CHAPTER 4

INTERACTIONS INVOLVING BIOTRANSFORMATION REACTIONS

Almost all drugs, insecticides, and other foreign compounds are metabolized by living organisms. The extent of this metabolism often limits the rate of toxicity or biological activity. Therefore, alterations in the metabolism of a given chemical can lead to profound changes in the extent and/or duration of its biological effects.

Clearly, exposure of an animal to combinations of more than one chemical (either simultaneously or in close succession) can result in interactions whereby one chemical can markedly modify the metabolism of another. Such an interaction may synergize (potentiate) or antagonize the toxicity of one or more components of the mixture.

Drug interactions occur primarily through the ability of one chemical (A) to inhibit or to stimulate the enzymes responsible for the metabolism of another (B). When toxicant B is inactivated (detoxified) by a given enzyme system, compound A may interact to inhibit or stimulate enzyme activity, thereby leading to either potentiation or antagonism of toxicity, respectively. If, on the other hand, B is metabolically activated, the opposite result will be observed.

Many potentially hazardous toxic interactions can occur through quantitative and qualitative modifications in biotransformation. Several result from the increased use of multidrug therapy, and others have been demonstrated in the laboratory. There is growing concern over the potential hazard to humans of toxic interactions resulting from the large number of drugs, pesticides, and other chemicals to which they are inadvertently or occupationally exposed.

Discussions of interactions involving biotransformation cover an extremely broad area for which there is a vast amount of literature. Current knowledge comes mainly from in-vivo studies that have been conducted under carefully controlled laboratory conditions and are often combined with basic in-vitro investigations directed towards establishing mechanistic details at the subcellular or enzyme level. The information available at this level is considerable. However, few attempts have yet been made to determine whether the interactions observed under laboratory conditions (acute doses, nonphysiological routes of exposure, etc.) can occur in human populations exposed to "real-life" environmental or occupational conditions (low level, chronic, oral, dermal, or inhalation exposure), and if they can, whether they constitute a real toxic hazard.

This synopsis contains an outline of some basic mechanisms through which the biotransformation of various toxicants can be modified and an assessment of how these can be evaluated in vivo.

The importance of the hepatic microsomal oxidase system as the primary site for biotransformation of lipophilic foreign compounds has been established. Therefore, the interactions involving this system are emphasized below. However, interactions may involve any enzyme that plays a role in toxicant metabolism.

THE MICROSOMAL MONOXYGENASE SYSTEM

Foreign compounds may interact to induce or to inhibit monoxygenase activity.

Induction

Microsomal enzyme induction has been discussed at length in several recent reviews (Bock and Remmer, 1978; Conney, 1967, 1971; Gelboin, 1971; Remmer, 1972; Sher, 1971; Testa and Jenner, 1976). It may lead to a decrease in toxicity (detoxication) by enhancing the rate of metabolism of a toxicant to inactive products or to an increase in toxicity (intoxication) through enhanced formation of active metabolites.

Inducers. Almost all types of lipophilic compounds will cause some degree of induction (Bock and Remmer, 1978; Conney, 1967, 1971; Gelboin, 1971; Remmer, 1972; Sher, 1971; Testa and Jenner, 1976). In general, high levels of inductions result from exposure to lipophilic materials (e.g., many of the chlorinated hydrocarbon insecticides), which have prolonged biological half-lives. Compounds with low lipophilic character or with short half-lives are not effective inducing agents. Except for the generally established importance of lipophilic character, no structure-activity relationships are apparent in inducing agents.

Inducing agents can be divided into two major groups. Thus, phenobarbital and a large number of other drugs and insecticides cause a rather general increase in the oxidative metabolism of a wide variety of substrates. In contrast, polycyclic hydrocarbons, such as 3-methylcholanthrene, cause an increase in metabolic activity in a more limited spectrum of compounds (Conney, 1967, 1971; Testa and Jenner, 1976).

The different metabolic responses to these two groups of inducing agents are generally attributed to their ability to induce intrinsically different forms of cytochrome P-450, the terminal oxidase of the microsomal electron transport pathway and the catalytic center of the system (Conney, 1967, 1971; Mannering, 1971; Testa and Jenner, 1976). A detailed discussion of these differences is beyond the scope of this report.

Conditions Effecting Induction. The major factors relate to the degree, duration, and route of exposure vis-a-vis the tissue under consideration.

For induction of the hepatic monoxygenase system, there seems to be a general requirement for a prolonged hepatic exposure of the inducing agent (Remmer, 1969). In most induction studies, relatively high concentrations of the inducer are used and little attention is given to evaluating dose-response relationships or to defining the threshold levels of exposure that are required to elicit an effect.

The presence of a threshold concentration of the inducing agent in the tissue is presumably also required for monoxygenase induction in extrahepatic organs and tissues such as lung, skin, intestine, etc. In recent years, considerable attention has been focused on metabolism in these extrahepatic tissues. In some cases, they may be of critical importance in toxicological interactions resulting from inhalation or dermal exposures to combinations of chemical agents. Morever, there is some evidence that qualitative and quantitative differences may exist between the enzymes in these tissues and those in the liver, and including, perhaps, differences in response to inducers (Alvares, 1977; Grafström et al., 1977; Wollenberg and Ullrich, 1977).

To evaluate potential hazards from cargo vapors, it is important to obtain information on the rate and level of accumulation of inhaled chemical vapors, degree and duration of exposure, and the threshold concentrations required to elicit an inductive response in the lung.

Another factor to be considered is the duration of the induction process itself. The inducing action of different compounds begins at different rates and continues after cessation of exposure for various lengths of time. The time at which maximum induction is observed varies from approximately 24 hours after exposure (for 3-methylcholanthrene) to approximately 1-2 weeks after exposure (for the insecticide chlordane) (Testa and Jenner, 1976). Following cessation of exposure to phenobarbital, enzyme activity returns to normal levels within several days, but after exposure to more biologically stable inducers, enzyme activity continues for much longer periods. These effects are clearly related to the biological half-lives of different inducers and their continued presence in the tissue.

Several potent inducing agents actually inhibit drug metabolism during the first few hours after administration.

Tests for Enzyme Induction. Although the mechanistic aspects of induction are complex and incompletely understood and the precise conditions under which induction occurs are still not clearly defined, it is possible to predict the likely in-vivo effects of induction on the biological activity or toxicity of various compounds. Therefore,

if in-vivo tests to detect an induced state could be developed, the potential hazards of exposure to other compounds could be approximated.

