Modes of Action of Toxic Agents

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ABSTRACT

The principal modes of action of toxic agents are discussed in relation to the type of chemical bond formed between the poison and the target constituent of tissues. Alteration of enzyme activity, interference with the binding of poisonous chemicals to proteins, intercalation with nucleic acids, disturbances in electrolyte balance and the disorganization of cellular water and membrane lipids are illustrated as toxic processes involving ionic or van der Waals forces. The reactions of heavy metals with tissue nucleophiles and of exogenous nucleophiles with tissue metals are given brief attention in connection with coordinate-covalent binding. Covalent binding of poisons can arise from the incorporation of an antimetabolite into a larger molecule or reactions of electrophiles or free radicals with tissue constituents. These modes of action are illustrated by chemicals that produce necrosis, allergy or cancer.

The interactions of toxic agents with tissue constituents range from weak ionic and van der Waals forces to strong covalent bonds. The organization of this paper on the basis of types of chemical bonds represents a departure from the usual classifications of toxicology based on categories of chemicals, symptomatology, or organ pathology. This new framework is perhaps most useful for scientists concerned with devising therapy for the various kinds of poisoning, but it should be adequate for a brief review of the mode of action of toxic agents. It is important to emphasize at the outset that the strength of the binding of chemicals to tissue constituents correlates poorly with the seriousness of poisoning. The weakest interactions may be immediately lethal, whereas extensive covalent binding may have only minor consequences.

Noncovalent Binding

ALTERATION OF ENZYME ACTIVITY

The majority of toxic reactions encountered in connection with medical therapy are produced by the reversible binding of drugs to specific sites on cellular enzymes. Recent advances in the elucidation of the structure of cholinergic and adrenergic receptors have shown that these entities are proteins which share properties of enzymes such as allostery. Some toxic actions can be viewed as an extension of a therapeutic effect derived from a drug-enzyme combination. For example, an excessive dose of methacholine used to relieve atrial
tachycardia may produce cardiac arrest. Both the desired and unwanted effects are attributable to the action of the drug at muscarinic cholinergic receptors. The limited specificity of most drugs leads to alterations in the activity of enzymes not involved in the therapeutic response. Thus, tolbutamide given to promote the release of insulin from granules in the beta cells of the pancreas may, in the presence of alcohol, exert the toxicity characteristic of disulfiram. The mechanism appears to involve an inhibition of the aldehyde dehydrogenase-mediated oxidation of acetaldehyde by an aldehydic metabolite formed from tolbutamide.

Toxicity derived from the action of drugs on enzymes other than those participating in the therapeutic response is sometimes designated as a side effect. The enzymes most generally susceptible to alterations by drugs are those involved in the metabolism of compounds foreign to the body, particularly the mixed-function oxidases. The important implications of the induction or inhibition of the drug-metabolizing enzymes are now widely recognized in medical therapy and toxicology.

INTERCALATION WITH NUCLEIC ACIDS

Planar molecules such as the acridine dyes have been shown to insinuate themselves between the base pairs of double-stranded deoxyribonucleic acid (DNA) and, thereby, during DNA replication cause a misreading of the genetic code expressed as a frame-shift mutation. A number of drugs including actinomycin D, adriamycin, daunomycin, ethidium bromide, quinacrine, chloroquin and Miracil D also bind strongly to nucleic acids, most probably by intercalation, and many of these substances have exhibited mutagenic activity. Inasmuch as most of the available biological evidence has been obtained in microorganisms, the risk from mutagenic activity of these drugs in man cannot be accurately estimated.

In the past, considerable attention was given to the hypothesis that the carcinogenic activity of the polycyclic hydrocarbons was dependent on intercalation with DNA. It is now apparent that a more important feature of carcinogenesis is the formation of reactive metabolites and subsequent covalent binding to macromolecules. Recently, a variety of carcinogens known to lack mutagenic activity in microbial test systems have been
shown to become powerful mutagens after activation by the 9,000 g supernatant fraction of homogenates of rat liver. These findings have been reviewed as supporting the somatic mutation hypothesis of cancer.\textsuperscript{2}

**Alteration of Electrolyte Balance**

The serious consequences of disturbances in water and electrolyte balance are too widely recognized by physicians to warrant detailed discussion here. Of particular interest to the toxicologist are metabolic acidosis and alkalosis. The former condition is known to result from the ingestion of a variety of organic acids (e.g., salicylates) or alcohols that are metabolized to acids (e.g., methanol). The consumption of high doses of old preparations of paraldehyde may lead to serious metabolic acidosis, which has been attributed to the conversion of the drug to acetic acid in the presence of light and air.\textsuperscript{55} Metabolic alkalosis has been encountered in persons who consume large quantities of bicarbonate for the relief of gastric hyperacidity. This same abnormality of electrolyte balance may occur indirectly after poisons that cause protracted vomiting, diuresis or diarrhea.

