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Potential Indirect Mechanisms of Carcinogenesis
A Preliminary Taxonomy

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A preliminary taxonomy of possible indirect mechanisms by which chemicals might increase the incidence of tumors was presented. An attempt was made to determine the expected shapes of dose response relationships for agents acting by the different processes. Indirect processes that can increase the frequency of observed tumors were listed including changes in basic transport processes, changes in metabolic processing, changes in the effective amount of target DNA for reaction, and changes in the efficiency of repair of initial DNA lesions. Indirect processes that alter the frequency with which initiated cells progress through subsequent stages in the carcinogenic process were discussed, including the induction of subsequent genetic changes along the pathway to carcinogenesis, changes in the removal of initiated cells by terminal differentiation, the release of initiated cells from growth control by neighboring cells, and changes in the rates of proliferation or survival of initiated cells relative to the proliferation of normal cells. Indirect processes that might change the survival, growth, and spread of tumors or the progression of tumors included changes in hormonally mediated processes that speed up growth of specific cell types, changes in the efficiency of immune surveillance, and changes in local tissue conditions that favor colonization of new tissues by metastases.

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Introduction

It is now widely appreciated that basic considerations of fundamental mechanisms of carcinogenesis make it unlikely that there are thresholds in the dose response relationships for chemicals that cause tumors by direct reaction with DNA (Ehrenberg, 1983; Dedrick, 1979). Indeed, detailed modelling of the various processes of transport, metabolic activation and detoxification, DNA reaction and repair indicates that so long as the somatic mutations induced by a chemical are not totally distinct from the mutations that are responsible for producing "background" cancers in particular cell types, there should be an expectation of low dose linearity in dose response relationships (see discussion in Appendix A)*.

For a number of years it has been hypothesized that there are also a number of "indirect" or "epigenetic" mechanisms by which chemicals can increase the frequency of tumors in either animal test populations or humans. Because the basic transport/metabolism/DNA reaction/repair reasoning would not apply directly to these agents, some have suggested

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*Although a number of transport, metabolism, and repair processes can produce non-linearities at high doses in carcinogenesis dose response curves all of the nonlinearities arising from these processes disappear at the limit of low dosage. The basic reason these processes all "go linear" at low doses is that at low doses their rates directly depend on the number of collisions between an input chemical/activated metabolite/DNA adduct and a "hole in a membrane"/activating or deactiating enzyme molecule/repair enzyme molecule. At low doses the number of "holes in a membrane"/activating or deactivating enzyme molecules/repair enzyme molecules does not change appreciably as a function of the concentration of the input chemical/activated metabolites/DNA adducts. Therefore the number of relevant collisions and the rates of the reactions and side reactions in the causal sequence at low dosage must be direct linear functions of the amounts of input chemical/activated metabolite molecules/DNA adducts.

that it would be appropriate to assume that there might be thresholds in their dose response relationships (Weisburger and Williams, 1983; Stott and Watanabe, 1982). The goal of this paper is to set fortn a very preliminary taxonomy of possible "indirect" mechansims by which chemicals might increase the incidence of tumors, and to lay the groundwork for a possible future assessment of what might be expected about the shapes of dose response relationships for agents acting by the different processes from theory and existing data.

Development of a Taxonomy

Ideally the categories of a taxonomy of indirect carcinogenic mechanisms should be (1) mutually exclusive (without overlaps) (2) exhastive (covering all possible mechanisms) and (3) predictive of different kinds of dose response behavior. To meet the goal of desigining an exhaustive ssystem while recognizing our current incomplete understanding of some portions of the carcinogenic process, we have arranged our categories into a hierarchy.

The full preliminary taxonomy and some exmaples we know about are summarized in Table 1. The highest level of the hierarchy is built around three broad stages of the carcinogenic process:

1. "Indirect processes" that enhance of the rate of "initiation" (the initial change or rearrangement of information in DNA that places a cell on a pathway that can lead to cancer)
2. "Indirect processes" that enhance the frequency with which "initiated" cells undergo subsequent stages of transformation to form tumors

3. "Indirect processes" that alter the survival, growth or spread of tumors, or the progression of tumors to increased malignancy.

Within each of these major divisions, we can define a number of component processes. Because we have a relatively clear understanding of the processes that lead up to initiation (group 1), the component processes by which chemicals can indirectly influence carcinogenesis can be arranged in an orderly sequence as they affect the series of events that in the primary action of initiating agents:

1.1 Changes in basic transport processes

1.2 Changes in metabolic processing (activation and detoxification)

1.3 Changes in the effective amount of "target DNA" available for reaction

1.4 Changes in the efficiency of repair of initial DNA lesions

Within each of these categories in turn, there are more specific types of processes that represent either (1) enhancement of processes along the patheway to harm (e.g. increases in the metabolic activation of a primary genotoxic agent) or (2) inhibition of processes that tend to reduce damage (e.g. decreases in the activity of enzymes that detoxify reactive intermediates).

As discussed by Williams (1984) it is important to see this type of taxonomy as a system for classifying processes, not chemical or physical agents. There is nothing to prevent a single agent from acting by more than one process, and this undoubtedly happens quite frequently in practice [e.g. formaldehyde may well act both by primary reaction with DNA, and by stimulating cell division and inhibiting repair in target tissues (Hattis et al., 1981). In such cases, the overall dose response relationship for an agent will reflect the combination of its actions by all relevant processes at different doses.

Occasionally in developing the taxonomy of processes we were unable to avoid some overlap among the categories. In particular, gross killing of cells and stimulation of cell division are processes that may act in multiple ways to alter the frequency of tumors (see categories 1.3.2, 1.4.3, 2.3.4, and 2.3.5 in Table 1).

Preliminary Inferences From the Taxonomy

It is clear that there is wide diversity in the types of processes by which chemicals can indirectly affect the final incidence of benign and malignant tumors observed in animal bioassays and exposed human populations. Because of this diversity in basic mechanisms, we believe it is likely that there will be a diversity in the shapes of the dose response relationships. Some may well be threshold type processes that are describable by basic probit or log probit functions, but others are likely to have quite different shapes.

As a concrete illustration of this, imagine that an agent is a simple competitive inhibitor of an important DNA repair enzyme*. In the absense of the inhibitor, but in the continuous presence of agents that damage DNA there will be an equilibrium between the generation and repair of DNA lesions. The generation of lesions will depend on the concentration of relevant DNA and DNA reactive substance(s) [C]:

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*It should be noted in passing that this specific process is not one of those that is considered to be "epigenetic" in the taxonomy of Weisburger and Williams.

(1) $dL/dt = k[C][DNA]$

The repair of lesions will depend on the concentration of repair enzyme and the concentration of lesions in DNA at any one time [L]:

(2) $-dL/dt = [L]V_{max}/(K_m + [L])$

or at low amounts of [L], simply

(3) $-d[L]/dt = [L]V_{max}/K_m$

At equilibrium, these two must be equal

(4) $k[C][DNA] = [L]V_{max}/K_m$

and it follows that the concentration of lesions that is present in DNA at any one time and available to be fixed into the genome with DNA replication is

(5) $[L]_{eq} = k[C][DNA]K_m/V_{max}$

Now if we add a competitive inhibitor of the repair enzyme, to a first approximation this will simply tie up some number of enzyme molecules that would otherwise be available to do repair. At low doses of the inhibitor, the fraction of repair enzymes tied up in this way would be directly proportional to the concentration of inhibitor, and the effective V'_{max} with inhibition would be proportionately reduced.

