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Geosciences and chemistry and the scientific discipline of toxicology itself have been on parallel, often intertwined paths for many years. Issues related to toxic substances from natural sources, such as arsenic, lead and other metals, and from the contamination of soil and groundwater, have been recognized from the historical beginnings of the discipline.

Toxicology obviously plays a central role in medical geology. The scientific principles of toxicology apply to medical geology in three broad areas: clinical toxicology, risk assessment, and hazard control and monitoring. Clinical toxicology is the recognition, diagnosis, and management of human toxicity and in environmental medicine reflects the outcome of environmental chemical exposures. Toxicology plays an essential role in risk assessment both in characterizing the potential toxicity of a chemical hazard, the first step in the process, and in providing the conceptual framework upon which an estimate of safe exposure is derived for regulatory purposes. People may encounter chemical hazards in the course of daily life or in their jobs that are natural or that result from some disturbance of naturally-occurring hazards. Hazard control is more in the domain of occupational and environmental health and is necessarily based on an understanding of the physicochemical characteristics of the chemical hazard. Here toxicology provides the essential information needed to design a control system and to set priorities for control.

Toxicology engages other sciences as well as “medical geology”, pharmacology, medicine, and chemistry. Toxicologists often work closely with epidemiologists, who use statistical methods to determine the distribution of diseases, their risk factors, and health characteristics of populations rather than individuals. Because the delineation of “safe” levels of exposures assumes a socially determined level of acceptable risk (which is implicit in the definition of “safety”), toxicology has been adapted, together with epidemiology, and applied in the form of “risk assessment” to

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provide guidance to regulatory bodies. Risk assessment, as the term is used here, is the identification and characterization of the level of risks resulting from exposure to hazards, including the uncertainties.

Toxicology has a long and colorful history. Historically, toxicology developed in Europe as a forensic science, because poisons were used in “trials by ordeal” (God was thought to protect the innocent from the effects of poison), for executions, and, notoriously, for murder. It later became a subdiscipline of pharmacology, the science of medications, as the mechanisms of drug effects were elucidated. Many important drugs have been derived from classical toxins and toxic agents were extensively used in the early modern days of physiology and neuroscience to identify basic mechanisms of the body. From its early preoccupation with particularly toxic chemicals, from which it gained its essential definition as the science of poisons, toxicology has expanded its scope to include biological mechanisms of toxicity and host defences (or “resistance”) against toxicity. In the twentieth century, momentum for its development as an independent discipline has come (in roughly historical order) from food safety, chemical warfare, defense, product safety (especially cosmetics and food additives but also industrial chemicals), radiation biology, pesticide research, concern for environmental quality, environmental medicine, recent refinements in methodology of epidemiology and risk assessment, materials science and biocompatibility, molecular genetics and carcinogenesis research, and immunology. In recent years, toxicology has become highly specialized in the area of risk assessment, identifying the level of hazard associated with a particular chemical exposure and the limits of acceptably safe exposure. These issues go far beyond characterizing the effects of poisons, since most of the chemicals of modern concern are not classically “poisons”, in the sense of being potentially lethal at low doses.

For convenience in terminology, toxicologists often refer to all substances not normally present in the body and which are introduced from an outside source as “xenobiotics” (from the Greek *xeno-*, meaning foreign). Xenobiotics may be drugs, food constituents, natural chemical exposures, or anthropogenic environmental chemical exposures.

In this Chapter, the basic principles of toxicology will be presented briefly, followed by a general framework for clinical toxicology, a general framework for toxicology as applied to risk assessment and to hazard control, and finally a longer discussion of the interpretation of trace element analysis, which is a practical matter of great concern in medical geology. The science of toxicology can be divided into toxicokinetics, the study and description of how xenobiotics enter and are handled by the body, and toxicodynamics, the study and description of what the xenobiotic does to the body. (see also Chaps. 9, 22 and 24, this volume).

26.1 Toxicokinetics

Regardless of their effect or origin, the behavior of xenobiotics in the body can be described by accepted terms and general models reflecting the mechanisms by which exposure occurs and of how the body handles the chemical, which collectively is called “toxicokinetics”. From the standpoint of evolutionary biology, it is supposed that most of these mechanisms developed in response to natural selection pressures reflecting either of two biological needs: to detoxify and excrete harmful substances ingested in foods (especially in spoiled or putrefied foodstuffs) and to metabolize endogenous chemical compounds (such as steroid hormones).

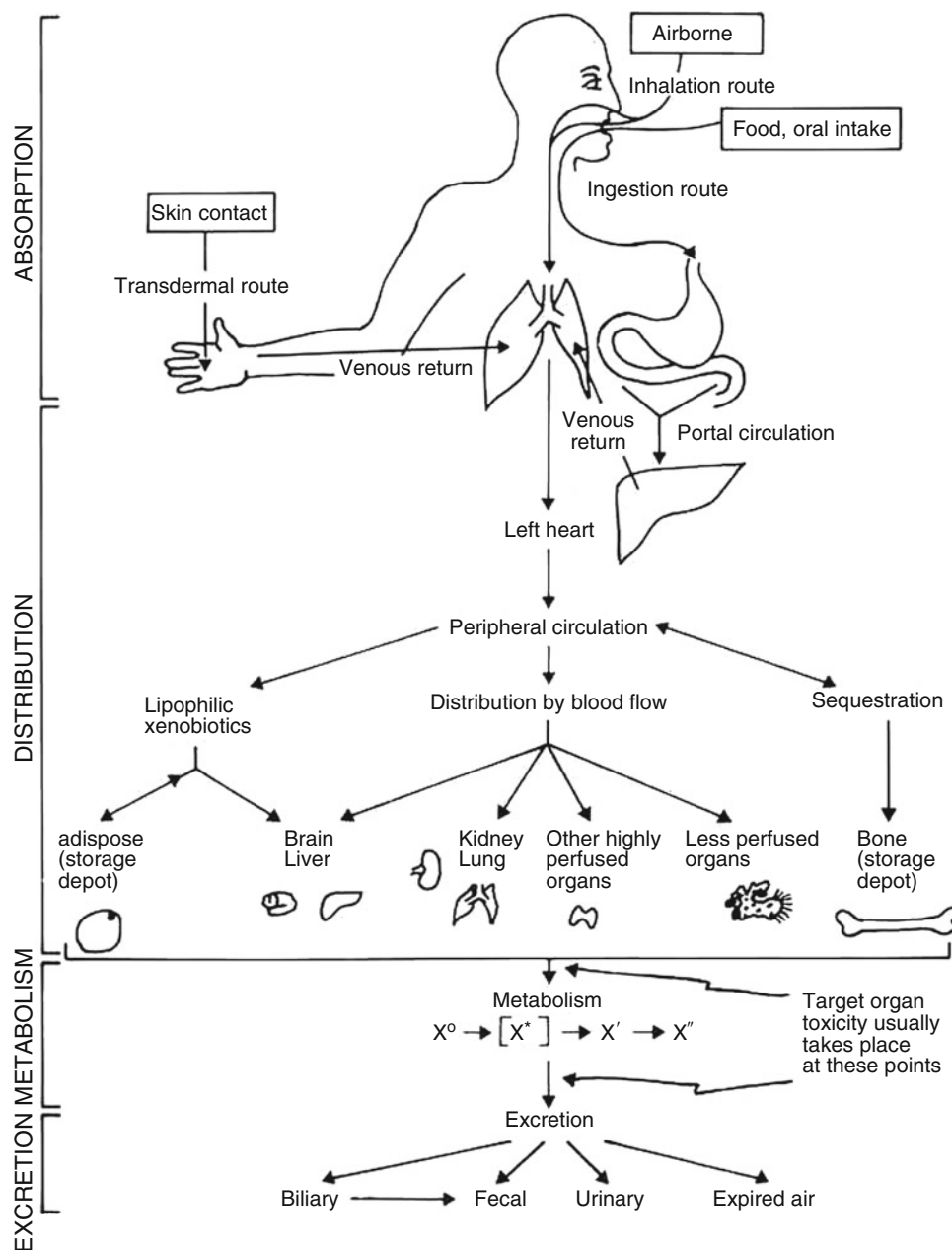
Toxicokinetics is the toxicological analogy to pharmacokinetics and is based on identical concepts. Four terms describe the disposition of xenobiotics, whether a drug or a toxic agent: absorption, distribution, metabolism, and excretion. Modelled together, the terms describe the entry, local and overall accumulation, transformation and removal from the body of the xenobiotic. Because tissue levels depend on transport of the xenobiotic to the target organ and the degree to which the xenobiotic partitions or is sequestered into the tissue, the kinetics of the xenobiotic determines the presentation of the xenobiotic or its metabolite to the target organ at the receptor level, where the toxic effect occurs. Figure 26.1 is an illustration of the principles of toxicokinetics.

