ O ₆ |

Figure 2. Major types of detoxication.

METABOLISM

Metabolism or biologic transformation of the toxic agent is one of the prime determinants for toxicity. These bio-transformations can be classified into three types of destructive transformation (oxidation, reduction and hydrolysis), synthetic transformation (conjugation) and enzyme induction.

OXIDATION

Oxidation is the most common form of bio-transformation reaction that occurs in response to a toxic substance. It includes the oxidation of alcohols and aldehydes to corresponding acids, the oxidation of alkyl groups to alcohols, the oxidation and hydroxylation of carbon ring compounds and ring-splitting, oxidation deamination of amines, oxidation of sulfur compounds to sulfoxides and sulfones, and dehydrohalogenation, all of which result in the body's excretion of highly oxygenated acids or acidic substances.

Although the intimate mechanism of oxidation is not precisely known in all instances, two general types are recognized: oxidation by direct addition of oxygen to the carbon, nitrogen, sulfur, or other bond; and oxidation by dehydrogenation. In either case, the energy required for the action is supplied by enzymes, resulting in free-radical reactions for the most part.

One of the most important examples in which oxidative mechanisms play a decisive role in the ultimate toxic response is the recently discovered metabolic formation of arene oxides. Arenes is the general term that applies to all aromatic nuclei including benzene and the polynucleated hydrocarbon carcinogens such as benzpyrene. The oxidation proceeds by the addition of an oxygen atom to two adjacent carbon atoms in the aromatic ring forming the so-called epoxide group. These epoxides are very unstable and highly reactive, and they, rather than the parent hydrocarbon, are believed to be the initiators of tumor production. In addition to subsequent isomerization to phenols, epoxides readily react with cellular macromolecules such as DNA, RNA, and protein, believed to be essential elements in tumorigenesis.

REDUCTION

Reduction of foreign substances is a less common body function than oxidation. It does occur, however, for those substances whose oxidation-reduction potential exceeds that of the body, such as nitro groups, certain aldehydes, certain oxidized forms of metal ions, and in certain reactions such as hydrogenation of carbon and nitrogen double bonds and reduction of disulfides to sulfhydryl derivatives.

In this connection, it must be noted that the entire body metabolism operates on an oxidation-reduction (O-R) system, poised at about the

potential of Vitamin C, which assumes the obvious fact that for each oxidation of a *natural body constituent*, there must be a reduction. Whether a toxic substance will be reduced depends upon its O-R potential relative to that of the body.

HYDROLYSIS

This reaction, which involves cleaving of a bond with the addition of water, is another means by which the body metabolizes and degrades toxic substances. If organic in nature, hydrolysis is performed by enzymes; if inorganic, by simple chemical action, as beryllium sulfate hydrolyzes to the (colloidal) hydroxide on entering the body.

Typical foreign substances that undergo hydrolysis are esters of all types; compounds of carbon, nitrogen, sulfur, and phosphorus, resulting in the formation of component acids and alcohols; amides which are hydrolyzed less well to the corresponding acid and ammonia; and ethers which are split by different enzymes, depending upon the nature of the alkyl group (the rate of splitting of aromatic ethers depends upon the nature and position of the substituents on the ring). Simple aliphatic ethers are excreted unchanged mainly via the respiratory tract; however, more complex ethers such as benadryl are split.

CONJUGATION

Synthetic mechanisms directly involved in the normal metabolic processes provide major pathways for disposing of toxic substances. These synthetic pathways involve conjugation of the toxic substance (any that the body cannot readily oxidize to carbon dioxide and water) with a limited number of defined body constituents. Figure 2 shows eight major types of conjugation and examples of the types of substances involved in each conjugation. These conjugation reactions, performed enzymatically, involve carbohydrate (glucoside formation), amino acids, methyl and acetyl groups, and sulfur derived indirectly from sulfur-containing amino acids.