(6) $V'_{max} = V_{max}/(k'[I])$

The equilibrium concentration of DNA lesions then would be proportionately increased:

(7) $[L]_{eq} = k[C][DNA]K_m/(V_{max}/k'[I] = kk'[C][DNA][I]K_m/V_{max}$

Therefore under these conditions one should expect a linear no threshold dose response relationship at low doses for agents that indirectly enhance the carcinogenic process in this way.

In summary, we believe it would be unwise to generally assume that compounds that might act by one or more of these "indirect" processes will have thresholds in their dose response relationships. Further work (both theoretical and experimental) is needed to explore the kinetic implications of the different processes in Table 1. Appendix B gives the results of a search of the scientific literature indexed in Toxline (1981+) for papers with potentially relevant dose response and mecahnism information.

Table 1
Preliminary Taxonomy of "Indirect" Processes that Can Increase the Frequency of Observed Tumors

1. "Indirect Processes" that Enhance of the Rate of "Initiation" (the initial change or rearrangement of information in DNA that places a cell on a pathway that can lead to cancer)

   1.1 Changes in Basic Transport Processes

      1.1.1 Facilitation of the absorption of a "genotoxic" agent or transport to sites of action [e.g., dimethyl sulfoxide enhances the skin permeability of many compounds; a high fat diet is likely to increase the lipid content of the blood and hence facilitate absorption of hydrophobic compounds from the diet. Williams, 1984 cites some examples of differential carcinogenesis arising from the administration of carcinogens in oily vs. saline vehicles (Homburger and Tregier, 1969; Hriono and Shibuya, 1972)].

      1.1.2 Inhibition of physical elimination of genotoxic agents through urine, feces (e.g., low fiber diets may prolong the residence time of feces in the gut, leading to greater exposure of the intestinal epithelium to reactive agents)

   1.2 Changes in Metabolic Processing

      1.2.1 Induction of enzymes that activate other chemicals to forms that can react directly with DNA.

      1.2.2 Inhibition of enzymes that detoxify a "genotoxic" agent (e.g. inhibition of epoxide hydrase by trichloropropen oxide--Berry et al., 1977), or depletion of co-factors in detoxification such as glutathione.

      1.2.3 Induced release of DNA reactive substances due to body defensive processes--E.g. possibly the release of strong oxidizing agents like O_2^- as a result of the ingestion of asbestos or silica particles by macrophages. Macrophages may also release such substances in the course of responding to infections, or cleaning up debris due to tissue damage by irritants or solids in unusual locations. See also the observations of Elcombe et al., 1985 on the proliferation of "peroxisomes" in response to trichloroethylene.

   1.3 Changes in the Effective Amount of "Target DNA" for Reaction

      1.3.1 Simple hyperplasia of the target tissue--enhancing the number of relevant cells that are potentially available for transformation

1.3.2 Changes in the amounts and types of DNA undergoing transcription or replication, thus increasing the amount of DNA that is single-stranded (and/or less protected by histones) and therefore more available for reaction

1.4 Changes in the Efficiency of Repair of Initial DNA Lesions

1.4.1 Induction of "error prone" DNA repair enzymes

1.4.2 Inhibition of normal DNA repair enzymes (e.g. for metal ions, Zakour et al., 1981)

1.4.3 Enhancement of the rate of cell proliferation, thereby decreasing the time available for DNA repair before lesions can become "fixed" into the genome at DNA replication

2. "Indirect Processes" that Alter the Frequency with Which "Initiated" Cells Progress Through Subsequent Stages in the Carcinogenic Process

2.1 Induction of subsequent genetic changes along the pathway to carcinogenesis [e.g. for a "recessive" mutation abolishing growth control on one chromosome, deletion or inactivation of the remaining normal growth control gene on the homologous chromosome as in the classic case of retinoblastoma (Knudson, 1971)]. Many promoters appear to be capable of inducing the expression of Epstein-Barr virus antigens (Takada and Zur Hausen, 1984). Another example may be the observations of Shirai et al. (1985) that the enhancement of diethyl nitrosamine-induced liver foci by phenobarbital is more pronounced at lower doses of diethyl nitrosamine. Finally, some "promoters" reportedly lead to the generation of active oxygen species that may damage DNA and lead to subsequent somatic mutations along the pathway to carcinogenesis (Kinsella and Radman, 1978). Caveat: It seems questionable to classify this as an "indirect" mechanism of carcinogenesis.

2.2 Changes in the Frequency with Which Initiated Cells are Effectively Removed by Terminal Differentiation (e.g. differentiation of squamous epithelium or red cells; see also Hanania et al., 1985--treatments of myeloid leukemia cells with various inducers of cell differentiation reduced the transcription of tumor-specific DNA sequences)

2.3 Release of Initiated Cells from Growth Control by Neighboring Cells

2.3.1 Mimicry of the action of a growth regulator or hormone by an introduced substance [e.g. phorbol esters alter the binding and phosphorylation of epidermal growth factor receptors (McCaffrey et al., 1984; Friedman et al, 1984). Also the observations of Degen et al. (1985) on the induction of plasminogen activator synthesis]. Hanania

et al. (1985) have observed specific enhancement of the transcription of genes involved in expression of tumor phenotypes.]

2.3.2 Inhibition of the action of normal growth suppressing substances. E.g. tumor promoting phorbol esters inhibit the binding of somatostatin (Zeggari et al., 1985).

2.3.3 Inhibition of the passage among cells of substances responsible for growth repression--e.g. Trosko's inhibition of cell cell communication by polychlorinated aromatic compounds (Tsushimoto et al., 1983). See also the observations of Mazzoleni et al. (1985) on an oncogenic beta-blocker using a dye transfer system.

2.3.4 Killing of neighboring cells responsible for repression of initiated cells.

2.3.5 Induction of cell replication among initiated cells, interfering with the ability of repressors to pass tight junctions, or isolating some daughter cells from tight junctions.

2.4 Changes in the Rates of Proliferation or Survival of Initiated Cells Relative to the Proliferation of Normal Cells (theoretical mechanism suggested by Moolgavkar and Knudson, 1981)

3. "Indirect Processes" that Might Alter the Survival, Growth and Spread of Tumors, or the Progression of Tumors to Increased Malignancy

3.1 Changes in Hormonally-Mediated Processes that Might Speed up the Growth of Specific Cell Types (e.g. estrogens and breast cancer)

3.2 Changes in the Efficiency of Immune Surveillance in Destroying Incipient Tumors at Early Stages. Some observations suggest that tumor promoters may alter the functioning of "natural killer" cells (Kabelitz, 1985). Immunosuppressive effects have also been observed for some promoters in vivo (Paquinelli et al., 1985).

