Methods to Assess Drug–Drug and Drug–Chemical Interactions: Problems that Arise in Their Application to the Prediction of Health Hazards from Exposure to Multiple Environmental Chemicals

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ABSTRACT

Drugs and chemicals to which humans are exposed commonly interact once they are in the body. Diverse in nature and sequelae, interactions can occur at multiple sites, and in complex ways can modify substantially the biological effects produced by each chemical individually. Interactions among drugs and chemicals are conventionally classified according to their effects on processes of drug and chemical absorption, distribution, metabolism, secretion, and receptor binding.

While sufficiently sensitive and specific methodologies are currently available to measure concentrations of most interacting drugs and environmental chemicals, ethical problems prevent many such studies being carried out in the tissues of living human subjects. Therefore, other approaches were developed to indicate indirectly in man the presence of and interactions among drugs and environmental chemicals. All such methods have certain limitations that need to be recognized. Extrapolation to man of results from other methods based on laboratory animals, tissue culture, or bacteria is valid for some chemicals, but invalid for others, partly because there are marked quantitative and qualitative differences among species in drug and chemical disposition. Other considerations invalidate attempts to extrapolate to all human subjects results of toxicity studies on certain environmental chemicals performed in small numbers of carefully selected laboratory animals. When investigated in sufficiently large numbers human subjects are exceedingly heterogeneous for numerous critical host factors

that influence response to environmental chemicals. By contrast, laboratory animals used in such tests are often selected for as much homogeneity as possible. In conclusion, when formulating regulations on permissible concentrations of environmental chemicals, we need to resist the temptation to believe that a single concentration of a particular chemical is equally toxic for all people. Quite the reverse is true; multiple host factors described in this paper render certain people more and others less susceptible to toxicity from a particular concentration of an environmental chemical.

1 INTRODUCTION

The development of sensitive, accurate methods to measure minute concentrations of drugs and chemicals and to identify their interactions has outdistanced our understanding of how to use such information to predict and hence prevent health hazards from such chemicals. The situation is much more complex than indicated by the following scenario which, unfortunately, is too generally followed as an acceptable routine: concentrations of several environmental chemicals or drugs are measured and a reference consulted to predict: (1) what interactions would be expected to occur *in vivo*; (2) how much interactions influence the *in vivo* concentrations of the participating chemicals; and (3) what toxicological consequences would ensue in all subjects who exhibited certain specific concentrations of the identified interacting chemicals. These simple sequential steps, ideal from a public health point of view, are indeed practicable for several chemicals such as bilirubin or malicyclic acid. However, such expectations are currently unrealistic and indeed may never be attainable for many commonly encountered, potentially toxic chemicals such as polycyclic hydrocarbons, herbicides, or insecticides. Numerous reasons for this conclusion have been elaborated by Gillette (1984). Not the least is the lack of a uniformly applicable threshold concentration for most chemicals that produce a specific health hazard. The threshold for toxicity of many chemicals is not uniform for all subjects, but rather varies among different subjects, and even within the same subject, depending on some of the factors shown in Figure 1. In fact, in laboratory animals large strain differences exist in sensitivity to the toxicity of many chemicals, including amphetamine, antipyrine, chloroform, hexobarbital, histamine, pentylenetetrazole, and strychnine (Meier, 1962).