Since monoxygenase induction leads to changes in the metabolism of several endogenous materials as well as foreign compounds, several noninvasive tests have been studied for possible use as in-vivo indicators of induction in humans. These tests are based largely on observed changes in steroid and glucose metabolism following induction.

• Steroid Metabolism. Under normal (uninduced) conditions, cortisol, a major adrenal-cortical hormone, is excreted in the urine as its 17-hydroxy derivative. Following treatment with several monoxygenase inducers, the rate of cortisol excretion in the urine is enhanced mainly by an increase in 6β-hydroxycortisol, a product of hepatic oxidase activity that is usually of minor significance. Thus, the ratio of 6β- to 17-hydroxycortisol in the urine has been suggested as a potentially useful indicator for detecting enzyme induction (Roots et al., 1977; Testa and Jenner, 1976) and has been shown to increase significantly following treatment of animals with phenobarbital, phenyl butazone, diphenylhydantoin, and several other drugs (Roots et al., 1977; Testa and Jenner, 1976). No increase in 6β-hydroxycortisol was detected in animals treated with 3-methylcholanthrene (Roots et al., 1977; Testa and Jenner, 1976), suggesting that this indicator cannot be of general utility for all types of inducers. On the other hand, this may indicate its potential usefulness in distinguishing between the effects of the phenobarbital or 3-methylcholanthrene type of inducers.

The potential use of the test in humans has been demonstrated by the increased excretion of 6β-hydroxycortisol (Roots et al., 1977; Testa and Jenner, 1976).

● Glucose Metabolism. A further consequence of exposure to monoxygenase inducers is an increase in the activity of the microsomal enzyme uridine-5'-diphosphate (UDP)-glucuronyl transferase and an increased urinary excretion of D-glucaric acid. This has been observed in several species of animals (Aarts, 1965; Marsh and Reid, 1963; Roots et al., 1977; Testa and Jenner, 1976) and humans (Aarts, 1965; Testa and Jenner, 1976) following treatment with drugs. Although it is only indirectly related to monoxygenase activity per se, it might prove to be a useful in-vivo empirical indicator of induction.

Several other indicators of monoxygenase induction have been investigated in laboratory animals hut are unsuitable for routine application to humans. These include measurement of ascorbic acid excretion, which occurs in many species but not in humans, administration of a test drug such as aminopyrine and measurement of its half-life, and in-vitro tests for direct measurement of cytochrome P-450 levels in liver biopsy samples.

In summary, some tests for enzyme induction in humans show promise, but as yet there is no single test or combination of tests that can be used to predict all induction effects likely to be encountered. Furthermore, apparent differences between species preclude simple extrapolation to humans of data obtained with laboratory animals.

A major problem in developing satisfactory in-vivo tests for enzyme induction in humans is the apparently substantial interindividual variation in enzyme activity and the difficulty of establishing a common control level for the various test parameters. Although much of this interindividual variation is due to genetic factors (Vesell, 1977), a large number of variables associated with both the external and internal environment have been identified as potential modifiers of drug metabolism. In many cases these factors could obviate any changes resulting from induction.

Inhibition

The monoxygenase system may be inhibited by several different mechanisms. Numerous compounds have been shown to inhibit the metabolism of other systems both in vivo and in vitro (Anders, 1971; Mannering, 1971; Testa and Jenner, 1976). Since the onset of inhibitory effects is usually observed quite rapidly after administration of a compound, interactions occurring through this basic mechanism may be encountered more often than those resulting from induction.

Alternative Substrate Inhibition. The microsomal enzyme system is noted for its low degree of substrate specificity. When two oxidizable substrates are presented simultaneously to the microsomal system, one can competitively inhibit the other by a process termed alternative substrate inhibition (Anders, 1971; Mannering, 1971; Testa and Jenner, 1976). This is probably a rather short-lasting effect in vivo and will become significant only under acute conditions when the combined concentration of the substrates temporarily

overloads the system causing the substrates to compete for binding sites. This type of inhibition is competitive; the K_m (metabolism constant) of each component when considered as a substrate must equal its K_i (inhibition constant) when considered as an inhibitor. The extent to which this type of inhibition occurs depends on the relative affinities of the two compounds for the binding sites. Compounds with high affinities and low rates of metabolism can be expected to be the most effective inhibitors of this type. Indeed, some of the best alternative substrate inhibitors are those that undergo little or no metabolism, e.g., perfluorinated hydrocarbons, which cannot be hydroxylated.

Noncompetitive Inhibitory Interactions. The action of most monoxygenase inhibitors occurs at least initially through alternative substrate inhibition. However, several types of compounds are able to exert additional, more intense noncompetitive inhibitory interactions through the formation of a variety of active metabolites which form inhibitory complexes with cytochrome P-450. These compounds are well recognized for their potent inhibition of drug oxidation *in vitro* and for their ability to modify the action of many drugs and insecticides *in vivo*. The well-known drug potentiator SKF-525A owes its activity in part to the formation of such a complex (Schenkman *et al.*, 1972). Other compounds, such as 1,3-benzodioxoles (commercially used as insecticide synergists) (Franklin, 1971; Philpot and Hodgson, 1971) and several amphetamines (Franklin, 1977) form similarly active oxidative metabolites, which complex with and reduce the amount of cytochrome P-450 that is available for further drug

oxidation. The formation of active inhibitory metabolites during drug oxidation is currently receiving much attention. It is likely that other examples will be discovered.

Another group of compounds that undergo monoxygenase-catalyzed activation to form reactive intermediates are those containing thiono-sulfur groups (thiourea, thioacetamide, carbon disulfide, thiobarbital, thiouracil, phosphorothionates, etc.). Several of these compounds inhibit both in-vitro and in-vivo drug oxidation mediated by cytochrome P-450 through the oxidase-catalyzed release of atomic sulfur, which binds covalently to available nucleophiles. The loss of cytochrome P-450 is associated with covalent sulfur binding (Neal et al., 1977).

Covalent binding of a metabolically formed radical intermediate ($^{\cdot}Cl_3$) is also thought to inhibit drug oxidation following exposure to carbon tetrachloride (Diaz Gomez et al., 1973).

Other Inhibitors. Another group of potentially important inhibitors are compounds that can undergo direct ligand binding to cytochrome P-450 because of the unhindered nitrogen atom in their structure. These compounds include several groups of imidazoles and other nitrogen-containing heterocyclic compounds, which are potent inhibitors of drug metabolism both in vitro and in vivo (Wilkinson et al., 1974 a,b).

