0031-6997/79/3004-0351$02.00/0
PHARMACOLOGICAL REVIEWS
Vol. 30, No. 4

Toxicology: Has a New Era Dawned?

LEON GOLBERG
Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina

I. Introduction

MUCH of the recent history of toxicology has involved routine investigation. The pioneers and innovators have always been there, crying out to be heard, but largely unnoticed. To this day the all too prevalent attitude persists that the experimental animal is a sort of "black box" into whose inner workings one need not and should not inquire. Test materials are introduced through every available orifice, into the conjunctival sac, onto the skin, or by various parenteral routes. The effects on the animal are then observed with ever-increasing degrees of elaboration and sophistication. More and more minutiae are noted, more and more man- and woman-power is engaged in checking and cross-checking the data, signing and countersigning the notebooks and worksheets. More and more computer programs are brought into action, more and more acres of printout generated, while the final reports increasingly attain gargantuan proportions.

Amid all this frenetic (and expensive) activity, does anyone anywhere pause to wonder what goes on inside the "black box"? Of course some people do, but they are in the minority, and most often they are offered little encouragement or support in their eagerness to probe further into the phenomena that they observe. The "black box" outlook persists in industry, government, and even in some sectors of academia. The reasons call for no elaboration; one may point to the justifications put forward for that archetype of the "black box" approach—and in all probability the last bastion of its stouthearted defenders—the NCI Carcinogenesis Bioassay Program. Preserving inviolate the secrets locked up in that particular "black box" has assured perpetuation of the mystical belief in the one-molecule theory of carcinogenesis.

Two hundred or even one hundred years ago the search for mechanisms by which the body handles toxic agents was a slow and tedious undertaking (table 1). Today we possess tools of exquisite and sometimes overpowering sensitivity that have accelerated, in fact made possible, the study of metabolism and pharmacokinetics. At the same time, the contributions from the basic sciences promise to revolutionize both the methods used and the study of mechanisms in toxicology. What encourages the belief that we really are emerging from the Dark Ages into a new era of enlightenment is the quickening interest of basic scientists in the relevance and application of their research efforts to toxicology. The general public's concern about toxic hazards and environmental pollution has permeated into the fastnesses of academia, so that increasingly it is those scientists who pose the questions and seek to provide answers, rather than leaving it to toxicologists to apply the findings stemming from basic research. More and more scientists in a variety of disciplines are heeding Claude Bernard's message that toxic agents are keys with which to unlock the secrets of nature.

There is irony and paradox in the fact that the new era in toxicology, with all this wealth of potential resources at its disposal, faces the prospect of progressive asphyxiation by proliferating regulatory guidelines,

TABLE 1
*Time-scale in discovery of metabolic conjugation**

Type of Conjugation	Substrate	Initial Observation	Identification	Time Lapse
				yr
Glycine	Benzoic Acid	1773	1845	72
Sulfuric acid	Phenol	1867	1876	9
Glucuronic acid	Euxanthic acid	1846	1879	33
Acetic acid	Aromatic amines	1887	1893	6

* Based on Conti and Bickel (21).

hypertrophied requirements based on cookbook recipes, and legalistic stereotyped testing protocols. As the noose draws tighter, there is less and less scope for the exercise of expertise and judgment to obtain meaningful answers to relevant questions, as distinct from negative data intended to satisfy government authorities.

Even worse, the dawning era is characterized by public disillusion concerning the benefits conferred by new technologies on society. This pessimism applies particularly to chemicals, where, as Harvey Brooks (11) has stated, "There is a growing domain of research which might be said to have a vested interest in the exaggeration of disbenefits. . . . " What until just recently was popularly regarded in the academic world as the pedestrian and mundane pursuit of safety testing has acquired a new glamour. Even the lowliest amateur can now climb on the bandwagon by proving something harmful with a minimum of skill and effort, and then bask in the radiance of instant media exposure.

It would thus be an understatement to say that we desperately need the help of genuine scientists. The area in which the greatest contribution can be made is that of long-term effects brought about by chronic low-level exposure to toxic agents: the nature of those effects, the mechanisms by which they arise, and the assessment of the risk to man of such effects seen in animals, in lower organisms, or in vitro systems. The no-threshold one-molecule philosophy—once exclusively prevalent in the domain of carcinogenesis—is increasingly encroaching into other areas of toxicology as part of the zero-risk attitude that the public is encouraged to adopt and demand as a basis of public policy. Looked at scientifically, even in the instance of mutagenic/carcinogenic potential, important factors need to be taken into account in assessing the problem posed by a chemical agent that has elicited positive results (table 2). In effect, therefore, we have an unsolved equation for the determination of the mutagenic/carcinogenic impact of a particular level of exposure (table 3). The equation omits to mention possible interactions with oncogenic viruses and bacteria. Moreover, this analysis makes no allowance for reversibility of the early lesions as a result of the action of repair mechanisms of the body, some of the evidence for which is discussed below; but it emphasizes the imponderables inherent in the situation.

II. Homeostasis and Adaptation

Since toxicity constitutes a breakdown in homeostatic mechanisms, this would be a good point to begin our discussion on the needs of the new era. The maintenance of stability in the internal environment of an organism was expressed by the term "homeostasis," introduced by Cannon (16). As shown schematically in figure 1, homeostasis involves regulation within the cell, as well as the action upon regulated cells of regulatory cells situated in particular loci possessing specialized functions. Wiener (177) was the first to point to the fact that this delicate modulating and integrating effect is a necessary condition for life in a multicellular organism and is achieved by a system of communication involving feed-

TABLE 2
Overall carcinogenic/mutagenic impact

Source	Factor(s)	Susceptibility	Resistance
I. Chemical agent	C/M* potency	Strong	Weak
	Level and duration of tissue exposure	High, prolonged	Low, transient
II. Body defense and adaptive mechanisms	Metabolism	Activation, covalent binding	Detoxication, inactivation
	Repair and restoration of damage	Delayed or error-prone	Accelerated, accurate and complete
	C/M agents	Increased	Decreased
III. Endogenous synthesis			
IV. "Natural" food	C/M synergists	Increased	Decreased
V. Rest of environment	C/M antagonists	Decreased	Increased

* C/M, carcinogenic/mutagenic; *synergists* include syncarcinogens, cocarcinogens, comutagens, promoters, modifying factors; *antagonists* include anticarcinogens, antimutagens, protective factors and other tumor suppressants.

TABLE 3
The unsolved equation

Total cancer impact of compound
= a (Carcinogenic potential of compound)
+ b (Action of syncarcinogens, cocarcinogens, promoters, modifying factors, etc.)
− c (Protective action of body defense and adaptive mechanisms)
− d (Action of anticarcinogens and other tumor suppressants)
+ e (Carcinogens in "natural" food)
+ f (Carcinogens formed endogenously)

TABLE 4
Sequence of DNA repair systems responding to alkylation (in bacteria; possibly also in mammalian cells)*

State of Cells	Systems Brought into Action		
	Error-free repair		Error-prone repair
	1. Constitutive	2. Adaptive responses	3. Inducible
Normal	+	Trace	0
Adapted	+	+	0
Low exposure†	+	Trace	0
Medium exposure	+	+	0
High exposure	+	+	+‡

* Jeggo et al. (71, 72), Lehman (90), Rupp et al. (133), Samson and Cairns (134), Witkin (181).

† Exposure to mutagen = Concentration × time; e.g. ethyl methane sulfonate (EMS), methyl methane sulfonate (MMS), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

‡ Error-prone system is liable to "fix" the mutation (unless excessive damage to the chromosome stops replication). Mispairing during replication is another possible mechanism of mutagenesis.

back mechanisms. Adaptation to exposure to a variety of chemical and physical agents, as well as to excess or deficiency of an essential element such as oxygen, is an important feature of the homeostatic mechanism. Thus the components of homeostasis may be divided into two parts: constitutive regulatory mechanisms; and various adaptive mechanisms for protection against the toxicity of endogenous and exogenous chemicals. The defense mechanisms may be mustered in sequence, according to the nature and severity of the challenge presented to the organism.

An interesting example of this sequence of adaptive developments is illustrated by the order of DNA repair systems responding to alkylation (table 4). What needs to be stressed here is that the inducible error-prone repair system is capable of decaying when the exposure to alkylating agent diminishes, so that the inducible mechanism is no longer needed and the constitutive and adaptive mechanisms are capable of dealing at an adequate rate with the need to repair alkylated DNA. Of course, there is no guarantee that occasionally some molecules of the alkylated DNA do not slip through the chinks in the defensive armour and a mutation results in the course of

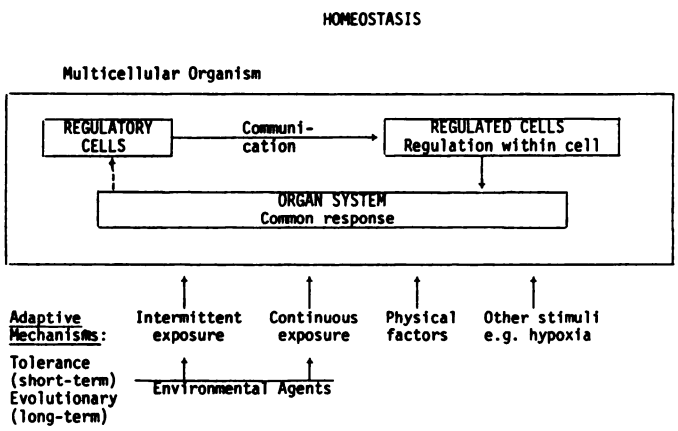

FIG. 1. Schematic representation of the interrelationships between homeostatic mechanisms of a multicellular organism and its adaptive responses to external exposures to physical and chemical agents.

postreplication repair. This hypothetical possibility needs to be weighed against the history of man's exposure, from time immemorial, to a host of environmental carcinogens, as discussed below.

More general aspects of the host defense mechanisms have been discussed by Apffel (2), who divided them into mechanisms of containment, directed against already transformed cells, and mechanisms of surveillance, intended to prevent or inhibit the initial events of neoplasia. Among the different aspects of endogenous chemical surveillance, two are worthy of special mention here: the natural anticarcinogenic and antimutagenic protective agents; and the microsomal enzyme systems. Under the first heading, reference should be made to the work of Wattenberg and Loub (170) and Wattenberg (171) and of Kallistratos and Fasske (77), and Kallistratos and Kallistratos (78). Kallistratos defined three categories of agents that were capable of inhibiting, partially or completely, the carcinogenic effect of benzo(a)pyrene on mice: polyamines such as putrescine; unsaturated polycarboxylic acids such as *cis*-aconitic acid; and sulphur compounds such as mercaptosuccinate. It is interesting that the observations of Kallistratos with putrescine have recently been fully duplicated and confirmed (47). In addition, the anticarcinogenic actions of indoles (170), vitamin A, retinol and synthetic retinyl esters (59, 151), and selenium, which is also antimutagenic (69), have been receiving increasing attention.

III. Metabolic Activation

A further aspect of endogenous chemical surveillance arises through the action of microsomal monooxygenase and other biotransforming enzyme systems. These generate the so-called "hockey-stick" dose-response curve (22) characterized by an initial portion at low levels of exposure possessing a slope that is zero or approaches zero (indicative of effective adaptation), and another portion of the curve at higher levels of exposure where the slope increases sharply as the threshold dose is passed (deactivation system saturated, adaptive reserves exceeded). Examples of such curves are illustrated in figures 2 and 3. In this context the term "threshold" may be defined as a level and/or rate of exposure that exists for every compound such that a certain maximum limit may be attained without overwhelming or impairing the defense mechanisms of the organism.