26.1.1 Absorption

Xenobiotics may enter the body through any of several “portals” or routes of entry. In environmental medicine, the most significant portals of entry include ingestion, absorption through skin and inhalation. Ingestion results from eating contaminated foods or placing objects such as cigarettes in the mouth in a situation where the object or the hands may have been contaminated. Splashes into the eyes are more often associated with local eye irritation and only rarely with absorption and systemic toxicity. Other routes of exposure, such as intravenous infusion or implantation, are artificial and seldom seen outside of medical care and experimental studies.

The toxicity of the xenobiotic may or may not involve the organ of first contact or site of entry. For example, carbon monoxide enters the body by the inhalation route but causes no toxicity whatever to the lung. Other chemicals may cause local toxicity without significant absorption into the body, such as strong irritants applied to the skin. These routes of entry are not mutually exclusive. Inhalation of poorly

Fig. 26.1 Principles of toxicokinetics: absorption, distribution, metabolism, excretion (Reproduced, with permission, from: Guidotti (1994). By permission of Elsevier/Mosby)



soluble dusts such as silica, for example, may result in ingestion of the same material because of clearance from the lung bringing the material up the primary particle clearance mechanism of the lung, called the mucociliary escalator to the throat, where it is swallowed or expectorated.

The rate at which a xenobiotic enters the bloodstream is determined by absorption across the barrier presented by the given route of exposure. Absorption of xenobiotics across membranes is determined for the most part by the chemical and physical properties of the agent. In general, lipid-soluble (lipophilic) substances are absorbed more readily than

water-soluble substances across barriers such as skin. The rate of absorption is the most important determinant of the peak levels that will be reached in plasma. For many toxic substances, this is the prime determinant of acute toxicity.

The skin is sufficiently permeable to be a major route of entry of many chemicals into the body, particularly those that are readily lipid-soluble. Absorption across the skin is highly variable, depending on skin characteristics and the solubility of the xenobiotic in fat. Most transdermal absorption occurs directly across the superficial layers of the skin, the stratum corneum, which consists of nonliving, keratinized cells, and

the other living cell layers of the epidermis, to be absorbed in the capillary bed of the dermis. Some chemicals applied to the skin may gain entry through a short cut, passing more rapidly through hair follicles and sebaceous gland ducts. When the skin is injured with open wounds or abrasion, or in the presence of a skin rash, absorption across the skin is much faster. Transcutaneous absorption is a problem in the toxicology of pesticides, solvents, and halogenated hydrocarbons generally. Some agents may be significantly metabolized by enzyme systems in the skin, but most gain entry into the bloodstream unchanged.

Exposure by inhalation is relatively efficient absorption and the lung itself is vulnerable to damage from inhaled xenobiotics. The lungs are the organ of gas exchange and are positioned in the circulation just before the heart. The organ receives venous blood from the body, oxygenates it, and returns it to the heart which pumps it out via arteries. Thus, blood reaching the lungs is initially low in oxygen and consists of mixed blood from many tissues, but the oxygen tension in lung tissue itself is very high, which makes the organ susceptible to damage-causing free radicals derived from oxygen. Exposure by inhalation results in relatively efficient absorption of gases if the gas can penetrate to the deep lung where gas exchange occurs, the alveolar-capillary bed. Whether the gas will penetrate efficiently depends on its solubility in water, reflecting clearance rates in the bronchial tree. Once having penetrated to the alveolar level, however, gases are readily absorbed into the blood stream across the alveolar-capillary membrane by simple passive diffusion. Absorption across the alveolar membrane in the lung is usually very efficient and complete and entry into the bloodstream is limited only by the xenobiotic's solubility in plasma, an aqueous medium. Particles, on the other hand, are subject to a number of host defense mechanisms in the respiratory tract that limit the efficiency of penetration to the alveolar level. Once there, their size prevents them from passing directly into the bloodstream and they must dissolve or be digested by macrophages (scavenging cells) before their constituent chemical contents can be absorbed and enter the bloodstream. Particles may contribute to systemic toxicity if they are composed of a soluble material, such as lead or polycyclic aromatic hydrocarbons. For this reason, inhalation of toxic gases is usually associated with acute systemic toxicity or vascular injury to the lung (resulting in pulmonary edema, a very dangerous condition of fluid accumulation in the lungs). Particle deposition in the lung is usually associated with localized pulmonary effects and chronic systemic toxicity if the particle is absorbed. Increasingly, inhalation toxicologists are learning about the effects of nanomaterials, both natural and synthetic. Particles at nanoscale (on the order of 100 nm or so) have very different characteristics than larger pieces of matter of the same composition and how they affect the lung and body

cannot be extrapolated from the physical and chemical characteristics of the bulk material.