These conjugates have two important properties essential for detoxication. They are, in general, considerably less toxic than the parent substances (with certain exceptions) and they are readily excreted in the urine. The well-known synthesis of phenylsulfate, which was one of the earliest synthetic mechanisms to be discovered (1876), converts highly toxic phenol to a substance which is practically nontoxic. Cyanide, both inorganic and organic forms, is synthesized to thiocyanate, a structure many times less toxic than cyanide. Certain toxic metal ions may react with sulfur of the body to be excreted as insoluble, and, thus nontoxic, metal sulfides.

It should be pointed out that these synthetic detoxifying mechanisms are not entirely free of injury to the body. In contributing some of its constituents, the body may deprive itself of vital amounts of these substances if synthesis is prolonged, and thus injure itself (Figure 1).

DETOXICATION AND BIOLOGIC INDICATOR

There are several important benefits to be derived from a knowledge of detoxication mechanisms. Such knowledge offers a biologic means, through analysis of appropriate body fluids or excreta, for positively identifying the agent to which the worker was exposed. If the analysis is performed quantitatively, it permits a reliable estimate of the degree of exposure. This capability permits, in turn, the development of biologic threshold limits for the control of worker exposure on an individual basis, because biologic estimates of exposure take into account absorption of the industrial substance by all routes, personal work habits, and hereditary characteristics. Furthermore, biologic analyses help evaluate the extent of the worker's exposure to a new product.

ENZYMES

The ultimate regulators of metabolism are enzymes and their associated factors of trace elements, vitamins, hormones, and antimetabolites. Substances that act chemically to produce injury to organs and tissues of the body usually do so by two basic means: either by depressing or by stimulating the activity of the enzyme systems. Severe, acute effects such as destruction of cell membrane integrity by corrosive agents or protein coagulants, etc., are obvious exceptions. A single substance may have more than one pathway and site of action. Multiple pathways of action may be invoked simply by differing doses of the toxic agent; low doses may stimulate enzyme action, high doses depress and inhibit the same or different enzyme systems. This is a characteristic action of most, if not all, toxic substances, including arsenic, benzene, chloroform, cobalt, fluoride and vanadium. A number of aspects of toxicity are shown in Figure 1.

1) Systemic toxicity is, by and large, a matter of the activity of enzyme systems, either by inhibition or overstimulation (removal of a natural inhibitor system), all accomplished at the free-radical level.

2) Substances display differing toxicities and have selective sites of action because different substances affect, to differing degrees, the various metabolic compartments and, thus, raise or lower the level of "observed toxicity." Different substances have differing chemical affinities for tissue sites.

3) Potentiation and synergism, the enhanced toxicity of two or more simultaneously acting substances, can be explained by the action of one preventing the elimination or the metabolism of the other, wholly or in part, thus maintaining elevated systemic levels of the toxic agent, resulting in an observed toxicity greater than the additive toxicity of the combined components (Figure 1).

4) Antagonistic action is explained by one component preventing, wholly or in part, the toxic action of another. This occurs when one component induces or supplies additional amounts of a critical enzyme system or factor that is being attacked by another component, the net result being to greatly reduce or even completely eliminate toxicity. A

similar mechanism appears to explain the antagonism of ethyl alcohol for methyl alcohol toxicity. In this case the liver alcohol dehydrogenase preferentially attacks ethyl alcohol, thus slowing down or preventing the oxidation of methyl alcohol to neurotoxic metabolites (Figure 1).

As mentioned previously, most of the metabolic activity of the body is a result of the activity of enzymes, which are biologic *catalysts* formed by living cells throughout the body. Consequently, it is reasonable that the bulk of all toxic mechanisms should involve interference in some way with normal enzyme activity.

All enzymes have a basic protein structure composed of 20 or more amino acids grouped in various chain arrangements in a three-dimensional structure. To perform the myriad of metabolic reactions of the body requires an estimated million diverse enzymes. This diversity of structure and function makes any simple classification inadequate. However, just as the major types of metabolism and detoxication were classified (Figure 2), so can the major metabolic reactions catalyzed by enzymes be classified.