Biphasic Interactions

Although we tend to discuss inducers and inhibitors of monoxygenase activity as distinct classes of compounds, this distinction should be evaluated carefully.

One important factor to be considered in distinguishing the two effects is the length of time after administration before the effect is observed. The effects of SKF-525A and 1,3-benzodioxoles such as piperonyl butoxide are clearly biphasic. Their initial acute inhibitory action on microsomal metabolism is usually followed by a marked stimulation of the metabolism (Testa and Jenner, 1976; Wilkinson, 1976). Thus, a compound classified as an inhibitor when its effect is measured from 0.5 to 12 hours after administration would be termed an inducer if its effect was not measured until 24 to 48 hours afterward. Similarly, as mentioned earlier, several compounds classified as potent inducers of monoxygenase activity actually inhibit enzyme activity during the period immediately following administration.

We must conclude, therefore, that time after exposure is an extremely important factor that is often given little or no attention in interaction studies. This adds to the complexity of the problem by suggesting that we have concerned ourselves mainly with compounds at either extreme of the spectrum. The compounds we believe to be good inducers may simply be those with a relatively short inhibitory phase, while those we believe to be inhibitors may simply be inducers that exhibit a longer inhibitory phase before induction becomes obvious. There may be many shades of gray between these two extremes.

CHANGES IN ACTIVITIES OF OTHER ENZYMES INVOLVED IN BIOTRANSFORMATION

The precise mechanism by which induction of mixed-function oxidase activity occurs is not fully understood. One complication is that the induction process is not specific for the system mediated by cytochrome P-450. Thus, exposure of animals to established inducers

of mixed-function oxidase activity often results in the coinduction of several other enzymes that appear to be unrelated to the microsomal oxidase system. The complete spectrum of enzymes that are induced by various foreign compounds has not yet been studied in detail, and there is currently no way to predict them.

Since some of the other induced enzymes may also play a role in the detoxication of various toxicants, an entirely new area of potentially important interactions remains to be studied. For example, there is a marked induction of serum and liver aliesterase activity in animals exposed to several drugs and insecticides (Cohen and Murphy, 1974) that are known inducers of the hepatic microsomal oxidase system. This leads to several unexpected interactions between such inducers as paraoxon and other organophosphate insecticides that are bound to aliesterase. Exposure of animals to the chlorinated insecticides aldrin or DDT (both known inducers of mixed-function oxidase) causes an increase in aliesterase titer which in turn protects animals from poisoning by paraoxon (Triolo et al., 1970).

Aliesterase is itself an important detoxication enzyme for several foreign compounds containing carboxyester functional groups, and its inhibition by organophosphates is a well-known and well-studied mechanism by which the toxicity of compounds such as malathion can be potentiated (Wilkinson, 1976).

Toxicological interactions could also occur through depletion by one chemical of a cofactor or cosubstrate that is required for the biotransformation of another. Thus, the anesthetic fluroxene ($CF_3CH_2OCH{=}CH_2$), but not several related compounds, caused a

significant dose-dependent reduction in tissue levels of glutathione, which could affect glutathione-dependent metabolism of a variety of other materials, e.g., 1,1-dichloroethylene). However, in view of the high endogenous levels of glutathione in tissue it is unlikely that such interactions will occur except under conditions of unusually high acute exposures.

CONSEQUENCES OF INTERACTIONS INVOLVING BIOTRANSFORMATION

The consequence of chemical interactions may be to potentiate or to antagonize the toxicity of one or more components (Gillette and Mitchell, 1975; Shand et al., 1975). Toxicological interactions occur when one chemical, A, inhibits or stimulates enzymes responsible for the metabolism of another, B. Where toxicant B is inactivated (detoxified) by a given enzyme system, compound A may interact to inhibit or stimulate enzyme activity, thus leading to potentiation or antagonism of toxicity, respectively. If, on the other hand, B is metabolically activated to a toxic species, the opposite will result.

INTERACTIONS WITH FORMATION OF REACTIVE INTERMEDIATES

Reaction Mechanisms

Recent evidence indicates that an increase in the formation of toxic metabolites may be a particularly important interaction after repeated low-level exposures to multiple chemicals (Jerina and Daly, 1974; Magee and Barnes, 1967; Miller, 1970; Mitchell and Corcoran, 1977; Mitchell et al., 1976, 1977). Other endogenous or exogenous compounds that alter the formation of these reactive species or that react with the biologically active form to spare critical tissue sites of action may modify the toxicity of chemicals that form reactive intermediates as the following examples illustrate.

Epoxides are among the reactive intermediates formed from a wide variety of aromatic compounds. Toxicity due to epoxide formation requires the P-450-mediated conversion of an unsaturated compound to a reactive epoxide, followed by reaction of the epoxide with critical cellular components. Normal cellular reactions of an epoxide include nonenzymatic conversion to a phenol (for benzenoid epoxides), enzymatic and perhaps nonenzymatic reactions with glutathione and with water (epoxide hydratase), and possibly nonenzymatic reactions with other thiols. As far as is known, radical mechanisms of reaction are not involved. Toxic nonenzymatic reactions with critical cell components are likely to occur only under specific circumstances. Depletion of glutathione or inhibition of epoxide hydratase should greatly increase the toxic effects of a reactive epoxide.

Many o- and p-quinones and, recently, quinonimines have been shown to react readily with nucleophiles such as thiols and amines to form isolable addition products. Although reactions of this type have been unquestionably valuable to the synthetic organic chemist for many decades, their significance in the disposition of chemicals and drugs by the body has been appreciated only recently.

Numerous drugs and their metabolites contain catechol or 1,4-dihydroxy functions within their structure. Although these reduced forms do not react readily with nucleophiles, cellular oxidation of these agents to reactive quinone structures followed by addition reactions with endogenous nucleophiles has led to the isolation of glutathione, cysteine, and mercapturic acid conjugates in bile or urine. On the other hand, endogenous cellular nucleophiles such as

glutathione should play key roles in the pathogenesis of cellular injury caused by such metabolites. Finally, further oxidation of diol drugs or their metabolites to specific triol configurations may result in striking exacerbation of the toxicity of these agents through mechanisms not involving adduct formation (see discussion of triols below).