Ingestion is an important route of exposure for water and food and sometimes soil, and is therefore of interest in medical geology. Absorption through the gastrointestinal (GI) tract depends for many organic compounds on pH (because passage is increased when they are in a nonionized state) and therefore on location in the GI tract: the stomach is acid and the small intestine is basic. There are specialized transport mechanisms in the GI tract, among them facilitated diffusion to absorb glucose and a divalent-metal ion transporter that increases absorption of metals such as calcium and iron, as well as electrochemically similar ions. The GI route of exposure is unique in another important respect. Absorbed xenobiotics do not pass directly into the systemic circulation, as they do by transcutaneous and inhalation exposure, to be returned to the heart (via the lungs). Rather, veins draining the stomach and intestine conduct the blood to the liver by a specialized circuit (the "portal circulation"). The liver then metabolizes many xenobiotics before they pass into the systemic circulation and stores many xenobiotics. The veins draining the liver conduct blood to the main vein of the lower body and into the systemic circulation. Thus, when a xenobiotic is ingested, it may produce a toxic effect on the GI tract, it may produce a toxic effect on the liver, it may be metabolized, sometimes to a more toxic product, and it may pass in an altered form into the general circulation. (see also Chaps. 6, 9 and 25, this volume.

26.1.2 Distribution

Once the xenobiotic is absorbed and enters the bloodstream, it is transported to the capillary level in tissues of the body where it becomes available for uptake by the target organ. After one pass through the circulation the xenobiotic is uniformly mixed in arterial blood regardless of its route of entry. When a bolus is absorbed, the peripheral tissues are therefore presented with an increasing concentration in the blood which peaks and then declines as the xenobiotic is distributed to tissues throughout the body and removed by metabolism, excretion, or storage.

When a xenobiotic is dissolved in plasma, some fraction of the total usually binds to circulating proteins, particularly albumin (an abundant, soluble protein which binds many organic compounds as well as calcium, copper and zinc). Metals may also be bound to specialized proteins in the plasma, such as ceruloplasmin (copper) and transferrin (iron). Binding occurs quickly and an equilibrium is established between the fraction of the xenobiotic bound to plasma protein, which cannot leave the vascular space, and that dissolved in the plasma, which is free to diffuse or be

taken up by tissues. As the concentration of free xenobiotic falls in plasma, some molecules will separate from their binding sites until a new equilibrium is reached. Binding therefore acts as both a storage and distribution mechanism, maintaining a more even blood concentration than would otherwise be the case and reducing the peak concentration that would otherwise be presented to tissues.

Bound xenobiotics may be displaced by other xenobiotics. Some drugs, such as barbiturates or sulfonamides, compete with others for binding sites and may increase the concentration of free xenobiotic in the plasma and therefore increase toxicity. As a practical matter, therefore this phenomenon is of greatest significance in drug-related toxicology as a mechanism of drug interaction and overdose and is seldom a consideration in environmental toxicology.

The persistence of free (unbound) xenobiotic in the bloodstream is an important determinant of the duration of its action and the penetration that may occur into tissues less avid in their uptake of the particular agent. However, the most important determinant of uptake by the target organ is the uptake of the xenobiotic from plasma into the tissue.

Uptake of a xenobiotic by an organ from the plasma depends on the blood flow to the organ and the avidity of the tissue for the material. Special transport mechanisms exist at the cellular level for some xenobiotics. As mentioned above in the context of absorption into the body, absorption of a xenobiotic from the bloodstream into the tissue depends importantly on the solubility of the xenobiotic in fat (how “lipophilic” it is); lipophilic agents will be accumulated in adipose tissue or lipid-rich organs such as the nervous system or in liver. Where the physicochemical properties of the organ attract and bind metals, as in bone, a metal xenobiotic will be sequestered and will accumulate over time.

Entry into some tissues is restricted by special barriers to passage, such as the blood–brain barrier and the placenta. In most cases, however, delivery of a xenobiotic depends on the blood supply to a tissue relative to its weight. When the xenobiotic is neither particularly lipophilic nor sequestered nor preferentially taken up by some organ-specific mechanism, it is largely distributed on the basis of blood flow to the target organ; organs with greater perfusion will tend to accumulate the xenobiotic because of the increased total amount presented to it. The lung, a very lightweight organ, is the only organ of the body to receive 100% of the cardiac output at a tissue level. (The heart, functioning as a pump, moves blood in bulk but is itself nourished by a much smaller coronary artery system.) Not surprisingly, the lung is a principal target organ for blood-borne as well as air-borne xenobiotics. The liver and kidneys each receive massive fractions of the cardiac output and are therefore presented with circulating xenobiotics in quantity. The brain also receives a disproportionate fraction of the cardiac output but is partly protected by the blood–brain barrier;

this barrier works well for most polar xenobiotics but is permeable to lipophilic compounds.

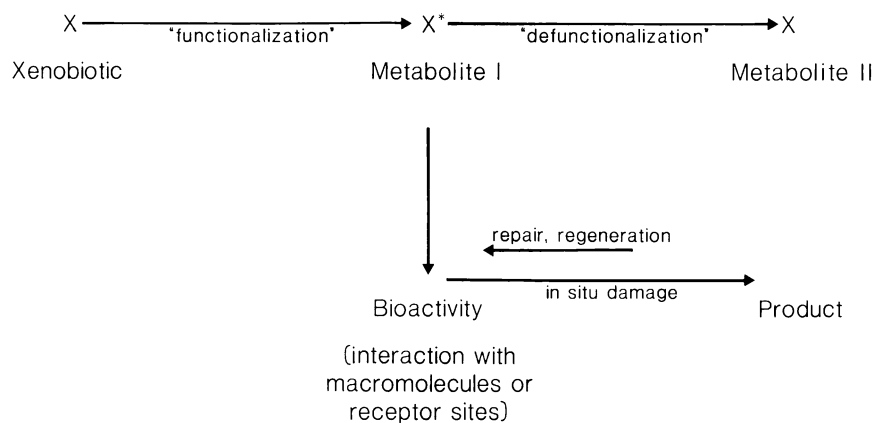
Between the stomach and the liver there are blood vessels called “portal veins” that bring nutrient-rich blood directly, without passing through the general circulation, to the liver for processing before distribution throughout the body by way of the general circulation. The portal circulation, however, also delivers ingested xenobiotics at high concentrations from the stomach and small intestine directly to liver cells often causing liver toxicity. The liver is the principal metabolic site for xenobiotics, as it is for nutrients. The portal delivery of xenobiotics therefore provides an opportunity for metabolism of some xenobiotics to take place before the xenobiotic enters the general circulation. Xenobiotics metabolized in the liver may even be taken up and reprocessed through biliary excretion and reabsorption through the enterohepatic circulation, such as many pesticides and metals, including mercury.

