18 June 1965, Volume 148, Number 3677 SCIENCE

Toxicology and the Biomedical Sciences

New programs are recommended for increasing research and training in toxicology and pharmacology.

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. . . When we reach the limits of vivisection we have other means of going deeper and dealing with the elementary parts of organisms where the elementary properties of vital phenomena have their seat. We may introduce poisons into the circulation, which carry their specific action to one or other histological unit . . . poisons are veritable reagents of life, extremely delicate instruments which dissect vital units. I believe myself the first to consider the study of poisons from this point of view, for I am of the opinion that studious attention to agents which alter histological units should form the common foundation of general physiology, pathology and therapeutics. [Claude Bernard, 1865 (*1*)]

The number and variety of chemicals that affect man has increased at an alarming rate and created a public health problem of major proportions. We are confronted with a profusion of chemicals in the form of industrial and municipal wastes, air and water pollutants, herbicides, pesticides, cosmetics, food additives, as well as drugs administered over extended periods of time, and yet we do not know what these substances do to biological systems. In effect, we are thrust into global experiments for which we are not prepared.

For some of these hazards, such as automobile exhaust fumes or cigarette smoke, we are unlikely to find more compelling evidence of their deleterious effects. It remains for industrial and governmental bodies to utilize in the public interest all the information now available, and for the scientific community to continue experimentation on the basic mechanisms of their effects and to find ways of preventing or attenuating their hazard.

There remains, however, a major problem with the vast number of chemical compounds whose possible poisonous effects are not known or cannot be predicted. It is this area which is the subject of our article.

It seems futile to record one by one the biological effects of millions of chemical entities without the development of unifying and simplifying generalizations. It is evident that new means must be sought to accelerate the acquisition of new knowledge on the effects of chemicals on living materials, and to develop a system for the rapid dissemination of such information. In this article we outline some of the problems in toxicology and offer recommendations as to how these problems should be approached.

LIMITATIONS OF TOXICITY TESTS

Investigations of drugs (*2*) are frequently complicated by the difficulties of eliciting their subtle, often unusual deleterious effects, and of evaluating these effects against the beneficial actions. Even members of a single species can vary in their response to a particular substance, yet large numbers of people may be exposed to a drug on the basis of toxicity studies in relatively few animals.

Predictability of Data from Animals to Man

At present, a potential therapeutic agent is first screened for biological activity in laboratory mammals. If the substance shows potentially useful pharmacological or therapeutic activity, then the toxic effects are determined in experimental animals before the substance is tested in man. Thus the pharmacological and toxic effects exerted by a drug must be predicted from the effects in laboratory animals. Our modern system of drug development, therefore, depends on the assumption of a high degree of correlation between effects in animals and man. That such predictions are often unreliable raises serious questions regarding these tests.

Sensitivity of receptor sites in animals and man. The projection of animal data directly to man is made on the assumption that the same dose of drug (per unit of body weight) will attain the same concentration at drug receptors in man as in animals. However, drug receptors are responsive not to the total dose but to the concentration to which they are exposed, and many factors can alter the final concentration at the receptor site.

In the past, variations among species in the response to a drug were attributed to differences in the sensitivity of receptor sites, and the prospects of obtaining data from animals that would be applicable to man were bleak. However, variation in drug metabolism within and between species is now known to be the rule rather

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than the exception; consequently, the variations in response might well be due to differences in amounts of drug available to the sites of action. If the relation between the effect of a drug and its concentration in the plasma were to be compared among various species, differences in concentrations of the drug at receptors could be distinguished from differences in responsiveness of receptors. Differences in responsiveness would be of particular interest since they might reveal phylogenetic changes in function.

In discussing species differences in response to drugs, most antitumor agents should be considered apart from agents which affect complex homeostatic systems. Antitumor drugs, usually antimetabolites or alkylating agents, interact with pathways of metabolism common to normal as well as neoplastic tissues. These drugs exert an antitumor action because, in being metabolized, they become enmeshed in mechanisms essential to the normal economy of the body; usually they are toxic for the same reason. Recent studies indicate that the toxicity of antitumor agents in animals agrees well with the maximum tolerated doses in man when expressed on the basis of weight to the 0.7 power (*3*).

Toxicity from Excessive Action on Physiological Control Systems

Most drugs used medicinally change the *intensity* of body function by acting on physiological control systems which mediate the adaptive response of the living animal. Drugs of this type are designated pharmacodynamic agents. Many act by mimicking or releasing hormones or neurohormones, or by blocking the action, synthesis, release, storage, or metabolism of these hormones. Untoward effects are due to an exaggeration of the desired action, or to an unwanted effect on a second physiological control system (*4*). In the case of a barbiturate, overdosage can produce respiratory arrest; a ganglionic blocking agent can elicit fainting by interfering with mobilization and utilization of energy fuel; amphetamine can cause hyperactivity, and chlorpromazine can produce tremor. In other words, to understand the side effects of these drugs, it is first necessary to know the nature of the physiological control system on which they act. Fairly reliable extrapolation of data from animals to man may be expected when this sort of side effect is related to the concentration of the drug in the plasma rather than to the dose administered. In general, these drugs are not metabolized by processes acting on substances normally present in the body; usually they are not even metabolized in the organ where they act. Instead, their action is terminated by specialized enzymes in liver microsomes, which have a predilection for liposoluble compounds (*5*).