CLASSES OF ENZYMES

The enzymes that perform oxidation-reduction reactions constitute one of the larger groups. The oxidases, which reduce the inhaled oxygen carried throughout the body by hemoglobin and myoglobin, reduce oxygen directly. One of the most important of these is cytochrome oxidase. Other important oxidases are xanthine oxidase with riboflavin as a prosthetic group, the polyphenol oxidases, with copper as prosthetic group, and tyrosinase responsible for the oxidation of tyrosine to the dark melanin pigment.

Closely related in action are the dehydrogenases, which catalyze the removal of hydrogen, and thus "oxidize" organic molecules. As body oxidations generally (respiration) proceed in this manner, there are several highly specific dehydrogenases. All cellular respiration involves three major classes of dehydrogenases: 1) pyridine-linked dehydrogenases, which require a dinucleotide as coenzyme, 2) flavin-linked dehydrogenases, which contain a flavin nucleotide, and 3) the cytochromes, which contain an iron-porphyrin ring system. More than 150 of the pyridine-linked dehydrogenases are known. One of these, glucose-6-phosphate dehydrogenase (G-6-PD), features prominently as the key system in rendering a worker hypersusceptible to hemolytic industrial chemicals. A genetic deficiency in G-6-PD can make a person susceptible to incurring a hemolytic crisis from exposure to such chemicals by either blocking the action of certain components of the G-6-PD system in the red cells or by the chemical's utilizing the hydrogen critically needed for cell respiration, resulting in loss of red cell integrity, and consequent cell lysis.

Another large and diversely acting group are the hydrolytic enzymes, chief among which are the phosphatases, which hydrolyze esters of phosphoric acid. These enzymes are involved in all catabolic (destruc-

tive) and anabolic (synthetic) reactions of the cells. Other representative hydrolytic enzymes are the esterases, such as liver esterases and pancreatic esterases. Others in this group are those that hydrolyze protein structures, proteolytic enzymes that break the common peptide bond of these structures. This group is further comprised of more specialized enzymes, the peptidases, the carboxy- and aminopeptidases, so named because of action on peptides with adjacent carboxyl (COOH) or amino (NH₂) groups; those that hydrolyze glycosidic linkages, the carbohydrases, which act on polysaccharides and glycosides.

The decarboxylases are a widespread group composed of keto-acid decarboxylase, which is responsible for the liberation of the end product of metabolism, carbon dioxide. Amino acid decarboxylases are responsible for the formation of amines by carbon dioxide liberation from amino acids. In the chain of metabolic end reactions, oxidative deaminating enzymes remove the amino group from these toxic amines, be they endogenous or of foreign origin, resulting in reduced toxicity, liberation of the end product, ammonia, and its excretion in the urine. Some of the ammonia, however, is transferred to other substrates by transferases. These transferases can also transfer other groups such as methyl, phosphate, and amino groups.

The above classes of enzymes, with other enzymes not classified, represent all the metabolic catalysts the body can muster to handle foreign chemical structures. As these structures may vary from closely similar to remotely related to the natural substrates of these enzymes, it is not difficult to see that destruction of a foreign toxic substance can range from nearly complete to scarcely perceptible.

ENZYMATIC ACTION

Enzymatic actions occur throughout the body without restriction to any particular organ site, although the liver cells perform a major proportion of the metabolic activity of the body. Similarly active, however, but less diversified, are the enzymes in the lung, kidney, intestine, brain and nervous tissue, and bone. From this, it may be inferred that enzymatic mechanisms may occur with the enzyme situated at cell surfaces or within the cell itself. *Although the activity of enzymes, in normal circumstances, occurs within or on cells which are inaccessible for measurement (except as biopsied tissue), toxic injury to cells may result in enzyme release in proportion to the injury into the blood and body fluids where they can be measured and serve as biologic indicators of exposure and/or response.*

In "metabolizing" a foreign substance, it is important to observe that the enzyme is merely performing a function that it normally performs in metabolizing natural foodstuffs; no special enzymes exist to metabolize toxic substances. Although "drug-metabolizing" enzymes are commonly mentioned, this does not mean that the body develops a new class of enzymes in response to the administration of a drug; drugs may, however, act to induce larger amounts of enzyme activity.

