Methods for Estimating Risk of Chemical Injury: Human and Non-human Biota and Ecosystems
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Implications for Risk Assessment of Genotoxic and Non-genotoxic Mechanisms in Carcinogenesis

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ABSTRACT

The genetic, somatic and psychological health of human beings depends on a hierarchy of homeostatic adaptive mechanisms at the molecular, biochemical, cellular and physiological levels. Possible ways are examined by which chemically induced mutagenesis, cytotoxicity or gene modulation might contribute to carcinogenesis, teratogenesis and reproductive dysfunction. The concepts of initiation and promotion are introduced to describe the process by which a single normal stem cell could be transformed to a stably altered cell, which is then clonally amplified to a critical mass of dysfunctional tissue.

Biological effects of chemicals cannot be accurately predicted without knowing the biological context of the chemical interaction with the target. Although it is important to characterize potential biological effects of chemicals, the amount of uncertainty built into individual biological organisms (including genetic, developmental, nutritional, and physiological factors) limits our ability to estimate the probable harm that exposures to chemicals may cause to human health.

1 INTRODUCTION

In order to develop a means for quantitative estimation of risk in humans from exposure to chemicals, it is necessary to put the problem into a clear conceptual framework. To this end we have summarized our working hypothesis in Figure 1. This conceptual scheme implicates three fundamental biological processes—mutagenesis, cytotoxicity and intercellular communication—in the genesis of genetic disorders, congenital defects, cancer, reproductive dysfunction and other chronic diseases. It is, therefore, essential to understand the basic mechanisms of these processes.

It should be obvious from the onset that this will not be an easy task since

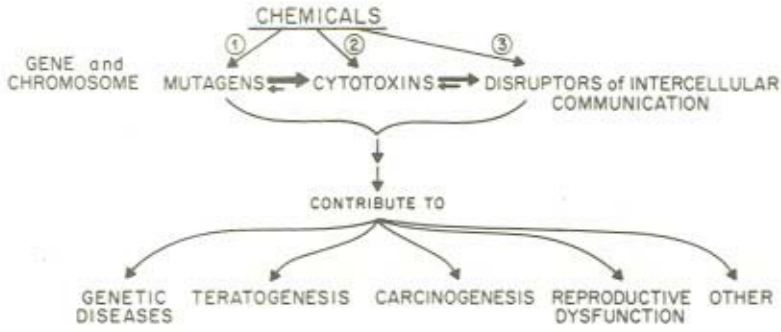

Figure 1 A heuristic scheme for classifying chemicals on the basis of three biological endpoints: mutagenicity, cytotoxicity and inhibition of intercellular communication. The arrows between the biological endpoints mean that chemicals can have multiple biological effects in the direction indicated. For example, mutagens can kill cells and the death of cells can cause the modulation of gene expression in some surviving cells. In addition, some chemicals which can inhibit intercellular communication at non-cytotoxic levels, could, at higher concentrations, kill cells, possibly also inducing some chromosomal mutations

(1) not all mutagens act in the same way (for example, gene mutations versus chromosomal mutations; mutagens which damage DNA versus those which modulate the fidelity of repair and replication of DNA);
(2) chemicals can be cytotoxic for various reasons (for instance, destroy membrane function; inhibit important enzyme functions; damage or mutate DNA, etc.);
(3) inhibition of intercellular communication by chemicals is probably as complex as mutagenesis and cell killing; and
(4) these processes are not mutually exclusive, i.e. chemicals which cause mutations can also be cytotoxic which could lead to inhibition of some forms of intercellular communication.

In summary, we will define chemicals which alter the genetic information of cells (at the gene and chromosomal level) as mutagens, whereas chemicals which alter the expression of the genetic information will be viewed as non-mutagenic (cytotoxins and inhibitors of intercellular communication). Since we are aware of the tendency to use the terms 'genotoxic' and 'epigenetic' in a very loose manner (without a clear definition or inconsistently), we will consider 'genotoxic' to be equivalent to the broadest meaning of 'mutagenic' (i.e., referring to mutations at both the gene and chromosomal levels), and 'epigenetic' to refer to the modulation of gene expression at the transcriptional to post-translational levels. Lastly, when these terms are used to describe chemicals as genotoxic or epigenetic, it is not assumed that it would preclude these chemicals from having

the opposite action under different circumstances. The terms genotoxic and epigenetic refer to specific mechanisms of action. Chemicals can elicit different biological consequences, depending on circumstances (for example, different metabolism in species, tissues, developmental stages or concentrations used).

2 MULTISTAGE NATURE OF CARCINOGENESIS AND ITS IMPLICATIONS

We will try to illustrate in the following discussion some of the factors thought to influence carcinogenesis in human beings. However, because of the limitations of this report, we will have to generalize some of what we feel are the important points. In-depth analysis of the following discussion can be found in other reports (Trosko and Chang, 1978a,b, 1979, 1980, 1981, 1983a,b).