Processes Affecting Intensity and Duration of Drug Response

To exert its characteristic effect, a therapeutic agent must gain access to its site of action, where the intensity of response is determined by the concentration of the drug. This concentration is seldom known, but drug-receptor interactions are generally in dynamic equilibrium with the free (unbound) drug in plasma; for a given drug, therefore, the concentration at receptor sites, and hence the intensity and duration of response, is related to the concentration of unbound drug in the plasma. For this reason it is important to consider the processes which influence this concentration and to determine which of these are similar in animals and man, and which are variable.

Physicochemical processes common to animals and man. In order to act systemically, an orally administered drug must be absorbed from the gastrointestinal tract and be carried by the blood stream to the various tissues. Before it reaches its site of action, it must penetrate a succession of cellular membranes, such as the blood-brain barrier, the placenta, and the boundaries of various tissue cells. Cellular membranes are lipoid in character and most drugs traverse them by simple diffusion at a speed that depends on a physical property, lipid solubility, or more precisely, the lipid-to-water partition coefficient (*6*).

The duration of action of a drug depends in part on the extent to which it becomes localized in various tissues. Most drugs form reversible complexes with plasma proteins and with one or more intracellular components of cells. These nonspecific drug attachments provide reservoirs of drug, which are in dynamic equilibrium with the unbound drug in plasma, and are released as the plasma concentration declines. Most drugs with a long duration of action are characterized by being extensively and reversibly localized in tissues; such localization acts as a brake on metabolic transformation, and serves to lessen otherwise rapid fluctuations in the amount of drug in the plasma.

Since processes such as penetration across membranes, localization in tissues, and urinary excretion are dependent upon physicochemical properties of organic compounds, they seldom account for species differences in drug action (*7*, *8*).

Processes that vary among animals and man. Profound differences are found in pathways and rates of drug inactivation among various species and individuals, including man (*7*). A clue as to why such wide variations exist is provided by the kidney tubule, which is lined by a lipoidal membrane across which liposoluble substances are almost completely reabsorbed into the circulation by simple diffusion. Without mechanisms to convert these chemicals to more polar, less liposoluble derivatives, the effects of some drugs would last for years. Recent studies suggest that the enzymes which metabolize drugs are not the usual enzymes of intermediary metabolism. Rather, they are mute testimony of an evolutionary development that had to occur before animals could migrate from water onto land, in order that the organism would be protected from a multitude of liposoluble foreign compounds ingested in food (*9*).

Plasma concentrations. Certain drugs which are poorly liposoluble are not metabolized but are disposed of, largely unchanged, by urinary excretion. With such drugs, the same dose (per unit body weight) should attain similar plasma concentrations, since urinary excretion is not particularly different from one mammalian species to another. Hence, if the receptors for one of these drugs were equally sensitive, the pharmacological and toxicological responses would be similar in various animals and man (*10*). Among these compounds are many important drugs including a variety of quarternary ammonium compounds, certain antibiotics, the carbonic anhydrase inhibitor acetazolamide, the thiazide diuretics, and the adrenergic blocking agent priscoline.

Thus, the parenteral dose of tubocurarine that produces neuromuscular blockade varies by no more than 50 percent in man, cat, dog, and rabbit (*11*). When 300 ganglionic blocking

agents were screened in five species of animal, the effects were found to vary by only 100 to 200 percent (*12*). In some animal species, streptomycin damages the sensory cells of the vestibular apparatus in about the same dosage as that which produces otoxic effects in man (*13*).

If a drug affected the target organ equally in animals and man, dependable predictions might be made even with drugs whose metabolism differed among species, provided that the effects were expressed in terms of the concentration of the drug in the plasma or tissues rather than dosage (*14*). Studies with hexobarbital (and other barbiturates) show that, despite a 20- to 50-fold variation in the duration of action, when various animal species and man recover from hypnosis the concentrations of the drug in the plasma are about the same. In rats, variations in the effects of this drug caused by strain and sex differences can be circumvented if activity is based on plasma concentrations (*15*).

The compound, ICI 33828, inhibits pituitary gonadotrophic function at vastly different daily doses in various species, including man (*16*). Despite the 200-fold variation in dosage, each species shows an inhibitory response at a plasma concentration of 3 micrograms per milliliter.

Only a few months ago it was discovered that Sprague-Dawley rats at the National Institutes of Health are no longer the Sprague-Dawley rats of 4 years ago. For example, 100 mg/kg of hexobarbital now elicits the loss of righting reflex for 20 minutes, compared to 90 minutes 4 years ago. Similarly, desmethylimipramine is far less active than before. Fortunately, the concentrations of these drugs in the plasma have been recorded and the animals now available have a much greater capacity to metabolize the drugs. By relating the effects to plasma concentrations, many valuable data have been saved.