ENZYME CHARACTERISTICS

It is now recognized that certain enzymes, heretofore considered homogenous in composition and in action, may consist of several distinct components, each still acting, however, on the same substrate; these components are called isoenzymes, or isozymes. Isozyme components can differ in number and activity, depending on the tissue of origin, e.g., lactic acid dehydrogenase has as many as five different isozymes, depending upon whether originating from the heart, kidney, liver, or lung.

Many enzymes have additional specificity requirements, in that they require a metal or a vitamin, or both, as cofactor(s) or activator(s). For example, the enzyme cocarboxylase that splits carbon dioxide from certain organic acids, requires vitamin B₁ and magnesium ions as necessary constituents before it can function.

Because enzymes are proteins, they exhibit the physical and chemical properties of proteins. They undergo denaturation 1) by heat, as in burns; 2) by marked changes in acidity or alkalinity as effected, for example, by contact with corrosive agents; or 3) by chemical denaturing agents, such as urea in high concentrations. These agents alike cause structural and configurational changes in the protein, and the characteristic specificity is lost, and with it the catalytic activity of the enzyme.

Enzyme activity can be inhibited in a number of ways. For example, among the enzymes requiring a specific metal as activator, any agent that will displace or render inactive the metal will render the enzyme inactive to the degree that the metal was rendered inert or removed from the enzyme. Certain metals with similar spatial requirements for the specific metal required by the enzyme may do this. Certain poisonous metals such as beryllium are believed to act in this way. Cyanide may combine with the iron of an iron-dependent enzyme and inactivate or inhibit the enzyme.

Another common way an enzyme may become inhibited is from competition with a substance whose structure is sufficiently similar to the natural substrate, but does not quite fulfill the spatial requirements of the enzyme. This is probably the most common way in which toxic substances exert their effect on enzymes. Common examples are the competitive inhibitive actions of the various closely related organophosphate pesticides.

A third way by which enzyme activity is inhibited is by accumulation of the product of the enzyme's activity. This is one of the natural ways by which body enzyme activity is regulated and is known as metabolite inhibition.

The fundamental aspects of enzyme activity with respect to toxicity may be summarized as follows. Enzymes combine with the toxic substance. This combination may result in partial or complete inhibition of enzyme activity or the enzyme may act on the toxic substance more or less incompletely, possibly with the production of even more toxic substances, but generally with production of degraded, less toxic substances. If the enzyme whose activity is blocked is a critical one, there may be

slowing down of some vital function, resulting in alteration of cellular constituents in amount or type, even in cell death.

DIRECT COMBINATION

The simplest way by which a toxic substance can alter enzyme action is by direct combination of the substance with active groups on the enzyme structure. Such is believed to occur with metals such as mercury and arsenic which combine so tightly with the active group of the enzyme that further action is blocked. If the enzyme or enzymes represent critical systems for which there is no shunt mechanism, then cells may die or function subnormally resulting ultimately in injury to the cell, the organ, and the host. Similarly, nonmetallic substances such as cyanide can combine with and block the action of heavy metalbearing enzymes because of the production of an inactive metal-cyanide enzyme. The blocking of this enzyme system to a significant degree results in the well-known fatal cyanide poisoning.

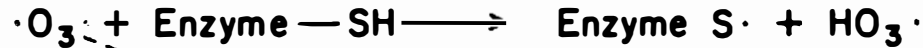
Another mechanism of poisoning by direct combination is illustrated by substances such as ozone and nitrogen dioxide, and possibly iodine and fluorine, that destroy enzymes by oxidation of their functioning groups (Figure 3). In these cases, specific chemical groups such as -SH and -SS- on the enzyme are believed to be converted by oxidation to nonfunctioning groups; or the oxidants may break chemical bonds in the enzyme leading to denaturation and inactivation.