3.3 Changes in Local Tissue Conditions that Favor Colonization of New Tissues by Metastases (e.g., the establishment of tumor blood supplies)

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APPENDIX A

QUANTITATIVE RISK ASSESSMENT FOR CARCINOGENS*

Excerpts from

Hattis, D., "From Presence to Health Impact: Models for Relating Presence to Exposure to Damage," in Analyzing the Benefits of Health, Safety, and Environmental Regulations, MIT Center for Policy Alternatives, CPA-82-16, October 1982.

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5. FROM PRESENCE TO HEALTH IMPACT: MODELS FOR RELATING PRESENCE TO EXPOSURE TO DAMAGE

5.1 Introduction

One of the hoary figures in the development of quantitative enzyme kinetics, J.B.S. Haldane, captured some of the spirit of basic science in general when he said: "Now my own suspicion is that the universe is not only queerer than we suppose, but queerer than we can suppose." (Haldane, 1927) For the basic scientist and the regulatory analyst alike, appreciation of the fact that the universe often behaves much more queerly than one might suppose can be a source of both humility and creative stimulation, as well as of day-to-day frustration.

In some ways, however, the basic scientist is in a better position to live with this mixture than the regulatory analyst. The scientist can choose to work in a specific narrow corner of the universe where there is reason to believe that a concentrated effort can reduce the apparent complexity of observed phenomena to some kind of order. The regulatory analyst, on the other hand, must follow the causal pathway from regulated activities to biological and other damage through whatever portions of the universe may happen to be involved, however odd or ill-defined the rules governing those areas may be. Where the basic scientist can limit the need for speculation to questions related to the likely productivity of different lines of research within the narrow area selected for study, the analyst is often called on to make multiple speculations on fundamental relationships between cause and effect in different fields of know-

ledge. Ironically, the "applied" work of the regulatory analyst requires the generation or adaptation of basic theories from first principles much more frequently than the "basic" work of the research scientist.

This discussion focuses on the issues that basic scientists usually have to think about least--the fundamental choices of "paradigms" (after Kuhn, 1962, basic patterns for organizing and interpreting data) which may be made without awareness of alternatives by analysts trained in particular disciplines.

5.3 Relating Exposure to Damage

The subject of dose-response relationships for hazardous substances has been an area of great controversy in recent years. The disputes primarily result when experts trained in the perspectives of different disciplines examine incomplete available data and are led to radically different expectations about the likely behavior of relevant biological systems in regions of dosage where information cannot be obtained from direct observations. The clash of expectations has been especially acute between people trained in traditional toxicology and people trained in the newer molecular biological disciplines. It will be helpful for later discussion to illustrate this particular disciplinary conflict at the outset.

A major theme, if not the central organizing principle of traditional physiology and toxicology, is the concept of the homeostatic system. Biological processes are seen as part of a complex interacting web, exquisitely designed so that modest perturbations in any parameter automatically give rise to adaptive negative feedback processes to restore optimal functioning. In this view, so long as an external stimulus does not push one or more parameters beyond a specified limit ("threshold"), adaptive processes can repair any damage which may have been temporarily produced

and completely restore the system to the functional state prior to the stimulus. This paradigm has enjoyed great success in guiding the design and interpretation of a wide range of experimental findings on acute responses to toxic chemicals, heat, cold, and other agents where the mechanism of damage does, in fact, consist of grossly overwhelming a particular set of bodily defenses.

Another type of damage mechanism dominates thinking in molecular biology and genetics. At the molecular level, some fundamental life processes are basically fragile--in particular, the integrity of the mechanism of inheritance depends on detailed fidelity in copying the massive amount of information coded within the DNA of each cell. An unrepaired error ("mutation") in copying will usually be passed on to all of the progeny of the mutated cell. Even if the mistake is confined to a single DNA base, massive adverse consequences may result if important genetic information has been altered in a functionally-significant way.

For the molecular biologist it is intuitively obvious that even a single molecule of a substance that reacts with DNA has some chance of producing a biologically significant result if it happens to interact with just the right DNA site. For the traditional toxicologist, intuition leads to just the opposite expectation; that for any substance there is some level of exposure that will not significantly affect a biological system. Clearly, application of either intuition to a particular biological response is appropriate only to the degree that the causal mechanism for that response resembles the paradigmatic damage-producing process which is the basis for the intuition.

This, of course, begs the question "What are the rules for deciding whether the causal mechanism for a particular response 'resembles' homeostatic-system-overwhelming, mutation, or some other type of damage process?" Section 5.3.1 below suggests a classification system for health effects with four broad categories defined by different properties of the fundamental damage-producing processes. We believe that a useful first step in considering dose-response information for any health effect is to classify it into one of these broad groups. Then the analyst can use sets of a priori assumptions/presumptions appropriate for the group in assessing the likely shape of the dose-response curve for the effect in question. Sections 5.3.2 through 5.3.5 discuss appropriate starting assumptions and available modelling techniques for each of the four categories of effects.

5.3.1 A Taxonomy of Biological Effects with Different Dose-Reponse Implications

In classifying particular toxic effects the analyst needs to focus on the kinds of events that are known or are likely to be occurring at subclinical dosage levels or at pre-clinical stages in the pathological process. The analyst should first ask,

- "Are the events that are occurring ordinarily completely reversible, given a prolonged period with no further exposure to the hazard?"

If the answer to this question is "Yes," then it will generally be appropriate to treat the condition within the framework of traditional toxicology. Examples of such reversible changes cited in Section 5.2.1

that require the use of time-weighting functions for accurately summarizing fluctuating exposures include:

- buildup of a contaminant in blood or other tissues,
- most enzyme inhibition, and
- induction of short term biological responses which act to maintain homeostasis (e.g., sweating in response to heat, tearing in response to irritations).

After assigning a particular effect to the province of traditional toxicology, it is then usually helpful to characterize the time-course over which the sub-clinical or pre-clinical events are likely to be reversed. If reversal is likely to be essentially complete within a few hours or days, it should be considered under the heading of acute toxicity. If reversal is likely to take longer than a few days before it can be considered substantially complete, (and longer-term modelling of toxicant buildup or of other effects is therefore required for accurate prediction of the response) the condition should be considered under the heading of classic chronic toxicity.

If the answer to the question above is "No" and events are likely to be occurring at subclinical exposure levels or preclinical stages that are not ordinarily completely reversible,* modelling of biological risks

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*Examples of such irreversible or poorly reversible events include:

- changes in genetic information or in the heritable pattern of gene expression after these are effectively "fixed" into a cell's genome by replication,
- death of non-replicating types of cells (e.g.neurons),
- destruction of non-regenerating structures (e.g., alveolar septa),
- generation and buildup of incompletely repaired lesions (e.g, atherosclerotic plaques), and
- apparently irreversible physiolgical changes produced by multiple, diverse fundamental mechanism (e.g., long term increases in blood pressure from "essential" causes).

will generally need to be based on fundamentally different concepts from the homeostatic system/threshold paradigm of traditional toxicology. As will be seen later, some traditional toxicological elements such as pharmacodynamic modeling are still helpful in the supporting role of determining the effective delivery of hazardous substances to the sites where irreversible or poorly reversible damage events can occur. However, appropriate modeling for conditions that are the result of irreversible or poorly reversible processes must fundamentally be based on the likely dose-response characteristics of the events which cause the basic irreversible changes.