Time-response relationships. Even with drugs whose metabolism differs widely among species, effects based on dosage are similar in animals and man if the time lapse between drug administration and measurement of response is so short that little or no drug is lost by inactivation. Thus the anesthetic dose of a particular barbiturate is practically the same in all mammals, indicating that drug receptors for these drugs show little species variability. On the other hand, in screening a drug for behavioral responses, considerable time passes before the test is completed and it is not surprising that results with these drugs may be difficult to extrapolate to man.

In tests of subacute and chronic toxicity, differences between animals and man in rates of drug metabolism are particularly important. Despite a large variability in metabolism, the acute lethal toxicity of many barbiturates (administered intravenously) is almost identical in various mammalian species because of the short time lapse between administration of drug and death. On the other hand, the lethal toxicity of a drug will vary considerably if time elapses between drug administration and death. A substance metabolized in rats 50 times more rapidly than in man may have the same acute toxicity in both species, but the chronic toxicity may be vastly different because of drug cumulation. Phenylbutazone, an antirheumatic agent metabolized much more rapidly in the rat than in man, causes the retention of sodium. Rats given a single dose of drug do not show this effect. To maintain the drug at a plasma concentration that produces sodium retention in man (about 150 μg/ml), the rat must be given a total daily dosage of 400 milligrams per kilogram of body weight compared with the 5 to 10 milligrams per kilogram required in man (*17*, *18*).

Much of the research on the teratogenic effects of thalidomide in animals is difficult to interpret. The drug is said to produce a long-lasting sedation in man and the horse but only a fleeting effect in most other species. We know of no studies that relate the plasma concentration to the teratogenic effects. From the short-lived sedative action in the rat, one would suspect that this animal might inactivate the drug much more rapidly than does man.

Thus, in toxicity studies it is important to compare in the various species the plasma or tissue concentrations at which a drug elicits an adverse effect. Until this has been done with a variety of agents we cannot know to what extent species variability in toxicity depends on differences in rate of drug metabolism or differences in inherent toxicity.

Drugs that act nonreversibly. Sometimes the response to a drug is clearly unrelated to its concentration in the plasma or tissues. In such instances the action of the drug may be mediated through a metabolic product; in fact, a number of therapeutic agents have been discovered by the study of such relationships (*19*). There are also drugs which show a complete lack of correspondence between plasma concentration and biological effect, but whose actions can not be explained by a metabolic product. Examples are drugs that remain irreversibly attached to receptors long after the drugs have vanished from the rest of the body. Such drugs are inherently dangerous since they can literally destroy an enzyme system or a target site which requires days or even weeks to recover; moreover, in small daily doses their effects are cumulative (*7*). Reserpine is a good example of such a "hit and run" drug. It rapidly enters the brain and in a short time seems to disappear from the body. But its effects are cumulative, so that small daily doses of the drug can induce "endogenous" depression (*20*).

Individual Variations in Drug Metabolism in Man

A common cause of toxic reactions arises from "overdosage" because of person-to-person variability in rates of drug metabolism (*21*); the same daily dose of a drug may cure, cause severe toxicity, or have no effect whatsoever (*22*). For example, a hypertensive drug may accumulate and finally cause a cerebral vascular injury or a coronary infarction; or an antidiabetic drug such as chlorpropamide may induce severe hypoglycemia. Examples of drugs with variable rates of metabolism are dicumarol and tromexan, which show a 14-fold difference among individuals. Each person seems to have his own pattern of metabolism for these drugs. The consequences of individual differences in drug metabolism are exaggerated in long-term therapy and may account for the variable time of onset for side effects such as Parkinson's syndrome induced by chlorpromazine (*23*).

The difficulties of screening drugs in man is illustrated by a study with the antidepressant drug desmethylimipramine (DMI). This drug was administered to three subjects in doses of 75 mg per day. One subject showed signs of euphoria that progressively increased over the 5 days on which the drug was given. The concentration of

DMI in the plasma of this person rose almost arithmetically, and after 5 days was 4 times higher than in the other two subjects (*24*).

Drug metabolism of the newborn. The importance of the drug-metabolizing enzymes in drug therapy is demonstrated by the prolonged action and high toxicity of many drugs in newborn infants, whose microsomal enzyme systems are not developed during their very early days of life (*25*). The accumulation and consequent toxicity of chloramphenicol in infants is mainly due to their inability to form a glucuronide. Because infants are ill-equipped to metabolize drugs, liposoluble substances should be administered only under exceptional circumstances.

Enzyme Stimulation and Inhibition

Many drugs can stimulate or inhibit the microsomal enzymes by which they are metabolized. The effects of such drugs are particularly important in screening and toxicological tests, and in clinical situations where one drug is followed by another in treatment. However, in the interpretation of drug action, measurements of drug concentrations in the plasma should correct for these phenomena.