It is now evident that carcinogenesis is a complex process involving the clonal expansion of a single altered cell (Fialkow, 1974; Cairns, 1975; Nowell, 1976; Baylin *et al.*, 1978). The concepts of initiation and promotion have been postulated to explain experimental carcinogenesis studies in animals (Berenblum, 1941; Rous and Kidd, 1941; Mottram, 1944; Boutwell, 1964) and epidemiological observations of human carcinogenesis (Armitage and Doll, 1954; Hakama, 1971; Weber and Hecker, 1978; Reddy *et al.*, 1978; Domellof, 1979; Moolgavkar *et al.*, 1980).

The initiation process appears to involve the permanent alteration of specific genes in a cell of the exposed organism, while promotion seems to include those processes which can allow the selective and clonal expansion of the initiated cell (Potter, 1980; Trosko and Chang, 1980). Clearly, the specific molecular mechanisms for either the initiation or the promotion phase of carcinogenesis are not known, although the characteristics of initiators and promoters appear to be distinct (Barrett and Sisskin, 1980). Chemical mutagens, of course, by definition can induce permanent alterations of genetic information, a property of initiators, and they, of course, have been shown in most cases to be carcinogens (Ames *et al.*, 1973; Trosko and Chang, 1979). On the other hand, many chemicals, which have not been shown to be mutagenic under biological conditions, can influence the promotion phase of carcinogenesis (Trosko *et al.*, 1982b). These promoting chemicals appear to enhance, at least, the selective hyperplasia of the initiated cell (Trosko and Chang, 1980) and to influence, among other things, the expression of genetic information, either by gene modulation through enzyme induction or by enzyme activation (Trosko and Chang, 1983a). One commonly, but by no means universally, accepted hypothesis is that initiators seem to be mutagens (genotoxins) while promoters appear to be 'epigenetic' (non-genotoxins) (Trosko and Chang, 1980). Alternative mechanisms by which initiators and promoters act involve initiators as inducers of genetic transpositions (Fahmy and Fahmy, 1980; Cairns, 1981; Renan, 1981) and promoters as inducers of recombination mechanisms (Kinsella and Radman, 1978; Nagasawa and Little, 1979; Gentil *et*

al., 1980; Varshavsky, 1981), chromosomal aberrations (Emerit and Cerutti, 1981) and aneuploidy (Parry *et al.*, 1981). For the sake of analysis, these alternative postulated mechanisms and their experimental basis will not be extensively discussed, since they have been reviewed previously (Trosko and Chang, 1983b).

It should be understood, however, that not all cells exposed to initiators are 'initiated'. An initiated cell is a cell which has a specific alteration in its genome which has converted it from a normal cell to a 'premalignant' cell. In addition, the terms 'initiator' and 'promoter' refer to the mechanisms of action of chemicals under specific conditions of use, and not to intrinsic properties of the chemical. In other words, a mutagenic chemical might be an initiator when applied in non-cytotoxic doses, whereas the same chemical, given to an initiated animal at high doses, might 'promote' the previously initiated cell to become a tumour by its cytotoxic action and the compensatory hyperplasia it would induce.

The hypothesis that carcinogenesis can be explained by the initiation and promotion concepts, if accepted, implies that the initiation and promotion processes could be prevented or suppressed (anti-initiation, anti-promotion) or enhanced (Trosko and Chang, 1978a, 1980). In addition, since it is well known that there are many variables which can alter the biological expression of a carcinogen in the host (for example, genetic factors (Trosko and Chang, 1978b; Trosko *et al.*, 1983), nutritional status of the organism (Newell and Ellison, 1981), synergisms or antagonisms with other drugs (Trosko and Chang, 1983a), psychosocial factors (Riley, 1981)), it seems to us that traditional concepts of 'thresholds' and 'carcinogens' (Trosko and Chang, 1983a) are not only misleading, they are, in effect, useless since they do not connote the specific mechanisms or imply the complex interplay of these factors. Based on the assumption that initiation and promotion concepts do adequately explain the carcinogenic process, there must be genetic, developmental, physiological, nutritional and emotional factors which can 'predispose' individuals to either or both of these processes (as well as the anti-processes). For example, if mutagenesis is at least one of the mechanisms for initiation of precancerous cells, then genetic factors, such as those which enhance the frequency of DNA damage or the error-proneness of DNA repair or replication, could enhance the risk associated with initiators. Xeroderma pigmentosum, Bloom's syndrome and albinos might fit into that category (Trosko and Chang, 1983a; Trosko *et al.*, 1983). In addition, chemicals which might either enhance or reduce another agent's potential to damage DNA (for example, photosensitizers or drug metabolizing chemicals) could either enhance or reduce the initiation and mutation frequency (Trosko and Chang, 1981).