One of the more commonly encountered enzyme inhibition mechanisms of direct combination in occupational exposures is that of the inhibition of the action of cholinesterase (acetylcholine esterase), an enzyme that regulates nerve-muscle action by destroying the muscle excitor acetylcholine. This muscle excitor is a powerful pharmacologic substance, which, if not destroyed when it is free, can act as a poison. The destruction is accomplished by the hydrolysis of the potential poison into its components, an acetyl group and choline. A large number of pesticides, chiefly organic phosphates and carbamates, act in the body by blocking this enzyme action, thus allowing excessive amounts of the muscle stimulator to accumulate. The excessive stimulation results in paralysis of the host.

COMPETITIVE INHIBITION

A second, and one of the more usual toxic mechanisms involving enzymes, is that of competition of the toxic substance with normal metabolites, or the cofactor(s) essential for enzyme action, for the site of action on the enzyme. This form of competition is highly effective, and thus injurious, only when the chemical structure of the competing toxic substance resembles that of the constituent normally used by the enzyme; the closer the structural similarity, the more effective the competition.

The successful competition of an unnatural or foreign toxic substance for the enzyme sites of action blocks normal action by not permitting either significant amounts of normal substances to be metabolized, or by preventing combination of a cofactor necessary for enzyme action.



Small reactive fraction Abnormal reacting free radicals



$\text{HO}_3\cdot + \text{Enzyme-S}\cdot \longrightarrow \text{HO}_2$ initiates a series of damaging chain reactions (large effect) ending in inhibited enzyme-S-S-enzyme.

Enzyme may be reactivated or active enzyme "protected" by:



Figure 3. Ozone-initiated free radical chain reactions in respiratory tract.

The cofactor can be a metal or a highly complex specific organic substance such as a vitamin.

Competitive inhibition, first shown to be the action of sulfanilamide by reason of its close similarity to the B vitamin, para-aminobenzoic acid, has been demonstrated to function similarly in many other drug actions; it is also the basis of the mechanism of action of a number of anticancer drugs, many of which are appreciably toxic, for example, the fluoropyrimidines.

Toxic mechanisms may operate also by metal-to-metal competition. For example it is believed that the poisonous action of beryllium results from its capacity to compete effectively for the sites of combination of magnesium and manganese on critical body enzymes, by which action the enzyme is no longer able to function at its normal rate or may be inactivated completely. This competitive inhibition of foreign metals is a very general way by which metals exert their toxic action.

A highly interesting example of a competitive mechanism is that recently found to explain the increased toxicity sustained following simultaneous exposure to two structurally similar economic poisons, malathion and EPN. EPN is highly toxic while malathion has a far lower order of toxicity. When the two substances are present in the body together in sufficient quantity, however, EPN prevents the elimination of malathion, maintaining its concentration, and raising its toxicity, so that the summated toxicities of both are far beyond expectation.

Inasmuch as both substances have chemically similar structures, EPN effectively competes for the same enzyme that hydrolyzes and thus would otherwise reduce the toxicity of malathion. By inhibiting this enzyme action, the concentration of the toxic form of malathion is maintained at a high level in the body, and consequently the toxicity is enhanced.

This is not an isolated instance of such a competitive mechanism. A number of other combinations of economic poisons are believed to produce enhanced toxicities by similar mechanisms, for example, the combinations malathion and Dipterex, and Guthion and Dipterex.

LETHAL SYNTHESIS

Another means by which enzymes are involved in toxic mechanisms concerns the synthesis of a new toxic product by enzyme action on the toxic substance originally taken into the body. The newly synthesized product then exerts its toxic effect by interfering with normal metabolic processes.