The reactivity of free radicals covers a wide spectrum. Individual reactivity is ultimately determined by the structure of the radical and its environment. Alkyl and acyl radicals, which have been suspected as metabolites of certain drugs, have been found to be particularly reactive. When generated under biological conditions, alkyl radicals can undergo numerous reactions, including hydrogen abstraction (to produce alkanes), addition to multiple bonds, and cross-linking reactions. Although newly formed radicals resulting from propagation reactions (e.g., hydrogen abstraction) may react with oxygen to form hydroperoxyl radicals, reaction with neighboring groups, such as unsaturated sites, would be expected to predominate.

Evidence indicates that alkyl and acyl radicals are the important toxic metabolic products of P-450 oxidation of alkyl- and acylhydrazines. The reaction of O_2^- with alkyl halides (e.g., alkyl bromides, carbon tetrachloride, trichlorobromomethane, and certain general anesthetic gases), sulfates, and phosphates may also result in the formation of alkyl radicals. Although vitamin E is recognized as a radical acceptor, it is likely that alkyl radicals would react very rapidly with neighboring molecules and would not necessarily react selectively with vitamin E. Glutathione should react with alkyl radicals and might have some

protective effect, but this may also not be a selective or biologically important reaction. Exogenous compounds that act as radical scavengers may also be expected to modify the toxic action of compounds that act through a free radical mechanism.

The generation of hydroperoxyl radicals within the body is likely to lead to a high degree of toxicity and a variety of products. The initial step is presumably a P-450 oxidation to generate ROO·, which can react with unsaturated compounds to form a variety of products and undergo cross-linking reactions. Secondary reactions of radicals with polyunsaturated fats may produce hydroperoxides, hydroxy acids, and hydroxyepoxy acids. Reactions of these species with membrane phospholipids could be critical to the normal functions of the cell and cellular response to toxic insult by reactive oxygen species.

Vitamin E should protect phospholipids in membranes against oxidation by hydroperoxide. It is doubtful that vitamin E could protect against reactions occurring at sites other than those associated with membranes. Glutathione may have some protective effect through a termination reaction.

Reactive Oxygen Species Produced from Reactive Metabolites

The generation of reactive oxygen species is most likely based upon the reaction of cellular oxygen with a one-electron intermediate that may be acidic, basic, or neutral, but which will transfer an electron to oxygen to form O_2^-. Examples of toxicity of this type include nitrofurantoin, in which the observed toxicity is apparently due to O_2^- formation, certain triols with a 1,2,4 or 1,3,4 relationship,

which may be capable of generating superoxide ions through a one-electron intermediate that corresponds to a semiquinone, and paraquat for which toxicity is presumably related to the one-electron reduction of oxygen to the superoxide anion.

It is apparent that an interdisciplinary, integrated approach is necessary in order to correlate the formation of chemically reactive metabolites with the incidence, types, and severities of toxicities caused by drugs and other foreign compounds. Studies of covalent binding should also be useful in determining whether alterations in the incidence and severity of various toxicities are due to differences in the metabolism of the foregin compound or to changes in the events that follow the formation of the reactive metabolite.

On the other hand, a survey (Table 4-1) of the different types and locations of the tissue lesions produced by reactive drug metabolites indicates that at least four types of reactive species causing tissue lesions can be postulated: electrophilic intermediates showing significant glutathione conjugation in vivo (e.g., bromobenzene, acetaminophen, 2-furamide); electrophilic species not showing significant glutathione conjugation in vivo (e.g., furosemide, dimethylnitrosamine); alkylating radicals (e.g., carbon tetrachloride, alkyl- and acylhydrazines); and nonalkylating reactive intermediates whose toxicities are potentiated by vitamin E-deficient diets. Thus, it is clear that additional tools and approaches are needed for the study of chemically induced tissue lesions caused by the formation of reactive, nonalkylating species.

TABLE 4-1

Nature of Chemically Reactive Metabolites

Electrophiles Alkylators GSH[a] Threshold Sulfhydryl Protection	Electrophiles Alkylators No GSH Threshold No Sulfhydryl Protection	Radicals and Hydroperoxides Alkylators No GSH Threshold Vitamin E Protection[b]	Reactive Oxygen Species Nonalkylators No GSH Threshold Vitamin E Protection
Acetaminophen	Furosemide	Hydrazines	Paraquat
Phenacetin	Dimethylnitrosamine	Haloalkanes	Nitrofurantoin
Acetanilide			Methyldopa[b]
Halobenzenes			Salicylates[b]
Simple Furans			
Simple Thiophenes			

[a]GSH = reduced glutathione.

[b]Protection is questionable.

Careful consideration of the types of electrophilic and radical metabolites of chemicals that might be formed, and their likley chemical reaction mechanisms with tissue molecules and other exogenous chemicals, should make it increasingly possible to examine and define the molecular basis for chemically induced tissue injuries. Such knowledge should also make it possible to predict with greater certainty the likelihood of chemical-chemical interactions producing adverse health effects in humans from either simultaneous or sequential exposure.

REFERENCES

Aarts, E. M. 1965. Evidence for the function of D-glucaric acid as an indicator for drug induced enhanced metabolism through the glucuronic acid pathway in man. Biochem. Pharmacol. 14:359-362.

Alvares, A. P. 1977. Stimulatory effects of polychlorinated biphenyls (PCB) on cytochromes P-450 and P-448 mediated microsomal oxidations. Pp. 476-483 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Anders, M. W. 1971. Enhancement and inhibition of drug metabolism. Ann. Rev. Pharmacol. 11:37-56.

Bock, K. W., and H. Remmer. 1978. Introduction to hepatic hemoproteins. Pp. 49-80 in F. DeMatteis and W. N. Aldridge, eds. Handbook of Experimental Pharmacology, Vol. 44. Heme and Hemoproteins. Springer-Verlag, Berlin.

Cohen, S. D., and S. D. Murphy. 1974. A simplified bioassay for organophosphate detoxification and interactions. Toxicol. Appl. Pharmacol. 27:537-550.

Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacol. Rev. 19:317-366.

Conney, A. H. 1971. Environmental factors influencing drug metabolism. Pp. 253-278 in B. La Du, H. G. Mandel, and E. L. Way, eds. Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins, Baltimore.

Diaz Gomez, M. I., J. A. Castro, E. C. de Ferreyra, N. D'Acosta, and C. R. de Castro. 1973. Irreversible binding of ^{14}C from $^{14}CCl_4$ to liver microsomal lipids and proteins from rats pretreated with compounds altering microsomal mixed-function oxygenase activity. Toxicol. Appl. Pharmacol. 25:534-541.