The issue of threshold should be viewed in the context of the relationship between metabolic biotransformation and pathological changes brought about by chemical compounds (fig. 4). In the case of vinyl chloride (VC), the polar excretable metabolites of VC identified in rat urine suggest the involvement of a chemically-reactive

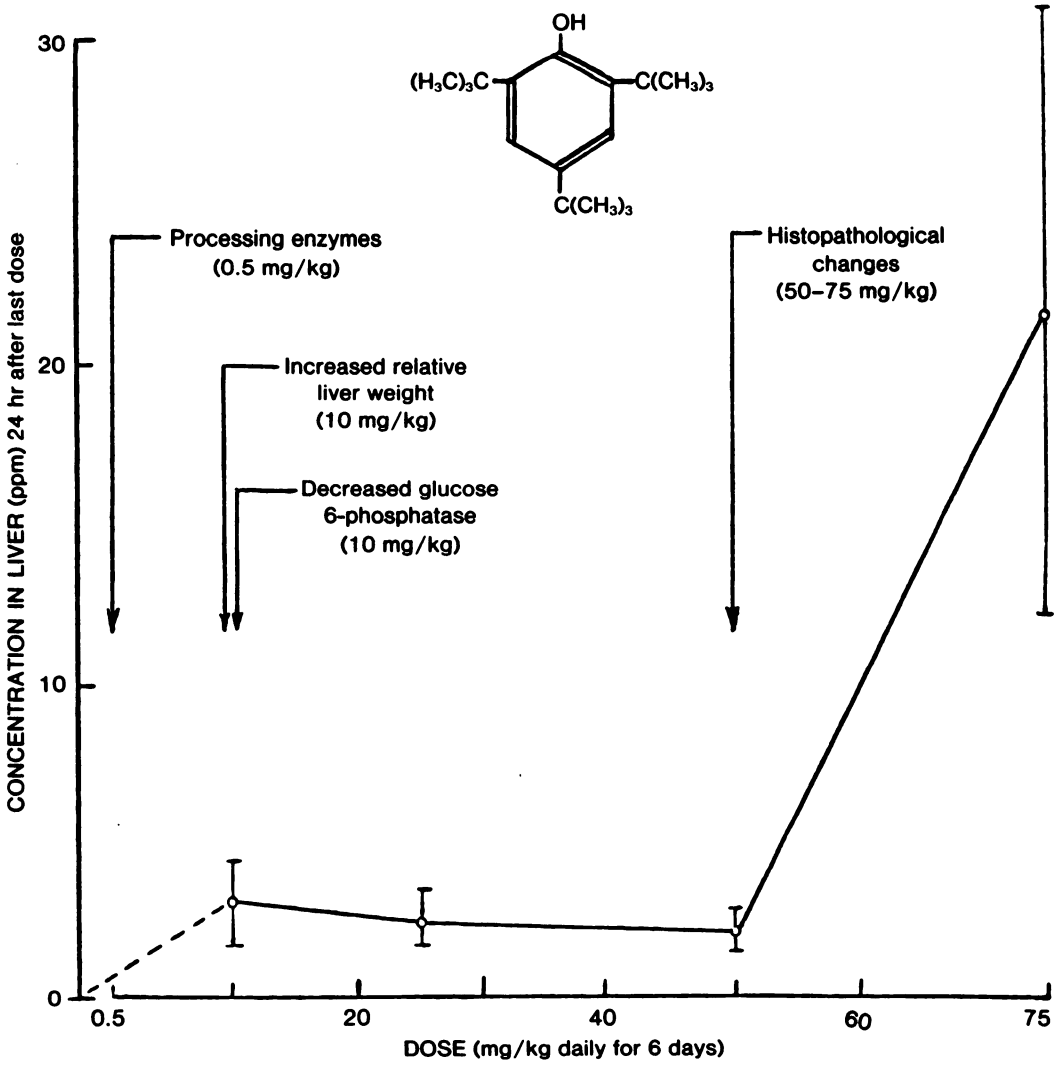

FIG. 2. Adaptive changes in the livers of rats, receiving daily doses of 2,4,6-tri*tert.*-butylphenol, that permit maintenance of a low concentration of the toxic agent in the liver (52).

intermediate in the hepatotoxic and carcinogenic actions of this compound. The metabolites concerned are N-acetyl(S-2-hydroxyethyl)cysteine and thiodiglycolic acid, probably formed from S-(2-hydroxyethyl)cysteine (63). Subsequent work has revealed the presence of S-(carboxymethyl)cysteine and N-acetyl-S-vinyl-cysteine in rat urine after inhalation or oral administration of VC, lending further support to the suggestion that chloroethylene oxide and thence chloroacetaldehyde are the reactive intermediates (55). What is of greater importance is the degree of covalent binding to nucleophilic groups in DNA, protein, and other macromolecules within the cell (80, 119, 163). Evidence has been sought for indices of exposure along these lines by analyzing the degree of alkylation of the globin moiety of hemoglobin as a dose monitor (118). Legator (89) has pursued work along similar lines, while also measuring substituted histidine and cysteine derivatives in urine as evidence of alkylating exposure in vivo. A further step in the delineation of the interaction products of VC with DNA has been the work of Bolt et al. (6, 87) and Green and Hathway (56) and

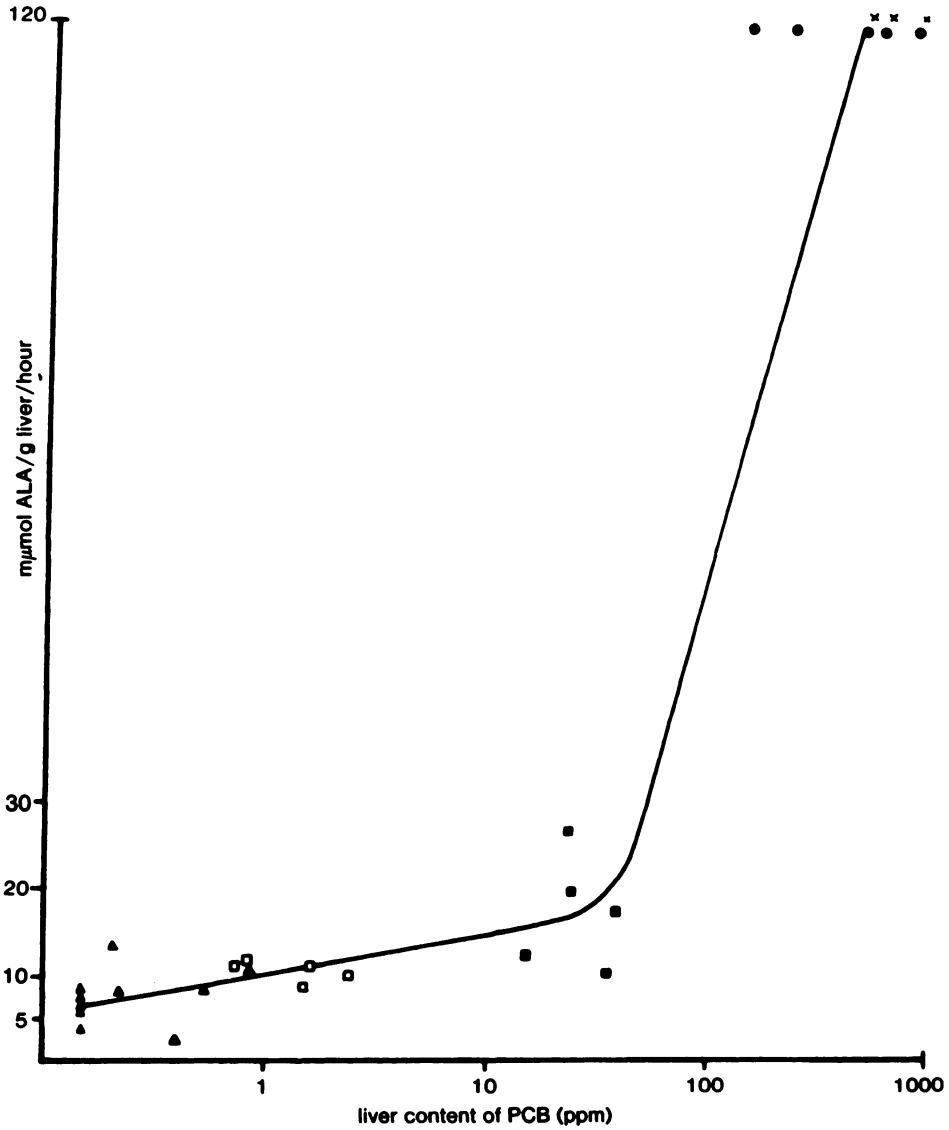

FIG. 3. Correlation between the liver content of polychlorinated biphenyl (PCB) (in ppm) and the mitochondrial δ-aminolevulinic acid (ALA) synthetase activity (μmoles ALA/g liver/hr). △, control animals; ▲, 0.1 mg/kg; □, 1 mg/kg; ■, 10/mg/kg; ●, 100 mg/kg; x = birds showing fluorescence under ultraviolet light. [From Vos et al. (166), with permission.]

Hathway (62) demonstrating the formation of etheno-derivatives, probably through the oxirane and/or chloroacetaldehyde pathways:1,N^6-ethenoadenosine and 3,N^4-ethenocytidine are postulated as forming from RNA and the corresponding imidazopyrimidine and purine derivatives from DNA in vitro. The relationship between detoxification and covalent binding of vinyl chloride is illustrated in figure 5, taken from the work of Watanabe et al. (169). Modulation of microsomal and other enzyme activity involved in the metabolism of vinyl chloride may be brought about by fasting of the animals. The striking influence of an 18-hr fast on the excretion of vinylidene chloride has been demonstrated by McKenna et al. (103, 104).

The nature and direction of covalent binding are influenced by trapping of reac-

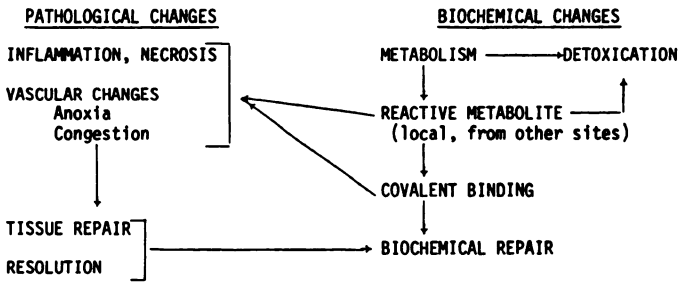

FIG. 4. Schematic representation of the interrelationships between the sequence of biochemical changes undergone or brought about by a toxicant and the associated pathological events.