Some tissues have an affinity for xenobiotics with certain characteristics. Organs with a high adipose or lipid content accumulate much larger concentrations of highly lipophilic xenobiotics, such as organohalide pesticides or the PCBs, than occurs in plasma or in other organs. When an obese individual in whose adipose tissue is stored a high level of a fat-soluble toxic chemical rapidly loses weight as a result of dieting, food deprivation, unaccustomed exercise, or cachexia, the xenobiotic may be mobilized and a rapidly climbing circulating level of the agent may rise to toxic levels. In general, however, the principal significance of adipose and intracellular lipid is as a storage depot, in that the blood concentration comes into an equilibrium with release from the tissue where it is stored, remaining fairly constant for the remaining life of the individual. The xenobiotic can rarely be effectively purged from the body in this situation because of the extent of the storage, although strategies exist to steadily reduce the body burden over time by vigorous removal from plasma to force mobilization. Another important implication of storage in fatty tissues is accumulation in breast tissue and subsequent excretion into breast milk. This is the major route of exposure to a variety of xenobiotics for newborns who breast feed. Metals such as lead are sequestered in bone; mobilization from depots in bone by chelating agents may substantially increase blood levels and create a risk of renal toxicity. (see also Chap. 25, this volume).

26.1.3 Metabolism

Many xenobiotics are substrates for intracellular enzyme systems, most of which appear to have evolved as mechanisms for clearing endogenous mainly steroid, hormones or foreign substances taken in with food. These

Fig. 26.2 Metabolism of organic xenobiotics often follows a pattern of chemical “functionalization” in which the intermediate product may be more reactive and toxic than the original xenobiotic, followed by de-activation and defunctionalization or conjugation to make the product more water soluble (From Zenz (1994). By permission of Elsevier/Mosby)



enzyme systems transform the xenobiotic in a series of steps from the original compound to a series of stable metabolites, often through intermediate unstable compounds. For many xenobiotics there are many pathways of metabolism, resulting in numerous metabolites. These transformations may have the effect of either “detoxifying”, by rendering the agent toxicologically inactive, or of “activation”, by converting the native agent into a metabolite that is more active in producing the same or another toxic effect. An active xenobiotic may be transformed into an inactive metabolite, effectively removing the agent from the body in its toxicologically active form. However, an inactive precursor may also be transformed into an active metabolite.

In general, the enzyme systems available for the metabolism of xenobiotics tend to convert nonpolar, lipid-soluble compounds into polar water-soluble products that are more easily excreted in urine or bile. The general pattern consists of two phases. These are illustrated in Fig. 26.2.

Phase I of the metabolic process involves the attachment of functional chemical groups to the original molecule. This usually results in activation, especially in the very important “mixed function oxidase” (MFO) system, and results in a metabolite capable of interacting with macromolecules, such as DNA in the early steps of carcinogenesis. The mixed function oxidase system requires a great deal of metabolic energy and is closely linked with the cytochrome oxidase system, which provides it. Because the particular cytochrome most closely linked with the system has a spectral absorption peak at 450 nm, there is frequent reference in the literature to “P450” as an indicator of MFO activity.

Most important of the metabolizing systems, the mixed function oxidase (MFO) system also is known by other names: aryl hydrocarbon hydroxylase, arene oxidase, epoxide hydroxylase, cytochrome oxidase. It is a complex of membrane-associated enzymes closely linked to the cytochrome P450 system (and other cytochromes) that acts on organic compounds with aromatic or double bonds. The system attacks these bonds, creating first an epoxide and then an alcohol and in the process first activating the compound and then deactivating it and rendering it more easily

excreted. The MFO system is virtually ubiquitous in the body but activity is particularly concentrated in liver and lung, and can be found and conveniently studied in circulating lymphocytes. The MFO system has a huge capacity and acts on a wide variety of substrates. It also has the property of being inducible; when presented with suitable substrate, the cell synthesizes more MFO enzymes, increasing the capacity of the system, and preparing itself for a greater load. The degree of inducibility and the level of baseline activity in a given tissue is genetically determined, so that at any one time MFO activity in a particular tissue reflects heredity combined with exposure in the recent past.

Phase II involves the removal or conversion of chemical groups in such a way as to render the molecule more polar and therefore more easily excretable by the kidney (and less easily diffused back across the renal tubular epithelium after filtration). In the process, the activated xenobiotic metabolite from Phase I usually becomes inactivated. This process frequently involves “conjugation”, the attachment of a functional group such as sulfonate or glucuronic acid that makes the molecule much more hydrophilic.

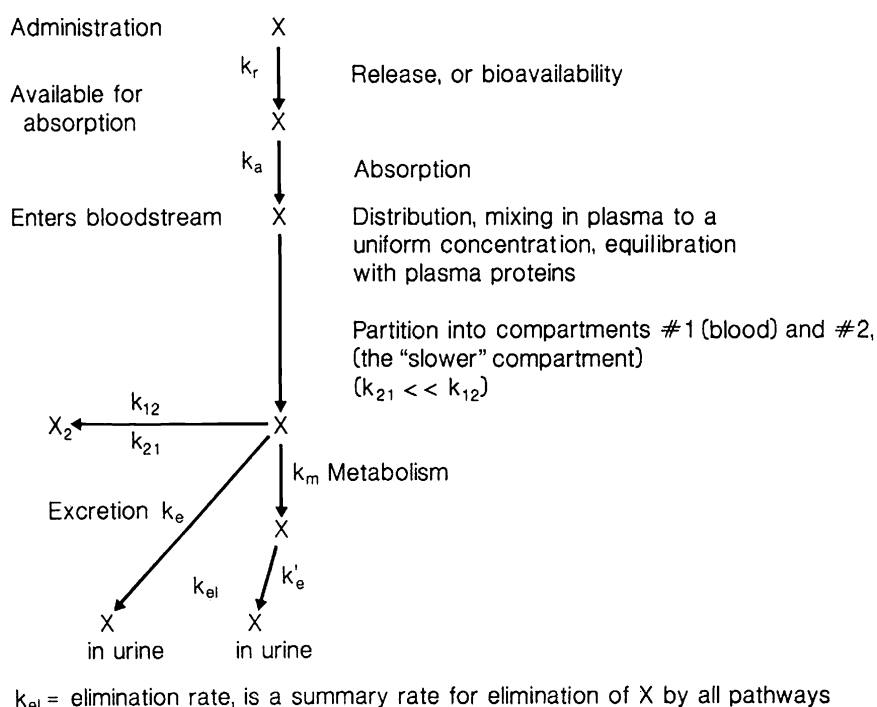
The most complicated metabolic pathways are those for organic compounds. Metals may also be metabolized, however. The methylation of mercury and arsenic, especially, plays a major role in their toxicity. The methylation pathway of arsenic is species specific and this is thought to be the reason why arsenic is a carcinogen in humans but not in animals.

26.1.4 Excretion

The xenobiotic or its metabolites would remain and accumulate within the body if there were no mechanisms for excretion. Elimination is the term used for removal of the xenobiotic from the bloodstream, whether by excretion or metabolism or sequestration (storage).