Once the primacy of such irreversible changes is established for a particular event, one should ask whether clinical manifestations are likely to be the direct result of only a few, or of very many individual irreversible damage events. If it is thought that only a few events directly contribute to a particular clinical manifestation (e.g., a small number of heritable changes within a singe cell line leading to cancer*) the effect can be considered to be a molecular biological disease. If thousands, millions, or billions of individual irreversible events directly contribute to a particular condition (e.g., very large numbers

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*Over the past several years considerable progress has been made in scientific understanding of the fundamental mechanism of carcinogenesis. It now seems virtually certain that the basic event which gives rise to most (if not all) tumors is a heritable change in the DNA of a single cell. Up until recently there were three basic lines of evidence that supported this somatic mutation theory of carcinogenesis.

o First, it appears that cancer originates within single cells. Women carry two X chromosomes but only one is functional in any one cell any time after a point early in fetal life. At this time one (

*cont'd from 5-29

chromosome in each cell (different ones in different cells) becomes irreversibly inactivated. In women who carry a genetic difference between their two X chromosomes, it has been found that tumors generally exhibit activation of the same X chromosome in all cells (Fialkow, P.J., 1977; Knudson, A.G., 1977, 1973). If events within more than one cell line were usually involved in tumor production, it would be expected that different cells of individual tumors would be a mixture of cells exhibiting activation of both X chromosomes, like body cells in general.

o Second, some well characterized deficiencies in DNA repair appear to lead to greatly increased cancer risk (Cleaver, J.E. and Botsma, D., 1975; Vogel and Motulsky, 1979).

o Third, there is a the general association between mutagenic and carcinogenic activity in many chemicals (Vogel and Motulsky, 1979; McCann, J., et al., 1975).

Very recently, a fourth and apparently conclusive line of evidence has been provided by gene transfer experiments (Marx, J.L., 1982; Cooper, G.M., 1982). Carcinogenic transformation has been produced by incorporating specific small lengths of purified DNA from many different lines of cancer cells into a standard strain of non-malignant tissue culture cells. A low frequency of transformation evidently can also be accomplished by transferring DNA from normal cells, provided the normal DNA has been broken up into small pieces by sonication. This latter result suggests that one kind of DNA change capable of inducing transformation is a breakage-and-rejoining event, in which a specific gene may be separated from its normal control region.

of individual alveolar speta must break in order to produce serious impairment from emphysema), we think it should be dealt with under the category of chronic cumulative conditions.*

The aim of creating these four broad categories (acute toxicity, classic chronic toxicity, molecular biological, chronic cumulative) is to help distinguish among types of risk analysis problems which must be approached from first principles in basically different ways. The next four subsections examine some of the analytical approaches and generalizations that are possible for each category.

5.3.4 Molecular Biological Effects

Under this heading come carcinogenicity, mutagenicity, and at least some forms of teratogenicity. We do not, however, attempt to deal specifically with the problems of modelling mutagenic and teratogentic risks in this writing.

We also do not review in any detail the extensive literature describing different statistical approachaes for fitting various hypothetical curves to animal dose-response data and projecting human risk. For standard procedures in this area, the reader should consult: Carcinogen Assessment Group (1980), IRLG (1979), Crump, et al. (1976, 1977), Guess and Crump (1977), and Whittemore (1976, 1979). These procedures have yielded useful insights;* however, as applied in the past they have also had substantial limitations. The most widely used approach (Carcinogen Assessment Group, 1980; Crump, K.S., et al., 1977) incorporates an hypothesis about the nature and dynamics of the

*particulary about the linearity of "upper confidence limit" risk projections at low doses almost regardless of the input data or the shape of the "maximum likelihood" (best fit) dose response relationship (Guess, H.A. and Crump, K.S., 1977)

fundamental carcinogenic process (Crump, K.S. et al., 1976) [the "multi-stage" theory of Armitge and Doll (1961)], but little else. There is no provision in the procedure to incorporate any specific information about the biology of the carcinogen in question other than the numbers of tumors observed at different dose levels and their time of appearance. Any departure from linearity in the observed dose response relationship is implicitly attributed to a departure in linearity of the fundamental carcinogenic process. In reality, though, such departures might very well arise from dose dependent changes in a number of ancillary processes, rather than the core carcinogenic mechanism. For example, nonlinearities might arise from changes in the ratio of pre-carcinogen processed by different metabolic routes (some of which may lead to DNA reaction and carcinogenesis and some of which may not) or changes in the efficiency of DNA repair at different dose levels.

We suspect that the field of carcinogenesis risk assessment has reached a turning point. It has been appreciated for some time that the inherent statistical limitations of carcinogenesis dose-response experiments in small groups of animals generally prevents selections among alternative dose-response models on the basis of experimental data--many alternative models with vastly different implications for the magnitude of low dose risk will fit the experimental data about equally well (Haugh, 1978). Moreover, as described in references cited earlier and discussed in Chapter 3, purely statistical techniques for fitting curves to the meager available data are now well developed, and it seems unlikely that further improvements along this line will markedly alter the confidence limits that can be derived from present procedures. Recently there have

been efforts to incorporate more biochemical sophistication into the interpretation of carcinogenic dose response information and the projection of human risk. Theoretical simulation models have been developed from principles of chemistry and enzymology and analyzed for their low dose behavior (Gehring, P.J. and Blau, G.E., 1977). Our own work projecting the carcinogenic risk of formaldehyde (Hattis, D. et al., 1981) considered alternative biochemical interpretations of the highly non-linear relationship observed at high doses in that case. Thus, a major hope for more accurate risk projections for individual carcinogens appears to lie with more detailed, quantitative modelling of the many steps that occur between carcinogen exposure and the eventual manifestation of clinically-detectable tumors.

This section offers some theoretical conjectures about the likely influence of various factors on the ultimate shape of carcinogen dose-response relationships. Our hope is that these conjectures will assist both policy analysts and basic scientists in discerning research approaches that are likely to yield significant new information in response to analytical and experimental efforts. We first describe the general theoretical basis for suspecting that the production of primary genetic lesions by carcinogens should be a linear function of the concentration of the ultimate carcinogenetic substance at the active site. Then

we survey a number of mechanisms that may modify the linearity in ultimate cancer dose-response that one would expect from this fundamental process. These modifying factors include:

- dose-dependent changes in metabolic handling of the carcinogen (or pre-carcinogen)
- the properties of DNA repair systems
- contributions to cell division (and hence susceptibility to carcinogenic transformation) by overt toxic responses to carcinogens at high dose levels
- differences among individuals in primary sensitivity to carcinogenic action from numerous sources
- the number of sequential mutations within a cell line required to produce carcinogenic transformation

5.3.4.1 The Expected Linearity with Dose of the Primary Production of Genetic Lesions

Suppose that there is some way of adding to the nucleus of a cell a known concentration of substance that reacts with DNA. For these conditions the rate of reaction would ordinarily be expected to be given by ordinary bi-molecular reaction kinetics:

$$\frac{dL}{dt} = k[C]\,[DNA] \qquad (5.1)$$

where $\frac{dL}{dt}$ is the rate of productions of DNA lesions,

$[C]$ is the concentration of the reactive substance,

$[DNA]$ is the concentration of "exposed" or available DNA, and

k is a constant.