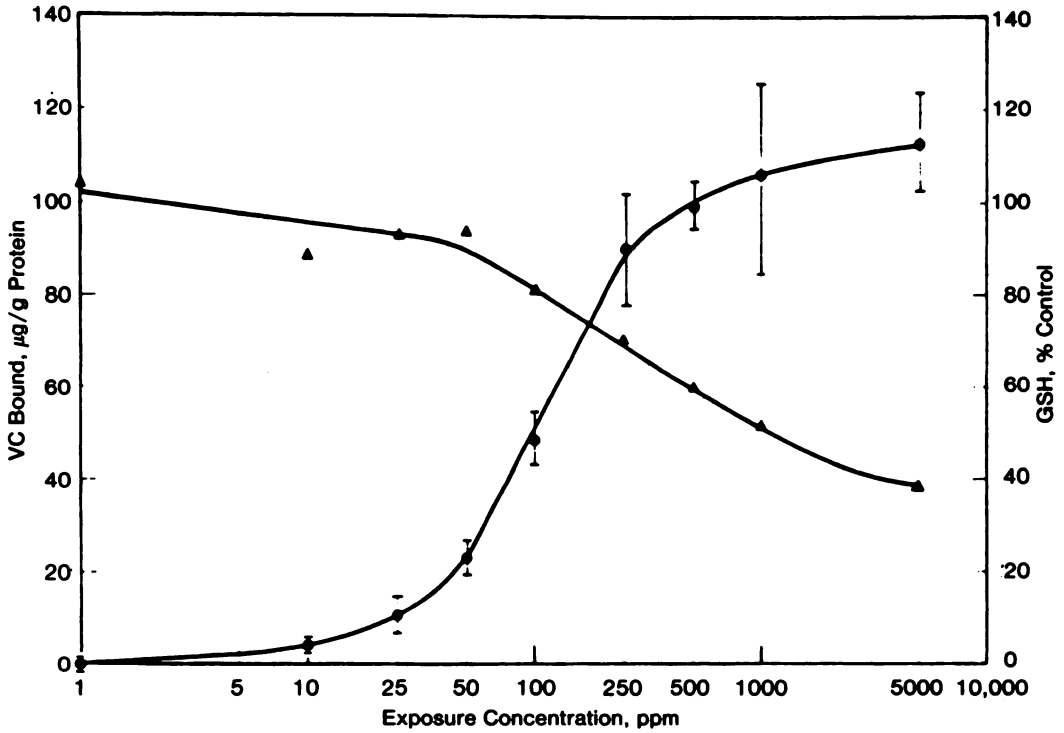

FIG. 5. Hepatic macromolecular binding and glutathione (GSH) depression expressed as a function of the logarithm of the exposure concentration of vinyl chloride (VC). Macromolecular binding (●) is expressed as microgram equivalents of VC bound per gram of protein (mean ± SD) GSH (▲) is expressed as percentage of control. [From Watanabe et al. (169), with permission.]

tive metabolites by small nucleophiles such as glutathione, cysteine, methionine, or ascorbic acid (108). Thus cysteine inhibits the in vitro covalent binding of chloroform to microsomal protein, concomitantly trapping a reactive metabolite, presumably phosgene, as 2-oxothiazolidine-4-carboxylic acid (126). Within a given target organ, the duration of persistence of particular activated metabolites of a compound and, more especially, the time course of covalent binding to components in particular parts of those organs constitute very important information. Examples are available from the study of the metabolism of benzene, demonstrating the persistence of catechol and hydroquinone within the bone marrow (66, 131) and the persistence of 2,5-hexanedione in sciatic nerve of rats exposed to *n*-hexane (174). Mudge et al. (107) have studied the

time course of covalent binding of tritiated *p*-acetylaminophenol given in a dose of 200 mg/kg to CD-1 mice. The authors demonstrated that the slope of disappearance is less for renal papilla than for renal cortex or liver. This represents a longer duration of covalent binding to protein in the renal papilla following administration of acetaminophen.

Some special features of metabolic activation have emerged from the study of halothane metabolism (19) intended to correlate the formation and macromolecular binding of reactive intermediates with the known and unusual hepatotoxic properties of this anesthetic agent (165). There appears to be a balance between oxidative metabolic pathways and reductive pathways (fig. 6), the latter giving rise to unstable reactive products (12), including trifluorocarbene that forms a complex with the ferrous iron of cytochrome P-450 (113). Covalent binding takes place predominantly with lipids, especially the phospholipids phosphatidylethanolamine and lecithin (147, 148, 164). Sipes and his colleagues (12, 70, 96, 148) have developed an anoxia model involving exposure of animals to 14% oxygen and 1% halothane for 2 hr, which makes possible a response that is predominantly in the reductive metabolic pathway, thereby eliciting liver necrosis. In view of the key role of phospholipid in the monooxygenase system (93, 183), the fact that covalent binding of halothane metabolites is two-thirds to phospholipid and only one-third to protein underscores the importance of studying phospholipid binding in other contexts.

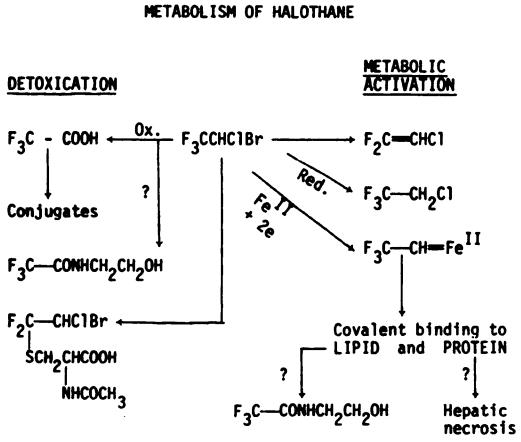

FIG. 6. Schematic representation of the oxidative and reductive pathways of halothane metabolism. Although the formation of trifluoroacetylethanolamine is shown as possibly stemming from the oxidative pathway, covalent binding of halothane metabolites to phosphatidylethanolamine and other microsomal lipids is greatly accentuated by a hypoxic environment (70).

Interactions with microsomal cytochrome P-450, for instance by high doses of cyclophosphamide, can lead to denaturation and depression of monooxygenase activities (98). 4-Hydroxycyclophosphamide or acrolein bring about 40 to 50% denaturation, apparently by binding with sulfhydryl groups. Interference with heme biosynthesis by allylisopropylacetamide (27) and heme destruction by VC (60) are other mechanisms of depression of cytochrome P-450 activity.

It would be a mistake, however, to think of alkylation and covalent binding exclusively in terms of drugs or other synthetic chemicals. Thus, enteric bacteria activate cholesterol, cholic acid, and deoxycholic acid to products that are bound covalently to DNA (187). Alkylating agents are derived from contaminants in air and water, formed from the volatile hydrocarbons of deciduous forests by photooxidation and degradation. These hydrocarbons include terpenes and particularly isoprene, which is also present in human alveolar air (26). From these terpenes is produced 3-methylfuran, which is metabolized in mice to an alkylating agent binding covalently to lung macromolecules and capable of producing severe bronchiolar necrosis (7).

Historically, both Williams (179) and Brodie (10) regarded the function of endogenous metabolizing enzymes as responsible for detoxication, making possible the elimination of xenobiotics from the body. James and Elizabeth Miller are the principal architects of our current concepts of metabolic activation in the conversion of procarcinogens (and promutagens) to the ultimate

reactive metabolites considered to be responsible for the long-term "irreversible" effects of such compounds. They postulated the general theory of conversion of chemical agents to electrophilic reactants through the action of microsomal mixed function oxidases, and demonstrated the capacity of such electrophiles to interact covalently with cellular nucleophiles in tissue macromolecules such as proteins and nucleic acids. By a series of brilliant investigations they identified the biotransformation products of 2-acetylaminofluorene and established previously unknown routes for the biosynthesis of the specific electrophilic reactant forms of this carcinogen. Subsequently, they have applied these approaches to other carcinogens such as safrole (105). The impact on toxicology of these theories, and the studies on which they are based, has been immense. Today, increasing emphasis is placed on the significance of metabolic mechanisms in the conversion of compounds to more toxic moieties, including the "ultimate" toxicants thought to be responsible for specific effects. Some examples of neurotoxic activation are shown in figure 7. L-Allylglycine is converted to 2-keto-4-pentenoic acid through the action of L-amino acid oxidase; 2-keto-4-pentenoic acid is one to two thousand times more potent as an inhibitor of cerebral glutamic acid decarboxylase, resulting in a convulsant action in mice (65). The conversion of *n*-hexane to 2,5-hexanedione through the intermediate methyl-*n*-butyl ketone has been established by the work of DiVincenzo et al. (31) and further studies along these lines have been carried out by White et al. (174). Finally, the neurotoxicity of Versalide (AETT) is thought to involve the possible activation step shown in figure 7, full details of which have not been clearly established. Why the metabolic products referred to above are neurotoxic is still unknown. Penetration of the blood-brain barrier may be a factor, as postulated for the neurotoxicity seen occasionally with azaribine (triacetyl 6-azauridine), where 6-azauracil formed by enteric organisms in the lower intestine is thought to be the active agent (34).

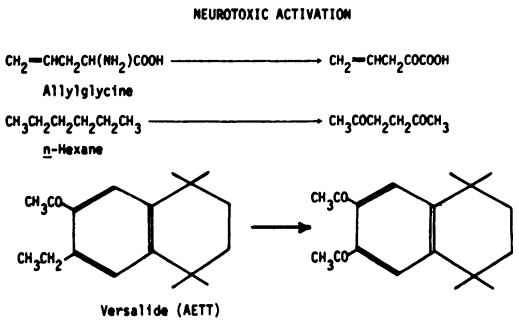

FIG. 7. Examples of neurotoxic activation: L-allylglycine to 2-keto-4-pentenoic acid (65), *n*-hexane to 2,5-hexanedione (31, 174) and 6-acetyl-1,1,4,4-tetramethyl-7-ethyl-1,2,3,4-tetralin (AETT) to the 6,7-diacetyl metabolite suspected to be the active neurotoxic agent (117).

Although the explanation is often not readily apparent, activation may involve cyclization in order to produce a highly toxic compound. Examples are provided by the case of lysinoalanine, which is acted upon by rat kidney L-amino acid oxidase in order to produce 1,7-diazabicyclo[4:3:0] nonane 6,8-dicarboxylic acid (fig. 8). This derivative appears to be the responsible agent that brings about cytomegalic alterations in the lining cells at the pars rectae of the proximal tubules of the rat kidney, thus accounting for the unique nephrotoxic effects of lysinoalanine in the rat, which are not observed in other species (88). A further example (fig. 9) of cyclization is the conversion of the food coloring Brown FK, probably through the action of azo reductases of intestinal microflora, to a tetraaminobenzene and thence to 2,4,7-triaminophenazine (52).

IV. Environmental Mutagens and Antimutagens

The availability of short-term tests (15) and notably the Ames test, for detection of mutagenic potential has provided a fascinating insight into the types of cyclization that occur during cooking of food. The outstanding products formed from foodstuffs by pyrolysis are heterocyclic nitrogen-con-

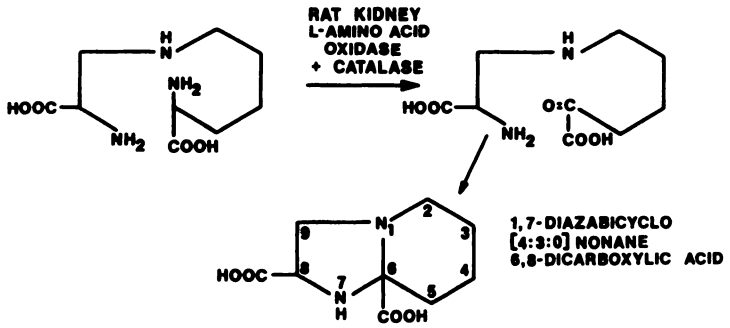

FIG. 8. Activation of lysinoalanine (N$^{\epsilon}$-(DL-2-amino-2-carboxyethyl)-L-lysine) by the action of rat kidney L-amino acid oxidase, followed by cyclization to 1,7-diazabicyclo[4:3:0]nonane-6,8-dicarboxylic acid, the suspect ultimate nephrotoxic agent (88).

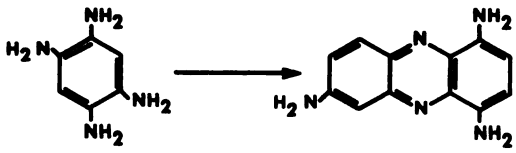

FIG. 9. Conversion of the food coloring Brown FK by the microflora of the rat cecum, via a tetraaminobenzene, to 1,4,7-triaminophenazine (52).

taining materials derived from amino acids such as tryptophan, glutamic acid, and protein or peptide sources (fig. 10). Not only are they potent mutagens in bacterial and mammalian cells in vitro, but the pyrolysate of L-tryptophan also transforms Syrian hamster embryonic cells and brings about sebaceous gland suppression in mice (160). The pioneering efforts of Sugimura and Nagao (156) have been joined by other groups studying a variety of pyrolysates of foodstuffs (77, 79, 101, 102) as well as mutagenic components in food (table 5). It is interesting to point to the similarity of some of these pyrolysis products to a natural plant material, ellipticine, which has been studied as a tumor-inhibitory agent (9, 17).