A drug may enhance its own metabolism as well as that of many other drugs by stimulating the activity of drug-metabolizing enzymes in liver microsomes (*26*). These enzymes are activated by many kinds of liposoluble compounds, including pesticides, hypnotics, antihistamines, tranquilizers, analgesics, and antidiabetic and uricosuric agents. The effect is not permanent; it may last a few days in rats, but persist for months in dogs (*27*). The mechanism of this adaptive phenomenon is not known but it may be inferred that it involves the synthesis of new enzyme protein, since the increased activity is prevented by actinomycin and puromycin. Recent reports show that in addition to genetic variants among the drug-metabolizing enzymes themselves, there are even genetic variants in the control of the drug-induced formation of enzymes.

Enzyme induction makes it extremely difficult to interpret the results of testing over long periods. In fact, these tests may defeat their very purpose, since drugs that stimulate their own metabolism will become less and less toxic, thus engendering a false sense of security. This will be true not only in the usual toxicity tests but in screening for carcinogenic, mutagenic, or teratogenic effects. As an example of this dilemma, the daily administration of large doses of phenylbutazone to dogs provokes ataxia and anorexia for the first 5 days. These effects then subside as the phenylbutazone-metabolizing enzymes are activated and the concentration of the drug in the plasma declines (*26*). Which is more important—the side effects during the first 5 days or the lack of side effects seen after months or perhaps years of treatment?

The validity of toxicity tests from laboratories that spray their animal quarters with insecticides, such as chlordane and DDT, should be viewed with some suspicion. It has, for example, been shown that a number of drugs are metabolized by rats and dogs at an accelerated rate for some months after the quarters are sprayed with chlordane (*28*).

If a drug acts through a metabolic product, enzyme induction will increase rather than decrease its toxic effects. For example, the anticholinesterase activity of a number of organophosphorous insecticides is mediated through a metabolic product. The toxicity of the insecticides will be magnified if animals are first treated with drugs that activate the metabolizing enzyme.

By inhibiting certain enzymes, one drug can potentiate the effects of other drugs. For example, SKF 525-A, a simple ester having almost no pharmacological action of its own, potentiates such diverse families of drugs as hypnotics, analeptics, and analgesics by inhibiting the drug-metabolizing enzymes in liver microsomes (*7*). These enzymes are blocked by many other organic compounds. The mechanism of inhibition is not known, but it is evident that the sites of drug action are not sensitized, since animals recover from the effects of various drugs at the same plasma concentration whether or not they have received SKF 525-A.

Inhibition of drug metabolism can lead to gross errors in drug screening procedures. When Tremorine was used to produce tremor in animals so that potential agents for the treatment of extrapyramidal disorders in man could be screened, a number of drugs, including desmethylimipramine and certain SKF 525-A analogues, were found to be effective. But Tremorine itself does not produce tremors; it acts through its metabolite, oxotremorine, and the apparent antitremor action of various drugs was shown to be due to blockade of the conversion of Tremorine to oxotremorine (*29*).

Other Types of Toxicity Caused by Drug Combinations

The increasing use of two drugs together can produce undesirable effects which are sometimes predictable, but often are not. Especially serious effects may occur if both drugs act on the central nervous system. Particularly hazardous are combinations of a monoamine oxidase inhibitor, which protects norepinephrine against inactivation, and drugs that release or potentiate the action of norepinephrine. If a drug that releases norepinephrine is given together with such an inhibitor, a hypertensive crisis or intense central excitation may arise depending on the degree to which the enzyme is blocked (*30*). Likewise, adverse and sometimes fatal effects may occur in persons who eat ripened cheese (which contains large amounts of tyramine) after receiving a monoamine oxidase inhibitor (*31*). Imipramine, an antidepressant drug which potentiates the action of norepinephrine, can also produce toxic effects if given to animals or patients previously treated with a monoamine oxidase inhibitor (*32*).

Tranquilizers and antidepressants given together do not cancel each other out. Desmethylimipramine given before reserpine can produce excitation by potentiating the effects of norepinephrine released by reserpine (*33*). Thiazide diuretics and digitalis administered together can also elicit synergistic toxic effects because of the hypokalemia associated with the diuretics (*34*).

An interesting effect of a drug combination may occur with drugs which become extensively bound onto tissue and plasma proteins. Ordinarily such binding is readily reversible and the complex formed is in dynamic equilibrium with the unbound drug in plasma. One drug may be displaced from its binding sites by another chemical, thereby increasing the concentration of the unbound drug at target sites. For example, strongly bound phenylbutazone displaces weakly bound sulfonamides from plasma proteins and enhances their antibacterial activity (*35*). Simi-

larly, phenylbutazone and other nonsteroidal antirheumatic agents displace corticosteroids from their plasma binding (*36*). The displacement of corticosteroid by these agents might explain, in part, their anti-inflammatory, sodium-retaining and ulcerogenic effects.