Franklin, M. R. 1971. The enzymic formation of a methylenedioxyphenyl derivative exhibiting an isocyanide-like spectrum with reduced cytochrome P-450 in hepatic microsomes. Xenobiotica 1:581-591.

Franklin, M. R. 1977. The inhibition of mixed-function oxidation reactions by amphetamines in liver and lung microsomes. Pp. 284-291 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Gelboin, H. V. 1971. Mechanics of induction of drug metabolism enzymes. Pp. 279-307 in B. La Du, H. G. Mandel, and E. L. Way, eds. Fundamentals of Drug Metabolism and Drug Disposition. Williams and Wilkins, Baltimore.

Gillette, J. R., and J. R. Mitchell. 1975. Drug actions and interactions: Theoretical considerations. Pp. 359-382 in J. R. Gillette and J. R. Mitchell, eds. Handbook of Experimental Pharmacology, Vol. XXVIII, Part 3. Springer-Verlag, New York.

Grafström, R., S. J. Stohs, M. D. Burke, P. Moldeus, and S. Orrenius. 1977. Benzo(α)pyrene metabolism by microsomes and isolated epithelial cells from rat small intestine. Pp. 667-674 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Jerina, D. M., and J. W. Daly. 1974. Arene oxides: A new aspect of drug metabolism. Science 185:573-582.

Magee, P. N., and J. M. Barnes. 1967. Carcinogenic nitroso compounds. Adv. Cancer Res. 10:163-246.

Mannering, G. J. 1971. Inhibition of drug metabolism. Pp. 452-476 in B. B. Brodie and J. R. Gillette, eds. Handbook of Experimental Pharmacology, Vol. 28, Concepts in Biochemical Pharmacology, Part 2. Springer-Verlag, New York.

Mannering, G. J. 1971. Properties of cytochrome P-450 as affected by environmental factors: qualitative changes due to administration of polycyclic hydrocarbons. Metabolism 20:228-245.

Marsh, C. A., and L. M. Reid. 1963. Changes in D-glucaric acid excretion induced by stimulators of ascorbic acid biosynthesis. Biochim. Biophys. Acta 78:726-728.

Miller, J. A. 1970. Carcinogenesis by chemicals: An overview-- G. H. A. Clowes Medmorial Lecture. Cancer Res. 30:559-576.

Mitchell, J. R., and C. B. Corcoran. 1977. Macromolecular binding in assessing drug and chemical-induced tissue lesions. Proc. of the Conference on the Status of Predictive Tools in Application to Safety Evaluation: Present and Future (Carcinogenesis and Mutagenesis). J. Environ. Pathol. Toxicol. 1, Special Issue:101-115.

Mitchell, J. R., S. D. Nelson, S. S. Thorgeirsson, R. J. McMurtry, and E. Dybing. 1976. Metabolic activation: Biochemical basis for many drug-induced liver injuries. Pp. 259-279 in H. Popper and F. Schaffner, eds. Progress in Liver Diseases, Vol. V. Grune & Stratton, New York.

Mitchell, J. R., R. J. McMurtry, C. N. Stathan, and S. D. Nelson. 1977. Molecular basis for several drug-induced nephropathies. Am. J. Med. 62:518-526.

Neal, R. A., T. Kamataki, A. L. Hunter, and G. Catignani. 1977. Monooxygenase catalyzed activation of thiono-sulfur containing compounds to reative intermediates. Pp. 467-475 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Philpot, R. M., and E. Hodgson. 1971. A cytochrome P-450-piperonyl butoxide spectrum similar to that produced by ethyl isocyanide. Life Sci. 10, Pt. II:503-512.

Remmer, H. 1969. The induction of hydroxylating enzymes by drugs. Pp. 125-141 in D. Shugar, ed. Biochemical Aspects of Antimetabolites and Drug Hydroxylation. Federation of European Biochemical Societies Symposium Vol. 16. Academic Press, London.

Remmer, H. 1972. Induction of drug metabolizing enzyme system in the liver. Eur. J. Clin. Pharmacol. 5:116-136.

Roots, I., B. Ley, and A. G. Hildebrandt. 1977. In vivo parameters of drug metabolism--differences in specificity towards inducing agents. Pp. 581-588 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Schenkman, J. B., B. J. Wilson, and D. L. Cinti. 1972. Diethylaminoethyl 2,2-diphenylvalerate HCl (SKF 525-A)-in vivo and in vitro effects of metabolism by rat liver microsomes--formation of an oxygenated complex. Biochem. Pharmacol. 21:2373-2383.

Shand, D. G., J. R. Mitchell, and J. A. Oates. 1975. Pharmacokinetic drug interactions. Pp. 272-314 in J. R. Gillette and J. R. Mitchell, eds. Handbook of Experimental Pharmacology, Vol. XXVIII, Part 3. Springer-Verlag, New York.

Sher, S. P. 1971. Drug enzyme induction and drug interactions: literature tabulation. Toxicol. Appl. Pharmacol. 18:780-834.

Testa, B., and P. Jenner. 1976. Chapter 2.2 Induction and inhibition of drug-metabolizing enzyme systems. Pp. 329-350 in B. Testa and P. Jenner, eds. Drug Metabolism: Chemical and Biochemical Aspects. Marcel Dekker, New York.

Triolo, A. J., E. Mata, and J. M. Coon. 1970. Effects of organochlorine insecticides on the toxicity and in vitro plasma detoxication of paraoxon. Toxicol. Appl. Pharmacol. 17:174-180.

Vesell, E. S. 1977. Effects of disease states on drug deposition in man. Pp. 628-645 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

Wilkinson, C. F. 1976. Chapter 15. Insecticide interactions. Pp. 605-647 in C. F. Wilkinson, ed. Insecticide Biochemistry and Physiology. Plenum Press, New York.

Wilkinson, C. F., K. Hetnarski, G. P. Cantwell, and F. J. Di Carlo. 1974a. Structure-activity relationships in the effects of 1-alkylimidazoles on microsomal oxidation in vitro and in vivo. Biochem. Pharmacol. 23:2377-2386.

Wilkinson, C. F., K. Hetnarski, and L. J. Hicks. 1974b. Substituted imidazoles as inhibitors of microsomal oxidation and insecticide synergists. Pestic. Biochem. Physiol. 4:299-312.