Since at low doses of C the concentration of exposed DNA would not be altered by changes in dose, the expectation is that the rate of lesion production should be a linear function of the concentration of the reactive substance at the site of the genetic action.

5.3.4.2 Dose-Dependent Changes in Metabolic Handling of the Carcinogen (or Pre-Carcinogen)

P. Gehring of Dow and some other toxicologists have repeatedly pointed out that where the body has more than one way of processing and excreting a substance, the proportions of an administered dose that are handled in different ways may change at different dose levels (Gehring, P., 1977, 1979; Watanabe, P.G., et al., 1976; Hefner, R.E., et al., 1975; Anderson, M.E., et al., 1980). This is because metabolic processes generally have finite capacities. As concentrations of a substance are increased, metabolic routes with relatively small capacities asymptomatically approach "saturation" and larger proportions of the administered substance are left to be processed by routes with larger capacities. Where some metabolic routes lead to production of DNA-reactive intermediates, and other metabolic routes lead to excretion without DNA damage, it is clear that the amount of DNA damage produced per unit of dose will change at different dose levels, and that the resulting cancer dose-response curve must depart from linearity.

This general phenomenon is often cited in discussions which, at the very least, leave the casual reader with the impression that this is a general mechanism which can ordinarily be expected to produce thresholds in carcinogenic dose-response curves (Gehring, 1977; Cornfield, 1977).* In fact, this mechanism will only produce a true threshold in one highly unlikely limiting case; that is, where the harmless metabolic route is

*This effect is produced in Cornfield's model by an assumption of full equilibrium between the carcinogin and a "detoxifying system" before any carcinogen is allowed to potentially react with DNA.

both infinitely fast relative to the route which produces the DNA-reactive metabolite, and yet still somehow has a finite capacity (Cornfield, 1977). Where the two competing processes both have finite reaction rates, straightforward application of the same Michaelis-Menten enzyme kinetics that Gehring and others frequently cite leads directly to the expectation that at very low doses the fraction of an administered substance which is handled by each competing route becomes constant.*

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*Consider two competing metabolic routes, both with Michaelis-Menten reaction kinetics. The rate of production of the potentially harmful (DNA-reactive) metabolite will be given by:

$$\frac{dR}{dt} = \frac{k_1 C}{K_R + C}$$

where C is the concentration of the administered pre-carcinogen, K_R is the "Michaelis constant" for the reaction, and k_1 is the maximum possible rate of the reaction. Similarly, for the "safe" metabolic route:

$$\frac{dS}{dt} = \frac{k_2 C}{K_S + C}$$

Clearly at low doses, where C is very much less than either K_R or K_S, these relationships become approximately:

$$\frac{dR}{dt} = k_1 C / K_R$$

$$\frac{dS}{dt} = k_2 C / K_S$$

and the ratio of the two rates (the rate of production of the reactive metabolite divided by the rate of production of the safe metabolite) becomes a simple constant

$$\frac{\frac{dR}{dt}}{\frac{dS}{dt}} = \frac{k_1 K_S}{k_2 K_R} = \text{Constant.}$$

This result means that, far from leading to a general expectation of thresholds, this mechanism must generally produce linear dose/response kinetics at very low doses. The linear slope will generally be different from that observed at high doses, but any balanced presentation of the subject should include the observation that the high-dose slope may be either greater or less than the slope at low doses, depending on whether the harmless or the harmful metabolic route begins to approach saturation first. Indeed, for the case of vinyl chloride, the substance which Gehring and coworkers used to demonstrate their point, it is in fact the harmful pathway which saturates first. This has been used to explain the observation that although the initial Maltoni vinyl chloride experiments produced essentally linear dose-response relationships for tumors below about 500 ppm, above that level the tumor response was essentially flat--up to 6-10,000 ppm little additional tumor risk was observed. (Maltoni and Lefemine, 1975) If, as is usually the case, only data at the two high dose levels had been available from the Maltoni experiments, and if an analyst had followed recommended standard procedures and done a linear interpolation between zero and the 6000 and 10,000 ppm dose levels to estimate cancer risk at low doses, the actual risk to rats at "low" dose (250 ppm) would have been underestimated by several fold. This example therefore indicates that, contrary to popular belief, linear interpolations of risk from high dose levels sometimes may not be "conservative" enough in projecting low dose risks.

5.3.4.3 Properties of DNA repair systems.

The existence of natural repair systems for various kinds of DNA lesions is another item which has been used by some to suggest that there

may be thresholds for carcinogens. The substance of the argument usually is that amounts of DNA damage below some given repair capacity per unit time are likely to be repaired with perfect efficiency, and that tumors result only when the rate of production of lesions exceeds the maximum rate of repair.

It is certainly true that at least some kinds of DNA repair systems significantly reduce the frequency of at least some kind of tumors in normal humans. There is at least one well-characterized set of human genetic disease, grouped under the general heading of "xeroderma pigmentosum," which results from inherited deficiencies in DNA repair systems (Cleaver and Bootsma, 1975). Patients with xeroderma pigmentosum are abnormally sensitive to ultraviolet light,* and sooner or later develop multiple malignant skin tumors unless very rigorous precautions are taken to avoid exposure to sunlight and other sources of ultraviolet radiation. (Vogel and Motulsky, 1979). DNA repair defects of various kinds are also suspected to contribute to some other genetic diseases characterized by high spontaneous rates of malignancies (Hirsch-Kauffman, et al., 1978; Poon, et al., 1974).

The argument for the general existence of thresholds from this source is most likely to be erroneous in its assumption of perfectly efficient repair at low rates of DNA damage. It seems more likely that (1) if a finite number of the enzyme molecules is capable of recognizing a particular kind of DNA lesion and initiating the repair process and (2) if

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*Ultraviolet light induces specific types of DNA lesions which can be repaired.

there is only a finite time between lesion generation and potential completion of the mutagenic process through the completion of cell division, then in general some finite fraction of repairable lesions at low doses must escape repair. Assuming that lesion recognition and repair is governed by the same basic principles as other enzymatic reactions, we would expect that if there were a single burst of lesion generation at time t = 0, the rate of lesion repair ($-\frac{dL}{dt}$) would be given by:

$$-\frac{dL}{dt} = \frac{k\,[L]\,[\text{Repair enzyme system}]}{K_m + [L]} \qquad (5.2)$$

At low doses this becomes approximately

$$-\frac{dL}{dt} = \frac{k\,[L]\,[\text{Repair enzyme system}]}{K_m} \qquad (5.3)$$

Rearranging and integrating, the amount of lesion that persists in a cell at a later time t_1 when the cell completes its next division would be given by:

$$L_{t_1} = L_0 e^{-kt_1 \cdot [\text{Repair enzyme system}]/k_m} \qquad (5.4)$$

In other words, at low doses we should expect that a constant fraction of repairable DNA lesions will escape repair and be available to affect future generations of cells adversely. At low doses, the amount of unrepaired lesions should be a simple linear function of the initial amount of lesions generated, Lo, although the slope of the relationship may well be less than the slope observed at high doses.