By direct implication of this hypothesis, initiation or mutation of specific genes in a cell is a necessary, but not sufficient, step in carcinogenesis (Potter, 1980; Trosko and Chang, 1980). Clearly, the pathogenesis of cancer involves the evolution of phenotypes which give a precancerous cell the ability to escape the

regulatory mechanisms of the body to divide and differentiate properly and to have uncontrolled invasive properties (Foulds, 1975; Nicolson, 1979; Poste and Fidler, 1980; Nicolson and Custead, 1982). If the initiated cell occurs in an organism whose genetic, developmental, nutritional or physiological state favours or suppresses the clonal expansion of that cell, a tumour will either appear or not.

We have discussed elsewhere that initiation probably occurs in every organism ('spontaneous' or 'induced'), and that the rate-limiting step is probably the promotion phase (Trosko and Chang, 1983a). Thus, it is imperative that we understand the mechanisms and factors which can either enhance or suppress the clonal expansion and evolution of a single initiated cell to a frank invasive tumour. The very fact that a single initiated cell can remain 'quiescent' for long periods of time and that many genetic (Trosko *et al.*, 1983), nutritional (Cruse *et al.*, 1978), physical (Argyris and Slaga, 1981), including foreign bodies (Ryan *et al.*, 1981), developmental (Goerttler and Loehrke, 1976), physiological (Yager and Yager, 1980) or exogenous factors (Hecker *et al.*, 1982) can promote these initiated cells seems to undermine the notion that an 'immune surveillance' system can eliminate many potential tumour cells (although it does not imply that the immune system plays no role in cancer development).

3 POTENTIAL ROLE OF INTERCELLULAR COMMUNICATION IN TUMOUR PROMOTION, TERATOGENESIS AND REPRODUCTIVE DYSFUNCTION

There are various mechanisms by which cells can communicate (see Potter, 1983). The function of all these mechanisms is to regulate, in multicellular and differentiated organisms, the cells of tissues to proliferate and differentiate (Loewenstein, 1979; Pitts, 1980; Revel *et al.*, 1980). Figure 2 diagrammatically illustrates, in broad general terms, two forms of intercellular communication, i.e., over a distance via a molecular signal such as a hormone, growth factor or chalone, and via gap junctions between physically contiguous cells.

Clearly if we, in principle, accept the fundamental role which intercellular communication plays in the delicate control that stem cells have on their terminally differentiated daughter cells and in the coordination of different tissues and of cells within a tissue, then we can logically deduce that chronic disruption or disruption at a critical stage of this cybernetic or homeostatic feedback control system will cause potential adaptive dysfunction. We and other investigators have speculated that chronic inhibition of intercellular communication in initiated tissue can, in part, lead to tumour promotion (Murray and Fitzgerald, 1979; Yotti *et al.*, 1979; Potter, 1980). In addition, we have speculated that disruption of intercellular communication during critical periods of development might be one of the causes of teratogenesis (Trosko *et al.*, 1982a). Experimental evidence in the form of the well known 'carcinogenesis/

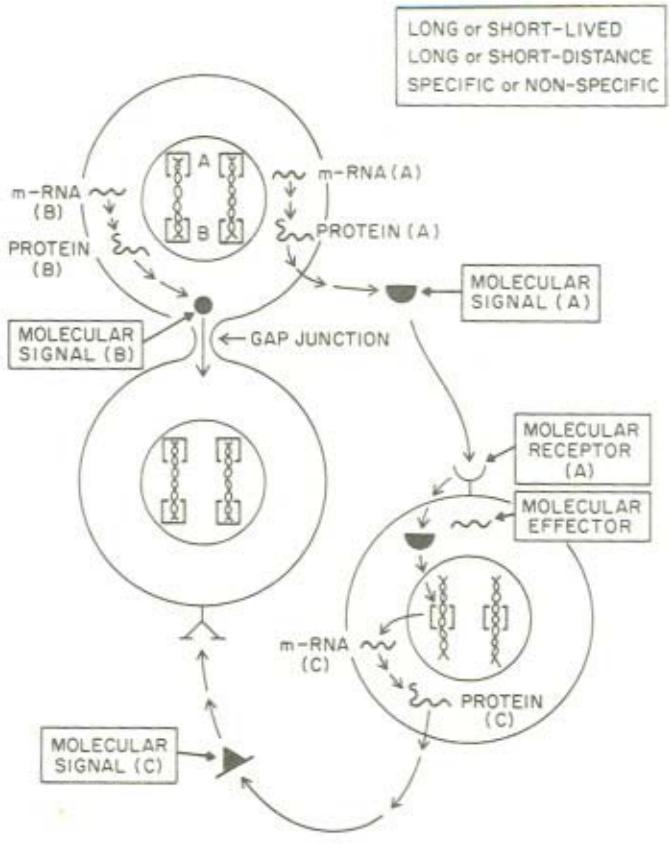

Figure 2 This diagram illustrates two general forms of intercellular communication. One involves the production and transmission of 'signal' molecules over a distance through extracellular space to a target tissue. The other involves the transfer of 'signal' molecules via permeable intercellular junctions between coupled cells

teratogenesis' connection (Miller, 1977; Nomura, 1977), and the demonstration that many chemicals which inhibit intercellular communication are either tumour promoters or teratogens or both (Figure 3), are consistent with that hypothesis.