**Alteration of the Structure of Water**

Molecules of diverse chemical structure alter the properties of water,\textsuperscript{64} and this action has been considered responsible for anesthetic activity.\textsuperscript{20,65} This hypothesis is not universally accepted, and some investigators place greater stress on the association of lipophilic substances with cell membranes.\textsuperscript{62,76} The diversity of opinions is too broad to warrant an attempt to summarize them here, but the toxicologist has frequent occasions to recall that weak, readily reversible bonds between xenobiotics and cellular water or lipids can have fatal consequences. The central nervous system depressants, including barbiturates and ethyl alcohol, are responsible for more deaths than any other category of poisons. Chemicals with this kind of pharmacological activity are not confined to useful drugs but embrace certain solvents and industrial gases.

**Disorganization of Cells**

Recent research has refined our understanding of the action of detergents on cellular membranes. It is now recognized that the effects produced by these chemicals cannot be attributed to massive emulsification and removal of lipids. Detergents such as saponin, filipin and lysolecithin have been shown to increase cellular permeability by binding within membranes and thereby altering the structure of the membranes.\textsuperscript{69,77} Lysolecithin is of practical interest in connection with bites from certain snakes and insects owing to its formation from lecithin by the action of phospholipase A contained in the venom.\textsuperscript{48} The increased permeability of cells caused by the lysolecithin not only allows the leakage of cellular constituents but permits other components of the venoms, notably hydrolytic enzymes, to enter the cells. The antifungal drug, amphotericin B, is thought to bind to cholesterol in cell membranes in a fashion similar to saponin.\textsuperscript{4} Its ability to produce anemia and kidney damage may depend on this interaction. The action of detergents extends to membranes other than the plasma membranes; thus, subcellular organelles such as the nucleus, mitochondria, lysosomes and biogenic amine storage granules may ultimately undergo lysis.

Although this review is emphasizing bond formation, the breakage of chemical bonds is a more prominent feature of certain toxic reactions. The hydrolytic action of acids or alkalis on the skin or mucous membranes is an obvious example of bond breakage.
Coordinate Covalent Binding

HEAVY METAL—NUCLEOPHILE REACTIONS

The usual mode of action of metal poisons is to bind to a variety of nucleophilic chemical groups, notably carboxyl, thiol, phosphate, imidazole and purine groups. The metals which are of greatest importance and have received most study in relation to toxic mechanisms are lead, mercury and arsenic. Cadmium, chromium and nickel (or nickel carbonyl) are of interest in connection with pulmonary carcinogenesis, and manganese, in relation to a Parkinsonian syndrome. The ubiquity of nucleophilic groups with different affinities for metals has greatly retarded identification of the key reactions in specific toxicities, although the anemia produced by lead is now rather well understood. The nature of heavy metal toxicity may vary with the chemical form, the route of entry into the body and dose-time relationships. Thus, methyl mercury and tetraethyl lead have more prominent effects on the central nervous system than inorganic salts of these metals. Also, inhaled cadmium exerts its toxicity primarily on the pulmonary system, whereas after absorption from the gastrointestinal tract, hepatic and renal toxicity are more common. Generally speaking, all the metals can inhibit a large number of enzymes and affect the conformation of nucleic acids, but the exact relationship of these processes to necrosis, carcinogenesis and teratogenesis remains uncertain.

The metallothioneins may be of some importance in connection with cadmium poisoning, but persuasive evidence is not available. These substances normally present in kidney and liver are proteins with low molecular weight and a very high content of cysteine. They bind cadmium strongly and appear to be inducible by this and other metals.

NUCLEOPHILE—TISSUE METAL REACTIONS

The possibility that chelating agents may exert toxicity by complexing trace metals, which are subsequently lost through excretion, has been considered, but no clear-cut example has been documented. Chelating agents are not restricted to the agents used in the therapy of heavy metal poisoning but embrace such widely used drugs as the tetracyclines. The inhibition of metal-containing enzymes by chemicals that bind the metal is a well-known phenomenon. The inhibition of dopamine-beta-hydroxylase, which contains copper, by diethyl-dithiocarbamate (the reduction product of disulfiram) is an example. The hemoproteins can react with a variety of nucleophiles that displace oxygen to impair cellular respiration. Carbon monoxide acts particularly on the ferrous iron in hemoglobin. The cyanide and sulfide ions and carbon disulfide act preferentially on the ferric iron in cytochrome c oxidase.