A striking example of a substance involved in this type of mechanism is the rat poison 1080, sodium fluoroacetate. After it is absorbed into the body, an enzyme transfers the fluorine atom in fluoroacetate so as to form fluorocitrate from citric acid, an important intermediate in the cycle of terminal metabolism. This fluorocitrate is unable to function to a significant degree in this important metabolic cycle and breaks the metabolic chain of activity, with the result that tissue respiration ceases, and death ensues.

TOXIC ENZYMES

A rather unusual type of toxic mechanism results when the toxic substance itself is an enzyme. Toxic enzymes are associated with the introduction into the body of substances such as snake and bee venoms and bacterial toxins. Although these substances exhibit a variety of toxic manifestations, the mechanisms of some of which are as yet unknown, the venoms of bees and certain snakes possess enzymes (phosphatidases) that lyse red blood cells, destroying the oxygen-carrying power of the blood, as well as enzymes (proteolytic) that destroy cells and inhibit blood coagulation. In addition, bee venom contains a substance that inhibits the dehydrogenases, enzymes important in the metabolism of many body functions.

INDUCIBLE ENZYMES

Thus far all of the mechanisms discussed have been depressant in action, but the response to toxic substances may under certain conditions stimulate metabolic activity. Inducible (adaptive) enzymes are those enzymes which permit the physiologic synthesis of additional amounts of the enzyme in response to the presence of an inducing agent. In this instance, the inducing agent may also be toxic.

Because inducible enzymes are difficult to demonstrate in the mammalian host (although a number have been so demonstrated in bacteria and yeasts), only few instances of industrial health interest are presently known in detail. High sucrose diets fortified with vitamins fed for 3 weeks to rats stimulate the enzymatic production of additional amounts of protein sulfhydryl groups in the kidney, which enables the rats to withstand otherwise lethal doses of mercury. The newly-formed sulfhydryl binds the mercury firmly, thus effectively reducing its toxic potential. High sucrose diets similarly protect against ozone lethality.

A mechanism exemplifying stimulation, probably mediated through inducible enzymes, is the increased production of serum alpha globulins by cobalt when absorbed into the body at relatively low levels of intake. At slightly higher levels of intake, cobalt stimulates the production of increased amounts of red blood cells (polycythemia production); associated with the polycythemia is increased production of hemoglobin. The exact mechanism of this stimulation is not known, but a hormone, erythropoietin, whose production is stimulated by cobalt, is believed involved. It appears also that the action of erythropoietin is not entirely restricted to stimulating bone marrow to increased production of red cells, but may include stimulation of other centers as well.

SECONDARY ENZYMATIC MECHANISMS

In this category are grouped those pathways of metabolism and mechanisms of injury that are not effected by the direct action of the toxic substance but develop either 1) as a result of metabolic alteration of the toxic substance following its entrance into the body or 2) as a consequence of an accumulation of toxic by-products from the initial,

direct action of the toxic substance. In the second instance, further injury occurs at a site in the body different from that of the original toxic action. Most, if not all, of the mechanisms considered here are performed by enzymes.

The body does not always act to its own advantage when handling a foreign, and sometimes toxic, substance. These peculiarly disadvantageous reactions result, however, merely because the body is equipped with certain definitive pathways of metabolism derived from the biochemical processes concerned with the utilization of food components or other environmental substances. These are its only resources when confronted with nonfood substances, and accordingly, these mechanisms are used insofar as they can act on foreign substances bearing chemical structures similar in some respects to food substances or other natural environmental materials. Whether this indiscriminate action by the body's enzymes results in an outcome favorable or unfavorable to the body depends only on the nature of the resultant modified foreign substance and not on any selective or guided action of enzymes.

METABOLISM

Mechanisms grouped here comprise all those metabolic activities that the body performs on a toxic substance in contradistinction to the actions that the toxic substance performs on the body. Broadly, the so-called "detoxication" mechanisms are those performed by the body, whether as a primary or secondary mechanism, in the process of attempting to eliminate the toxic substance, namely, oxidation, reduction, and synthesis. A few examples of each of these as a secondary mechanism will be given for well-known industrial substances of a toxic nature.