More knowledge concerning health hazards of many chemicals is required to define the extent of and causes for these variations among members of the same species. Also we need to ascertain whether threshold concentrations for toxicity of many chemicals do, indeed, exist in man. If thresholds do exist for certain chemicals, we should determine how broad these thresholds for toxicity are and how much they may be affected by some of the host factors shown in Figure 1.
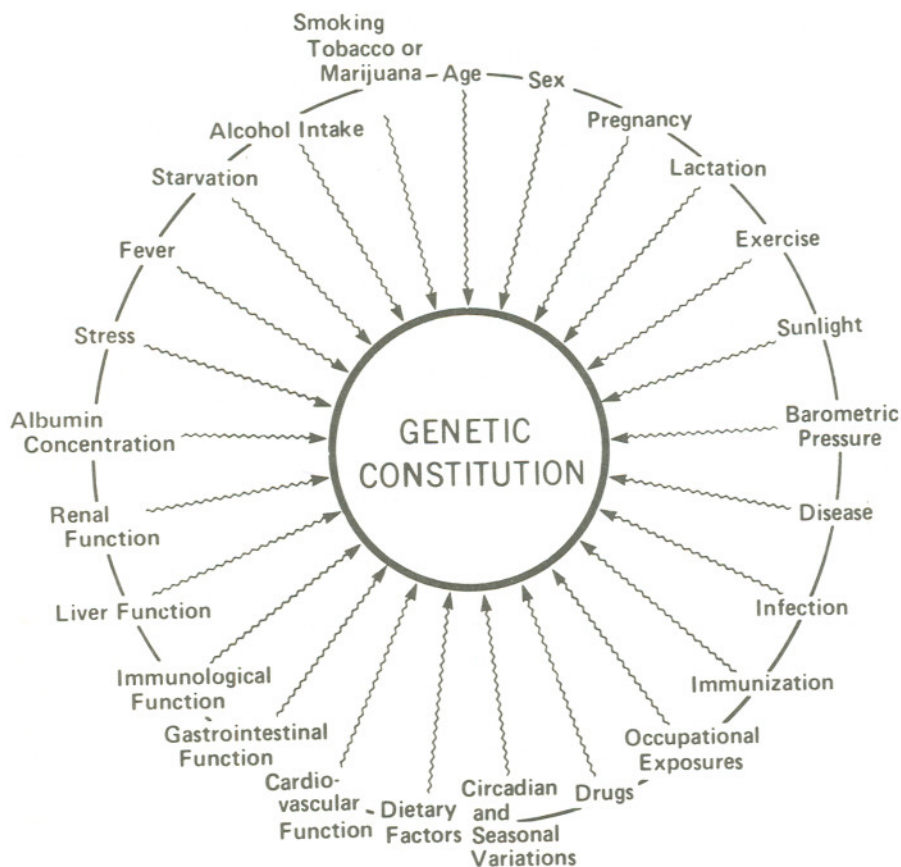
Figure 1 This circular design suggests the multiplicity of either well-established or suspected host factors that may influence drug response in man. A line joins all such factors in the outer circle to indicate that effects of each host factor on drug response may occur at multiple sites and through different processes which include drug absorption, distribution, metabolism, excretion, receptor action, and combinations thereof

2 LARGE VARIATIONS AMONG NORMAL HUMAN SUBJECTS IN THE DISPOSITION OF DRUGS AND CHEMICALS ADMINISTERED SINGLY AND IN COMBINATION

For most toxic chemicals, just as documented above for laboratory animals (Meier, 1962), health risks are not uniform for all human subjects equally exposed, i.e. exposed to similar concentrations of potentially harmful chemicals for similar periods of time and by the same route. Far from uniformity, human

subjects vary widely in susceptibility on exposure to equivalent doses of many chemicals (Vesell, 1973). This variation in susceptibility is frequently observed; two examples will be cited. Cirrhosis can occur in subjects who imbibe ethanol chronically in lower doses than others who, although they ingest higher doses of ethanol, somehow remain healthy. The same paradox applies to the seeming lack of relationship in certain subjects between dose and duration of cigarette smoking, on the one hand, and risk of developing bronchiogenic carcinoma, on the other. The generalization emerges that some subjects, for reasons largely unknown, are at greater health risk than others, even though the total dose of the offending chemical they receive is appreciably less than that of subjects who escape injury.

One discovery relevant to the subject of this Workshop is that simultaneous exposure of certain subjects to other chemicals, such as asbestos or ethanol, increases their risk of developing certain forms of cancer if they are cigarette smokers and vice versa; for example, subjects exposed to asbestos in their work are at greater risk of developing cancer if they smoke cigarettes or imbibe ethanol chronically. But even within each subgroup of subjects exposed to several noxious chemicals, susceptibility to toxicity is non-uniform and paradoxical from solely a dosage consideration. Some subjects succumb, whereas others appear to possess resistance conferred by as yet unknown factors. A better understanding of the intimate mechanisms of chemical toxicity might help to dispel confusion created by the illusion of a uniformly applicable threshold concentration of a chemical required to produce toxicity. Firmer understanding of mechanisms could reveal more fruitful methods to test for risks of health hazards from environmental chemicals.