The kidney is the major route of excretion for most xenobiotics. Those that are water-soluble may be filtered or excreted unchanged. The reserve capacity of the kidney is

Fig. 26.3 Kinetics of elimination are determined by the behavior of the xenobiotic in different toxicokinetic phases and can be modeled (From Zenz et al. (1994). By permission of Elsevier/Mosby)



very great and this mechanism is rarely saturated in healthy people but individuals with renal insufficiency may show accumulation and persistence of the xenobiotic and, consequently, prolonged and more severe toxicity. Other xenobiotics may be metabolically transformed into more water-soluble metabolites before renal clearance occurs. Xenobiotics that are themselves nephrotoxic may injure the kidney and reduce their own clearance, enhancing their own toxicity by causing further accumulation.

The liver, besides being an important metabolizing organ, secretes some xenobiotics into bile, including heavy metals such as lead and mercury. These may recirculate by the enterohepatic circulation, persisting in the body much longer than otherwise, or they may pass out of the body in feces. Forced biliary excretion is not presently possible but interruption of the enterohepatic circulation by binding agents such as cholestyramine is a practical clinical intervention to hasten excretion and reduce the body burden of xenobiotics excreted in the bile and reabsorbed in the gut; this was first demonstrated for kepone. Although hepatotoxic agents may interfere with their own excretion by the liver, they are more likely to interfere with metabolism and as a practical matter this effect is rarely significant.

Volatile gases are readily excreted by the lung through passive diffusion from the blood, crossing the alveolar-capillary barrier in “reverse” direction. Gases that are poorly soluble in blood, such as ethylene, are rapidly and efficiently eliminated by this route. Those that are readily soluble in blood, such as chloroform, are less efficiently eliminated and may be detectable in expired air for days or even weeks.

Xenobiotics and their metabolites are also eliminated by various minor routes that matter little with respect to reduction of the total body burden but may have toxicological implications. Lipid-soluble agents may be secreted in breast milk; this is a major route of exposure of neonates and young children to substances such as the organohalides, including PCBs. Water-soluble agents are excreted in saliva and tears and are filtered through sweat glands, the latter functioning much like miniature nephrons. Lipid-soluble agents may also be found in cerumen and sebum. These minor elimination pathways permit noninvasive monitoring techniques for the detection of the agent but are rarely reliable enough to quantify exposure.

26.1.5 Kinetics

Metabolism and excretion define the rates of elimination, the change in the concentration of the xenobiotic in the plasma with time. Elimination may occur (1) because the xenobiotic is excreted, (2) because it is converted to something else by metabolism, or (3) because it is stored somewhere inaccessible to the bloodstream. The description of the rates of elimination of the agent is an important tool in understanding its behavior in the body. Each phase of the kinetics of a xenobiotic is governed by rates determined by properties of the agent and characteristics of the biological system, as illustrated in Fig. 26.3. Each rate is described by a rate constant (k) that determines how rapidly the process proceeds.

Rate constants are described by their “order”. A zero-order rate constant describes an elimination curve in which the rate is limited intrinsically by the fixed ability of the body to eliminate the agent, regardless of its concentration. In practice, the only important example of this is, ironically, the most common metabolizing system of toxicological concern: alcohol dehydrogenase, which metabolizes ethanol and other alcohols. Regardless of how much alcohol a person ingests, elimination will occur at a fixed rate regardless of the dose or plasma concentration. (The kinetics of the enzyme are not truly zero-order and it behaves as a first-order elimination curve at low levels of alcohol consumption; the capacity of the enzyme is simply saturated quickly as a person consumes more than a minimal amount.)

A first-order rate constant describes a process in which the rate of elimination is independent of the dose and the concentration of the xenobiotic in plasma is proportional to the concentration of the agent in tissue, the most common situation. The concentration of the xenobiotic in plasma decreases over time by a constant proportion per unit time. This is called a “one-compartment” model because the agent behaves as if it is restricted to one compartment of the body, the vascular space. In reality, it may not remain in the vascular space but equilibrates freely with it from its tissue depots. Water-soluble xenobiotics usually show first-order kinetics, except for alcohols.

A multi-compartment, or “multiexponential”, function of elimination suggests that the agent equilibrates in more than one compartment and is eliminated at different rates from each. The rate of elimination varies with the concentration in plasma and the initial dose and is biphasic. The elimination will not fit a simple exponential decay (or straight line on a logarithmic scale) but will be described by a more complex equation with two rate constants, a “fast” rate constant and a “slow” one. Organohalides typically show two-compartment kinetics because of their storage and slow release from fatty tissue. (The term “second order” is not used because it would imply that elimination rate is a function of the square of the concentration, which is not the case.) Increasingly, the behavior of such xenobiotics is modeled using “physiologically-based pharmacokinetic” (PBPK) models, so-named because they were first worked out for drugs. Metals often have multiple compartments and complicated toxicokinetics.

First-order kinetics are most common for water-soluble xenobiotics. In such systems, elimination of the agent, being proportional to concentration, results in an exponential decay or reduction in plasma concentration over time. The period required for the plasma concentration to drop by half is called the “half life” ($t_{1/2}$). The $t_{1/2}$ can be calculated easily and accurately and is related to the elimination rate for first order systems by the equation: $t_{1/2} = 0.693/k_{el}$.

26.2 Toxicodynamics

The mechanisms of toxic injury are too numerous to accommodate simple classification or generalizations. There are, however, a few general principles that are useful in understanding toxic effects.

26.2.1 Mechanisms of Toxicity

Xenobiotics exert toxic effects by interfering with the normal functions of the body. These effects occur at the molecular and cellular level. Thus, an understanding of normal function and biochemistry is essential for understanding toxicodynamics. The toxic effect is an interaction between the xenobiotic and the cellular and biochemical mechanism, usually involving the interaction between the xenobiotic and a macromolecule, as illustrated in Fig. 26.2. This has not always been understood: for centuries, poisons were considered to be a special class of chemicals and the toxicity of poisons were understood to be intrinsic properties of the chemical, or magic.

Certain organs of the body are in harm’s way. Because they may be the first to encounter a toxic exposure, receive a large blood flow, are highly metabolically active, actively metabolize xenobiotics themselves, concentrate toxic substances or their metabolites or have biochemical characteristics that render them vulnerable, the liver, kidney, lungs, skin and bladder are particularly susceptible to toxic effects.

Although there are as many potential mechanisms of toxic effects as there are reactions in biochemistry and functions in physiology, there are a few processes that play special roles. They include the following:

- **Inflammation.** The body has natural mechanisms to repair and limit damage. Many xenobiotics are irritating to human tissues and induce local inflammation. For others, inflammatory reactions may contribute to systemic toxicity. A particularly important phenomenon associated with inflammation is the production of reactive oxygen and nitrogen free radicals, which cause intracellular damage as a byproduct of inflammation.
- **Immune responses.** The body also has natural mechanisms to respond to specific foreign substances (“antigens”) or cells, by producing antibodies or by mobilizing special cells that destroy the foreign material, and in the process set off inflammatory responses. These immune responses require that the body has seen the antigen first or that it is persistent, and are triggered by subsequent exposure to low levels of the antigen. When the immune response is dysfunctional, it may cause

allergies, diseases collectively called “immunopathies” and self-directed autoimmune diseases.