Wollenberg, P., and V. Ullrich. 1977. Characterization of the drug monooxygenase in the mouse small intestine. Pp. 675-679 in V. Ullrich, A. Hildebrandt, I. Roots, R. W. Estabrook, and A. H. Conney, eds. Microsomes and Drug Oxidations. Pergamon Press, New York.

CHAPTER 5

INTERACTIONS AT STORAGE SITES

Various parts of the body serve as sites for the storage of selectively accumulated chemicals. These sites include plasma proteins and other extracellular depots such as connective tissues, bone, intracellular fluids, and fat. During storage, no biological reactions are expressed. Consequently, such reservoirs may be considered "silent receptors" or "sites of loss" (Levine, 1973).

The portion of a chemical that is bound to a silent receptor is in equilibrium with the active portion in the plasma, and the release of the bound chemical occurs as plasma concentrations are reduced through biotransformation or excretion. Thus, the effective plasma level of a chemical may be maintained for a prolonged period, and the physiological and potential toxicological effect may be correspondingly prolonged.

The propensity of a chemical to bind to a silent receptor is governed by the same principles that determine its reaction with active tissue binding sites (Fingl and Woodbury, 1975). These include the affinity of the specific chemical for that receptor as well as the strength and reversibility of the bond formations involved. In addition, such properties as lipid solubility and affinity for active transport processes may determine the amount of chemical found in tissue storage sites. When two or more chemicals with similar properties bind to the same storage site, toxicological

interactions between those chemicals are governed by the extent to which the chemicals compete with each other for that site.

PLASMA PROTEIN AS A STORAGE SITE

When plasma protein, such as albumin, is the site of storage for two or more chemicals, the extent of interaction depends principally upon the strength of the reversible bonds that are formed between the chemicals and the silent receptor and the affinities of the chemicals for that binding site. This point is particularly important if a chemical is strongly bound to a storage site receptor. Thus, if the affinity of a chemical is such that 90% or more is bound to plasma protein, displacement of that chemical by another, producing even a minor percentage change in protein binding, could result in a doubling or tripling of the plasma concentration of the chemical. That increased concentration would be free to exert a toxicological effect. In contrast, a chemical with only a slight affinity for a storage tissue receptor site may be completely displaced by a chemical of higher affinity with relatively minor toxicological consequences.

INTRACELLULAR ACCUMULATION

Many chemicals accumulate in higher concentrations within cells than they do in extracellular fluids. If the intracellular concentration of a chemical is high, the tissue involved may serve as a large storage depot. Accumulation within cells may be brought about by binding of the chemical to intracellular tissue constituents, such

as proteins or phospholipids. It sometimes involves active transport into the cell. Toxicological interactions between chemicals that bind principally to intracellular storage sites may occur when one chemical displaces or prevents another from binding in a manner similar to that involving plasma proteins. They may also occur when a chemical that is normally transported to intracellular storage sites by active processes is prevented from being transported by other chemicals, which block or compete for those processes.

STORAGE IN NEUTRAL BODY FAT

Finally, many chemicals have a high lipid solubility and are stored to a large extent in neutral body fat. Chemicals such as DDT and many nonpolar organic solvents, which have high lipid water partition coefficients, are particularly prone to storage in fatty tissues. In this case, the possihility of toxicological interactions between two or more such chemicals can be measured in terms of their relative lipid solubilities and the extent to which displacement of one chemical by another from fatty tissues increases the availability of unbound chemical, which is free to exert toxicity to the host.

SUMMARY

In general, storage reservoirs within the body permit binding of most chemicals without ensuing toxicity from saturation of silent receptors. Therefore, toxicological interactions are most commonly manifested as a result of exposure to high concentrations of chemical mixtures or during prolonged or continuous exposure to such substances.

REFERENCES

Fingl, E., and D. M. Woodbury. 1975. Chapter 1. General principles. Pp. 1-42 in L. S. Goodman and A. Gilman, eds. The Pharmacological Basis of Therapeutics, 5th edition. Macmillan, New York.

Levine, R. R. 1973. Chapter 5. How drugs reach their site of action. II: Absorption and distribution. Pp. 71-111 in Pharmacology: Drug Actions and Reactions. Little, Brown, and Company, Boston.

CHAPTER 6

INTERACTIONS AT TARGET SITES

This chapter describes the principles governing identification of toxicological interactions involving target enzymes and covalent binding at sites of action.

CHEMICAL BINDING AT TISSUE RECEPTORS: GENERAL CONSIDERATIONS

Chemicals, alone or in combination, produce effects on living organisms primarily through reactions with functionally important receptor molecules that act as target sites for those chemicals. Most chemicals act selectively by combining only with certain receptors, such as enzymes or other macromolecular tissue elements, and showing specific binding characteristics with that receptor. The ability of chemicals to alter either the physicochemical nature of these tissue receptors or the specific binding characteristics of other chemicals forms the basis for defining the principles of toxicological interactions between two or more chemicals within cells.

Before specific toxicological interactions between chemicals can be considered, the nature of the biological interaction between a chemical and its tissue receptor must be defined. A principal characteristic of such an interaction is that it is sufficiently strong to initiate an action-effect sequence. For most chemicals, this would mean a reversible reaction requiring the synchronous operation of various binding forces. The first force to be exerted as a chemical approaches a receptor must overcome the random thermal

agitation of the chemical molecule and draw it to its site of action. The binding forces usually involved in this reaction are ionic bonds formed by electrostatic attraction. Although the formation of one or two ionic bonds may be sufficient to initiate a chemical-receptor combination, the strength of these bonds by themselves is insufficient to hold the molecule in combination long enough to promote an action-effect sequence. Therefore, the additional attraction of other forces, such as hydrogen bonds and Van der Waal's forces, are also required to give the chemical-receptor combination the stability that is essential for chemical action.

The formation of one or even two ionic bonds is also insufficient to confer significant specificity or selectivity upon a chemical-receptor interaction because the receptor requires not only unique physicochemical properties, such as charge, but also a definite structural conformation in order to account for specificity. Thus, the operation of electrostatic attractions of hydrogen bonds and the binding of Van der Waal's forces are also required to maintain these conditions. Although, Van der Waal's forces are the weakest of all the binding attractions, they have the most critical dependence upon the interatomic distance between reactive molecules. Hence, they are the major contributing forces in determining the specificity of chemical-receptor interactions (Levine 1973 a,b).