Another approach to this problem is to identify distinguishing biochemical characteristics of certain subjects closely associated with, or possibly even causing, their special susceptibility to certain environmental chemicals. According to this approach, biochemical individuality in the disposition of certain chemicals arises from different subjects having different types of distribution and metabolism of these toxic chemicals. For example, claims have been made for high correlations between susceptibility to various tumours and activities of various drug-metabolizing enzymes (DMEs), such as those reflected by antipyrine half-life or the inducibility of aryl hydrocarbon hydroxylase in cultured lymphocytes (Kellermann *et al.*, 1973, 1978, 1980). Such claims have been contradicted by several well-designed and controlled studies (Paigen *et al.*, 1977; Tschanz *et al.*, 1977). Nevertheless, intersubject variations in the aryl hydrocarbon hydroxylase inducibility of cultured lymphocytes (Atlas *et al.*, 1976) or monocytes (Okuda *et al.*, 1977) are controlled primarily by genetic factors in subjects who themselves are uninduced and who are examined under carefully controlled, stable conditions. Furthermore, the conviction persists that, if the right expression of DME activity were selected, this approach would be successful.

DMEs in hepatic smooth endoplasmic reticulum are particularly susceptible to perturbation by many host factors identified in Figure 1. A better understanding of the molecular mechanisms by which these host factors influence DME should reveal why certain subjects are more resistant and others more sensitive to many environmental chemicals. Another reason for intense interest in the state of activity of DMEs is explained by the fact that many exogenous chemicals are themselves non-toxic but require activation to highly reactive intermediates which covalently bind to multiple cellular macromolecules. This activation is largely the result of DMEs. Covalent binding initiates an as yet unknown cascade of events which terminates in cell damage and death (Gillette, 1984). The potential of many environmental chemicals for toxicity could presumably be largely reduced or even eliminated if DMEs were entirely inhibited. This step would decrease or entirely prevent production of reactive metabolites. Such a rationale has been successful in acute toxicity experiments in laboratory animals (Drummond and Kappas, 1982a,b) but as yet remains to be applied in man. A single dose of tin–haem virtually obliterated hepatic drug metabolism in rats for several weeks.

Numerous host factors markedly influence the disposition of drugs and environmental chemicals (Vesell, 1979, 1980, 1982). Figure 1 illustrates several characteristics of these host factors:

(1) Factors in the internal and external environment of a given subject that affect the disposition of drugs and environmental chemicals are most often interdependent, not isolated from one another. To suggest such close connections, environmental factors in the outer circle of Figure 1 are connected. For example, with advancing age, many of the other factors in the outer circle change concomitantly.

(2) The environmental factors in the outer circle of Figure 1 can alter the independent process of drug or chemical absorption, distribution, metabolism, excretion, tissue binding, or various combinations of these.

(3) At different times in the life of a normal subject the extent to which these environmental factors, both alone and in combination, influence drug and carcinogen pharmacokinetics and pharmacodynamics can change markedly. Even in the same subject, kinetic values that characterize how that subject eliminates drugs and carcinogens can differ appreciably not only from day to day, but also from week to week and month to month. Accordingly, certain host factors that might render a subject susceptible to chemicals under one set of environmental conditions could, under a different constellation of circumstances, be neutral or even make the subject resistant.

(4) The expression of the underlying genetic factors that produce and maintain large interindividual variations among subjects in rates of drug and chemical elimination can be markedly altered by several environmental factors shown in the outer circle of Figure 1.

(5) New environmental factors that affect drug and chemical disposition are continually being identified, thereby increasing the size of the outer circle in Figure 1.

These considerations illustrate some limitations of attempts to interpret how single host factors affect drug or chemical disposition. In the real world, in living subjects, such single factors rarely act in isolation but rather in complex interactions with continuously changing additional factors, as suggested in the outer circle of Figure 1. The temporal component of these differential risk effects needs to be stressed: the relative roles of these host factors can change dramatically with time. Hence the influence of each host factor on the disposition of a chemical fluctuates even in a single subject with time. In some cases this period can be very short, a matter of hours or less in certain patients. But even in subjects of the same age, sex, and species, marked interindividual differences can occur in susceptibility to the toxic effects of an environmental chemical.

Identification of host factors responsible for interindividual differences in toxicity and elucidation of their mechanisms of action can help to reduce or prevent morbidity and mortality that afflict susceptible subjects after exposure to drugs or toxicants. However, failure to recognize complex dynamic interactions among such factors *in vivo* and investigation instead of each factor in complete isolation from the others have yielded oversimplified and misleading relationships.