Figure 10 also serves to draw attention to the comutagens, harman and norharman, whose actions have been studied extensively (100, 110–112, 162). There is also increasing evidence of antimutagenic principles (desmutagens) in food, especially uncooked vegetable juices (75, 76, 106). Although some flavonoids are mutagens (table 5), and quercetin brings about neoplastic transformation of hamster embryo cells (161), other naturally-occuring flavones are suppressants of mutagenicity, for example of benzo(a)pyrene (29). There are other instances such as cysteine (132) of the modulating action of food components on mutagenicity. Ironically, nitrite present in pyrolyzed proteins and related materials acts as a protective agent, decreasing appreciably their mutagenic activity, as well as that of the mutagenic aminobenzoquinolines (185). The ubiquity of antagonists to mutagens and to carcinogens (referred to above) is matched by some evidence of agents protective against teratogens. Cysteamine inhibits oncogenesis (61, 99) and also prevents the teratogenic action of 5-azacytidine (158). It would indeed be interesting to test the effectiveness of cysteamine against such teratogenic actions as that of hyperthermia (18, 44) or of potato preparations (82, 138).

In addition to the mutagens in food, the short-term tests have revealed the presence of a number of hitherto-unsuspected environmental mutagens (table 6). Not surprisingly, known environmental carcinogenic factors such as ultraviolet light, including sunshine, and ionizing radiation have also proved to be mutagenic. To these findings should be added the observations on the endogenous formation of N-nitrosamines in the body (41) and the presence of volatile

Cooked Food	Pyrolyzed Component	Mutagens (M) or Comutagens (C)
Broiled meat and fish	*L*-Tryptophan	Amino-γ-carbolines (M,I) (102, 109, Harman, norharman (C,II) 156)
	L-Glutamic acid	Dipyridoimidazoles (M,III) (182)
Soybean	Soybean globulin	Amino-α-carbolines (M,IV) (186)

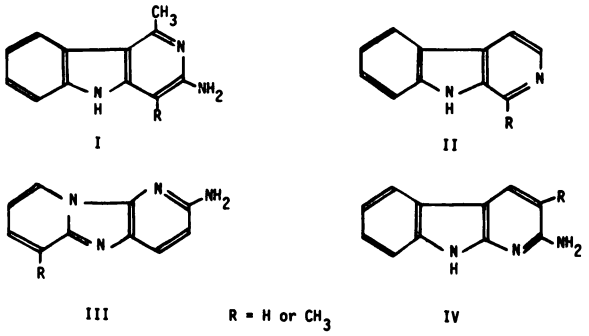

FIG. 10. Pyrolysis products isolated from various proteins, peptides, and their constituent amino acids. In addition to those structures depicted, phenylalanine pyrolysate has been reported to yield the mutagen 2-amino-5-phenylpyridine (153).

TABLE 5
Mutagens in food

Mutagens	Foodstuffs
Heated carbohydrates (caramels from various sugars.)	Molasses, honey, caramel candy, wine, brown sugar, soy sauce (115, 136)
Malonaldehyde	Beef (2–15 μg/g) (137)
Cholesterol (autoxidized)	Various (1)
Flavonoids	Plant foods (30, 155)
Ascorbic acid ($+Cu^{+}$), triose reductone	Processed, stored, cooked (116, 152)
Benzo(a)pyrene	Bread (up to 1·1 μg/kg) (57)
	Charcoal-broiled or smoked meat (46, 91)
Nitrosamines	Bacon, cheese, other foods; (57, 58) beer (67, 150)
Nitrosatable amines (methylguanidine, agmatine)	Various (81)
Unknown	Cooked beef, beef extract, hamburgers (20)
Unknown (?diethylnitrosamine)	Salted fish (China) (64)

TABLE 6
Sources of environmental mutagens

Water (? from pipelines) (135)
Oxygen (physiological concentrations) (13)
Ozone (ambient) (184)
Intestinal bacteria (157)

nitrosamines in human feces (167). It seems appropriate to quote a phrase from Sugimura et al. (154) that speaks for itself: "Finally, we must say that it really seems peculiar that the importance of mutagen-carcinogens in foods has not been the main subject throughout the long history of cancer research."

V. The Importance of Saturating Doses

Reference was made above to constitutive, adaptive, and inducible DNA repair mechanisms in bacterial cells and possibly also mammalian cells. These mechanisms have relevance to observations of the formation of alkylated derivatives of DNA in whole animals (37). The work that ushered in a new era in consideration of DNA and RNA alkylation was carried out by Loveless (94) who demonstrated O^6-methylguanine or O^6-ethylguanine formation through the action of methylating or ethylating agents respectively. Recently this has been shown to result in base-pair substitutions, with

resultant mutagenic effect (51). Emphasis was laid by Rajewsky, Kleihues, and others (14, 54, 84, 85, 97, 114) on the persistence of O^6-alkylguanines in the brain as an explanation of the origin of tumors of the central nervous system resulting from transplacental effects of ethylnitrosourea and similar alkylating agents (35, 36). Persistence of unrepaired O^6-methylguanine in the brain, and to a lesser extent in the kidney, has been followed for as long as 6 months after single or repeated (weekly) injections of methylnitrosourea (97). Evidence has recently been provided by Pegg et al. (114, 121, 122) that dimethylnitrosamine in high doses inhibits the removal of O^6-methylguanine from brain. Accordingly, while placing emphasis on the capacity of different organs to repair alkylation damage to DNA, and the speed with which low levels of damage are restored to normal, one must bear in mind the possibility that in high doses the toxic agent itself may exercise an inhibitory effect. Singer et al. (143–146) have stressed that alkylation at the O^6 position of guanine may merely indicate more critical reactions at other sites, for example the oxygens of pyrimidine bases. They have also drawn attention to the formation of phosphotriesters. In fact, Shooter et al. (140–142) have postulated that the presence of phosphotriesters may be a more critical indicator of mutagenic/carcinogenic activity than the formation of alkylated purine and pyrimidine bases. These observations should be brought to bear on problems of the association of persistent DNA damage with organotropic carcinogenic potential (92).

Considerations of the capacity and the rate of repair mechanisms assume great importance when, as is often the case, one is dealing with processes characterized by nonlinear pharmacokinetics. The common source of nonlinearity is the limited capacity of a key system (metabolic, tissue-binding, distributory, or excretory). Saturation of such systems may be brought about by high doses of compounds possessing low acute toxicity, thus influencing for example monooxygenase metabolism or glutathione conjugation. Applying Michaelis-Menten kinetics, one finds that at low doses or concentrations approaching real-life situations the rate of change of the concentration of a test compound is proportional to the concentration, whereas at high doses (e.g., in the range of maximum tolerated doses used in carcinogenesis bioassays) the rate of change is a constant—that is, zero order rate (48). The practical implications of this difference in pharmacokinetics of high and low doses have emerged most clearly from the work of Gehring et al. (48–50, 130, 168, 169), Bolt et al. (5, 6), Kensler (83), and others. Several of these authors have also drawn attention not only to the fallacies that can result from exclusive reliance on high-dose studies but also to the importance of defining "dose" as that amount of the reactive metabolite present in covalently bound form linked to tissue macromolecules; or, failing that, the amount of labeled compound present at the target site (50, 169). This is a far cry from the practice of basing dose-response extrapolations on the amount administered to the animal or present in an inhalation chamber in which exposure occurs.

VI. Lessons Learned from the MTD

Disregard of the critical considerations concerning homeostatic and defense mechanisms of the body has been evident in the use of the maximum tolerated dose (MTD) in carcinogenesis bioassays. Predicting the appropriate MTD calls for experience and judgment (149). A careful set of criteria for selection of the MTD may be cited (43) as follows:

1. Induces no overt toxicity, i.e., appreciable death of cells or organ dysfunction as determined by appropriate clinical pathological, pathological, or biochemical methods.

2. Induces no toxic manifestations which are predicted to shorten the lifespan of the animals except as the result of neoplastic development.

3. In two generation studies, is not det-

rimental to conception rates, fetal or neonatal survival, or postnatal development.

4. Does not retard weight gain during the subchronic test by greater than 10% as compared to control animals.

5. Takes into consideration metabolic and pharmacokinetic data, and if dose-dependent qualitative or quantitative differences occur, at least one test dose should be set above the metabolic shift (provided the level(s) does not exceed the criteria listed above 1 through 4).

As indicated in these criteria, it is clearly inappropriate to select a MTD that produces massive tissue toxicity, which has happened not infrequently in the past, even right from the start of exposure. The fallacies that can result from nonspecific tissue damage were discussed at the First International Congress on Toxicology (53). In particular, the practice of fluctuating dosage is scientifically undesirable because those doses producing substantial mortality are liable to result in survivors with severe toxic tissue damage, a fact conveniently cloaked in the final report by expressing the dose in terms of the overall time-weighted average. Assays in which this approach was used should be evaluated with great caution and, preferably, repeated using an acceptable protocol.

The historic debate concerning the role of injury and repair (25) or "chronic irritation" (4) in the etiology of cancer has attracted renewed interest through the accumulating evidence of the heightened susceptibility of proliferating tissue to the action of carcinogens. Such proliferation may be the result of mechanical perforation or freezing of the serosal surface of the bladder in the rat (74, 139) or of partial hepatectomy, or occur during the regenerative phase after hepatic necrosis (23, 24, 127, 128).

One of the consequences of nonspecific tissue damage (129) that may be produced by compounds administered at the MTD is carcinogenesis by a "secondary" mechanism. Here is a well-recognized concept that was utilized by the Food and Drug Administration to account for the production of liver cancer by high doses of selenium, which is an essential nutrient at low doses (40). The important principle of secondary carcinogenesis is much more widely applicable (86), although its implications have not been adequately explored. Suffice it to say that the production of cancer by secondary mechanisms is an instance in which the end-result is not necessarily indicative of carcinogenic potential of the compound responsible for the effect. In some cases of this sort, a threshold is demonstrable for the production of the primary effect that elicits this secondary neoplastic change. Thus, in considering selenium, the Commissioner of the Food and Drug Administration pointed out that consumption of beverage alcohol "is associated with a higher incidence of liver cirrhosis, which in turn, is associated with a higher incidence of liver cancer. Other common agents, at high levels, may produce the same result." He drew the conclusion that such substances "are not, by reason of their capacity to induce liver damage when abused by being consumed at high levels, properly classified as carcinogenic because of their potential association with a higher rate of liver cancer."

One may extend the illustration of secondary mechanisms of carcinogenesis to the area of antithyroid action that results in thyroid epithelial hyperplasia which, if continued long enough, eventually leads to neoplasia. This is the mechanism by which iodine deficiency elicits thyroid cancer in rats (33, 95) but, apparently, does not lead to thyroid cancer in people (32). Thyroid hyperplasia is produced in rats by dietary goitrogens, certain sulfonamides, and other antithyroid agents (3). What is more, the high rate of apparently spontaneous follicular hyperplasia in the thyroids of old rats makes this species an unsuitable model for man. A further example of "secondary" carcinogenesis involves the production of bladder stones by compounds like diethylene glycol (172, 173). In rats and mice, the presence in the urinary bladder of such stones

or other foreign bodies is associated with the development of urothelial hyperplasia, papillomas, and ultimately carcinomas (159). These illustrations emphasize the need for careful analysis and expert judgment in interpreting observations on carcinogenesis, in view of the importance of distinguishing primary from secondary pathological events. Only in this way can one assess the role played by the particular MTD of the compound used in any given bioassay in eliciting the primary changes.