In addition, in cases where the maturation of specific cells, such as sperm cells, depends on an intimate communication process with neighbouring but different cells (Sertoli and Leydig cells) (Sharpe *et al.*, 1981), the interference with

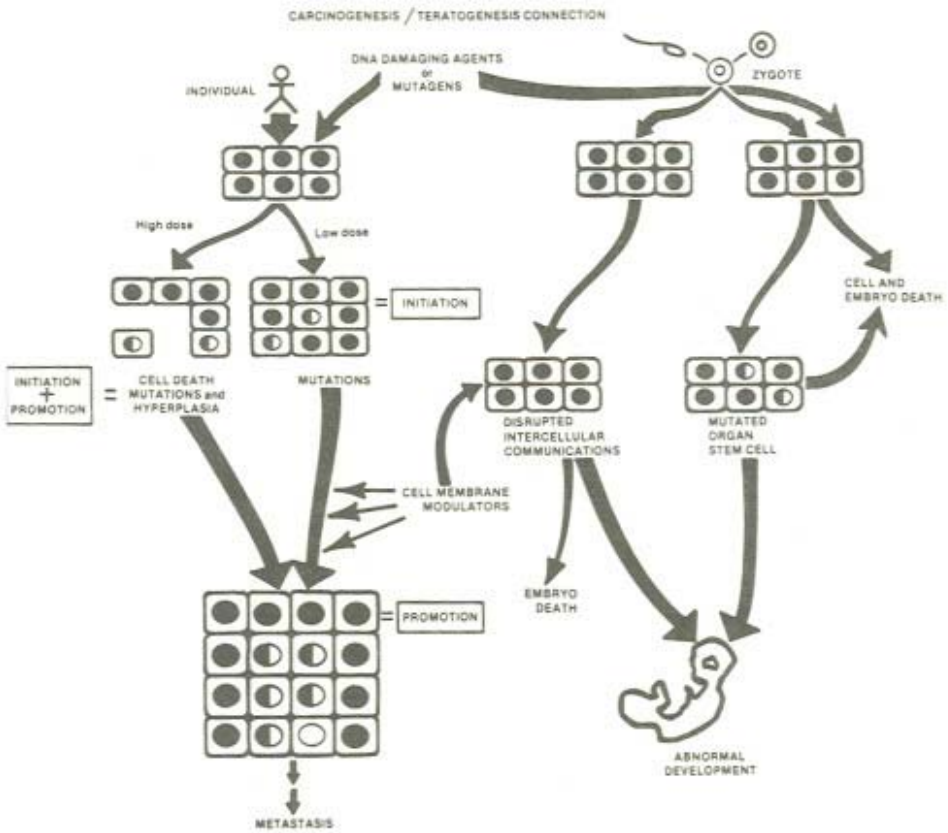

Figure 3 This diagram illustrates a hypothesis linking mutagenesis, cell death and disrupted intercellular communication to the initiation/promotion of carcinogenesis and to teratogenesis. An early conceptus or fetus, exposed to a mutagen at a low dose could conceivably have a critical gene mutated in a stem cell leading to a congenital defect. At high, cytotoxic doses of a mutagen, fetal toxicity would be expected. Exposure to non-genotoxic, but cytotoxic chemicals could also lead to congenital defects or fetal toxicity. Chemicals which inhibit intercellular communication could disrupt regulation of proliferation and differentiation of tissues if given at a critical period of development. The consequences would be either congenital defects or embryo- or fetotoxicity. The same chemicals and actions of chemicals can influence carcinogenesis as either complete or 'incomplete' carcinogens

intercellular communication could block germ cell maturation. One would predict that chemicals which interfere with this specific form of intercellular communication could lead to reproductive dysfunction.

Clearly, even if intercellular communication plays little or no direct role in the

tumour promotion process, the fact that there are chemicals which disrupt this important biological process at concentrations that do not kill the cells, leads us to speculate they can be harmful to human health

(1) if the concentration is high enough to inhibit intercellular communication;
(2) if the exposure to the chemicals is regular and chronic so as to prevent normal homoeostatic control between stem and daughter cells, between regulatory and target tissue or between functioning cells of a given tissue; and
(3) if present during critical periods of development.