- Carcinogenesis. Cancer is the prototype for “stochastic” or probabilistic toxic effects, in which the response depends on the probability of an interaction rather than the magnitude of exposure and degree of response. This is discussed below. There is some evidence that certain other classes of toxic response, such as neurotoxicity, follow similar patterns.
- Endocrine mimics. Many xenobiotics interact with hormonal receptors, sometimes by simulating the effect of hormones and sometimes by inhibiting them.

26.2.2 Exposure-Response Relationships

The exposure-response relationship is a concept fundamental to an understanding of toxicology. Paracelsus, the great medieval toxicologist, said “It is the dose that makes the poison” and thereby established that poisons were not a mystically benighted form of matter but that all chemicals have toxic properties that become apparent as increasing quantities are consumed or absorbed. It follows from this simple observations that there may be “safe” levels of exposure to even the most toxic substances, a much more controversial assertion. Obviously, there are several dimensions to this seemingly straightforward concept. There are three distinct varieties of the exposure-response relationship that need to be distinguished conceptually. These are the toxicological dose-response relationship, the clinical dose- or exposure-response relationship, and the epidemiological exposure-response relationship. These are illustrated in Fig. 26.1.

“Dose” is generally understood to mean the total quantity of a toxic substance administered; “exposure” is generally considered to be the level of concentration available for absorption by any or all routes at or over a given period of time. Thus, dose is best understood as total or cumulative exposure over a relevant time period. If the dose is given all at once, the dose-response relationship is most meaningful, as it is when the toxic substance is accumulated in the body. If the exposure takes place over a prolonged period of time, the internal dose at any given time tends to vary and it is more useful to think of an exposure-response relationship. When a xenobiotic accumulates and persists in the body, such as lead over a period of weeks or dioxin and pesticides over a period of months and years, cumulative exposure approximates dose in toxicological terms. When a xenobiotic does not readily accumulate and is quickly eliminated, cumulative exposure over a long time period does not equate to effective dose in toxicological terms, although there may be cumulative effects if each exposure produces permanent

injury. For example hydrogen sulfide, a highly toxic gas that is often a problem in medical geology because of its association with volcanoes, vents, and petroleum and gas, is acutely toxic with a toxicity that is a high exponent of exposure (on the order of concentration to the fifth power) and does not accumulate; for hydrogen sulfide, duration of exposure does not matter much but the concentration at first contact is critical.

The most fundamental building block of toxicology is the dose-response relationship demonstrable in the laboratory, often called the “toxicological” dose- or exposure-response relationship. The fundamental principle is that the physiological response depends on the amount of the agent presented to the tissue. In a given individual, exposure to an increasing amount of a toxic substance leads to the progressive appearance of new and usually more severe health problems leading to death, a sort of stepladder to lethality.

This gives rise to another type of dose- or exposure-response relationship, which might be termed the “clinical” exposure-response relationship. At a given level of exposure, often referred to clinically (if colloquially) as a “threshold”, one can usually expect a given constellation, or “toxidrome”, of symptoms and signs. This clinical exposure-response relationship depends importantly on the strength of the host defenses of the individual (which can be very variable) and whether the individual has an acquired condition or genetically-determined phenotype that renders him or her more vulnerable than others. In a given exposure situation, one person may show one symptom and another a different symptom, based on personal susceptibility. At relatively low levels of lead toxicity, some patients show elevated uric acid levels because of reduced renal clearance and develop gout, most do not. As well, the detection of the expected clinical response depends on the sensitivity of clinical examination and laboratory tests. Clinical tests are often inadequate for early detection of equivocal cases because they were designed for making specific diagnoses in people known to be sick in a way that strongly suggested a particular type of disease.

The third type of exposure-response relationship relates exposure levels to the frequency of the response in a population. If one is interested in what personal characteristics of those exposed render them vulnerable to a toxic effect or in how frequently a response is associated with a given level of exposure in a population, one may do a “nose-count” of the observed response among individuals exposed. This is the essential method of epidemiology and yields what is usually called the “epidemiological” exposure-response relationship. To be meaningful, however, the outcome must be experimentally or clinically detectable. This removes from study many types of response that cannot be directly measured and which are usually considered “subclinical”

or “adaptive responses”. In this system, a “threshold” means the level of exposure associated with the first appearance of an excess of the health outcome representing the toxic response. It is this threshold for response that generates the most controversy in regulatory policy. However, interpretation of this type of threshold depends on understanding the basis for selecting and detecting the health outcome.

At higher levels of exposure, the exact shapes of these exposure-response relationships are not critical and the general relationship is usually obvious. At lower levels of exposure, however, interpretation of the population response is very dependent on an interpretation of the general mechanism of the toxic effect and extrapolation to low exposures is very sensitive to the biological model applied.

A particularly important, if confusing, term in toxicology is “threshold”, which means the level of exposure at which an effect is first observed. The existence of thresholds for certain types of response (particularly carcinogenicity) are controversial and arguments surrounding identification of a threshold for response frequently neglect to specify the type of threshold under consideration.

26.2.3 Interaction

Some xenobiotics interact with others to produce disproportionate effects. For example, exposure to sulfur oxides in the presence of particulate air pollution in combination causes worse lung irritation than would be predicted by the individual effects of each added together. This is because the sulfur oxide adsorbs onto the surface of the particle and is carried deeper into the lung than it would be as a gas.

Interaction may be positive (often called “synergy”) or negative (often called “inhibition”). Different models of interaction are applied to the interpretation of data. When the effects of two or more are additive, no interaction occurs. This may suggest that both are acting by the same pathway or mechanism and are simply adding their proportionate share to the total magnitude of the effect. When the effects multiply, this is strong evidence for extensive interaction, and suggests that the xenobiotics are acting by different pathways that potentiate each other’s effects. When the effects are less than additive, it is evidence that in some way the effects of one exposure are reducing or blocking the effects of the other or are acting by a similar pathway that has only limited capacity.

Toxicologists are very concerned that exposure to mixtures, such as cigarette smoke or heavily contaminated groundwater, could present the potential for numerous interactions and unpredictable effects. In practice, except for drugs relatively few examples of interaction producing significant health effects have been documented. Some of the most important involve cigarette smoking and persistent

carcinogens, including asbestos, silica and radon daughters, which greatly increase the risk of lung cancer compared to the sum of the separate risks of either smoking alone or exposure to the other carcinogen alone.