Displacement of a drug can be dangerous if it is so highly bound to plasma proteins that the unbound moiety is only a small fraction of the total. Displacement of only a fraction of the drug may then double or treble its unbound concentration at the target site. Thus, strongly bound sulfonamides can induce hypoglycemic coma by displacing antidiabetic drugs from protein (*37*). In the premature baby these sulfonamides can produce toxic effects by displacing bilirubin from albumin which is present in relatively small amount (*38*). The unbound bilirubin is then free to diffuse into the brain and produce harmful effects (kernicterus). Also sulfonamides and salicylates displace methotrexate from serum albumin (*39*).

A particularly dramatic example of drug displacement occurs in the treatment of malaria when pamaquine is given to patients previously treated with quinacrine (atabrine). Since pamaquine is displaced from organ tissues, its plasma concentration is increased five- to tenfold (*40*), and it may become toxic.

Toxicity Caused by Structural or Biochemical Changes

Drugs used medicinally may produce adverse effects by causing biochemical lesions and cellular damage, rather than by exaggerating the actions of physiological control systems.

Some drugs will invariably produce cellular damage if the concentration in the plasma is high enough. For example, isoniazid at almost the same plasma concentration in animals and man reacts with pyridoxal to produce adverse effects on the nervous system. In fact, isoniazid produces a neuropathy in patients who, by genetic predisposition, metabolize the drug excessively slowly and therefore receive the maximum antituberculous effects of the drug. Now that the mechanism of toxicity is known, patients are usually given a large dose of isoniazid together with a liberal amount of pyridoxal.

Most adverse effects in this category are not recognized in animals and occur only occasionally in humans, suggesting that genetic differences in responsiveness of the reactive site are involved. It will be necessary to know the intimate mechanisms of these toxic effects before specific animal tests can be devised to predict their occurrence in man. The term idiosyncrasy is sometimes assigned to this type of toxicity. By this definition, an idiosyncrasy would be unique to man and would not be anticipated in animals. But such a term should not obscure the possibility that the adverse effect is caused by a definite substance possessing reactive chemical groups. In a given individual, a toxic metabolic product might be formed in exceptionally large amounts, or an inherited defect might make a particular tissue or chemical process vulnerable to the parent drug or to a metabolic product. Other things being equal, a slowly metabolized drug is more likely to elicit one of these rare adverse effects, since more of the toxic metabolite is likely to form. Rapid metabolism of these drugs may account in part for the difficulties of obtaining the effects in animals.

Metabolic products that act on red blood cells. Aromatic amines are converted in part to *N*-hydroxylated amines (aniline) or quinones (pamaquine) by the action of microsomal enzymes. These metabolites may cause the hemolysis of red blood cells or the formation of methemoglobin. The extent of these reactions depends upon two factors, the concentration of the metabolite and the susceptibility of the red cells (*17*). The erythrocytes of certain individuals, especially Negroes, have an inherited defect of an enzyme, glucose-6-phosphate dehydrogenase, that generates the reduced form of triphosphopyridine nucleotide (TPNH). This defect is unimportant until the red cell is exposed to the drug metabolites. The defective cells may then perish, presumably because the metabolites compete for TPNH which prevents oxidative destruction. Thus, hemolysis depends on two factors—the concentration of the oxidant formed from the drug and the susceptibility of red cells (*41*). The same drug metabolites can also convert massive amounts of hemoglobin to methemoglobin in persons who possess certain hereditary variants of hemoglobin (*42*). Thus, two kinds of toxicity, formerly classified as examples of drug hypersensitivity or idiosyncrasy, can be explained by a confluence of environmental and hereditary factors.

Allergic responses. In recent years, biochemical studies have thrown some light on the mechanisms underlying allergic responses to drugs (*43*). Sensitization occurs if the drug reacts with a body protein to form hapten-protein conjugates that induce the formation of antibodies. For example, although penicillin itself does not form covalent bonds, two of its metabolites react with protein to form an antigen whose antibody reacts with penicillin.

Delayed drug reactions. Certain drugs cause delayed toxic reactions. Carcinogenesis and mutagenesis are important aspects of chemical toxicity. Three to four weeks after they are administered to animals, certain new cytotoxic agents (the nitrosoureas) cause hepatic necrosis with no warning, and at a time when the animals apparently are well (*44*). Another group of chemotherapeutic compounds, the terephalanalides, produce without warning an irreversible, fatal renal lesion 1 to 2 months after they are administered (*45*). The production of cataracts by dinitrophenol and triparanol also occurs late and without warning (*46*).

Photosensitivity to drugs. Certain drugs which usually possess condensed, unsaturated ring systems can elicit photosensitive reactions. These substances (or their metabolites), perhaps in the form of complexes with protein or nucleic acid, absorb light at 290 to 320 millimicrons. When the energy captured by drug electrons becomes great enough to break chemical bonds, free radicals are formed which cause tissue damage.

A great deal is now known about the mechanism by which these substances produce phototoxic effects. But little is known of the mechanism by which hypersensitivity to these effects is produced in certain subjects. It is possible that the free radicals are in some way transformed into a true allergen (*47*).

Tissue damage related to drug accumulation. Drugs such as chloroquine and certain phenothiazines, which are characterized by a high degree of tissue localization, become incorporated into melanin. Small amounts of these drugs may be retained by the body for months and even years. The complexes formed with melanin have been associated with certain noninflammatory diseases of the retina (*48*).