Infrequently, covalent bonds are also involved in chemical-receptor binding. Because the covalent bond is many times stronger than the ionic bond and the other forces usually involved in chemical-receptor interactions, covalent bonds involve reactions that are essentially irreversible at ordinary body temperatures. Thus, covalent binding

to tissue receptors is characteristic of long-lasting chemical reactions and usually requires synthesis of new tissue receptors before normal biological function can be recovered.

The tendency, or affinity, of any chemical for binding to a tissue receptor is inherent in its molecular structure. In reversible reactions, which involve all but covalent binding forces, that property is governed by the law of mass action, i.e., that the fraction of a chemical that is bound to a receptor site is in equilibrium with the fraction of chemical that is free (Barrow, 1961 a,b). The relationship between the concentration of the chemical and the chemical-receptor complex, which produces its biological effect, may be shown as follows:

$$C + (100 - X) \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X$$

where C is the chemical concentration, X is the percentage of the total number of receptors occupied by the chemical, and (100 - X) is the percentage of unoccupied receptors.

The rate at which the chemical combines with unoccupied receptors is proportional to the product of the chemical concentration and the concentration of unoccupied receptors:

$$k_1 C(100 - X)$$

where k_1 is a constant of proportionality.

The rate of dissociation of the chemical receptor complex is proportional to X:

$$k_2X$$

where k_2 is the specific constant for the reverse reaction. At equilibrium, the rate of combination is equal to the rate of dissociation:

$$k_1C(100 - X) = k_2X$$

Thus,

$$C = \frac{k_2}{k_1} \times \frac{X}{(100 - X)} \text{ or } C = \frac{X}{K_e\ (100 - X)} ,$$

where $K_e = \frac{k_1}{k_2}$ = equilibrium (affinity) constant of the particular reaction.

Thus, the greater the affinity constant of an agent for its receptor, the greater its propensity to bind with that receptor and to produce a subsequent biological effect. This principle is essential in order to explain or predict the effects of interaction of two or more chemicals that act at the same tissue receptor (Goldstein et al., 1968). Given equal intrinsic activities, the substance possessing the greatest physicochemical affinity for receptor binding would be expected to elicit the most pronounced biological response at that receptor. Moreover, because of its ability to displace other chemicals from sites of activation or deactivation, the agent possessing the higher binding affinity would also be expected to antagonize or act synergistically with chemicals producing those effects (Fingl and Woodbury, 1970).

ENZYMES AS TARGET SITES OF TOXICOLOGICAL INTERACTIONS

The principles discussed in the previous section are directly applicable to the role of enzymes as cellular target sites for chemical interactions. A principal biological manifestation of chemical interactions at enzymatic receptor sites is the alteration of biological transformation reactions involving either acceleration or deceleration of these processes. The physicochemical forces that regulate the binding of chemicals to enzyme receptors and the ensuing enzymatic reactions dictated by the law of mass action underlie the basic principles by which interactions of chemicals at enzymatic target sites may be understood.

To catalyze a reaction, an enzyme must be able to combine with its substrate. Hence, any agent that interferes with the access of a substrate to active enzyme binding sites will also decrease the rate of metabolism, even if the concentration of the enzyme is normal. The metabolic rate of one chemical may be decreased by another in several ways. In competitive enzyme-substrate interactions, the metabolic rate of one chemical may decrease when another chemical is also a substrate for and successfully competes for the same active site of that enzyme. Competitive inhibition may also occur when one chemical combines reversibly with the active site of the enzyme by virtue of its structural similarity to another chemical, which also acts as substrate. In both cases the extent of inhibiton is dependent upon the concentration of each chemical at the active site of the

enzyme as well as on their respective binding affinities for that site. Such competitive interactions may be represented as follows:

$$E + S_1 + S_2 \rightleftharpoons ES_1 + ES_2 \rightleftharpoons E + P_1 + P_2$$

$$E + S_1 + I \rightleftharpoons ES_1 \pm EI_2 \rightleftharpoons E + P_1 + I$$

where E is enzyme, S_1 and S_2 are chemical substrates, I is a non-substrate, and P_1 and P_2 are end products of the metabolism of E_1 and E_2, respectively.

Inhibition reactions involving enzymes as target sites for chemical interactions may also occur when one chemical is unrelated in structural or physical properties to another but is capable of binding with an enzyme, thereby preventing formation of an enzyme-substrate complex. Such noncompetitive inhibition is typically observed in chemical interactions involving heavy metals or organic phosphate insecticides. It may be either reversible or irreversible and may, therefore, involve covalent binding of chemicals to enzyme target molecules. Since noncompetitive inhibitors do not combine with the enzyme in the same manner as the chemical that acts as substrate, such inhibition depends only on the concentration of the inhibitor. However, neither the binding affinities of the various chemicals nor their respective concentrations in the cell greatly affect the nature of the direct interactions between the chemicals.

Stimulation of the enzymatic metabolism of one chemical by another is an additional mechanism by which enzymes may act as target sites for chemical interactions. This process is usually the

consequence of an increased rate of protein synthesis resulting in new enzyme formation and, hence, an increase in the concentration of enzyme in the cell. However, a change in the structural conformation of an enzyme by one chemical, leading to increased enzymatic activity, may also occur. The former situation generally involves reversible binding of inducing chemicals to nuclear binding sites. Therefore, the extent of induction of new enzyme synthesis would be dependent upon the physicochemical properties of the chemicals that determine their access and affinities for binding to those sites. This manifestation of chemical interaction reflects the properties of both the inducing chemicals and the substrate in interacting with the enzyme. Any direct interaction between inducing and substrate chemicals would also be a determinant of the outcome of an enzymatic process involving more than one chemical substance.

Finally, amplification of biological processes resulting from the action of two or more chemicals may reflect the operation of more than one enzyme as target sites for those chemicals. For example, if two chemicals form reactive complexes with two different enzymes or with different sites on the same enzyme, a combination of the effects of those chemicals should be observed. In this case, the principles governing the binding of chemicals to their respective enzyme binding sites and the law of mass action regarding the ensuing reactions would dictate the extent of the combined effects, as they do with other types of reversible chemical interactions.

COVALENT BINDING AS AN INDEX OF TARGET SITE FOR TOXICOLOGICAL INTERACTIONS

A covalent bond is formed when two atoms share a pair of electrons. It has a typical bond energy of approximately 100 kcal/mol. Because of their high binding energy, covalent bonds are essentially irreversible at ordinary body temperature unless a catalytic agent such as an enzyme intervenes. Such reactions represent chemical interactions that often result in toxic consequences of prolonged duration.