3 TOXICOLOGICAL TESTS THAT SUPPRESS EXPRESSION OF INTERINDIVIDUAL VARIATION

In contrast to the concepts developed in the preceding section are those expressed in certain mathematical models that attempt to extrapolate, in a given species or strain of animal, toxicological responses from high to low doses of environmental chemicals or from laboratory animals to man. Although useful for many other purposes and still widely employed, these models, exemplified by the conventional graphical representation of the LD_{50} determination, are overly simplistic and misleading in important ways. Typical graphical representations of these data (Figure 2) disregard and indeed even conceal variations among the animals. Instead of showing the magnitude of diversity in response to an environmental chemical, a single mean value is plotted, thereby lumping the different responses of each animal into a single average value that falsely implies numerical identicality. The fallacy of this concept is disclosed in Figure 2 where a marked alteration in chloroform LD_{50} and TD_{50} occurs after alteration of only a single host factor, genetic constitution. If other host factors shown in Figure 1, such as age, sex, disease, diet, etc., were to be investigated in similar fashion, there would probably emerge a large family of curves for chloroform toxicity. Such experiments would document the extent to which toxicity from an environmental chemical developed in association with numerous host factors.
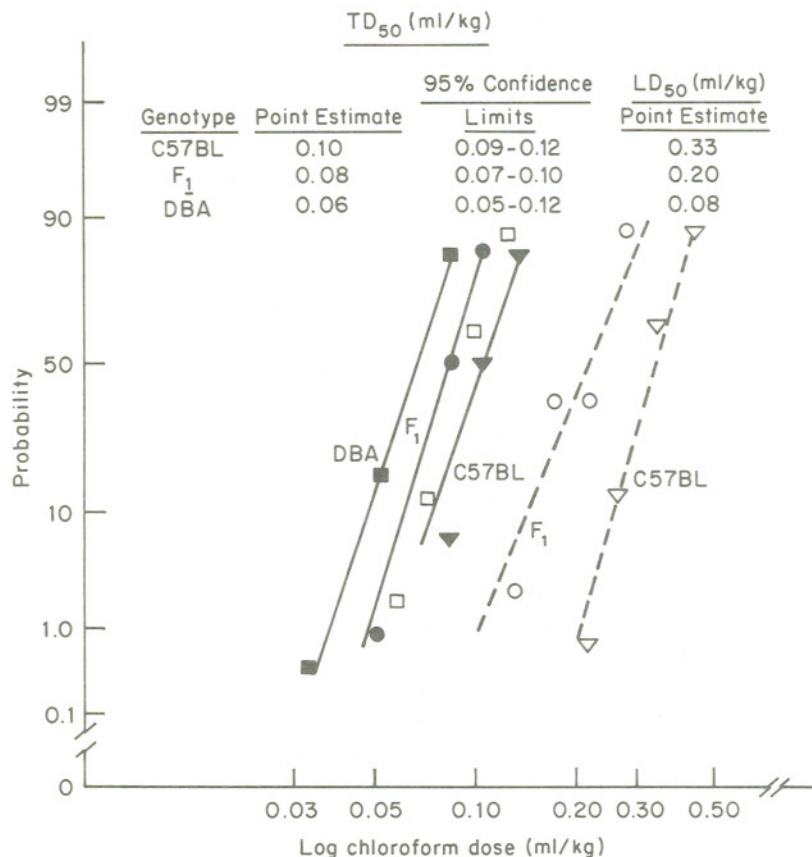
Figure 2 Dose–effect curves for chloroform-induced kidney damage (closed symbols) and death (open symbols) on DBA (squares), C57BL (triangles), and their F_1 hybrid, B6D2F_1 (circles), male mice. The line representing the LD_{50} for DBA mice (□) has been deleted for the sake of clarity. Dose is plotted on a log scale. A single dose of chloroform was administered by gavage and renal function 24 hours later, or number of animals dying after 10 days, was evaluated. Five or six animals were studied at each dose. The LD_{50} data have been reported previously (Hill *et al.*, 1975). From Clemens *et al.* (1979). *Reproduced by permission of Academic Press*

Furthermore, such an approach might permit identification of new causes of special susceptibility and of those combinations of chemicals that produce marked changes in response.