4 MUTAGENESIS, MUTAGENS AND MUTATIONS

It should be apparent that mutations in the germ line contribute to genetic diseases. Although a review of the possible role of mutations in somatic tissue to other disease states will not be made here, it is highly likely that they play some role in carcinogenesis (Trosko and Chang, 1978c), atherosclerosis (Benditt, 1977; Trosko and Chang, 1980), teratogenesis (Wilson, 1977) and 'ageing' (Trosko and Chang, 1976; Trosko and Hart, 1976). Consequently, it is essential that we understand the mechanisms of mutagenesis and the techniques to measure various classes of mutations and mutagens as well as the specific mutations which might contribute to these diseases. Clearly, that kind of comprehensive review is not possible here. Suffice it to say that we know very little about the molecular details of mutagenic mechanisms especially for mammalian and human mutations.

First, we must agree on a working definition of a mutagen. For our purposes a mutagen is an agent which can, in practical terms, irreversibly alter the original quality or quantity of genetic information. This can be achieved by chemicals which may directly or indirectly damage DNA bases, DNA strands, cause additions or deletions, or translocations or transpositions of chromosome structure. Operationally, techniques used to measure DNA damage can usually detect this class of mutagens.

In addition, agents which might not damage DNA, but which could affect membrane/cytoskeleton structure or arrest normal DNA synthesis, could cause karyotypic changes (Huang *et al.*, 1983). Furthermore, there are chemicals which do not damage DNA *per se*, but can alter the DNA information content by interacting with other non-mutagens (comutagens, Nagao *et al.*, 1978), by inhibiting DNA repair enzymes, by changing the fidelity of polymerases (Weymouth and Loeb, 1978), by altering nucleotide pools (Hopkins and Goodman, 1980; Anderson *et al.*, 1981), and by activating/deactivating procarcinogens/carcinogens (Langenbach *et al.*, 1978). This does not include the possible synergistic interaction between two non-mutagenic or weakly mutagenic agents or mutagenic affectors (for example, interaction of visible light with 8-methoxypsoralen, Burger and Simons, 1979). Consequently, tests to detect

mutagens which do not damage the template DNA directly will have to be developed. DNA damage and DNA repair assays will not detect agents affecting spindle fibres or agents which modulate the fidelity of normal DNA replication.

There are many kinds of mutations at the gene and chromosome levels. The mechanisms and kinetics of induction of various kinds of mutations are probably quite different and have not been worked out in fine detail for human mutagenesis. Most test systems, *in vitro* or *in vivo*, which are used to measure mutational change are 'indirect' in the sense that they measure phenotypic changes, from which we infer a change in the quality or quantity of genetic information. Many of these test systems have severe limitations (Trosko *et al.*, 1981a,b). For example, using a bacterial system to detect possible human mutagens, can lead to some false positives, if the chemical is activated to mutagenic form by nitroreductases (enzymes active in bacterial cells) (Warren *et al.*, 1982). Also, environmental, physiological and technical factors related to the assay can cause gross artefacts in the measurement of mutations, using the recovery of specific phenotypic changes (such as expression times of mutations, selective disadvantage of mutant cells in the population tested, 'metabolic cooperation', phenocopy induction) (Trosko *et al.*, 1981a,b). Techniques to study the various types of gene mutations at the molecular level are only now being developed, and the application of these approaches to widespread quantitative and rapid screening is not yet possible.

5 GENE MODULATION, GENE MODULATORS AND INHIBITED INTERCELLULAR COMMUNICATION

The cells of a multicellular organism respond to changes in the environment for normal development, differentiation and function. However, the chronic and 'critical time' disruption of homoeostatic mechanisms can have adverse consequences as previously suggested. The general model of a transmembrane–gene modulation mechanism is thought to allow specific cells to respond to various environmental signals in such a manner that adaptive gene or gene product function comes into play. The role of intercellular communication in maintaining homoeostatic regulation of proliferation and differentiation is beginning to be understood in some detail (Loewenstein, 1979). However, exact details of how chemicals modulate specific gene or gene product activity is still under investigation. It may be assumed that any chemical which interferes with these intercellular communication–membrane/gene triggers can have detrimental consequences, depending on circumstances (for example, susceptible target cells, critical periods of interference).

Chemicals which can cause a potential reversible alteration in the expression of genetic information could be considered 'gene modulators' (epigenetic agents). They might function at different levels (DNA, or protein, or cellular) to cause phenotypic changes. Stem cells, which have a 'choice' of proliferation or

differentiation, are normally held in a state of quiescence. Various mechanisms (for example, DNA methylation, hormones, cyclic AMP, calcium, cell–cell communication) are thought to be involved in the regulation of the processes of proliferation and differentiation. Chemicals which change or block normal regulatory processes without killing or mutating the target cell (for instance hormones or PBBs) can cause potential harm by altering the patterns of development and differentiation, as well as by disrupting normal organ function.