26.2.4 Carcinogenesis

Much of environmental toxicology is oriented toward the etiology and prevention of cancer. This emphasis is not misplaced, as cancer is a leading cause of death and results in the largest total loss of quality-adjusted years of life, among all causes, in developed countries.

Carcinogenesis is not a straightforward, deterministic process. Rather, at each step in the sequence there is a finite probability of events leading to the next step. Chemical carcinogenesis is thus a stochastic, or probabilistic process, like a roulette wheel or radioactive decay, not a certain prediction based on chemical structure and properties. In any one individual, an exposure may increase the odds of getting cancer but do not make it certain in absolute terms that this will happen.

Chemical carcinogens are demonstrable by their effect in increasing the frequency of cancers observed in exposed subjects as compared to unexposed. They may produce malignant tumors that are often different in tissue type and wider in diversity than those usually observed among unexposed subjects, produce malignant tumors at characteristic or unusual sites in the body, and produce these malignant tumors earlier in the life span of subjects than they would otherwise be seen. Often, however, chemical carcinogens produce malignancies identical in tissue type, location, and onset to those seen in unexposed populations and the only clue is an increased frequency of cancers in exposed groups.

Recent advances in research on carcinogenesis, especially the identification of the oncogene, may have identified new and rather complicated mechanisms but the effect has been to simplify our understanding by providing common pathways and unitary, comprehensible mechanisms by which many causes may act. The principal categories of causes of cancer are fairly conclusively identified as heredity, chemical exposure, viral infection, and radiation exposure. Other categories of causes may be identified, but these appear to be primary.

The contemporary model for induction of cancer by chemicals that is most consistent with available evidence for most chemicals and for radiation is that called the “two-stage model of carcinogenesis”. (The model is insufficient to account for some other types of cancer induction - these are discussed below.) The two-stage model assumes the introduction of a carcinogen into the body (or the metabolic activation of a procarcinogen) and its distribution in the body in such a way as to be presented to a tissue at levels

in which it is likely to be taken up intracellularly and to react with cellular constituents, most specifically nuclear DNA. Transformation of the cell does not occur with every interaction between a carcinogen and DNA, however. Only in a relatively small fraction of such interactions will the critical sites on DNA be affected, resulting in a probabilistic phenomenon. When it occurs, the process is called "initiation" because those cancers that may ultimately result are initiated at this step. In many cases, things presumably go no further than initiation. The mechanism for much, if not most, initiation activity is oncogene activation.

Among these relatively few but numerically many interactions with DNA are a handful that may cause the cell to behave in a manner more appropriate to a primitive, embryo-like state and these are thought to be the mechanism for transforming normal cells into neoplastic cells. Oncogenes are capable of being activated by chemical exposure. They are latent within the genetic structure of all humans (and probably all advanced life) and at least some probably play a physiological role in normal embryonic and fetal growth and development. Activated in the absence of regulation, however, the oncogenes trigger malignant transformation of the cell, causing a previously differentiated cell to regress to a more primitive state abnormal for that stage of the life of the organism.

Next in sequence is the growth of a clone of transformed cells from a single cell altered in its growth characteristics to a small focus *in situ*. The transformed cell, having been altered in its DNA blueprint, does not necessarily begin to multiply at once. Rather, it may be held in check by host factors or cell-specific factors, such as the need for further DNA reorganization or oncogene activation to take place. The abnormal cell may rest for a very long time, contributing the greater part of the latency period before appearance of the clinically evident tumor. Additional exposures may trigger the conversion of the initially abnormal cell into a transformed or preneoplastic cell capable of giving rise to a tumor. This process may be facilitated by exposure to chemicals that also have genotoxic potential, either simultaneously or after the action of the primary carcinogen. This ancillary process is called "cocarcinogenesis", implying that the second or combination exposure may not be the initiator but participates in the genotoxic cell events and either leads to expression of the critical event, resulting in oncogene activation, or overrides mechanisms that would otherwise inhibit oncogene activation and cell transformation. In general, the same chemicals that are primary carcinogens are likely also to be cocarcinogens. The distinguishing feature is not which chemical reaches the DNA first or which exposure preceded which, but which chemical actually participated

in the critical event that specifically altered the DNA in such a way as to activate the oncogene.

Exposure may occur at this stage to chemicals that are capable of triggering proliferation by removing the inhibitory factors that are suppressing the transformed cell. This is called "promotion" and it is the second stage in the two-stage model of carcinogenesis. Promoters are sometimes primary carcinogens themselves and probably act through genetic mechanisms, such as the polycyclic aromatic hydrocarbons, but others are weakly or not carcinogenic and presumably act by nongenetic mechanisms. The most well known are the plant-derived phorbol esters (specifically tetradecanoyl phorbol acetate, TPA), constituents of croton oil that are chemically extremely complex and seem to act at least in part pharmacologically by activating certain specific receptors on the cell surface. Chlorinated hydrocarbon species are often potent promoters, including the PCBs, DDT, PBBs, and certain dioxins. They seem to act by nongenetic means and have variable primary carcinogenic activity, depending on the species.

By whatever mechanism, promotion results in deregulation and progression of the neoplasm by proliferation into a clone of cells. The transformed cell has now become a cancer cell with the essential features of a malignancy: unresponsiveness to regulation, loss of contact inhibition, potential for sloughing and migration of cells, and the potential for inducing growth of new nutrient blood vessels.

Because this all takes time at each step, there is a delay between the initiation (commonly assumed to be at first exposure) and earliest clinical presentation of a tumour. This is called the "latency period". For most chemically-induced cancers it is on the order of 20–30 years but may be as long as 50 or more (in the case of mesothelioma and asbestos) or as short as 5 years (for radiation or radiomimetic exposures and some bladder carcinogens).

Not all chemically-induced cancers act by this genetic mechanism. "Epigenetic" refers to the actions of cancer-inducing agents and exposures that do not directly interact with DNA. At least some probably act by inducing intracellular free radicals that damage DNA in a nonspecific manner, however. Asbestos is an example. Others are more obscure in their mechanisms. None are adequately explained by the conventional two-stage model of carcinogenesis but subsequent refinements in theory will almost certainly result in a unitary model demonstrating a final common mechanism for most cancers.

Metal-induced carcinogenesis occurs by a variety of mechanisms and often strongly depends on the chemical composition, redox state and solubility: arsenic (lung, bladder and skin), beryllium (lung), cadmium (lung), chromium (hexavalent ion: lung), nickel (sulfide: lung).

26.3 Interpretation of Trace Element Levels

By definition, trace elements are normally present in very low concentrations in the body. A good working definition of trace elements in medicine would include an element that is present in minute amounts and that is not under tight physiological regulation. Iron and magnesium, for example, are not true trace elements in the body because substantial quantities are normally present and concentrations are kept within a normal range by mechanisms of biological control, reflecting the importance of these elements in metabolic and physiological processes. Other trace elements are not kept under control by the body and therefore blood and tissue concentrations vary widely. Even trace elements that are essential elements for various metabolic functions (e.g. cobalt, manganese, selenium) may not be physiologically regulated because they are present only in minute quantities. Most trace elements are metals, although two are metalloids (arsenic and selenium).