4 THE ROLE OF GENETIC FACTORS

In animals, approximately 100 genetic conditions have been identified that control large variations in susceptibility to toxicity from xenobiotics (Nebert and

Felton, 1976). In human beings, more than a dozen different pharmacogenetic conditions have been identified and investigated intensively (Table 1) (Vesell, 1973). Each condition is genetically distinct from the others, i.e. each condition involves a separate, single genetic locus, thereby explaining the designation monogenic. Table 1 divides these monogenic conditions into those that affect drug metabolism and those that affect pharmacodynamic, rather than pharmacokinetic, processes.

Genetic control of variations among human subjects in response to drugs and toxicants extends well beyond the conditions listed in Table 1. Twin studies disclosed predominantly genetic control of large interindividual variations occurring among normal, uninduced human subjects in elimination rates of amobarbital, antipyrine, bis-hydroxycoumarin, ethanol, halothane, nortriptyline, phenylbutazone, phenytoin, and salicylate (Vesell, 1973, 1979). Generalization of these results leads to the hypothesis that genetic factors may also influence large interindividual variations in the metabolism of many other drugs and toxicants. The initial twin investigations mentioned above were followed by family studies which suggested polygenic control of interindividual variations in the disposition of some of these drugs, but monogenic control of interindividual variations in that of others.

Almost all these studies described only kinetic changes in the parent drug with time after its administration. Since these parent drugs are metabolized by several biochemically and genetically distinct hepatic cytochrome P-450s, the sensitivity and specificity of the information obtained could be improved if the primary gene product were approached directly by measuring the rates of formation of each metabolite. Accordingly, 20 pairs of uninduced monozygotic (MZ) and dizygotic (DZ) twins were measured for rate constants for formation of each of the three main metabolites of antipyrine (Penno *et al.*, 1981). Genetic factors primarily controlled the two-fold differences among these 40 uninduced healthy adults in rates of formation of each antipyrine metabolite. The rate constant for formation of each metabolite of a drug had previously been advocated as a useful index of the gene product in a family study of amobarbital metabolism (Kalow *et al.*, 1977).

Our studies were performed in uninduced twins and later (Penno and Vesell, 1983) in families of only two generations, carefully controlled with respect to most factors in the outer circle of Figure 1. Each subject was tested on at least two separate occasions to establish reproducibility and stability of kinetic values. Since multiple host factors can alter rates of drug disposition and a subject's rate of drug elimination is easily perturbed by slight environmental changes (Figure 1), careful selection of subjects and demonstration of stability through repeated measurements in each subject are essential in pharmacogenetic studies. If a subject took various drugs, smoked cigarettes or imbibed ethanol, then the underlying genetic factors could be obscured. In 13 families whose stability was suggested by both history and reproducible kinetics, we obtained data compatible with monogenic control of variations in the rate constant for formation of

Table 1 Pharmacogenetic conditions with putative aberrant enzyme, mode of inheritance, frequency and drugs that can elicit the signs and symptoms of the disorder

Name of condition	Aberrant enzyme and location	Mode of inheritance	Frequency	Drugs that produce the abnormal response
GENETIC CONDITIONS PROBABLY TRANSMITTED AS SINGLE FACTORS ALTERING THE WAY THE BODY ACTS ON DRUGS				
1. Acatalasia	Catalase in erythrocytes	Autosomal recessive	Mainly in Japan and Switzerland, reaching 1 % in certain small areas of Japan	Hydrogen peroxide
2. Slow inactivation of isoniazid	Isoniazid acetylase in liver	Autosomal recessive	Approximately 50 % of US population	Isoniazid, sulphamethazine, sulphamaprine, phenelzine, dapsone, hydralazine, procainamide
3. Suxamethonium sensitivity or atypical pseudocholinesterase	Pseudocholinesterase in plasma	Autosomal recessive	Several aberrant alleles; most common disorder occurs 1 in 2500	Suxamethonium or succinylcholine
4. Diphenylhydantoin toxicity due to deficient p-hydroxylation	? Mixed function oxidase in liver microsomes that p-hydroxylates diphenylhydantoin	Autosomal or X-linked dominant	Only 1 small pedigree	Diphenylhydantoin
5. Bishydroxycoumarin sensitivity	? Mixed function oxidase in liver microsomes that hydroxylates bishydroxycoumarin	Unknown	Only 1 small pedigree	Bishydroxycoumarin
6. Acetophenetidin-induced methaemoglobinemia	? Mixed function oxidase in liver microsomes that de-ethylates acetophenetidin	Autosomal recessive	Only 1 small pedigree	Acetophenetidin