One view of primary changes, such as thyroid hyperplasia, that progress to neoplasia, regards them as precancerous processes. The question then arises: Are such primary changes irreversible, or does lowering or cessation of exposure to the agent that elicited them cause them to disappear? The latter course is observed with antithyroid agents (73). Evidence also exists that, in the liver, bladder, or other organs, changes seeming to be preneoplastic can be reversed in their early stages, depending on dose and duration of exposure, by discontinuing administration of the chemical responsible for eliciting these effects (38, 39, 45, 68, 123–125, 178). Finally, reference should be made to the frequency with which neoplastic cells are found in rodents, possibly associated with the presence of oncogenic viruses. Thus DeOme et al. (28) have reported the detection of inapparent nodule-transformed cells in the mammary gland tissues of virgin female BALB/cfC3H mice infected with mammary tumor virus. Brand (8) has furnished convincing evidence that "solid-state" carcinogenesis involves the existence of preneoplastic properties in mesenchymal pluripotential stem cells of the microvasculature in subcutaneous connective tissue. The implications of these few observations are potentially momentous. They may not be found to be universally applicable, but they do emphasize that measured caution and a search for understanding are more appropriate in the interpretation of specific observations than insistence upon rigid criteria.

VII. Risk Assessment and Acceptable Risk

Two problems are addressed here: that of extrapolation from high-dose responses to the likely consequences of exposure at the (usually) low environmental levels; and interpretation of human risks from the effects observed in laboratory animals. The fallacies to which the use of the MTD may give rise in the course of carcinogenesis bioassays have been discussed above. But, assuming that valid data have in fact resulted from the high-dose experiments, risk assessment dependent upon the downward extension of the dose-response curves is still fraught with considerable potential for error. Numerous authors have stressed the uncertainties that stem from different shapes of the dose-response curves in the low-dose region and attempts have followed to use various quantitative theories of oncogenesis as a basis for the development of mathematical approaches to high-dose low-dose extrapolation. Discussions of this subject will be found in the report of the Food Safety Council (43), in Wilson (180), and in Cornfield (22).

Whittemore (176) has summarized the data of White (175), pointing to the importance of the possible discrepancy between the exposure of the animal (the applied dose) and cellular concentrations of the test material. Whittemore suggests that "future modeling efforts should emphasize incorporation of physiological, pharmacological, and biochemical information ... there is need for study of the distribution, excretion and metabolism of chemical carcinogens for the purpose of establishing effective dose in experimental work." Unfortunately, no reference is made by Whittemore to the advances in this field in recent years. The advantages of such mathematical models that incorporate pharmacological and biochemical information, as predicted by Whittemore in regard to "reducing uncertainty due to interspecies differences", are already beginning to be realized through

the work of Gehring, Watanabe, McKenna, and their colleagues referred to above (49, 50, 103, 104, 130, 168, 169).

In the face of advancing knowledge about the constant exposure of the human body to exogenous and endogenous carcinogens and mutagens, the protagonists of the one-molecule theory of carcinogenesis attack on another front, namely the proportion of human cancer attributable to workplace exposure. But they maintain a discreet silence over the widening gap between their theory and the facts of life. An interesting dilemma faces them in trying to explain away the observation that a number of elements, such as chromium and selenium, are essential nutrients and yet are carcinogenic at high doses. Rather than concede that there must be a threshold below which these potentially carcinogenic elements are harmless, the incredible suggestion is put forward that some parts of the body benefit, at the cost of cancer induced by the same levels (in the case of chromium, 50 ppb) of the same elements in other parts of the body. Thus, Wilson (180) submits that "it is possible that a substance can be necessary for one part of a body's function while providing the risk of destruction of another." As biologists, let us recognize this philosophy for what it is: the death rattle of a flat-earth hypothesis!

The risk involved in any activity, including that associated with chemical exposure, is perceived differently by those responsible for creating it, those who control it, or those who experience it. Accordingly, the issue of acceptability of such risk requires some objective standard of comparison. Just as the natural level of background radiation is used to assess the hazard of low-level radiation exposures, Flowers (42) has suggested that the natural level for chemicals can act as a basis for judging chemical hazards. However, the fallacious assumption is made that the natural levels are low and correspond to very low risks. On the contrary, while the evidence cited above concerning naturally-occurring mutagens/carcinogens in food and the environment is by no means an exhaustive presentation of the subject, it clearly points to much greater risks than most people have ever imagined. Thus the prevalent idea that a new period of mutagenic exposure of man began with the manufacture of chemicals may be correct; but, historically, this latest development was preceded by the greatly increased mutagenic exposures that occurred when our ancestors began to breathe oxygen, adopted a terrestrial environment, learned the secret of fire, and started to cook their food.

In order to render a judgment on the level of risk, essential prerequisites are: identification of the nature of the hazard, development of sound dose-response data, and application of appropriate mathematical treatment of the data. While no scientifically valid scale exists for a decision on acceptability of the risk, it is appropriate to consider how much is added to the existing background of hazard due to the environment and lifestyle of the individual. Obviously, the new era in toxicology should do much to make possible a more informed assessment of this background. It should also help in comparative risk analysis, affording a measure of the risks of alternative occupations involving exposure to chemicals, potential life-shortening agents, or stress factors that need to be taken into account.

VIII. Conclusion

The new era in toxicology has emphasized certain needs that are essential to an intelligent and informed approach to toxicological evaluation of a compound. A strong bridge between biochemistry and morphology is essential in such studies. Furthermore, adequate investigations along biochemical lines should precede the important toxicological tests, particularly those carried out on a long-term basis. The study of metabolism and pharmacokinetics should seek to define inflection-points in the dose-response curves. Covalent binding should be studied, and in this connection

better methods are needed that are less time-consuming and more accurate. Above all, a better definition of what is happening in the course of covalent binding will achieve a clearer understanding of the chemical conduct of the compound.

It has not been possible in this presentation to detail all the mechanisms of toxic action that are capable of being studied by the means at our disposal. Certainly, the participation of membranes, lysosomes, and immune mechanisms in toxic action are of vital importance. In the new era of toxicology, it is essential to apply whatever tools are appropriate to the study of a particular compound; and, if we do all this in an intelligent and well-informed manner, the new era will indeed have arrived. The public and the environment will be the beneficiaries of our objective and conscientious scientific efforts.

REFERENCES

1. Ansari, G. A. S., Smart, V. B., and Smith, L. L.: Mutagenicity of autoxidised cholesterol samples. Pharmacologist **20**: 155, 1978.
2. Apffel, C. A.: Nonimmunological host defenses: A review. Cancer Res. **36**: 1527–1537, 1976.
3. Astwood, E. B., Sullivan, J., Bissell, A., and Tyslowitz, R.: Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat. Endocrinology **32**: 210–225, 1943.
4. Berenblum, I.: Irritation and carcinogenesis. Arch. Pathol. Lab. Med. **38**: 233–244, 1944.
5. Bolt, H. M.: Pharmacokinetics of vinyl chloride. Gen. Pharmacol. **9**: 91–95, 1978.
6. Bolt, H. M., Filser, J. G., and Hinderer, R. K.: Rat liver microsomal uptake and irreversible protein binding of [1, 2-^{14}C]vinyl bromide. Toxicol. Appl. Pharmacol. **44**: 481–489, 1978.
7. Boyd, M. R., Statham, C. N., Franklin, R. B., and Mitchell, J. R.: Pulmonary bronchiolar alkylation and necrosis by 3-methylfuran, a naturally occurring potential atmospheric contaminant. Nature (London) **272**: 270–271, 1978.
8. Brand, K. G., Johnson, K. H., and Buoen, L. C.: Foreign body tumorigenesis. CRC Crit. Rev. Toxicol. **4**: 353–394, 1976.
9. Branfman, A. R., Bruni, R. J., Reinhold, V. N., Silveira, D. M., Chadwick, M., and Yesair, D. W.: Characterization of the metabolites of ellipticine in rat bile. Drug Metab. Disp. **6**: 542–548, 1978.
10. Brodie, B. B., and Hogben, C. A. M.: Some physiochemical factors in drug action. J. Pharm. Pharmacol. **9**: 345–380, 1957.
11. Brooks, H.: The problem of research priorities. Daedalus **107**: 171–190, 1978.
12. Brown, B. R., Jr., Sipes, I. G., and Baker, R. K.: Halothane hepatotoxicity and the reduced derivative 1, 1, 1-trifluoro-2-chloroethane. Environ. Health Perspect. **21**: 185–188, 1977.
13. Bruyninckx, W. J., Mason, H. S., and Morse, S. A.: Are physiological oxygen concentrations mutagenic? Nature (London) **274**: 606–607, 1978.
14. Buecheler, J., and Kleihues, P.: Excision of O^6-methylguanine from DNA of various mouse tissues following a simple injection of N-methyl-N-nitrosourea. Chem.-Biol. Interact. **16**: 325–333, 1977.
15. Butterworth, B. (ed.): Strategies for Short-Term Testing for Mutagens/Carcinogens. CRC Press, West Palm Beach, Fla., 1978.
16. Cannon, W. G.: Homeostasis. Cycloped. Med. **6**: 861, 1932; The Wisdom of the Body, Norton, New York, 1932.
17. Chadwick, M., Silveira, D. M., Platz, B. B., and Hayes, D.: Comparative physiological disposition of ellipticine in several animal species after intravenous administration. Drug Metab. Disp. **6**: 528–541, 1978.
18. Cockroft, D. L., and New, D. A. T.: Abnormalities induced in cultured rat embryos by hyperthermia. Teratology **17**: 277–284, 1978.
19. Cohen, E. N., Trudell, J. R., Edmunds, H. N., and Watson, E.: Urinary metabolites of halothane in man. Anesthesiology **43**: 392–401, 1975.
20. Commoner, B., Vithayathil, A. J., Dolara, P., Nair, S., Madyastha, P., and Cuca, G. C.: Formation of mutagens in beef and beef extract during cooking. Science **201**: 913–916, 1978.
21. Conti, A., and Bickel, M. H.: History of drug metabolism: Discoveries of the major pathways in the 19th century. Drug Metab. Rev. **6**: 1–50, 1977.
22. Cornfield, J.: Carcinogenic risk assessment. Science **198**: 693–699, 1977.
23. Craddock, V. M.: Liver carcinomas induced in rats by single administration of dimethylnitrosamine after partial hepatectomy. J. Nat. Cancer Inst. **47**: 899–907, 1971.
24. Craddock, V. M.: Effect of a single treatment with the alkylating carcinogens dimethylnitrosamine, diethylnitrosamine and methyl methanesulphonate, on liver regenerating after partial hepatectomy. I. Test for induction of liver carcinomas. Chem.-Biol. Interact. **10**: 313–321, 1975.
25. Deelman, H. T.: The part played by injury and repair in the development of cancer, with some remarks on the growth of experimental cancers. Proc. Roy. Soc. Med. **20**: 19–20, 1927.
26. DeMaster, E. G., and Nagasawa, H. T.: Isoprene, an endogenous constituent of human alveolar air with a diurnal pattern of excretion. Life Sci. **22**: 91–98, 1978.
27. DeMatteis, F.: Drug-induced destruction of cytochrome P-450. Drug Metab. Disp. **1**: 267–274, 1973.
28. DeOme, K. B., Miyamoto, M. J., Osborn, R. C., Guzman, R. C., and Lum, K.: Detection of inapparent nodule-transformed cells in the mammary gland tissues of virgin female BALB/cfC3H mice. Cancer Res. **38**: 2103–2111, 1978.
29. DeSerres, F. J.: Report of the conference on modification of mutagenic and carcinogenic activity. Mutat. Res. **54**: 197–202, 1978.
30. Dietrich, P. S., Bakner, C. M., and Brown, J. P.: Plant flavonols mutagenic in the *Salmonella*/microsome assay: Activation of flavonol glycosides by enzymatic extracts of rat cecal bacteria. Environmental Mutagen Society Abstr., p. 36, 1978.
31. DiVincenzo, G. D., Kaplan, C. J., and Dedinas, J.: Characterization of the metabolites of methyl *n*-butyl ketone, methyl *iso*-butyl ketone, and methyl ethyl ketone in guinea pig serum and their clearance. Toxicol. Appl. Pharmacol. **36**: 511–522, 1976.
32. Doniach, I.: Aetiological consideration of thyroid carcinoma. *In* Tumours of the Thyroid Gland, pp. 55–72, E. & S. Livingstone, Edinburgh, 1970.
33. Doniach, I.: Experimental thyroid tumours. *In* Tumours of the Thyroid Gland, pp. 73–99, E. & S. Livingstone, Edinburgh, 1970.
34. Doolittle, C. H., McDonald, C. J., and Calabresi, P.: Pharmacological studies of neurotoxicity in patients

with psoriasis treated with azaribine, utilising high-pressure liquid chromatography. J. Lab. Clin. Med. 90: 773–785, 1977.