Many chemicals in this category have been shown to be related to tumour promotion (for example, hormones) and antipromotion (cyclic AMP). Of particular interest is the demonstration that most suspected tumour promoters have the ability to block cell–cell communication. As previously hypothesized (Yotti *et al.*, 1979; Trosko and Chang, 1980), under the influence of the normalizing effect of surrounding and communicating normal cells, a single initiated (or genetically deficient) cell might not be able to express its tumour (or other genetically deficient) phenotype. The inhibition of intercellular communication (blockage of a critical gene product) by certain chemicals could eliminate this normalizing effect and convert the initiated (or genetically deficient) cell into a tumour (or phenotypically altered) cell. It should also be noted that inhibition of intercellular communication can also be brought about by cell removal (surgery, wounding), mutagen-induced cell killing, non-specific cytotoxins and physical blockage (plastic or metal surfaces).

The technology to detect chemicals which can interfere with the various forms of intercellular communication is being developed (for example, electrocoupling, metabolic cooperation, isotope or dye transfer (Murray and Fitzgerald, 1979; Yotti *et al.*, 1979; Umeda *et al.*, 1980; Kinsella, 1981; Newbold and Amos, 1981; Enomoto *et al.*, 1981; Trosko *et al.*, 1981a,b; Williams *et al.*, 1981; Warren *et al.*, 1982)). There will always be intrinsic problems in using any *in vitro* assay to predict *in vivo* higher order processes (such as species, tissue or developmental differences). An example of the problem of interspecies extrapolation has been recently analysed for hypolipidaemic drugs in rodents and man (Cohen and Grasso, 1981).

However, if it turns out that modulations of gap junctions are critical in part of the membrane-triggered shift in the cell's physiological and phenotypic state, then it might be that the use of most *in vitro* intercellular communication assays could detect all but the specific receptor-dependent membrane modulators. Although the exact structural and functional homologies of gap junctions in all multicellular species are not known, it would not be surprising if at least a high degree of functional homology has been highly conserved through evolution (Hertzberg, 1980; Nicholson *et al.*, 1981). Therefore, any agent which can affect the gap junction-dependent intercellular communication *in vitro* could (depending on the *in vitro* distribution, concentration and metabolism) be predicted to block intercellular communication *in vivo*. This might explain why the 'metabolic cooperation' assay, described by Yotti *et al.* (1979), has detected known

promoters of the skin, bladder, liver, lung, colon and breast of several species. Clearly, *in vivo* models to detect promoters have not done as well (for example, phenobarbitone or DDT are not mouse skin promoters, but rat liver promoters).

A potentially new problem, from our perspective, is that there are likely to be different kinds of tumour promoters, similar to different classes of initiators (direct/indirect acting; with short half-life, long half-life, etc.). In the classic studies on croton oil/phorbol esters mouse skin tumour promotion, it would appear that regular and long-term chronic exposure at a 'threshold' level of a promoter is needed in order for 'promotion' to occur (Boutwell, 1974; Verma and Boutwell, 1980). The metabolism and excretion of the chemicals which induce a potentially reversible and adaptive response (for example, membrane modulation of ion flux and redox potential, dissolution of gap junctions) would necessitate regular, chronic exposure to the chemical at a level which is high enough to trigger these events. This explanation seems to fit the observations of phorbol ester and phenobarbitone promotion (chemicals which are metabolized and excreted) or of promotion by saccharin (a chemical which is not metabolized, but only reaches high enough concentrations in specific organs such as bladder). However, there is now evidence that some chemicals, for example the polybrominated biphenyls, are not significantly metabolized (at least not the biologically active congeners) nor are they excreted easily. Once in the body, they are stored in the fat cells and an equilibrium is established in the body, thereby 'bathing' the body constantly. Mobilization of the stored but unmetabolized PBBs during physiological shifts (for example, pregnancy) or dietary shifts could conceivably cause long-term 'promotion' from a single exposure.

The possibility that there are 'thresholds' for individual promoters (Boutwell, 1974; Verma and Boutwell, 1980) does not, however, give us the licence to expose ourselves, willingly, to many subthreshold levels of promoters, since we have no scientific evidence that there would be no synergistic interaction of two or more subthreshold level promoters. If the organ distribution or concentration of different subthreshold promoters is different, then there might not be any problem. However, this is an area of research where no information is yet available. In addition, conceptually, antipromoters might also ameliorate the effect of one or more promoters. If our thesis is correct that promotion might be the rate-limiting step of carcinogenesis, one can see that in the 'real' world of chemicals to which humans are exposed, the 'net effect' might never be predicted from information on individual substances.

6 CYTOTOXICITY AND CYTOTOXINS

The role of cell death (or cell removal) in the tumour promotion process has been considered by Frei (1976) and Trosko and Chang (1980). Partial hepatectomy, virally induced cell killing, cell death caused by mutagens or non-specific

cytotoxins (agents destroying membrane function, inhibiting crucial enzymes) can bring about the elimination of intercellular communication without mutating the DNA or without altering gene expression directly.