Table 1 (*Cont'd*)

Name of condition	Aberrant enzyme and location	Mode of inheritance	Frequency	Drugs that produce the abnormal response
7. Deficient *N*-glucosidation of amobarbital	? Mixed function oxidase in liver microsomes that *N*-glucosidates amobarbital	Autosomal recessive	Only 1 pedigree: screening of over 100 unrelated, normal volunteers revealed that approximately 2% were homozygous affected	Amobarbital
8. Polymorphic hydroxy-lation of debrisoquine in man	? Mixed function oxidase in liver microsomes that 4-hydroxylates debrisoquine	Autosomal recessive	94 volunteers and 3 families with a frequency of homozygous affected individuals of approximately 3%	Debrisoquine

GENETIC CONDITIONS PROBABLY TRANSMITTED AS SINGLE FACTORS ALTERING THE WAY DRUGS ACT ON THE BODY

Name of condition	Aberrant enzyme and location	Mode of inheritance	Frequency	Drugs that produce the abnormal response
1. Warfarin resistance	? Altered receptor or enzyme in liver with increased affinity for vitamin K	Autosomal dominant	2 large pedigrees	Warfarin
2. Glucose-6-phosphate dehydrogenase deficiency, favism or drug-induced haemolytic anaemia	Glucose-6-phosphate dehydrogenase	X-linked incomplete codominant	Approximately 100 000 000 affected in world; occurs in high frequency where malaria is endemic; 80 biochemically distinct mutations	Many different drugs

3. Drug-sensitive haemoglobins (a) Haemoglobin Zurich (b) Haemoglobin H	Arginine substitution for histidine at position 63 of the β-chain of haemoglobin Haemoglobin composed of 4 β-chains	Autosomal dominant	2 small pedigrees	Sulphonamides Many different drugs
4. Inability to taste phenylthiourea or phenylthiocarbamide	Unknown	Autosomal recessive	Approximately 30 % of Caucasians	Drugs containing N-C-S group such as phenyl-thiourmethyl and propylthiouracil
5. Glaucoma due to abnormal response to intraocular pressure to steroids	Unknown	Autosomal recessive	Approximately 5 % of US population	Corticosteroids
6. Malignant hyper-thermia with muscular rigidity	Unknown	Autosomal dominant	Approximately 1 in 20 000 anaesthetized patients	Various anaesthetics, especially halothane
7. Methaemoglobin reductase deficiency	Methaemoglobin reductase	Autosomal recessive heterozygous carriers affected	Approximately 1 in 100 are heterozygous carriers	Many different drugs

each antipyrine metabolite (Penno and Vesell, 1983). This represents a new genetic polymorphism of drug oxidation different in several ways from the one exemplified by debrisoquine and sparteine. As one notable distinguishing characteristic, the rare form of the gene in the antipyrine polymorphism involves increased rates of drug metabolism, whereas for debrisoquine and sparteine the rare allele confers decreased capacity to metabolize the drug.

Genetic and environmental factors that cause variations among normal subjects in rates of elimination of chemicals are often discussed as though such factors were entirely separate, unrelated entities without interaction or inter-dependence. However, genetic and environmental factors that control these variations interact dynamically at multiple levels. In fact, to achieve expression, genetic factors often require participation of environmental factors; the latter often require a genetic apparatus to exert their effects. For example, the pharmacogenetic conditions listed in Table 1 are expressed only in the presence of an environmental agent: a drug. Toxicity then develops only or predominantly in subjects whose genetic constitutions render them sensitive. The genetic factor may be a metabolic block that leads to drug accumulation or it may consist of a structurally altered receptor site that fails to bind normally the parent drug or its metabolites, thereby preventing anticipated therapeutic effects from occurring at usual drug concentrations.

Another example of dynamic interactions between the gene and environment involves near basal rates of hepatic drug metabolism in normal uninduced subjects. Such rates, as indicated by the twin studies described above, are controlled primarily by genetic factors, but are exquisitely sensitive to pertur-bation by numerous environmental factors, some of which are listed in the outer circle of Figure 1. Many of these factors act simultaneously and are interrelated. These factors can exert their effects by altering, at different steps and sites, a normal subject's near basal, genetically controlled rate of drug elimination. Environmental factors can change a subject's drug response by altering processes of drug absorption, distribution, metabolism, excretion, receptor action, or combinations of these. The wavy arrow joining factors in the outer circle of Figure 1 to the inner circle suggests these multiple sites. Pharmacogenetic studies require more careful selection of subjects to ensure that these subjects are under the basal, uninduced conditions necessary for identifying the operation and transmission of genetic factors.