35. Druckrey, H.: Specific carcinogenic and teratogenic effects of indirect alkylating methyl and ethyl compounds, and their dependency on stages of ontogenic developments. Xenobiotica 3: 271–303, 1973.
36. Druckrey, H., Preussmann, R., Ivankovic, S., and Schmahl, D.: Organotrope carcinogene Wirkungen bei 65 verschiedenen N-Nitroso-Verbindungen an BD-Ratten. Z. Krebsforsch. 69: 103–201, 1967.
37. Falk, H. S.: Biologic evidence for the existence of thresholds in chemical carcinogenesis. Environ. Health Perspect. 22: 167–170, 1978.
38. Farber, E.: On the pathogenesis of experimental hepatocellular carcinoma. *In* Hepatocellular Carcinoma, ed. by K. Okuda and R. L. Peters, pp. 1–24, Wiley, New York, 1976.
39. Farber, E., and Solt, D.: A new liver model for the study of promotion. *In* Carcinogenesis, vol. 2, Mechanisms of Tumor Promotion and Cocarcinogenesis, ed. by T. J. Slaga, A. Sivak and R. K. Boutwell, pp. 433–451, Raven Press, New York, 1978.
40. Federal Register: Selenium in animal feed. 38: 10458–10460, 1973; 39: 1355–1358, 1974.
41. Fine, D. H., Ross, R., Rounbehler, D. P., Silvergleid, A., and Song, L.: Formation *in vivo* of volatile *N*-nitrosamines in man after ingestion of cooked bacon and spinach. Nature (London) 265: 753–755, 1977.
42. Flowers, E. F.: Proposal: a non zero approach to environmental limits for carcinogens. Occupational Health and Safety, May/June 1977 [cited from Wilson, 1978 (180)].
43. The Scientific Committee, Food Safety Council: Proposed System for Food Safety Assessment, ed. by V. O. Wodicka, L. Golberg, and C. J. Carr. Food Cosmet. Toxicol. 16: suppl. 2, 1–136, 1978.
44. Fraser, F. C., and Skelton, J.: Possible teratogenicity of maternal fever. Lancet ii: 634, 1978.
45. Friedell, G. H., Jacobs, J. B., Nagy, G. K., and Cohen, S. M.: The pathogenesis of bladder cancer. Amer. J. Pathol. 89: 431–442, 1977.
46. Fritz, W.: Zur Bildung kanzerogener Kohlenwasserstoffe bei der thermischen Behandlung von Lebensmittelin. 7. Mitteilung: Untersuchungen zur Kontamination beim Räuchern. Arch. Geschwulstforsch. 47: 685–693, 1977.
47. Fujita, K., Nagatsu, T., Shinpo, K., Maruta, K., Takahashi, H., and Sekiya, A.: Increase in urinary putrescine in 3,4-benzopyrene carcinogenesis and its inhibition by putrescine. Cancer Res. 38: 3509–3511, 1978.
48. Gehring, P. J., and Blau, G. E.: Mechanisms of carcinogenesis: Dose response. J. Environ. Pathol. Toxicol. 1: 163–179, 1977.
49. Gehring, P. J., Watanabe, P. G., and Young, J. D.: The relevance of dose-dependent pharmacokinetics in the assessment of carcinogenic hazard of chemicals. *In* Origins of Human Cancer, Book A, Incidence of Cancer in Humans, vol. 4, ed. by H. H. Hiatt, J. D. Watson and J. A. Winsten, pp. 187–203, Cold Spring Harbor Laboratory, 1977.
50. Gehring, P. J., Watanabe, P. G., and Park, C. N.: Resolution of dose-response toxicity data for chemicals requiring metabolic activation: Example—vinyl chloride. Toxicol. Appl. Pharmacol. 44: 581–591, 1978.
51. Gerchman, L. L., and Ludlum, D. B.: The properties of O^6-methylguanine in templates for RNA polymerase. Biochim. Biophys. Acta 308: 310–316, 1973.
52. Golberg, L.: The amelioration of food. The Milroy Lectures. J. Roy. Coll. Physicians London 1: 385–426, 1967.
53. Golberg, L.: Introductory remarks. Rationale for high dose exposures. Proceedings of the First International Congress on Toxicology, Toronto, Canada, ed. By G. L. Plaa and W. A. M. Duncan, pp. 87–96, Academic Press, New York, 1978.
54. Goth, R., and Rajewsky, M. F.: Persistence of O^6-ethylguanine in rat brain DNA; correlation with nervous system specific carcinogenesis by ethyl nitrosourea. Proc. Nat. Acad. Sci. U.S.A., 71: 639–643, 1974.
55. Green, T., and Hathway, D. E.: The chemistry and biogenesis of the S-containing metabolites of vinyl chloride in rats. Chem.-Biol. Interact. 11: 137–150, 1977.
56. Green, T., and Hathway, D. E.: Interactions of vinyl chloride with rat-liver DNA in vivo. Chem.-Biol. Interact. 22: 211–224, 1978.
57. Griciute, L.: Les substances cancérogènes dans les aliments. Bull. Cancer 65: 53–58, 1978.
58. Gross, R. L., and Newberne, P. M.: Naturally occurring toxic substances in foods. Clin. Pharmacol. Ther. 22: 680–698, 1977.
59. Grubbs, C. J., Moon, R. C., Sporn, M. B., and Newton, D. L.: Inhibition of mammary cancer by retinyl methyl ether. Cancer Res. 37: 599–602, 1977.
60. Guengerich, F. P., and Strickland, T. W.: Metabolism of vinyl chloride: Destruction of the heme of highly purified liver microsomal cytochrome P-450 by a metabolite. Mol. Pharmacol. 13: 993–1004, 1977.
61. Harman, D.: Prolongation of the normal lifespan and inhibition of spontaneous cancer by antioxidants. J. Gerontol. 16: 247–254, 1961.
62. Hathway, D. E.: Comparative mammalian metabolism of vinyl chloride and vinylidene chloride in relation to oncogenic potential. Environ. Health Perspect. 21: 55–59, 1977.
63. Hefner, R. E., Jr., Watanabe, P. G., and Gehring, P. J.: Preliminary studies of the fate of inhaled vinyl chloride monomer (VCM) in rats. Ann. N. Y. Acad. Sci. 246: 135–148, 1975.
64. Ho, J. H. C., Huang, D. P., and Fong, Y. Y.: Salted fish and nasopharyngeal carcinoma in southern Chinese. Lancet ii: 626, 1978.
65. Horton, R. W.: The role of 2-keto-4-pentenoic acid in seizures induced by allylglycine. Biochem. Pharmacol. 27: 1471–1477, 1978.
66. Irons, R. D., Dent, J. G., Edgar, T. S., and Rickert, D. E.: Benzene is metabolized and covalently bound in bone marrow *in vivo*. Submitted for publication, 1979.
67. Ivankovic, S.: Gastric cancer in rats after chronic intraperitoneal application of sap of green parts of potatoes (*Solanum tuberosum L.*). Experientia (Basel) 34: 645, 1978.
68. Jacobs, J. B., Arai, M., Cohen, S. M., and Friedell, G. H.: Light and scanning electron microscopy of exfoliated bladder epithelial cells in rats fed *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide. J. Nat. Cancer Inst. 57: 63–66, 1976.
69. Jacobs, M. M.: Inhibitory effects of selenium on 1,2-dimethylhydrazine and methylazoxymethanol colon carcinogenesis. Cancer 40: 2557–2564, 1977.
70. Jee, R., Maiorino, R., Gandolfi, A. J., Sipes, I. G., and Brown, B. R., Jr.: Investigations of the hypoxic model for halothane hepatotoxicity. Pharmacologist 20: 258, 1978.
71. Jeggo, P., Defais, M., Samson, L., and Schendel, P.: An adaptive response of *E. coli* to low levels of alkylating agent: Comparison with previously characterized DNA repair pathways. Mol. Gen. Genet. 157: 1–9, 1977.
72. Jeggo, P., Defais, M., Samson, L., and Schendel, P.: The adaptive response of *E. coli* to low levels of alkylating agent: The role of *pol A* killing adaptation. Mol. Gen. Genet. 163: 299–305, 1978.
73. Jemec, B.: Studies of the tumorigenic effect of two goitrogens. Cancer 40: 2188–2202, 1977.
74. Johansson, S.: Urothelial hyperplasia of the urinary bladder of the rat induced by mechanical perforation and phenacetin treatment. Acta Pathol. Microbiol.