Toxic Effects from Enzyme Induction

The effects of certain drugs on the enzyme delta-amino levulinic acid (ALA) synthetase is of particular interest to toxicologists since it concerns another recognizable genetic difference in the human population. The offending drugs, which often possess an allyl side chain, can be fatal to subjects with latent porphyria. The metabolic disorder involves a defect in the gene responsible for the induction (or repression) of ALA synthetase. By inducing unrestricted formation of the enzyme, the drugs cause the normal pathways of porphyrin-formation to be clogged by excess ALA synthetase. As a result, abnormal porphyrins are formed and cause photosensitive reactions (*49*).

It is tempting to speculate on the possibility that other relatively rare toxic effects might involve a genetic defect in enzyme induction. A suspicion of this is raised by the events that accompany a drug-induced stimulation of the drug-enzymes in microsomes: (i) proliferation of endoplasmic reticulum to the point where nearly all the cytoplasm is filled by membranes; (ii) an attendant increase in phospholipids; (iii) a shunting of glucose metabolism into the pentose cycle; (iv) a large increase in the synthesis of ascorbic acid; (v) stimulation of protein synthesis; (vi) stimulation of liver growth.

It is probable that these changes in hepatic ultrastructure and biochemical function require the induction of a number of enzymes of intermediary metabolism. If an abnormal gene controls the induction of any one of these enzymes, structural damage might result by interference with cellular mechanisms. It seems pertinent that drugs having such diverse chemical structure and pharmacological actions should not only stimulate drug enzymes, but cause structural changes in liver and affect a number of metabolic pathways. An important consideration is that the drugs might also increase the endoplasmic reticulum and the various pathways of intermediary metabolism in organs other than the liver.

RECOMMENDATIONS

The following recommendations are offered to further research in those areas which seem most likely to provide results that will control the effects of a chemical environment that is hostile to man. It must be pointed out, however, that if a program of research were directed only to the solution of practical problems, it would prove a failure and might even retard progress by diverting manpower, funds, and resources from fundamental research. Implicit in the directing of funds to "targeted" research is the assumption that the foundation of basic research is adequate and that important problems in public health will be solved by the mere organization of available knowledge.

Information Processing and Communication

The rapid pace at which man is increasing his store of biological knowledge has raised the problem of how to compile and store the data in a form readily applicable to toxicology and pharmacology. The following needs are regarded as crucial.

1) *Storage and retrieval of information on biological effects of chemicals.* A system is required which will provide quickly, on request, an annotated bibliography of published information in response to a specific question formulated by the enquirer. To be of value to the creative scientist, such a system should be comprehensive yet selective; papers on chemical-biological interaction would have to be indexed under many more subject headings than provided by MEDLARS (*50*), in order to narrow the output to an inclusive list of relevant material.

Methods and theories for efficient retrieval of pertinent material are now under development. Such studies include automatic abstracting, logical question programs, citation indexing, and association indexing. Whatever system is used, a major difficulty will be the construction of a glossary of terms suitable for use with automated equipment. The various disciplines have different glossaries of terms and classifications of knowledge, and without a common language, the questions asked may retrieve the wrong answers.

A word of warning is necessary. In many reports automatic devices have been proposed for retrieving precisely what the interrogator requires. These proposals usually show little appreciation of the needs of creative biologists or of how scientific discoveries are really made. Because organic information is susceptible to storage it is often proposed that biological data can be stored and retrieved in a similar manner. But organic chemistry is concerned with structures and physical properties that are absolute, whereas biological data are intrinsically variable and are greatly influenced by seemingly trivial variations in experimental technique or environment. Despite automation, the creative scientist will continue to filter data through his own unique and selective mind to fit into the framework of his original thinking.

2) *Special problems related to the clinical use of drugs: Satisfying the needs for drug information.* At present, the main sources of information are the detail men, drug brochures, and the "Physicians' Desk Reference" —an incomplete but widely used digest of information prepared by various pharmaceutical companies (*51*). The cost of these services, paid by the public in one way or another, is staggering—the salaries of the detail men alone exceed $100 million annually—and the information is not likely to be completely objective or authentic.

Before World War II, when the list of useful drugs was relatively small, *The United States Pharmacopeia, New and Nonofficial Remedies,* and *Useful Drugs* provided authoritative and unbiased information. There is an urgent need today for an official source of drug information that the physician will learn to respect as a medical student and will use as a practitioner. Since the Food and Drug Administration is a repository of information on commercially available drugs, they might be authorized to establish boards of experts from universities, government, and industry to handle this problem.

In addition, there is a need for critical reviews of advances in pharmacology and therapeutics, written by authorities in a succinct and informative style. The American Society for Pharmacology and Experimental Therapeutics has announced its intention to meet this need.