Partial hepatectomy is a known promoter (Pitot and Sirica, 1980). Exposures to mutagens/carcinogens at doses which do not cause significant cell killing usually have to be followed by promoters ('incomplete carcinogens'), whereas at concentrations inducing cell killing, they are 'complete carcinogens' (Trosko and Chang, 1983a). Chemicals, such as chloroform and carbon tetrachloride and possibly TCDD, which are not thought to be mutagens, do kill cells and are promoters (Uehleke *et al.*, 1977; Reuber, 1979; Poland and Glover, 1979; Diaz Gomez and Castro, 1980; Pitot *et al.*, 1980; Geiger and Neal, 1981; Kirkland *et al.*, 1981). One potential explanation is that these chemicals, by their membrane-destructive properties, might induce a Ca^{2+} toxicity (Kroner and Plunker, 1980; Farber, 1981; Chenery *et al.*, 1981). Alcohol might be a tumour promoter, by killing cells via its membrane-altering regulation of Ca^{2+} (Goldstein and Chin, 1981; Schanne *et al.*, 1981). In any case, cell death or removal by any means, forces the surviving stem cells, including a few initiated cells, to proliferate.

7 CHEMICALS, CONTEXT AND CONSEQUENCES

If it were a simple matter to identify chemicals which either mutate genes, modulate gene expression or kill cells, and to know how mutagenesis, gene modulation or cytotoxicity influence various disease states, then our ability to predict the consequences of human exposure to these chemicals would be relatively easy. Alas, if such a deterministic relationship existed between specific chemicals and a specific biological disease state, life would probably be a very limited, if not precarious, state. The scientific evidence to date shows that there are many factors which can either enhance, reduce or ameliorate the potential effect of a specific chemical in a specific biological host.

We must consider the fact that a great number of 'defence' mechanisms must be overcome before a given chemical can affect a complex biological entity or process. In addition, once molecular or cellular targets have been reached or damaged, there are redundant repair and other compensatory mechanisms available to protect the organism from damage. The age at which the animal or human organism is exposed to the chemical makes a significant difference in the biological outcome. Killing a few critical cells with a cytotoxin or inhibiting a critical intercellular communication mechanism or mutating a somatic stem cell for a given organ during the early phases of embryonic development may be biologically more devastating than the same exposure during the adult stages of life. In general, experience with agents such as X-rays and thalidomide applied during critical stages of early development highlights that point.

Exposure of a biological host to the same chemical but in different circumstances, can again bring about opposite effects. Exposure of animals to chemical carcinogens after they were exposed to agents such as phenobarbitone or butylated hydroxytoluene seems to reduce cancer risk (see reviews by Trosko and Chang, 1978a, 1981).

On the other hand, the same chemicals given to the same animals after they have been exposed to a carcinogen enhance the occurrence of cancer (see reviews by Trosko and Chang, 1978a, 1981). The simultaneous administration of two or more 'harmless' chemicals can, in some cases, enhance the biological consequences by comutagenesis or sensitization.

Exposure of individuals of the same or different species to the same potentially harmful chemical can lead to different results because there can be a wide variety of genetic differences in the metabolism of chemicals to toxic and non-toxic forms, in the repair of biological damage and in the manifestation or expression of that damage (Trosko and Chang, 1979). The example of xeroderma pigmentosum (xp) and albino individuals can serve to illustrate this point. Both genetic impairments predispose the individual to sunlight-induced cancer. In the case of xp, sunlight-induced base damage in the DNA of exposed cells is not removed. As a result, these unrepaired DNA lesions seem to act as substrates for mutations which can 'initiate' the cells or which cause cell death (acting as an indirect 'promoter', Trosko, 1981). Albinos, on the other hand, would have more sunlight-induced DNA damage/unit dose since they lack melanin which absorbs the ultraviolet component of sunlight in normal individuals. There appears to be no defect in DNA repair in albinos. However, by saturating the normal repair system, some of the increased DNA damage could act as substrates for mutations (initiation) and cell death (indirect promotion).

Mutations will always occur because of the inevitability of exposure to environmental mutagens and of the small, but finite, chance of error-prone replication (repair) of DNA every time a cell divides. An increase of exposure to environmental mutagens will increase mutation risk in a critical gene of a given somatic cell. If the organism can compensate a defective or deficient cell either by redundancy, metabolic cooperation, removal or suppression, then a single dysfunctional cell will not have a physiological impact. However, if by clonal expansion of that dysfunctional cell a 'critical mass' of such cells is reached by the aforementioned mechanism of promotion, then the organ and organism will be physiologically affected (Figure 4). The biological consequence (disease state) of such clonal expansion of mutated or dysfunctional cells will depend on the stem cell and on the gene in the cell which is mutated.