Scand. 86: 333–335, 1978.
75. KADA, T., AND NAMIKI, M.: Screening for antimutagenic agents on environmental mutagens including nitro- and nitroso-compounds. Mutat. Res. 54: 239, 1978.
76. KADA, T., MORITA, K., AND INOUE, T.: Anti-mutagenic action of vegetable factor(s) on the mutagenic principle of tryptophan pyrolysate. Mutat. Res. 53: 351–353, 1978.
77. KALLISTRATOS, G., AND FASSKE, E.: Prevention of 3,4-benzopyrene carcinogenesis in presence of putrescine. Z. Krebsforsch. 87: 81–92, 1976.
78. KALLISTRATOS, G., AND KALLISTRATOS, U.: 3,4-Benzopyrene carcinogenesis and its inhibition by natural and synthetic compounds. Folia Biochem. Biol. Graeca 13: 1–10, 1976.
79. KANADA, T., MAZAKI, M., TAUE, S., AND UYETA, M.: Mutagenicity of food pyrolysates by Ames' test. Mutat. Res. 54: 239–240, 1978.
80. KAPPUS, H., BOLT, H. M. BUCHTER, A., AND BOLT, W.: Liver microsomal uptake of (^{14}C) vinyl chloride and transformation to protein alkylating metabolites in vitro. Toxicol. Appl. Pharmacol. 37: 461–471, 1976.
81. KAWABATA, T., OHSHIMA, H., AND INO, M.: Occurrence of methylguanidine and agmatine, nitrosatable guanidino compounds, in foods. J. Agr. Food Chem. 26: 334–338, 1978.
82. KEELER, R. F., YOUNG, S., BROWN, D., STALLKNECHT, G. F., AND DOUGLAS, D.: Congenital deformities produced in hamsters by potato sprouts. Teratology 17: 327–334, 1978.
83. KENSLER, C. J.: Personal communication, 1978.
84. KLEIHUES, P., AND MARGISON, G. P.: Carcinogenicity of N-methyl-N-nitrosoures: Possible role of repair excision of O^6-methylguanine from DNA. J. Nat. Cancer Inst. 53: 1839–1841, 1974.
85. KLEIHUES, P., LANTOS, P., AND MAGEE, P. N.: Chemical carcinogenesis in the nervous system. Int. Rev. Exp. Pathol. 15: 153–232, 1976.
86. KOLBYE, A. C., JR.: Cancer in humans: Exposures and responses in a real world. Oncology (Basel) 33: 90–100, 1976.
87. LAIB, R. J., AND BOLT, H. M.: Formation of 3,N^4-ethenocytidine moieties in RNA by vinyl chloride metabolites in vitro and in vivo. Arch. Toxikol. 39: 235–240, 1978.
88. LEEGWATER, D. C.: The nephrotoxicity of lysinoalanine in the rat. Food Cosmet. Toxicol. 16: 405–406, 1978.
89. LEGATOR, M.: Personal communication, 1978.
90. LEHMAN, A. R.: Post-replication repair of ultra-violet irradiated mammalian cells. J. Mol. Biol. 66: 319–337, 1972.
91. LIJINSKY, W., AND SHUBIK, P.: Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. Science 145: 53–55, 1964.
92. LILJA, H. S., CURPHEY, T. J., YAGER, J. D., JR., AND LONGNECKER, D. S.: Persistence of DNA damage in rat pancreas following administration of three carcinogens and/or mutagens. Chem.-Biol. Interact. 22: 287–295, 1978.
93. LOTLIKAR, P. D., DWYER, E. N., BALDY, W. J., JR., AND NYCE, J.: Phospholipid requirement for 2-acetamidofluorene *N*- and ring-hydroxylation by hamster liver microsomal cytochrome P-450 enzyme system. Biochem. J. 168: 571–574, 1977.
94. LOVELESS, A.: Possible relevance of O^6-alkylation of deoxyguanosine to the mutagenicity and carcinogenicity of nitrosamines and nitrosamides. Nature (London) 223: 206–207, 1969.
95. LUPULESCU, A., AND PETROVICI, A.: Ultrastructure of the Thyroid Gland, pp. 36–40, Williams & Wilkins Company, Baltimore, 1968.
96. MAIORINO, R. M., SIPES, I. G., JEE, R. C., AND BROWN, B. R., JR.: Volatile metabolites of halothane: Correlation with hepatotoxicity in the rat hypoxic model. Pharmacologist 20: 258, 1978.
97. MARGISON, G. P., AND KLEIHUES, P.: Chemical carcinogenesis in the nervous system. Preferential accumulation of O^6-methylguanine in rat brain deoxyribonucleic acid during repetitive administration of N-methyl-N-nitrosourea. Biochem. J. 148: 521–525, 1975.
98. MARINELLO, A. J., GURTOO, H. L., STRUCK, R. F., AND PAUL, B.: Denaturation of cytochrome P-450 by cyclophosphamide metabolites. Biochem. Biophys. Res. Commun. 83: 1347–1353, 1978.
99. MARQUARDT, H., SAPOZINK, M., AND ZEDECK, M.: Inhibition by cysteamine-HCl of oncogenesis induced by 7,12-dimethylbenz(a)anthracene without affecting toxicity. Cancer Res. 34: 3387–3390, 1974.
100. MATSUMOTO, T., YOSHIDA, D., AND MIZUSAKI, S.: Enhancing effect of harman on mutagenicity in Salmonella. Mutat. Res. 56: 85–88, 1977.
101. MATSUMOTO, T., YOSHIDA, D., MIZUSAKI, S., AND OKAMOTO, H.: Mutagenicity of pyrolyzates of amino acids, peptides and proteins in *Salmonella typhimurium* TA 98. Mutat. Res. 54: 219–220, 1978.
102. MATSUMOTO, T., YOSHIDA, D., MIZUSAKI, S., AND OKAMOTO, H.: Mutagenicities of the pyrolyzates of peptides and proteins. Mutat. Res. 56: 281–288, 1978.
103. MCKENNA, M. J., ZEMPEL, J. A., MADRID, E. O., AND GEHRING, P. J.: The pharmacokinetics of [^{14}C]vinylidene chloride in rats following inhalation exposure. Toxicol. Appl. Pharmacol. 45: 599–610, 1978.
104. MCKENNA, M. J., ZEMPEL, J. A., MADRID, E. O., BRAUN, W. H., AND GEHRING, P. J.: Metabolism and pharmacokinetic profile of vinylidene chloride in rats following oral administration. Toxicol. Appl. Pharmacol. 45: 821–835, 1978.
105. MILLER, E. C.: Some current perspectives on chemical carcinogenesis in humans and experimental animals: Presidential address. Cancer Res. 38: 1479–1496, 1978.
106. MORITA, K., HARA, M., AND KADA, T.: Studies on natural desmutagens: Screening for vegetable and fruit factors active in inactivation of mutagenic pyrolysis products from amino acids. Agr. Biol. Chem. 42: 1235–1238, 1978.
107. MUDGE, G. H., GEMBORYS, M. W., AND DUGGIN, G. G.: Covalent binding of metabolites of acetaminophen to kidney protein and depletion of renal glutathione. J. Pharmacol. Exp. Ther. 206: 218–226, 1978.
108. MULDER, G. J., HINSON, J. A., AND GILLETTE, J. R.: Conversion of the *N*-*O*-glucuronide and *N*-*O*-sulfate conjugates of *N*-hydroxy-phenacetin to reactive intermediates. Biochem. Pharmacol. 27: 1641–1649, 1978.
109. NAGAO, M., HONDA, M., SEINO, Y., YAHAGI, T., AND SUGIMURA, T.: Mutagenicities of smoke condensates and the charred surface of fish and meat. Cancer Lett. 2: 221–226, 1977.
110. NAGAO, M., YAHAGI, T., HONDA, M., SEINO, Y., MATSUSHIMA, T., AND SUGIMURA, T.: Demonstration of mutagenicity of aniline and *o*-toluidine by norharman. Proc. Jap. Acad. 53: 34–37, 1977.
111. NAGAO, M., YAHAGI, T., HONDA, M., SEINO, Y., KAWACHI, T., SUGIMURA, T., WAKABAYASHI, K., TSUJI, K., AND KOSUGE, T.: Comutagenic actions of norharman derivatives with 4-dimethylaminoazobenzene and related compounds. Cancer Lett. 3: 339–346, 1977.
112. NAGAO, M., YAHAGI, T., AND SUGIMURA, T.: Differences in effects of norharman with various classes of chemical mutagens and amounts of S-9. Biochem. Biophys. Res. Commun. 83: 373–378, 1978.
113. NASTAINCZYK, W., ULLRICH, V., AND SIES, H.: Effect of oxygen concentration on the reaction of halothane with cytochrome P450 in liver microsomes and isolated perfused rat liver. Biochem. Pharmacol. 27: 387–392, 1978.
114. NICOLL, J. N., SWANN, P. F., AND PEGG, A. E.: Effect of dimethylnitrosamine on persistence of methylated guanines in rat liver and kidney DNA. Nature (London) 254: 261–262, 1975.
115. OKAMOTO, H. S., MIZUSAKI, D., YOSHIDA, D., MATSU-

MOTO, T., AKIYAMA, A., AND FUKUHARA, Y.: Enhancing effect of sucrose pyrolysate on mutagenicity in *Salmonella typhimurium*. Mutat. Res. 54: 251, 1978.

116. OMURA, H., SHINOHARA, K., MAEDA, H., NONAKA, M., AND MURAKAMI, H.: Mutagenic action of triose reductone and ascorbic acid on *Salmonella typhimurium* TA 100 strain. J. Nutr. Sci. Vitaminol. 24: 185–194, 1978.
117. OPDYKE, D. L.: Monographs on Fragrance Raw Materials: AETT. Food Cosmet. Toxicol. 17: in press, 1979.
118. OSTERMAN-GOLKAR, S., EHRENBERG, L., SEGERBÄCK, D., AND HÄLLSTRÖM, I.: Evaluation of genetic risks of alkylating agents. II. Haemoglobin as a dose monitor. Mutat. Res. 34: 1–10, 1976.
119. OSTERMAN-GOLKAR, S., HULTMARK, D., SEGERBÄCK, D., CALLEMAN, C. J., GÖTHE, R., EHRENBERG, L., AND WACHTMEISTER, C. A.: Alkylation of DNA and proteins in mice exposed to vinyl chloride. Biochem. Biophys. Res. Commun. 76: 259–266, 1977.
120. PEGG, A. E., AND HUI, G.: Formation and subsequent removal of O^6-methylguanine from deoxyribonucleic acid in rat liver and kidney after small doses of dimethylnitrosamine. Biochem. J. 173: 739–748, 1978.
121. PEGG, A. E., AND HUI, G.: Removal of methylated purines from rat liver DNA after administration of dimethylnitrosamine. Cancer Res. 38: 2011–2017, 1978.
122. PEGG, A. E.: Dimethylnitrosamine inhibits enzymatic removal of O^6-methylguanine from DNA. Nature (London) 274: 182–184, 1978.
123. PITOT, H. C.: The natural history of neoplasia. New insights into an old problem. Amer. J. Path. 89: 402–411, 1977.
124. PITOT, H. C.: The stability of events in the natural history of neoplasia. Amer. J. Path. 89: 703–716, 1977.
125. PITOT, H. C., BARNESS, L., AND KITAGAWA, T.: Stages in the process of hepatocarcinogenesis in rat liver. *In* Carcinogenesis, vol. 2, Mechanisms of Tumor Promotion and Cocarcinogenesis, ed. by T. J. Slaga, A. Sivak, and R. K. Boutwell, pp. 433–442, Raven Press, New York, 1978.
126. POHL, L. R., BHOOSHAN, B., WHITTAKER, N. F., AND KRISHNA, G.: Phosgene: A metabolite of chloroform. Biochem. Biophys. Res. Commun. 79: 684–691, 1977.
127. POUND, A. W., AND MCGUIRE, L. J.: Repeated partial hepatectomy as a promoting stimulus for carcinogenic response of liver to nitrosamines in rats. Brit. J. Cancer 37: 585–594, 1978.
128. POUND, A. W., AND MCGUIRE, L. J.: Influence of repeated liver regeneration on hepatic carcinogenesis by diethylnitrosamine in mice. Brit. J. Cancer 37: 596–602, 1978.
129. POZHARISSKI, K. M.: The significance of nonspecific injury for colon carcinogenesis in rats. Cancer Res. 35: 3824–3830, 1975.
130. REITZ, R. H., GEHRING, P. J., AND PARK, C. N.: Carcinogenic risk estimation for chloroform: An alternative to EPA's procedures. Food Cosmet. Toxicol. 16: 511–514, 1978.
131. RICKERT, D. E., EDGAR, T. S., BUS, J. S., BARROW, C. S., AND IRONS, R. D.: Benzene disposition in the rat after exposure by inhalation. Toxicol. Appl. Pharmacol. In press, 1979.
132. ROSIN, M. P., AND STICH, H. F.: The inhibitory effect of cysteine on the mutagenic activities of several carcinogens. Mutat. Res. 54: 73–81, 1978.
133. RUPP, W. D., WILDE, C. E., RENO, D. L., AND HOWARD-FLANDERS, P.: Exchanges between DNA strands in ultra-violet irradiated *E. coli*. J. Mol. Biol. 61: 25–44, 1971.
134. SAMSON, L., AND CAIRNS, J.: A new pathway for DNA repair in *Escherichia coli*. Nature (London) 267: 281–283, 1977.
135. SAXENA, J., SCHWARTZ, D. J., AND KOPPLER, F. C.: Mutagens may form in water after treatment. Chem. Eng. News, August 14, p. 8, 1978.
136. SETLIFF, J. A., AND MOWER, H. F.: Mutagens in decomposition products of carbohydrates. Fed. Proc. 36: 304, 1977.
137. SHAMBERGER, R. J.: Antioxidants and cancer. VII. Presence of malonaldehyde in beef and other meats and its epidemiological significance. Trace Subst. Environ. Health 11: 36–43, 1977.
138. SHARMA, R. P., WILLHITE, C. C., WU, M. T., AND SALUNKHE, D. K.: Teratogenic potential of blighted potato concentrate in rabbits, hamsters, and miniature swine. Teratology 18: 55–62, 1978.
139. SHIRAI, T., COHEN, S. M., FUKUSHIMA, S., HANANOUCHI, M., AND ITO, N.: Reversible papillary hyperplasia of the rat urinary bladder. Amer. J. Pathol. 91: 33–48, 1978.
140. SHOOTER, K. V., AND SLADE, T. A.: The stability of methyl and ethyl phosphotriesters in DNA in vivo. Chem.-Biol. Interact. 19: 353–361, 1977.
141. SHOOTER, K. V., SLADE, T. A., AND O'CONNOR, P. J.: The formation and stability of methyl phosphotriesters in the DNA of rat tissues after treatment with the carcinogen *N,N*-dimethylnitrosamine. Chem.-Biol. Interact. 19: 363–367, 1977.
142. SHOOTER, K. V.: DNA phosphotriesters as indicators of cumulative carcinogen-induced damage. Nature (London) 274: 612–613, 1978.
143. SINGER, B.: All oxygens in nucleic acids react with carcinogenic ethylating agents. Nature (London) 264: 333–339, 1976.
144. SINGER, B.: Sites in nucleic acids reacting with alkylating agents of differing carcinogenicity or mutagenicity. J. Toxicol. Environ. Health 2: 1279–1296, 1977.
145. SINGER, B., BODELL, W. J., CLEAVER, J. E., THOMAS, G. H., RAJEWSKY, M. F., AND THON, W.: Oxygens in DNA are main targets for ethylnitrosourea in normal and xeroderma pigmentosum fibroblasts and fetal rat brain cells. Nature (London) 276: 85–86, 1978.
146. SINGER, B., KRÖGER, M., AND CARRANO, M.: O^2- and O^4-alkyl pyrimidine nucleosides: Stability of the glycosyl bond and of the alkyl group as a function of pH. Biochemistry 17: 1246–1250, 1978.
147. SIPES, I. G., AND BROWN, B. R., JR.: An animal model of hepatotoxicity associated with halothane anesthesia. Anesthesiology 45: 622–628, 1976.
148. SIPES, I. G., PODOLSKY, T. L., AND BROWN, B. R., JR.: Bioactivation and covalent binding of halothane to liver macromolecules. Environ. Health Perspect. 21: 171–178, 1977.
149. SONTAG, J. M.: Aspects in carcinogen bioassay. *In* Origins of Human Cancer, Book C, Human Risk Assessment, ed. by H. H. Hiatt, J. D. Watson, and J. A. Winsten, pp. 1327–1338, Cold Spring Harbor Laboratory, 1977.
150. SPIEGELHALDER, B., EISENBRAND, G., AND PREUSSMANN, R.: Contamination of beer with trace quantities of N-nitrosodimethylamine. Food Cosmet. Toxicol. 17: 29–31, 1979.
151. SPORN, M. B.: Retinoids and carcinogenesis. Nutr. Rev. 35: 65–69, 1977.
152. STICH, H. F., KARIM, J., KOROPATNIC, J., AND LO, L.: Mutagenic action of ascorbic acid. Nature (London) 260: 722–724, 1976.
153. SUGIMURA, T., KAWACHI, T., NAGAO, M., YAHAGI, T., SEINO, Y., OKAMOTO, Y., SHUDO, K., KOSUGE, T., TSUJI, K., WAKABAYASHI, K., IITAKA, Y., AND ITAI, A.: Mutagenic principle(s) in tryptophan and phenylalanine pyrolysis products. Proc. Jap. Acad. 53: 58–61, 1977.
154. SUGIMURA, T., NAGAO, M., KAWACHI, T., HONDA, M., YAHAGI, T., SEINO, Y., SATO, S., MATSUKURA, N., MATSUSHIMA, T., SHIRAI, A., SAWAMURA, M., AND MATSUMOTO, H.: Mutagen-carcinogens in food with special reference to highly mutagenic pyrolytic products in broiled foods. *In* Origins of Human Cancer, Book C, Human Risk Assessment, ed. by H. H. Hiatt, J. D. Watson, and J. A. Winsten, pp. 1561–1577, Cold