Covalent Binding

ANTIMETABOLITE INCORPORATION

The conversion of the rodenticide, fluoroacetate, into fluorocitrate, which can exert catastrophic effects on cellular metabolism by jamming the citric acid cycle, represents the classical example of the utilization of a false metabolite. Hypoglycin, a constituent of the unripe fruit of the ackee, provides another case of lethal synthesis of some practical importance. A large number of anomalous amino acids, purines, pyrimidines and sugar derivatives have been shown to produce toxic effects by participating in cellular reactions. Most of the vast literature on this subject involves synthetic chemicals but includes observations on unusual compounds elaborated by plants. Some antimetabolites of purines and pyrimidines have proved useful in
the chemotherapy of cancer. The majority of analogues participate in only a few of the reactions of normal metabolites. However, the incorporation of false amino acids into proteins, and purines and pyrimidines into nucleic acids, has been documented.19,54,80

**ELECTROPHILE—MACROMOLECULE REACTIONS**

The capacity of certain chemicals to react covalently with macromolecules is currently being intensively investigated in connection with carcinogenesis, mutagenesis, allergy and cellular necrosis.15,14,23,25,50,51,83 The sharpest distinction now apparent between toxic and therapeutic effects is the frequency of involvement of covalent bond formation in toxic processes. Indeed, this mechanism may be essential in the induction of allergy and cancer by small molecules. Excluding agents used in the chemotherapy of cancer, covalent bond formation in therapeutic effects is practically limited to diisopropyl phosphofluoridate and phenoxybenzamine. Although the importance of covalent binding in certain toxic reactions seems unquestionable, it is clear that this process does not always have serious consequences, even when the macromolecule involved is DNA. The animal body has a considerable capacity for the repair of DNA. In some research on poisons believed to act through covalent bonding, the evidence for covalency is far from adequate. Investigators must keep in mind that ionic and van der Waals forces may lead to complexes with very low dissociation constants. The binding of reserpine to adrenergic neurons1 and of the radiocystein medium, iophenoxic acid, to serum albumin7 are familiar examples of tight noncovalent associations.

Simple Alkylating Agents

Alkyl halides can react with nucleophilic groups on macromolecules, but the relationship of such reactions to toxicity has not been thoroughly explored. A review46 on the toxicology of methyl bromide states that use of this compound as a fumigant has been responsible for more deaths among occupationally exposed persons in California than all the organophosphate insecticides. Allyl and benzyl halides are more reactive, and urinary metabolites derived from the condensation of benzyl chloride with glutathione illustrate their capacity for reacting with thiol groups.84 The nitrogen and sulfur mustards, which owe their reactivity to cyclic intermediates, react extensively with carboxyl and thiol groups on proteins.75 Their binding includes cross-linking of the paired strands of DNA, a process that is regarded as fundamental in their chemotherapeutic effects and may be relevant to their mutagenic and carcinogenic actions. Another category of simple alkylating agents includes diazomethane and related compounds. Azaserine and 6-diazo-5-oxo-L-norleucine (DON) have found some use in cancer chemotherapy,17 while cyclasin and dimethylnitrosamine, which are metabolized to diazomethane, are of interest in connection with carcinogenesis.59 Dimethylnitrosamine has been shown to methylate DNA.89

**Epoxides**

The belief that the polycyclic hydrocarbons exert their carcinogenic effects by reaction of epoxide derivatives with DNA is continuously being strengthened, but emphasis may be shifting from K-region epoxides to other epoxides formed by the metabolism of these poisons. Work at the Chester Beatty Research Institute10,53,82 has provided strong evidence that the principal DNA reactant derived from benz(a)anthracene is the 8,9-dihydro-8,9-dihydroxy-10,11-oxide. This product is derived from the formation and hydrolysis of the 8,9-epoxide and subsequent epoxidation at the 10,11 double bond.
Likewise, the 7,8-dihydro-7,8-dihydroxy benz(a)pyrene, 9,10-oxide appears to be responsible for the binding to DNA seen after the administration of benz(a)pyrene. Presumably, the diol-epoxides are more effective than simple epoxides in reaching cellular DNA, because they are poor substrates for epoxide hydrase and glutathione transferase, the enzymes that normally inactivate epoxides.

The study of bromobenzene, which is also believed to exert its toxicity through an epoxide, has led to a classic in chemical pathology. Radiolabeled metabolites of the poison have been shown to be bound covalently to proteins in centrilobular necrotic regions of the liver. Pretreatment with phenobarbital, which induces the epoxidase more than the epoxide hydrase, augments the binding and the necrosis. Likewise, 3-methylcholanthrene, which has a greater effect on the hydrase, decreases binding and necrosis. Neither of these processes is prominent until the cellular supply of glutathione (which reacts with bromobenzene epoxide to form the precursors of a urinary mercapturic acid) is depleted. In rats given large doses of bromobenzene, sufficient amounts of the poison are bound to correspond to one molecule for each five molecules of protein in the entire liver. When localization of the necrosis to the centrilobular regions is taken into account, a one-to-one ratio is approximated.