Three biological fluids are assayed on a routine basis: serum, whole blood, and urine. These analyses typically report on a profile of several metals. The state of the art for trace element analysis in body fluids is a highly accurate and sophisticated technology called inductively-coupled plasma mass spectroscopy (ICP-MS) to analyze the concentration of minute amounts of metals in a sample. It is an exceptionally accurate method for low concentrations of trace elements, in the nmol/l or $\mu\text{mol/l}$ range. Metals that are present at higher concentrations, such as iron, overwhelm the system and are better determined using traditional methods. The technology works equally well for biological fluids and for water samples and is used routinely for both.

The clinical interpretation of trace element analysis has lagged behind the technology available to measure the elements in body fluids. It is easy to automate the determination of a dozen or so trace elements (metals or semimetals such as arsenic and selenium) in blood or urine. This is commonly done commercially by laboratories that offer their services in screening for purported disease through “alternative” health practitioners that do not necessarily practice in the mainstream of modern medicine. However, if health practitioners do not know what they are looking for or do not understand the principles of toxicology, they can be seriously misled. The use of trace element analysis by alternative medical practitioners and its conduct by questionable laboratories have somewhat discredited the method and is a frequent source of confusion in medical diagnosis.

Interpretation can be difficult to interpret and requires knowledge of toxicokinetics. Trace element analysis is best used for specific applications, such as establishing levels of exposure, epidemiological studies, occupational and environmental health surveillance, biomonitoring of populations,

and to confirm an association following a compatible diagnosis. Except for lead, trace element analysis is not well suited for screening individual subjects because the results are not easy to interpret, there is wide variation and a skewed distribution in the population, and even elevated levels carry limited prognostic value. Just because a level of, say, selenium is higher in one person than is usually seen in the general population does not mean that that person is suffering from selenium toxicity or needs to do anything about it. Some metals, such as manganese, do not lend themselves at all to analysis and results correlate very poorly with evidence of toxicity.

Trace element analysis reflects absorption from all sources, including diet (the major source for most of them), occupational exposure (a major source for elevated levels), smoking (a particularly important source for cadmium), hobbies, housing quality (the major source for exposure to lead in children being peeling paint chips), medication, and local soil-containing dust (another important source of lead). It may also reflect characteristics of the host in retaining or accumulating the trace element, as in the case of patients on dialysis (aluminium), receiving parenteral nutrition (in which nutrition is provided directly into the bloodstream through a catheter) with inborn errors of metabolism (such as Wilson’s disease, which involves copper but is not caused by excessive intake), or with impaired excretion (lead).

The essential “trace elements” are copper, cobalt, manganese, molybdenum, selenium and zinc. A balanced and regular diet generally keeps these trace elements roughly constant and consistent in well-nourished populations, but there is no homeostatic mechanism that does so in the individual. Deficiencies in the uptake or metabolism could result to sustained imbalance of trace elements. In addition, an excess intake could result in disease. Other trace elements, such as arsenic, cadmium, lead and mercury, have no known human body function and high exposure to these elements could result in both immediate and delayed adverse health effects.

Testing is performed on serum (the liquid fraction of blood) for metals that are carried in the blood in dissolved form or that are bound onto proteins that circulated in the blood. These include aluminium, antimony, barium, beryllium, copper, manganese, nickel, selenium, vanadium, and zinc. Serum samples cannot be used for lead or arsenic and are inaccurate for some trace elements such as bismuth.

Testing is performed on whole blood for metals that are present in serum and also for those that concentrate in the red cell fraction of blood. Concentrations in serum and whole blood for those metals present in serum are usually similar but in some cases, as for copper, the serum assay result may be somewhat higher because there is less in the red cells that make up approximately 40% of the volume of whole blood. Those metals that accumulate preferentially in red cells are

not accurately reflected in serum concentrations and only whole blood concentrations are valid for these metals: cadmium, cobalt, molybdenum, lead, and thallium.

Urine is tested to determine the excretion of metals over a 24-h period for aluminium, antimony, arsenic, barium, beryllium, bismuth, cadmium, manganese, selenium, lead, thallium, vanadium, and zinc. This is usually the most accurate reflection of the total body burden of the metal. Many factors affect excretions of metals over short periods: state of hydration, renal function, intake with foods, short-term exposures from other sources, renal blood flow. Over a longer period of time, however, these variations even out and excretion is then generally directly related to the average serum concentration during this period (in equilibrium with red cell concentration, in the case of metals that accumulate in red cells). For this reason, spot urine samples are not very useful in determining whether excessive exposure has occurred, although their toxicological value can be improved somewhat if the results are normalized to the urinary creatinine level (a marker of kidney function). Compliance is much more difficult to achieve in collecting a 24-h urine specimen but it is preferable for an accurate and valid result.

Hair analysis has been very popular among some practitioners because of its convenience in sampling. However, hair is a difficult matrix for trace element determinations because it is solid and so difficult to process, and is contaminated by dust, hair products, and other environmental exposures. Concentrations may also vary along the length of the hair. As a consequence, hair analysis is not recommended for trace element analysis except in highly controlled settings, when collecting body fluids is not possible or practical, and when forensic studies are conducted (for example, assessing changes over time along the length of the hair).

The reported value is compared against a reference range which is usually derived from the authoritative literature, matched against the experience of the local laboratory. In recent years, data have become available from large cross-sectional surveys, such as the National Health and Nutrition Examination Survey in the United States. Reference ranges for occupational exposure are often benchmarked against standard reference works. Trace element studies on populations must be interpreted with knowledge of the log-normal distribution pattern and suitable transformation

for statistical analysis. Always, the toxicokinetics of the trace element defines the meaning of the results obtained.

Chelation therapy is a potentially dangerous form of treatment in which trace elements (usually lead, mercury or arsenic) are eluted from tissue by chemical agents that coordinate with and trap the metal ion, solubilizing it and making it easier to excrete. The abuse of chelation treatment is intimately linked with misunderstanding of trace elements and the significance of laboratory levels. Greater care and understanding in interpretation of trace element analysis will lead to safer interventions and less potentially dangerous over-utilization of chelation. The danger, even in treatment for overt toxicity from metals, is that mobilization of the metals also enhances their toxicity, particularly to the nervous system and kidney.

See Also the Following Chapters. Chapter 6 (Uptake of Elements from a Biological Point of View) • Chapter 9 (Biological Responses of Elements) • Chapter 22 (Environmental Epidemiology) • Chapter 21 (Environmental Medicine) • Chapter 24 (Environmental Pathology)

Further Reading

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