One of the principal mechanisms of chemical interactions involving covalent bonding is observed in irreversible reactions with enzymes. Two types of irreversible enzyme inhibitors operate by formation of a covalent bond (Baker, 1970). The first type reacts with an essential functional group on the enzyme by a bimolecular process:

$$E + IX \xrightarrow{k_a} EI + X$$

where X is a leaving group of the inhibiting chemical, E is the enzyme, and I is the inhibitor.

This process has little specificity since all groups on the surface of all enzymes with the nucleophilic capability to do so will react at varying rates, depending on their rate constant, k_a.

The second type of irreversible inhibition involving covalent bond formation is:

$$E + IX \underset{K_i}{\rightleftharpoons} EIX \xrightarrow{k_b} EI + X$$

In this case, the enzyme, E, forms a reversible complex with the inhibitor, I, which bears the leaving group, X. K_i is the inhibitor

constant, and k_b is the bimolecular rate constant. If a nucleophilic group on the enzyme is closely juxtaposed with the reversible enzyme-inhibitor complex, EIX, then a rapid neighboring group reaction can occur within the complex. Such covalent bond formation can be highly specific since properly positioned neighboring groups can react many orders of magnitude more rapidly than the identical biomolecular reaction (i.e., $k_b > 10^3 k_a$). This highly specific reaction with an enzyme-inhibitor reversible complex has been referred to as "active site-directed irreversible inhibition" or "affinity labeling". Such reactions have an extra dimension of specificity dependent on k_b that does not exist with reversible inhibitors. This is known as the "bridge principle of specificity."

There are two classes of active site-directed irreversible enzyme inhibitors. The first class operates by forming a covalent bond within the active site (endomechanism). The second class forms a covalent bond outside the active site (exomechanism). An example of the first class is the L-glutamine antagonist, L-azaserine, which specifically alkylates a single cysteine in the active site of the enzyme that converts formylglycinamide ribonucleotide to its amidine. The exomechanism is illustrated by the inactivation of glutamate dehydrogenase by 4-(iodoacetamido)salicylic acid. Clearly, two or more chemicals reacting irreversibly with enzyme target sites by either of these covalent binding mechanisms could inibit or inactivate multiple biological processes with subsequent toxic effects throughout their presence in the organism.

Chelate formation is another mechanism by which covalent binding may be important as an index of target site for chemical interactions

in biological systems. This process, which entails the formation of five- or six-membered ring complexes involving coordinate covalent bonds, is especially important in chemical interactions with metals.

Coordinate covalent bond formation occurs when both electrons of the electron pair that forms the bond between two atoms are donated by the same atom. In biological systems, the donor atom is usually nitrogen, oxygen, or sulfur since these elements contain a pair of s-orbital electrons, usually unshared when the valence electrons have participated in bond formation.

The stability of chelate complexes may vary greatly depending on the nature of the chelating agent and the metal. Stability is expressed quantitatively by the stability (equilibrium) constant in the mass action law equation, discussed above, for the equilibrium relationship between the free and complexed reactants. For any given chelating agent, the magnitude of the stability constants is determined largely by the atomic structures of the various metals involved. A metal with a high stability constant would effectively compete with a metal of lower stabililty for the chelating agent. Given sufficient time, it would displace the less tightly bound metal from complexes already formed.

Naturally occurring chelates play an important role in biological systems. Perhaps the primary essentiality of some metals for life rests in their ability to form functional chelate complexes. Such natural chelates as heme and various metalloporphyrins, for example, are well suited to act as bridges to facilitate electron transfer, which must

occur in intermediary metabolism. Therefore, substitution of non-essential metals in chelation complexes that perform essential biological functions may either diminish or abolish that function. Moreover, they may produce biological reactions that are completely different from those that are compatible with the life of the cell.

The covalent binding of metals to tissue receptors as sites of potential interactions between chemicals is of particular importance when considering binding to sulfhydryl groups in proteins and other macromolecules. Bonds between sulfur and metal ions are very strong, as reflected in the essentially irreversible character of metal sulfides such as those of lead, mercury, and silver. When such reactions involve enzymes that require sulfhydryl groups as part of their active centers, profound biological consequences may ensue. Pronounced toxic effects may also result from the covalent binding of metals to sulfhydryl or specific sulfur-sulfur bonds, which are necessary to the maintenance of protein structure and configuration.

Finally, the formation of tightly bound chemical complexes, which affect biological processes without interacting with specific tissue receptors, represents another mechanism by which covalent binding might be important as an index of target site in chemical interactions. The extracellular formation of the complex between edetate (EDTA) and various metals illustrates how the biological effects of individual chemicals can be modified through such an interaction. Underlying this mechanism is the principle that the attractive force, i.e., the binding affinity, of EDTA for a metal

is many orders of magnitude greater than that between the chemical and the biological tissue. A corollary to this principle is that covalent binding may sometimes prevent or reverse the potential toxicity caused by chemical reactions with tissue components.

REFERENCES

Baker, B. R. 1970. Specific irreversible enzyme inhibitors. Ann. Rev. Pharmacol. 10:35-50.

Barrow, G. M. 1961a. Chapter 8. Introduction to the theory of chemical bonding. Pp. 185-221 in Physical Chemistry. McGraw-Hill, New York.

Barrow, G. M. 1961b. Chapter 9. The nature of the chemical bond. Pp. 222-247 in Physical Chemistry. McGraw-Hill, New York.

Fingl, E. and D. M. Woodbury. 1970. Chapter 1. General principles. Pp. 1-42 in L. S. Goodman and A. Gilman, eds. The Pharmacological Basis of Therapeutics, 4th edition. Macmillan, New York.

Goldstein, A., L. Aronow, and S. M. Kalman. 1968. Binding forces in the drug-receptor interaction. Pp. 3-25 in Principles of Drug Action: The Basis of Pharmacology. Harper and Row, New York.

Levine, R. R. 1973a. Chapter 3. How drugs act on the living organism. Pp. 27-47 in Pharmacology: Drug Actions and Reactions. Little, Brown, and Company, Boston.

Levine, R. R. 1973b. Chapter 10. Factors modifying the effects of drugs in individuals. Pp. 261-291 in Pharmacology: Drug Actions and Reactions. Little, Brown, and Company. Boston.