In passing it should be noted that not all DNA repair systems uniformly return damaged DNA to the status quo that existed before lesion generation. Some "error prone" repair systems exist which, though they correct most of the damage they encounter, occasionally make inaccurate repairs. Where different DNA repair systems exist with different repair capacities and different error rates, the situation is exactly parallel to the case of alternative metabolic pathways discussed in the previous section. Under these conditions, the low-dose slope of the relationship between lesion generation and the survival of unrepaired lesions may be either greater than or less than the slope at higher doses, depending on whether the more- or less-error-prone system begins to approach "saturation" first.

5.3.4.4 Effects of Overt Toxic Responses which Increase Cell Division.

One consequence of Equation (5.4) derived in the previous section may lead an analyst to validly infer in some cases that more cancers will be produced per unit of dose at high dose rates than at low dose rates. The current practice of conducting carcinogenic bioassays within two-fold of estimated "maximum tolerated doses" often leads to testing at doses

which induce appreciable cell death and enhanced rates of cell division in target tissues for carcinogenic action. This is likely to lead to a greater production of tumors per unit of dose if either (a) only actively replicating cells are susceptible to carcinogenic transformation, or (b) the time t_1 in Equation (5.4) between lesion generation and the completion of cell division is shortened.

The cell division responses of specific tissues to increasing doses of carcinogenic substances can be easily measured in relatively short-term experiments. We used data of this kind to help interpret the observed nonlinearity in the dose-response curve for formaldehyde carcinogenesis (Harris, D. et al., 1981). With greater difficulty, it may also be possible to produce information on the dynamics of DNA repair processes in animal bioassay systems. As an aid to risk analyses, it may be helpful to add studies in these two areas to existing animal cancer bioassay protocols. Further, in projecting risks to human populations, analysts may wish to consider the likelihood that predictable individual differences in cell turnover and DNA repair may lead to predictable differences among individuals in carcinogenic risks.

5.3.4.5 Effects of Individual Differences in Susceptibility to Carcinogenic Risk on the Relationship Between Overall Population Dose and Total Tumor Responses.

Individual differences in susceptiblity to carcinogenic risk can arise from many different mechanisms. What happens to the overall tumor response of a population as a result of this individual diversity? To

show this, we have constructed Table 5.2, using two very simple assumptions:

(A) The basic form of the dose-response relationship for the carcinogen is "one-hit" (linear at low doses, approaching a limit of 100% tumor incidence at very high doses)

$$P_{tumor} = 1-e^{-kd}$$

where P_{tumor} is the probability that a particular individual exposed to dose "d" of the carcinogen will get at least one tumor over his/her lifetime, "k" is a constant for any individual that varies among different individuals, and "d" is dose. "kd" is the expected number of "hits" (tumors) given a particular dose and sensitivity value.

(B) There is a log-normal distribution of sensitivity in the population to the carcinogen in queston. That is, the logarithm of k for different individuals shows a normal Gaussian distribution. Results based on four different standard deviations for this distribution are shown in Table 5.2 A log standard deviation of 0.5 means that about 95% of the population has an intrinsic sensitivity (slope of the dose-response function at low doses) within a tenfold range of the risk of the middle (median) person in the population--that is between tenfold less than the median risk and tenfold greater than the median risk (see Figure 5.7). By the same token, a log standard deviation of 1.0 means that 95% of the people will be between 100-fold less and 100-fold greater than the median risk; 1.5 and 2.0 represent distributions where 95% of the people are respectively within 1,000 and 10,000 fold on either side of the median risk.

It can be seen in Table 5.2 that if the dose received by the population is high enough to bring the expected number of tumors ("kd") for the median person to the very high level of .64 (bottom row of the table), the width of the distribution of sensitivity of the population does not matter very much; all the curves indicate that about half of the total population will get tumors. As one goes to lower doses and lower levels of median risk, however, it can be seen that the curves diverge greatly from each other. At the $1x10^{-5}$ risk level for the median person, the overall population risk spans a 50-fold range from about two in one hundred thousand to slightly over one in one thousand.

TABLE 5.2

Relationship Between Median Risk and Overall Populations Risks
For Populations with Log-Normal Distributions of Susceptibility
Having Various Standard Deviations

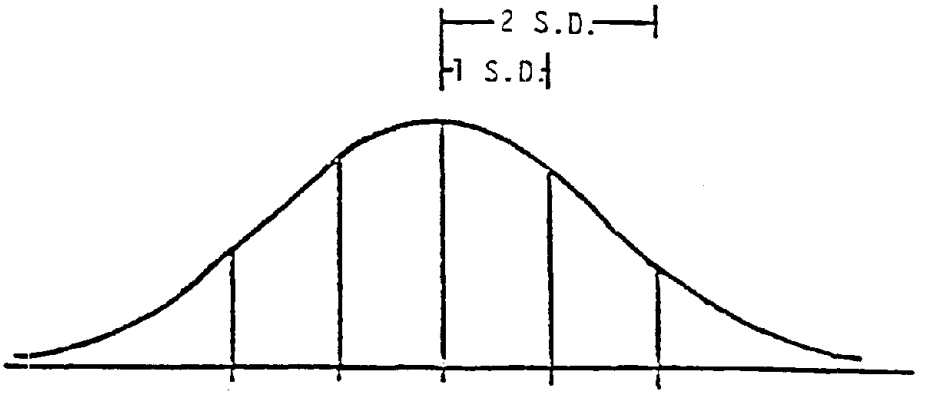
"kd" for Median Person**	Median Risk***	Overall Population Risk*			
	LOG S.D.=0	LOG S.D.=0.5	LOG S.D.=1.0	LOG S.D.=1.5	LOG S.D.=2.0
$1x10^{-11}$	$1x10^{-11}$				$3.35x10^{-7}$
$1x10^{-10}$	$1x10^{-10}$				$2.75x10^{-6}$
$1x10^{-9}$	$1x10^{-9}$				$2.01x10^{-5}$
$1x10^{-8}$	$1x10^{-8}$			$3.77x10^{-6}$	$1.27x10^{-4}$
$1x10^{-7}$	$1x10^{-7}$			$3.46x10^{-5}$	$6.84x10^{-4}$
$1x10^{-6}$	$1x10^{-6}$		$1.75x10^{-5}$	$2.84x10^{-4}$	$3.09x10^{-3}$
$1x10^{-5}$	$1x10^{-5}$	$1.94x10^{-5}$	$1.72x10^{-4}$	$1.95x10^{-3}$	.00116
$1x10^{-4}$	$1x10^{-4}$		$1.40x10^{-3}$	.0106	.0361
$1x10^{-3}$	$1x10^{-3}$	$1.93x10^{-3}$	.0113	.0441	.0931
$2x10^{-3}$	$2x10^{-3}$		.0196		.119
$5x10^{-3}$	$4.99x10^{-3}$		.0399	.100	.161
.01	$9.95x10^{-3}$	.0188	.0656	.137	.199
.02	.0198	.0364	.103	.182	.242
.04	.0392	.0688	.156	.235	.289
.08	.0769	.125	.224	.296	.341
.16	.148	.216	.310	.364	.359
.32	.274	.345	.408	.436	.452
.64	.473	.505	.512	.511	.510

* The overall population risk is the fraction of the total population that is expected to get at least one tumor.