Collection and analysis of adverse reactions. The urgency of such a monitoring system is underlined by recent events which have shown the difficulty of detecting certain types of reaction. The need is not only for a rapid warning system, but also for a means of processing and evaluating information so that the warning system will not

"cry wolf" (*52*). Although simple in concept, a monitoring system is complex in execution. To be effective it should detect any important but unexpected frequency of adverse reactions to a drug even though the particular agent is not directly under suspicion. It goes without saying that such a monitoring system would be put into practice only after considerable experience with pilot monitors.

Research and Training

Centers of research and training. Several "centers of research" in pharmacology and toxicology should be created to serve as focal points of basic research and training in the broad area of interactions of chemicals with biological systems. Each center should be an integral part of a university and should be of sufficient magnitude and scope to contribute substantially to important public health problems. A particularly important duty of the centers would be the training of pre- and postdoctoral students who would participate in the activities of the center in connection with their research interests and career objectives.

Grants. University departments should be encouraged to expand their research programs on studies of the interactions between animal organisms and chemical substances at the molecular, cellular, and ecological levels.

Contracts. Industrial research institutes and laboratories could be asked to supply scientific answers to questions generated by practical or clinical problems. Research that can be supported by this mechanism could concern specific problems such as the effects of pesticides on drug toxicity in animals or the disposition and fate of a particular type of chemical important as a health hazard. The resources of the pharmaceutical firms should not be overlooked because of their important role in the development, evaluation, and study of the toxicity of drugs.

Participation of foreign laboratories. The resources of foreign laboratories should be explored if the research is unique and the training opportunities and experience are unusual.

Intramural NIH fellowships. The National Institutes of Health plans to establish an intramural program to provide training in broad aspects of pharmacology and toxicology. Under such a program, a group of competent young scientists, as well as more mature investigators, will benefit from the resources and experience of the National Institutes of Health. This program is planned to provide training for as many as 30 fellows each 3-year period.

Pharmacology training in universities. There is considerable concern about the growing manpower shortage in pharmacology and toxicology. Over the next few years, about a dozen new medical schools must be staffed, a number of research institutes will require pharmacologists, and there is an increasing demand for pharmacological skills in clinical research and practice. In addition, the needs of the FDA and industry are putting additional strains on the manpower pool. Accordingly, in making recommendations for the rapid expansion of pharmacological research it must be recognized that the expansion cannot outpace the number of pharmacologists that will be trained.

Academic departments of pharmacology will continue to be the principal source of pharmacologists. Thus far, Ph.D. programs have not attracted enough outstanding students because pharmacology does not have the romantic image of biochemistry, physiology, or molecular biology. A major problem in bringing young people into pharmacology lies in the fact that high school and college students know very little about the subject. Perhaps the National Science Foundation, which is developing programs to enrich high-school science curricula, could include a segment describing pharmacology and indicating the nature of the research that concerns the interaction between chemicals and living material. This would not only aid in the future recruitment of pharmacologists, but also perform a unique service in providing the public with a background against which to consider high-pressure advertising, in pharmacologic terms, of over-the-counter drugs.

In addition, a more flexible program for graduate students should be considered. Many kinds of interests are needed in pharmacology and a graduate student with previous training in a related discipline such as biochemistry should not be deterred from entering the field of pharmacology by insistence that he take courses equivalent to the entire first 2 years of medicine. Some of these students might be better served by a grounding in general physiology, biophysics, molecular kinetics, calculus, and the biochemistry of physiological systems. Many creative students who have much to give to pharmacology might rebel against the constraint of memorizing their catechism instead of practicing their religion.

Serious thought should be given to combined M.D.-Ph.D. programs in which outstanding students could participate and for which they would receive an adequate stipend. For those students who wish to continue their clinical training and become clinical pharmacologists, financial support should be continued during their years as house officers.

It is particularly important that encouragement should be given to persons holding degrees in the biological or physical sciences to study problems related to the broad aspects of the interactions of chemicals with biological materials. Such scientists have already made telling contributions to pharmacology.

References and Notes

1. C. Bernard, *Leçons sur les Effets des Substances Toxiques et Medicamenteuses* (Baillière, Paris, 1857).
2. Throughout this article, any chemical compound that elicits a biological effect is referred to as a drug.
3. E. J. Freireich, D. P. Rall, L. H. Schmidt, H. E. Skipper, *Cancer Chemotherapy Rept.*, in press.
4. By definition, toxicity will be elicited by overdosage of any drug that acts on a biological control system.
5. Liver microsomes contain drug-metabolizing enzymes, which act on a multitude of foreign compounds along surprisingly few chemical pathways. These include oxidation (N- and O-dealkylation, sulfoxidation, ring- and side-chain hydroxylation, deamination, and exchange of O for S), reduction (nitro and azo compounds), de-esterification, and glucuronide conjugation. Drug metabolites are invariably less liposoluble than the parent compound and consequently are usually less toxic, because they are excreted more readily and are less able to penetrate membranes to reach sites of action.
6. B. B. Brodie and C. A. M. Hogben, *J. Pharm. Pharmacol.* **9**, 345 (1957).
7. B. B. Brodie, in *Absorption and Distribution of Drugs*, T. B. Binns, Ed. (Wiliams and Wilkins, Baltimore, 1964).
8. There are some species differences in binding of drugs to plasma proteins in the case of small rodents. For example, many drugs are bound much less in the mouse than in other species.
9. B. B. Brodie and R. P. Maickel, in *Metabolic Factors Controlling Duration of Drug Action, Proceedings of the 1st International Pharmacological Meeting, Stockholm, 1961*, B. B. Brodie and E. G. Erdös, Eds. (Pergamon, Oxford, 1962), vol. 6, p. 299.
10. A target site may show an equal responsiveness to a drug but the net effects may be different. Thus, the primary action of reserpine is to impair the processes that store catecholamines and serotonin, but the net effects of the drug may depend on the relative turnover of these amines after amine stores are depleted. Chlorpromazine com-