5 QUESTIONABLE ASSUMPTIONS IN CERTAIN METHODOLOGICAL APPROACHES TO THE IDENTIFICATION AND PREDICTION OF EXPOSING SUBJECTS TO DRUGS AND CHEMICALS, ALONE AND IN COMBINATION

The assumptions underlying some approaches to predicting and reducing the health hazards that arise from exposure of certain human subjects to toxic

chemicals should be re-examined. The goals of devising and applying methods to identify not only such toxic chemicals in our external and internal environments but also particularly susceptible subjects are laudable. However, certain approaches to achieving these objectives are based on dubious assumptions. Such questionable assumptions include the following:

(1) That a quantitative separation exists in the concentration of a specific chemical within subjects rendering them either susceptible or resistant to health hazards from potentially toxic environmental chemicals. In man this assumption has been largely untested, and the underlying concept of a single threshold (body concentration) for a certain chemical to produce a toxicological effect in *all* subjects may be incorrect for many chemicals. This may be the case for two reasons: (a) the threshold concentration may change with time and condition (Figure 1) even in the same subject; and (b) different subjects, because of the circumstances of their life-style, employment, or physiology, may have markedly different thresholds.

If (a) and (b) both applied to a particular chemical, then the approach of developing methods to measure threshold concentrations to indicate potential toxicity from such chemicals becomes useless. Other approaches to protect subjects from potential health hazards posed by these chemicals need to be devised. In some cases multiple, rather than single, causes of toxicity can be implicated, whereas in others a single cause may *seem* to be the only one involved, such as the single genetic lesion responsible for monogenically controlled conditions listed in Table 1. For each condition listed in Table 1, a single genetic lesion renders certain subjects particularly sensitive to adverse effects and toxicity from certain chemicals (drugs).

(2) That the toxicity associated with the conditions listed in Table 1 arises only from drug accumulation to toxic concentrations in certain subjects who by virtue of their genotype lack the enzyme that normally metabolizes the active drug to inactive product. Instead of the normal enzyme, they possess an aberrant or mutant form of the enzyme which is less efficient in converting the chemical to its primary metabolites. However, the same form of toxicity due to excessive accumulation of a specific chemical can also occur for different reasons in other subjects with normal genotypes. Thus, the same phenotype of toxicity can be produced for a given chemical by several distinguishable mechanisms. These other predisposing causes of chemical toxicity may include one or more of the host factors shown in Figure 1.

6 THE MODEL DRUG APPROACH: THE ANTIPYRINE TEST

While the antipyrine test has been widely accepted in clinical pharmacology as a sensitive method to quantitate the capacity of certain drugs, alone and in combination, to alter the basal activity of the hepatic DMEs, such effects are detectable only for a limited number of drugs possessing certain specific

pharmacological properties and administered in relatively high doses. Thus, this method is far from universally applicable as a test of exposure to all or even most environmental chemicals; quite the opposite, the antipyrine test is suitable only for identifying exposure to a very small fraction of environmental chemicals. Analytical measurement of these chemicals in host tissues and biological fluids is a more desirable approach, being more direct, sensitive, and accurate, even if it is longer, more tedious, and less convenient. As indicated in the preceding section, such concentrations of a chemical may not always be reliable guides to the toxicological consequences of that chemical in a particular person. The following discussion stresses not only the special characteristics of the chemical that make it appropriate for study with the antipyrine test but also the special host factors that determine the extent to which any given chemical influences the activity of the hepatic DMEs in a particular subject.