In effect, this hypothesis predicts that, as more mutations occur in different cells and genes, clonal expansion of these altered cells can occur in all our tissues that are exposed to promoters. The implication of this model is that promotion (or clonal expansion) of dysfunctional cells is the 'rate-limiting' step for many chronic diseases.

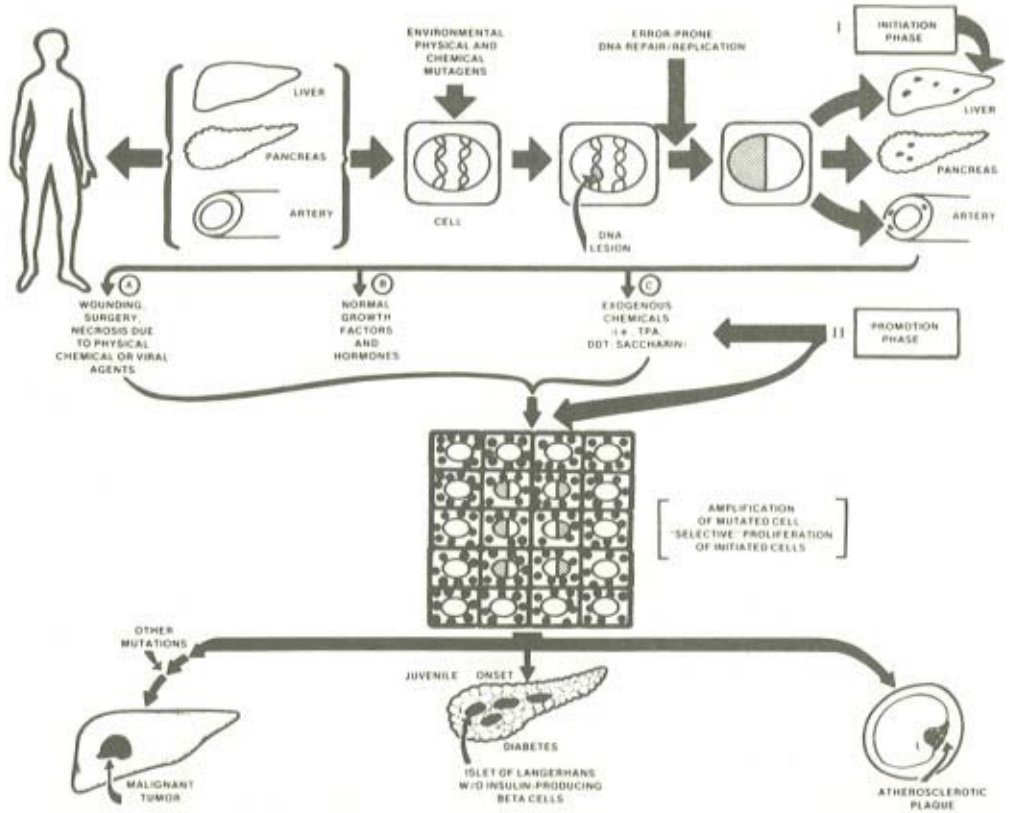

Figure 4 Initiation and promotion concepts in the genesis of chronic diseases such as carcinogenesis, atherosclerosis and diabetes. A mutation, depending on the gene and cell type, if clonally amplified can lead to a 'critical' mass of dysfunctional tissue in a given organ

8 SUMMARY

Chemicals can affect living systems by way of several mechanisms. Their ability to interact with cell membranes, cytosolic enzymes and DNA are means which send a signal to adaptive cellular and organismic responses. When this interaction leads to permanent damage of cell function, gene expression or genomic information (cell death, gene modulation and mutation, respectively), the consequences of cytotoxicity, gene modulation and mutagenesis can impact on the organism depending on a variety of factors. Some factors which could modify the consequences of a chemical exposure include:

(1) the cell or gene within the cell which is killed, altered, or mutated;
(2) the stage of development when the chemical interaction has occurred;
(3) genetic status;
(4) nutritional status; and
(5) the physiological conditions of the host.

In effect, the causes of human teratogenesis, carcinogenesis, reproductive dysfunction, genetic and other chronic diseases are multifactorial. No one thing 'causes' these diseases. Identifying the nature of potential biological harm a chemical can induce (for example, cytotoxicity, mutagenicity, gene modulation) will be of some help. Understanding the role of genetic, nutritional and physiological factors in modifying the effects of chemicals in the host is needed. However, it seems to us that even with an apparently complete understanding of some of these variables, there will be always some other unknown and uncontrolled (and uncontrollable) factors that would prevent a complete understanding of disease cause and progression.

Quantitative estimation of risk from exposure to chemicals seems far away, in spite of a better conceptual understanding of some disease states, such as the initiation/promotion model of carcinogenesis.

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