petitively inhibits the action of norepinephrine on brain adrenergic receptors; hence it may be less effective in an excited individual than in a placid one. Usually in drug screening, animals are brought to a common state by superimposing a stimulus (for example, electrical stimulation or another drug) and the effect of the drug under test in antagonizing the superimposed effect is determined. Similarly, the toxic effects of a drug may be increased by interaction with environmental factors. Thus amphetamine increases responses to outside stimuli; accordingly, the toxicity of this drug is increased by exciting stimuli. Likewise, the action of a convulsant is facilitated by light or noise.

11. W. S. Spector, Ed., *Handbook of Biological Data* (Saunders, Philadelphia, 1956), p. 371.
12. K. Nádor, in *Progress in Drug Research*, E. Jucker, Ed. (Interscience, New York, 1960), p. 297.
13. J. Wersäll and J. E. Hawkins, Jr., *Acta Oto-Laryngol.* **54**, 1 (1962).
14. An essential part of developing a drug is that of devising a method by which it can be assayed in vivo. For studies with animals this presents few problems since the agents are generally given in large doses. Methods of increased sensitivity are needed for clinical screening of psychiatric drugs which are given in small doses and tend to accumulate in tissues. Such methods would permit the physiological disposition of a drug in man to be determined early in its development and make possible a decision as to whether the drug might be clinically practical. The smaller the dose of drug the safer it will be to give the agent in single doses based on limited toxicity in animals.
15. G. P. Quinn, J. Axelrod, B. B. Brodie, *Biochem. Pharmacol.* **1**, 152 (1958).
16. W. A. M. Duncan, in *Viewpoints on the Study of Drug Toxicity, Proceedings of the European Society for the Study of Drug Toxicity, 1963* (Excerpta Medical Foundation, Amsterdam), p. 67.
17. J. J. Burns, T. F. Yü, P. G. Dayton, A. B. Gutman, B. B. Brodie, *Ann. N.Y. Acad. Sci.* **86**, 253 (1960).
18. Since organic compounds can disappear from mice as much as a thousand times as rapidly as from man, it is disconcerting to hear proposals that certain drugs should be administered in small daily doses over the lifetime of a mouse without it being known whether or not the agent is inactivated so rapidly that it is almost equivalent to a placebo.
19. Some examples are sulfanilamide, Paraoxon, *p*-hydroxyacetanilide, oxyphenbutazone, desmethylimipramine, sulfinpyrazone, chlorzoxazone.
20. The anticoagulant biscoumacetate (Tromexan) is also a "hit and run" drug. Only by giving Tromexan in accordance with its effect on plasma prothrombin can the drug be controlled. Certain insecticides are inherently dangerous because of their conversion in the body to cholinesterase inhibitors that act nonreversibly.
21. Although most drug investigations are conducted with normal, healthy animals, patients taking drugs are usually neither normal nor healthy. Many physiological and pathological conditions such as fasting, diabetes, jaundice, increased amounts of hormones, and conditions associated with regenerating liver alter both drug response and microsomal drug metabolism in various animals.
22. The practice of studying the physiological disposition of a drug in man, only after it is clearly the drug of choice in animals, may not only prove shortsighted and time consuming, but might also result in the rejection of a drug that would be best for man. Such studies of the drug in man would also facilitate the retrieval of drugs that would be active in animals if they did not disappear so rapidly. The administration of a drug in a single small dose on the basis of results obtained from toxicity tests conducted in animals for relatively short periods is safe in experienced hands. In the long run, by permitting the early development of rational dosage regimens, this procedure actually introduces a large element of safety. But to obtain information about the rate of disappearance of a drug after it has been given in small doses, sensitive methods of drug assay are necessary.
23. Other examples of drugs that are metabolized at widely variable rates in man are isoniazid, diphenylhydantoin, aminopyrine, succinylcholine, antipyrine, quinidine. The actions of some of these drugs are known to be related to plasma concentrations.
24. W. Hammer, personal communication.
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27. It is known that some substances in our environment, for example, 2-naphthylamine and tetraethyl lead are converted to carcinogenic compounds. Certain substances in cigarettes and smog may be harmless by themselves but may cause cancer through conversion in the body to carcinogens. Other drugs that induce drug-metabolizing enzymes might increase this hazard.
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