In patients, environmental factors in addition to those that affect normal subjects can alter rates of drug elimination. Even in the same patient the relative role played by each factor shown in Figure 1 in influencing drug disposition changes with time and other variables, such as the course and intensity of the disease (Vesell, 1973, 1982). The same disease that is subclinical in one patient may be rapidly lethal in another. Due to patient-dependent fluctuations in the course of many diseases, it may prove difficult to identify, even at a particular time, which factors are operating and what specific contribution each factor or chemical administered actually makes to the total drug-metabolizing capacity of a particular patient. Therefore, each factor is generally investigated in normal, unmedicated subjects who are near basal with respect to factors known to alter hepatic drug-metabolizing capacity. To estimate this basal capacity in each subject, measurements are then performed with a test drug, such as antipyrine, aminopyrine, acetaminophen, diazepam, phenytoin, or a combination of these drugs at different times—each drug being administered on several separate occasions to assure reproducibility. For antipyrine, a single oral dose of 18 mg/kg is generally administered, but doses as low as 1 mg/kg can now be used with the development of more sensitive methods, such as radioimmunoassay or nitrogen detection with gas–liquid chromatography (Shively *et al.*, 1979). A single environmental alteration is introduced, after which the subject's drug-metabolizing activity is remeasured by readministration of the same dose of antipyrine. A change from the reproducible basal values for antipyrine metabolism is taken to suggest the effect exerted by the single environmental variable (Vesell, 1979). Dose–response relationships can be obtained with such methods. It can be appreciated that the antipyrine test is best suited to detect enhancement (induction) or suppression (inhibition) of the activity of hepatic DMEs. To achieve such an alteration from normal, basal activities of the DMEs, a chemical or host factor must be present in sufficient dose. For many chemicals in man a minimum dose of 100 mg/day for several days is required. Chemicals vary in their capacity to induce or inhibit DMEs according to such properties as

their lipid solubility and capacity to bind to the DMEs in hepatic smooth endoplasmic reticulum. For chemicals that cannot be investigated in man due to their toxicity, the antipyrine test can be performed in laboratory animals, although extrapolation of such results to man may pose problems.

The antipyrine and aminopyrine tests (Hepner and Vesell, 1974, 1975, 1976) are sensitive, not only in detecting very small changes in the activity of the hepatic DME responsible for biotransforming antipyrine and aminopyrine, respectively, but also for detecting the effects on hepatic DMEs of some chemicals singly and in combination (Vesell, 1979; Vesell *et al.*, 1975). For use in man these tests enjoy the major advantages of safety in healthy volunteers, convenience, rapidity, and non-invasiveness (salivary concentrations of antipyrine in a normal subject have been shown in many laboratories to equal their plasma concentrations).

Special pharmacological properties uniquely qualify antipyrine for selection as a test drug. These include its rapid and complete absorption from the gastrointestinal tract, distribution in total body water with negligible binding to tissue or plasma proteins, almost complete hepatic biotransformation, mainly to three metabolites readily identifiable in urine, a low hepatic extraction ratio of the parent drug, and negligible renal elimination.

This widely used test has certain limitations which have been discussed at length (Vesell, 1979). In brief, these include the following:

(1) results cannot always be extrapolated with accuracy to other drugs;
(2) certain environmental factors which alter the disposition of other drugs may not always alter antipyrine pharmacokinetics; and
(3) results obtained in normal male subjects may not apply to patients with various diseases.

These drawbacks are often outweighed by the virtues of a carefully controlled approach using each subject as his own control. Such an approach permits investigation of a single factor independent of most others, thereby eliminating many interfering genetic and environmental variables that confound the results obtained with the alternative experimental design of comparing a control group with a different experimental group. For these reasons, the antipyrine test has been successfully utilized to identify the impact of many factors shown in Figure 1 on drug disposition. The specificity and sensitivity of the test can now be improved by using initial results as a screen and re-examining the factors proven positive by measuring the rate constants for formation of each of the three principal metabolites of antipyrine. This much more tedious method requires complete urinary collections for at least two, and preferably three, days. Close cooperation of subjects is necessary, but increased specificity of the information obtained may justify the extra trouble for both subject and investigator.

It now becomes clear why effects of a subject's genetic constitution on drug-eliminating capacity need to be measured under near basal, uninduced conditions where most environmental factors in the outer circle of Figure 1 are

carefully identified and controlled. Under these conditions, intraindividual variations determined by measuring at several different times either the same subject's rate of elimination of an appropriate test drug (Conney *et al.*, 1980; Vesell, 1980) or the rate constants for the formation of the test drug's principal metabolites (Penno *et al.*, 1981) are generally small, approximately 10 % of mean values. Under non-basal conditions, intraindividual variations can become large enough to reduce the validity of the interindividual or the before and after drug comparisons, thereby negating the virtues of the test in identifying how drugs and chemicals affect the activity of the DMEs in normal subjects.

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