Spring Harbor Laboratory, 1977.
155. SUGIMURA, T., NAGAO, M., MATSUSHIMA, T., YAHAGI, T., SEINO, Y., SHIRAI, A., SAWAMURA, M., NATORI, S., YOSHIHIRA, K., FUKUOKA, M., AND KUROYANAGI, M.: Mutagenicity of flavone derivatives. Proc. Jap. Acad. 53: 194–197, 1977.
156. SUGIMURA, T., AND NAGAO, M.: Mutagenic factors in cooked foods. CRC Crit. Rev. Toxicol. 6: in press, 1979.
157. SUZUKI, K., AND MITSUOKA, T.: Mutagenicity of intestinal bacteria. Mutat. Res. 50: 295–299, 1978.
158. TAKEUCHI, I. K., AND MURAKAMI, U.: The preventive influence of cysteamine on the teratogenic action of 5-azacytidine. Life Sci. 23: 897–900, 1978.
159. TOYOSHIMA, K., AND LEIGHTON, J.: Bladder calculi and urothelial hyperplasia with papillomatosis in the rat following insertion of chalk powder in the bladder cavity with subsequent trauma of the bladder wall. Cancer Res. 35: 3786–3791, 1975.
160. TSUDA, H., KATO, K., MATSUMOTO, T., YOSHIDA, D., AND MIZUSAKI, S.: Effects of L-tryptophan pyrolysate on mammalian cells in vitro and mouse sebaceous glands. Mutat. Res. 54: 260, 1978.
161. UMEZAWA, K., MATSUSHIMA, T., SUGIMURA, T., HIRAKAWA, T., TANAKA, M., KATOH, Y., AND TAKAYAMA, S.: In vitro transformation of hamster embryo cells by quercetin. Toxicology Lett. 1: 175–178, 1977.
162. UMEZAWA, K., SHIRAI, A., MATSUSHIMA, T., AND SUGIMURA, T.: Comutagenic effect of norharman and harman with 2-acetylaminofluorene derivatives. Proc. Nat. Acad. Sci. 75: 928–930, 1978.
163. VAINIO, H.: Vinyl chloride and vinyl benzene (styrene)—metabolism, mutagenicity and carcinogenicity. Chem.-Biol. Interact. 22: 117–124, 1978.
164. VAN DYKE, R. A., AND GANDOLFI, A. J.: Studies on irreversible binding of radioactivity from [^{14}C] halothane to rat hepatic microsomal lipids and protein. Drug Metab. Disp. 2: 469–476, 1974.
165. VERGANI, D., EDDLESTON, A. L. W. F., TSANTOULAS, D., DAVIS, M., AND WILLIAMS, R. : Sensitisation to halothane-altered liver components in severe hepatic necrosis after halothane anaesthesia. Lancet ii: 801–803, 1978.
166. VOS, J. G., STRIK, J. J. T. W. A., VAN HOLSTEYN, C. W. M., AND PENNINGS, J. H.: Polychlorinated biphenyls as inducers of hepatic porphyria in Japanese quail, with special reference to δ-aminolevulinic acid synthetase activity, fluorescence, and residues in the liver. Toxicol. Appl. Pharmacol. 20: 232–240, 1971.
167. WANG, T., KAKIZOE, T., DION, P., FURRER, R., VARGHESE, A. J., AND BRUCE, W. R.: Volatile nitrosamines in normal human faeces. Nature (London) 276: 280–281, 1978.
168. WATANABE, P. G., ZEMPEL, J. A., AND GEHRING, P. J.: Comparison of the fate of vinyl chloride following single and repeated exposure in rats. Toxicol. Appl. Pharmacol. 44: 391–399, 1978.
169. WATANABE, P. G., ZEMPEL, J. A., PEGG, D. G., AND GEHRING, P. J.: Hepatic macromolecular binding following exposure to vinyl chloride. Toxicol. Appl. Pharmacol. 44: 571–579, 1978.
170. WATTENBERG, L. W., AND LOUB, W. D.: Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. Cancer Res. 38: 1410–1413, 1978.
171. WATTENBERG, L. W.: Inhibitors of chemical carcinogenesis. *In* Advances in Cancer Research, vol. 26, ed. by G. Klein and S. Weinhouse, pp. 197–226, Academic Press, New York, 1978.
172. WEIL, C. S., CARPENTER, C. P., AND SMYTH, H. F., JR.: Urinary bladder response to diethylene glycol. Arch. Environ. Health 11: 569–581, 1965.
173. WEIL, C. S., CARPENTER, C. P., AND SMYTH, H. F., JR.: Urinary bladder calculus and tumor response following either repeated feeding of diethylene glycol or calcium oxalate stone implantation. Ind. Med. Surg. 36: 55–57, 1967.
174. WHITE, E. L., BUS, J. S., AND HECK, H. D'A.: Simultaneous determination of *n*-hexane, 2-hexanone, and 2 ,5-hexanedione in biological tissues by gas chromatography-mass spectrometry. Biomed. Mass Spectrom. in press, 1979.
175. WHITE, M.: *In* Proceedings of the Sixth Berkeley Symposium on Mathematical Statistics and Probability, vol. IV, ed. by L. M. LeCam, J. Neyman, and E. L. Scott, pp. 287–307, University of California Press, Berkeley, 1972.
176. WHITTEMORE, A. S.: Quantitative theories of oncogenesis. Advan. Cancer Res. 27: 55–88, 1978.
177. WIENER, R.: Cybernetics, or Control and Communication in the Animal and the Machine. Technology Press, New York, 1948.
178. WILLIAMS, G. M.: Enhancement of hyperplastic lesions in the rat liver by the tumor promoter phenobarbital. *In* Carcinogenesis, vol. 2, Mechanisms of Tumor Promotion and Cocarcinogenesis, ed. by T. J. Slaga, A. Sivak, and R. K. Boutwell, pp. 449–451, Raven Press, New York, 1978.
179. WILLIAMS, R. T.: Detoxication Mechanisms: The Mechanism of Detoxication of Drugs, Toxic Substances and Other Organic Compounds, 2nd ed., John Wiley & Sons, Inc., New York, 1959.
180. WILSON, R.: Risks caused by low levels of pollution. Yale J. Biol. Med. 51: 37–51, 1978.
181. WITKIN, E. M.: Mutation-proof and mutation-prone modes of survival in derivatives of *E. coli* B differing in sensitivity to ultraviolet light. Brookhaven Symp. Biol. 20: 17–55, 1967.
182. YAMAMOTO, T., TSUJI, K., KOSUGE, T., OKAMOTO, T., SHUDO, K., TAKEDA, K., IITAKA, Y., YAMAGUCHI, K., SEINO, Y., YAHAGI, T., NAGAO, M., AND SUGIMURA, T.: Isolation and structure determination of mutagenic substances in L-glutamic acid pyrolysate. Proc. Jap. Acad. 54: 248–250, 1978.
183. YANG, C. S.: The organization and interaction of monoxygenase enzymes in the microsomal membrane. Life Sci. 21: 1047–1058, 1977.
184. YAO, J. S., CALABRESE, E. J., AND DINARDI, S. R.: Does ambient ozone pose a serious public health concern as a widespread environmental mutagen? Med. Hypoth. 4: 165–172, 1978.
185. YOSHIDA, D., AND MATSUMOTO, T.: Changes in mutagenicity of protein pyrolyzates by reaction with nitrite. Mutat. Res. 58: 35–40, 1978.
186. YOSHIDA, D., MATSUMOTO, T., YOSHIMURA, R., AND MATSUZAKI, T.: Mutagenicity of amino-α-carbolines in pyrolysis products of soybean globulin. Biochem. Biophys. Res. Commun. 83: 915–920, 1978.
187. ZACHARIAH, P. K., SLAGA, T. J., BERRY, D. L., BRACKEN, W. M., BUTY, S. G., MARTINSEN, C. M., AND JUCHAU, M. R.: The ability of enteric bacteria to catalyze the covalent binding of bile acids and cholesterol to DNA and their inability to metabolize benzo(a)pyrene to a binding product and to known metabolites. Cancer Lett. 3: 99–105, 1977.