Oxidation

An example in which secondary oxidative mechanisms are believed to play a dominant role in the toxicity of an alcohol is that of methyl alcohol. Oxidation to formaldehyde, which subsequently interferes with oxidative enzyme synthesis, is believed to be the pathway by which methyl alcohol exerts its injurious effect on the optic nerve leading to blindness. Ethyl alcohol, and presumably other alcohols, proceed through this metabolic pathway of oxidation to the corresponding aldehyde, which is responsible, in part at least, for the toxic effects.

In this connection, it should be recognized that by no means do all metabolic alterations in the structure of toxic organic substances result in toxic by-products. A sizeable number of the metabolic products are detoxified in the process.

A striking example of the role of oxidative mechanisms in developing toxicity of organic substances is seen in the organic thiophosphate insecticides such as parathion. These substances, containing sulfur in the molecule, are relatively nontoxic until oxygen replaces the sulfur forming the "oxones" which are extremely toxic, completely inhibiting an important enzyme of nerve function, cholinesterase.

An example of oxidation among inorganic toxic substances is that

of uranium. The tetravalent form is unstable to the body's oxidation-reduction potential, and is oxidized to the more toxic hexavalent form. The hexavalent form then combines with active sites (phosphate groups) on the surface of cells, blocking normal metabolic processes necessary for cell survival.

Much, if not all, of the toxicity of the long-recognized poisoning action of aniline arises not from aniline itself, but from its various oxidation products formed in the body. The more important of these are para-aminophenol and, by further oxidation, the quinoneimine which is believed responsible for the methemoglobinemia that develops when aniline, or other aromatic amines, are absorbed into the body. The oxidized product of aniline oxidizes the ferrous iron of hemoglobin to the ferric form, resulting in methemoglobin, which is incapable of releasing oxygen.

Reduction

Although reduction is far less common a body function than oxidation, nevertheless several types of foreign organic substances are metabolized by this pathway to produce one or more substances that are more injurious than the parent substance. Among certain of the inorganic metal ions, reduction is also the pathway of metabolism. Organic nitro-groups are reduced by stages to amines. Some aldehydes are reduced to alcohols.

Unsaturated double bonds of carbon compounds may add hydrogen and thus become reduced. These types are not an exhaustive listing.

In general, however, reduction, contrary to oxidation, tends to result in products that are less toxic than the original substances, for example, reduction of aldehydes to alcohols, and are thus of lesser interest here. On the other hand, metabolism of nitrobenzene results in a number of products, one of which, para-aminophenol, is from 50-80 times more acutely toxic than the parent nitrobenzene.

Among inorganic ions, pentavalent arsenic is relatively inactive in the body until reduced to the trivalent state. The physiologically active form of manganese is trivalent. If manganese is taken into the body in the form of pyrolusite in which the manganese is tetravalent, reduction to the active form must occur, at least to that portion which is absorbed into the blood stream and later incorporated into active tissue components.

Synthesis

Synthesis is one of the more common means whereby the body contributes some tissue constituents in the conversion of the foreign substance to a new product, thereby disposing of the toxic agent. There are more than a dozen known synthetic mechanisms to accomplish this.

It should be pointed out, as noted previously, that these synthetic detoxifying mechanisms are not entirely free of injury to the body. In contributing some of its constituents, the body may deprive itself of vital amounts of these substances if synthesis is prolonged, and thus injure itself.

SECONDARY ORGAN INVOLVEMENT

A secondary mechanism of very general nature, and of considerable toxicologic importance, involves the indirect action of either the toxic agent or its metabolic by-products, or both. Once having injured a primary site, the substance may cause either the production or accumulation of deleterious products that in turn affect a secondary site.