Proteins contain a greater number of accessible nucleophilic groups than nucleic acids and are more extensively alkylated by epoxides. Aliphatic epoxides are more stable than arene oxides but undergo the same reactions. A compound of some toxicological interest is ethylene oxide, which is widely employed as a fumigant. Some bifunctional epoxides have been used in the chemotherapy of cancer.

Other Strained Rings

Epoxides and the nitrogen and sulfur vesicants mentioned owe their reactivity as alkylating agents to the strain in three-membered rings. Certain four- and five-membered strained rings act in the same fashion. The best known examples are beta-propiolactone and propanesultone (1-hydroxy-1-propane sulfonic acid sultone). Both compounds alkylate proteins and nucleic acids and exhibit carcinogenic activity.

Reactive Esters

Aromatic amines and their acetyl derivatives are metabolized to varying extents to N-hydroxy compounds, which after esterification in vivo (presumably with sulfuric acid) react as carbonium or amidonium ions with proteins and nucleic acids. These reactions have received intensive study in connection with the carcinogens, 2-acetylaminofluorene and N-methy1-4-aminoazo-benzene. Sulfonylates of aliphatic alcohols and glycols are sufficiently reactive as alkylating agents to be useful in the chemotherapy of cancer. Sulphonic acid esters of allyl and benzyl alcohol can also act as alkylating agents. The carcinogenic activity of safrole appears to depend on hydroxylation of the allyl group and subsequent esterification of the alcohol. Epoxidation of the double bond would then create a bifunctional alkylating agent. The senecio or pyrrolizidine alkaloids illustrate organic esters that can alkylate macromolecules.

Acylating Agents

The acylating agents of greatest importance in toxicology are the phosphorus insecticides. As is well known, these chemicals inhibit the cholinesterases by esterifying serine at the esteratic site of the enzymes, a reaction that in the absence of treatment with pralidoxime is irreversible. The action of the organophosphates on
cholinesterases has been regarded as highly specific, but it is now known that certain aliesterases, which are responsible for the detoxification of a variety of drug esters including pesticides, are more sensitive to inhibition.\(^3^0\) It has long been recognized that amides formed by the acylation of the epsilon-amino group of lysine in serum albumin are sufficiently stable to act as haptens. The spontaneous rearrangement of penicillin to penicillenic acid, which can acylate lysine in proteins, has been shown to be responsible for the allergic reactions from this antibiotic.\(^3^9\) The acetyl group of aspirin can likewise be transferred to serum albumin, but the relationship of this reaction to allergy has not been clarified.\(^6^7\) Interference with the aggregation of platelets by aspirin has been attributed to acetylation of the platelet membrane.\(^7^4\)

Aldehyde-amine Condensations

The reaction of chloroacetaldehyde with amino groups on macromolecules has been considered in connection with the carcinogenic activity of vinyl chloride, a very inert halide in respect to alkylation.\(^8^7\) This particular aldehyde is a probable metabolite of ethylene dichloride and ethylene chlorohydrin as well as vinyl chloride and is of interest in connection with toxicities other than the production of tumors.\(^4^3\),\(^5^7\) Beyond reactions with macromolecules, the condensation of acetaldehyde with catecholamines to form tetrahydroquinoline derivatives has been investigated in relation to acute and chronic alcoholism.\(^5^6\) It seems inevitable that this reaction would occur in vivo to some extent, but evidence that the tetrahydroisoquinolines exert appropriate pharmacological effects and are present in meaningful concentrations is inadequate. A number of foreign amines, particularly hydrazine and its derivatives, have been shown to form Schiff bases with pyridoxal phosphate. This reaction is responsible for the inhibition of a number of enzymes that utilize vitamin B\(_6\) as a coenzyme.

Free Radical Reactions

Homolytic fission to form free radicals is believed to be responsible for the hepatic damage produced by carbon tetrachloride and certain other halocarbons.\(^7^0\) The fission is catalyzed by the mixed function oxidase in the endoplasmic reticulum. The short-lived free radicals abstract hydrogen from the membrane phospholipids, thereby creating new free radicals and promoting the conjugation of double bonds and the cleavage of fatty acids that comprise lipid peroxidation. The autocatalytic decomposition then spreads to other parts of the cell, probably through the movement of lipid peroxides and hydroperoxides, which can yield new free radicals. In carbon tetrachloride poisoning, thiol groups on proteins are undoubtedly alkylated to some extent by the trichloromethyl free radical, but it appears likely that the major attack is made by lipid peroxides.

References

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