** Where $P_{tumor} = 1-e^{-kd}$, "kd" represents the average number of tumors expected per person in a population of people with absolutely uniform sensitivity. (At very low values of kd the ratios of the overall population risk to median risk become constant at approximately 2, 18, 380 and 34,000 for Log S.D. of 0.5, 1.0, 1.5, and 2.0 respectively.

*** Probability that a person of median sensitivity will get at least one tumor.

Figure 5.7



Log (risk)	-6	-5.5	-5	-4.5	-4
Risk	10^{-6} (one per million)		10^{-5} (one per 100,000)		10^{-4} (one per 10,000)

The general population risk estimates at low levels can be used to assess how much a simple linear extrapolation from high doses could underestimate the true risk to a population with diverse sensitivities. If the lowest dose available from epidemiological studies or animal testing were to yield about 50% tumors (corresponding roughly to the bottom line of the table), simple linear extrapolation would lead one to expect overall population risk estimates that are fairly close to the median risk levels shown in the second column. It can be seen that if the distribution of sensitivities in the population is fairly narrow (log S.D. = 0.5, third column) the linear extrapolation is not too bad--underestimating the true risk at low dose by less than 2-fold (Compare columns for Log

S.D.= 2.0 and 0.5). On the other hand, for successively broader distributions of sensitivity in the population the ratio of the overall population risk to the median risk gets quite large at very low doses. For log standard deviations of 1.0, 1.5, and 2.0, the linear extrapolation may be too low by 17-fold, 380-fold, and 34,000-fold respectively.

To date there has been no systematic effort to pull together available literature and attempt to assess the likely range of individual variation in susceptibility either for specific carcinogens, or for carcinogens in general. Analytical efforts in this area may produce useful information for future risk assessments.

5.3.4.6 The Number of Mutations Required for Ultimate Carcinogenic Transformation

This last consideration has been dealt with extensively in the existing literature, and we will not cover it in detail here. We should note however (1) that if n sequential mutagenic "hits" are required to achieve transformation in a particular cell type, the frequency of transformation will generally be proportional to the 'n'th power of the frequency of mutation, and (2) that if "one-hit" routes to transformation exist in competition with multi-hit processes for a particular tissue, the one-hit routes should be expected to contribute an increasing share of total transformation events as dosage is reduced.

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National Institute for Occupational Safety and Health, 1977b. Criteria for a Recommended Standard . . . Occupational Exposure to Ethylene Dibromide, DHEW (NIOSH) Publication No. 77-221.

National Institute for Occupational Safety and Health, 1977c. Criteria for a Recommended Standard . . . Occupational Exposure to Dioxane, DHEW (NIOSH) Publication No. 77-226.

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Whittemore, A., 1979. "Mathematical Models of Cancer and Their Use in Risk Assessment," Technical Report No. 27, SIAM Institute for Mathematics and Society, prepared for U.S. Department of Energy under contract No. EY-76-S-02-2874.

152
AU - STENBACK F
AU - PETRO R
AU - SHUBIK P
TI - Initiation and promotion at different ages and doses in 2200 mice: 3. Linear extrapolation from high doses may underestimate low dose tumor risks.
SI - HEEP/82/03897
SO - BR J CANCER; 44 (1). 1981. 24-34.
AB - HEEP COPYRIGHT: BIOL ABS. The dose-response relationships from the data previously described were analyzed. Among unpromoted animals, only doses sufficient to cause ulceration with subsequent promotion due to wound healing caused a rapid crop of tumors, so the dose-response curve exhibited strong upward curvature. Among promoted animals, the response of the skin to initiation appeared to be nearly saturated by all DMBA (dimethylbenzanthracene) doses tested, so that a 30-fold decrease in dose produced only a 3-fold decrease in effect. The dose-response relationship thus exhibited strong downward curvature. Among promoted animals, estimation of the risks associated with very low doses of carcinogen by linear extrapolation through the origin from the effects of larger doses (which is often assumed to be conservative) would underestimate the true risks by 10-fold or more. Whereas linear interpolation from the results of high doses may be reasonable for data on the effects of continuous treatment with non-toxic dose levels of carcinogen, it may be misleading when extrapolating from the effects of single larger doses.

APPENDIX B

Toxline Literature Search on Promoters and Epigenetic Carcinogenesis
(Plus some additional references from author searches)

dose(tw) and response(tw)

SS (2) PSTG (22289)

2 and carcinog:(tw)

SS (3) PSTG (1227)

promot:(tw) or epigenet:(tw)

SS (4) PSTG (12240)

3 and 4

PROG:
SS (5) PSTG (159)

1
AU - SHIRAI T
AU - HOSODA K
AU - HIROSE K
AU - HIROSE M
AU - ITO N
TI - Promoting effects of phenobarbital and 3-methyl-4-dimethylaminoazobenzene on the appearance of gamma-glutamyltranspeptidase positive foci in rat liver pretreated with varying doses of diethylnitrosamine.
SI - BIOSIS/86/01213
SO - CANCER LETT; 28 (2). 1985. 127-134.
AB - BIOSIS COPYRIGHT: BIOL ABS. The promotion potential of phenobarbital (PB) and 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) on liver carcinogenesis after initiation with various doses of diethylnitrosamine (DEN) was assessed using an in vivo short term system. Male F344 rats were pretreated with a single intraperitoneal injection of varying doses of DEN (0, 6, 12, 25, 50, 100 or 200 mg/kg body wt), and 2 weeks later were treated with 0.05% PB or 0.06% 3'-Me-DAB for 6 weeks. All animals were subjected to partial hepatectomy 3 weeks after the DEN treatment. Quantitation of gamma-glutamyltranspeptidase-positive (gamma-GT+) foci revealed a DEN dose-dependent response. Magnitude of promotion by PB and more pronounced by 3'-Me-DAB was, in contrast, strongest at the lower doses of DEN. The results suggest that quantitative differences with regard to initiation level may exist, influencing the promotability of initiated cells.