A striking example of this secondary mechanism is the action of hexavalent uranium, which first injures the kidney in such a way as to prevent normal elimination of waste products such as urea, ammonia, and other substances. These products accumulate in the blood stream and injure the liver, resulting in fatty degeneration of this organ.

Similar indirect injury occurs when the lung, through direct injury by some toxic substance, restricts blood flow thus placing undue stress on the heart (cor pulmonale).

There are numerous other examples; in fact, the function of the body is so organized that there are few alterations of significant magnitude in an organ or tissue site that do not have repercussions in some other organ even at a remote site. The interlocking activities of the endocrine glands, with their respective hormones and their dependence on vitamins and minerals for normal function, is the basis for this entire group of secondary mechanisms.

An interesting example of the involvement of these highly sensitive interlocking endocrine systems is the simple inhalation of nonlethal concentrations of ozone, which produces alterations in the activities of the adrenal glands and disturbs the normal uptake of iodine by the thyroid gland, which in turn alters the activity of the thyroid-stimulating hormone of the pituitary body.

IMMUNE MECHANISMS

A mechanism whose toxic significance remains to be fully evaluated, but which nevertheless has been recognized for many years, is the stimulation of immune mechanisms as a result of the production of a new antigenic structure from the combination of a toxic substance with body constituents, usually protein. This mechanism is thought to be the basis of skin sensitivity resulting from contact with certain reactive organic substances, for example, the chloronitrobenzenes.

Another substance that illustrates this mechanism strikingly is toluene diisocyanate and related aromatic isocyanates. These substances, upon inhalation, have unusual avidity for combining with body protein with resultant allergic sensitization of the respiratory tract.

ENZYMOLGY AND ITS USES

As the practice of industrial hygiene and occupational medicine are increasingly involved in the control of worker exposure and prevention of industrial disease, knowledge of how enzymatic mechanisms are involved in the toxic response becomes of increasing importance. Measurement of enzyme activity and identification of metabolic products have

been refined so that the clinical toxicologist can use these measurements as an aid in diagnosis of toxic injury and can identify early indicators of response, from the quantitative determination of which, biologic threshold limits can be developed.

These measurements fall into two broad categories: 1) those that measure *exposure*, as arsenic, fluorides, lead, mercury, and benzene, in blood, urine, hair, or breath; 2) those that measure *response* from exposure, as changes in amounts of a natural body constituent, a body metabolite, or changes in enzyme activity.

Some common examples of measurement of exposure: urinary arsenic, fluoride, lead, phenylsulfate from benzene exposure, dichlorodiphenylacetic acid (DDA) from dichlorodiphenyltrichloroethane (DDT). Among the better known measures of response are the measurement of inhibition of activity of plasma cholinesterase for effects from exposure to organic phosphate and carbamate insecticides, changes in urinary delta amino levulinic acid or its enzyme in the red blood cell as responses to overexposure to lead.

Measurement of changes in biochemical constituents as a result of exposure to toxic amounts of substances can be extended to many other industrial substances to which the worker may be significantly exposed, provided they are metabolized by the body. The following are exceptions: 1) Those substances which are constituents common to the body or which convert to same, e.g., sulfur dioxide, chloride, phosphate, or which create no alteration in body composition or function; 2) fast-acting substances such as (skin) irritants that decompose upon contact with body surfaces such as nitrogen dioxide, bis(chloromethyl) ether; 3) substances with predominantly sensitizing properties.

About 2,000 metabolites of substances of industrial interest are now recognized. Accordingly, to use them as biologic indicators, it is necessary to select the appropriate metabolite from this number, and to develop procedures for its quantitative determination, or in the case of a new substance without known metabolites, determine the readily measurable metabolite(s) and apply the procedure to the exposure situation.

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Editors —

Marcus M. Key, M.D.

Austin F. Henschel, Ph.D.

Jack Butler, M.D.

Robert N. Ligo, M.D.

Irving R. Tabershaw, M.D.

Manuscript Editor —

Lorice Ede, J.D.

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