1
UI - 85030333
AU - McCaffrey PG
AU - Friedman B
AU - Rosner MR
TI - Diacylglycerol modulates binding and phosphorylation of the epidermal growth factor receptor.
AB - Tumor promoters cause a variety of effects in cultured cells, at least some of which are thought to result from activation of the Ca2+-phospholipid-stimulated protein kinase C. One action of tumor promoters is the modulation of the binding and phosphorylation of the epidermal growth factor (EGF) receptor in A431 cells. To determine if these compounds act on the EGF receptor by substituting for the endogenous activator of C kinase, diacylglycerol, we compared the effects of the potent tumor promoter 12-O-tetradecanoyl phorbol 13-acetate (TPA) with those of the synthetic diacylglycerol analog 1-oleyl 2-acetyl diglycerol (OADG). When A431 cells were treated with TPA, the subcellular distribution of C kinase activity shifted from a predominantly cytosolic location to a membrane-associated state; OADG also caused the disappearance of cytosolic C kinase activity. The shift in the subcellular distribution of C kinase, caused by TPA or OADG, correlated with changes in binding and phosphorylation of the EGF receptor. OADG, like TPA, caused loss of binding to an apparent high affinity class of receptors, blocked EGF-induced tyrosine phosphorylation of the EGF receptor, and stimulated phosphorylation of the EGF receptor at both serine and threonine residues. No difference between the phosphopeptide maps of receptors from cells treated with OADG or TPA was observed. Thus, it appears that tumor promoters can exert their effects on the EGF receptors by substituting for diacylglycerol, presumably by activating protein kinase C. Further, these results suggest that endogenously produced diacylglycerol may have a role in normal growth regulatory pathways.
SO - J Biol Chem 1984 Oct 25;259(20):12502-7

2
UI - 84221899
AU - Friedman B
AU - Frackelton AR Jr
AU - Ross AH
AU - Connors JM
AU - Fujiki H
AU - Sugimura T
AU - Rosner MR
TI - Tumor promoters block tyrosine-specific phosphorylation of the epidermal growth factor receptor.
AB - Tyrosine-specific phosphorylation of the epidermal growth factor (EGF) receptor in hormonally stimulated A431 cells is blocked by three chemically distinct classes of tumor promoters. Tumor-promoting esters of the diterpene phorbol (phorbol 12-myristate 13-acetate, beta-phorbol 12,13-dibutyrate, and beta-phorbol 12,13-didecanoate), indole alkaloids (teleocidin and lyngbyatoxin A), and polyacetates (aplysiatoxin and debromoaplysiatoxin) all inhibited EGF-stimulated phosphorylation of the receptor. Non-tumor-promoting analogs (beta-phorbol, alpha-phorbol 12,13-didecanoate, and hydrolyzed teleocidin) had no effect on the levels of receptor phosphorylation. The ED50 values of the inhibitory effect (0.1-3 ng/ml) reflected the relative tumor-promoting abilities of these compounds in vivo. None of the tumor promoters tested significantly decreased the overall specific binding of 125I-labeled EGF to A431 cells. Scatchard analysis, however, revealed two apparent EGF receptors in this cell type. The dose-responses for tumor-promoter inhibition of EGF receptor tyrosine phosphorylation and high-affinity EGF binding were similar, suggesting that the same initial event is responsible for both effects. This demonstrates a correlation between modulation of EGF receptor binding and phosphorylation of tyrosine by tumor promoters. The data suggest a possible role for protein kinase C, the putative cellular receptor for these tumor promoters, in the mechanism of action.
SO - Proc Natl Acad Sci USA 1984 May;81(10):3034-8
4
AU - Dotto GP
AU - Parada LF
AU - Weinberg RA
TI - Specific growth response of ras-transformed embryo fibroblasts to tumour promoters.
SI - TOXBIB/86/065474
SO - Nature; VOL 318, ISS 6045, 1985, P472-5
AB - Chemical carcinogenesis is a process involving multiple steps, as shown in several in vivo experimental systems. Two early steps have been well characterized: initiation, achieved by a single, subthreshold dose of a carcinogen, and promotion, induced by repetitive treatments with a non-carcinogenic tumour promoter. At the cellular level, establishment of the transformed phenotype is also a multi-step process and activation of several, independent

genes appears to be required. Here we show that, like initiated cells, primary rat embryo fibroblasts (REFs) containing a ras but not a myc oncogene, are strongly and specifically stimulated to grow by tumour promoters. In the presence of these promoters, ras-containing REFs acquire the ability to overgrow normal cells in the monolayer and to form foci with 100% efficiency. Similar to the in vivo situation, promoter effects can be blocked by the concomitant application of retinoic acid.

7
AU - Hanania N
AU - Castagna M
AU - Shaool D
AU - Zeliszewski D
AU - Harel J
TI - Effects of tumor promoters on the expression of a tumor-related multigenic set in human cells.
SI - TOXBIB/86/053115
SO - Cancer Res; VOL 45, ISS 12 Pt 1, 1985, P6058-62
AB - We previously found that a minor subfraction of the human genomic DNA, corresponding to 2500-3000 nonrepetitive sequences of 3 kilobases each and designated as tumor-activated DNA (TaDNA) was transcriptionally active in Burkitt's lymphoma cells and almost inactive in normal lymphocytes growing in vitro following integration of the Epstein-Barr virus genome. Furthermore all the neoplastic cells in culture or primary neoplasms (leukemias, sarcomas, carcinomas) studied contained transcripts from most of the TaDNA sequences found in malignant lymphoblasts whereas normal cells growing in vitro contained only a few TaDNA transcripts. It is shown in the present study that treatments of the myeloid leukemia HL60 cells with various inducers of cell differentiation (dimethyl sulfoxide, retinoic acid, mezerein, 12-O-tetradecanoylphorbol-13-acetate, teleocidin) caused a dose-dependent reduction of the level of TaDNA transcripts, correlated with the diminution of c-myc transcripts. The 12-O-tetradecanoylphorbol-13-acetate treatment had this same effect on Burkitt's lymphoma cells (Raji or Namalwa) but the opposite effect on normal cells (Epstein-Barr virus-immortalized lymphocytes or fetal fibroblasts) where it enhanced the formation of Ta-DNA transcripts up to the levels found in untreated malignant cells. These data suggest two conclusions (a) TaDNA corresponds to a multigenic set which seems to be involved in modulation of the malignant phenotype and (b) depending on the origin of the cells, agents like 12-O-tetradecanoylphorbol-13-acetate may operate either as tumor promoters or as differentiation inducers through the control of TaDNA expression.

8
AU - Kitahori Y
AU - Konishi N
AU - Shimoyama T
AU - Hiasa Y
TI - Dose-dependent promoting effect of trisodium nitrilotriacetate monohydrate on urinary bladder carcinogenesis in Wistar rats pretreated with N-butyl-N-(4-hydroxybutyl)nitrosamine.
SI - TOXBIB/86/033284
SO - Jpn J Cancer Res; VOL 76, ISS 9, 1985, P818-22
AB - The dose-dependent effect of trisodium nitrilotriacetate monohydrate (Na3.NTA.H2O) as a promoter in 2-stage carcinogenesis in the urinary bladder of male Wistar rats was investigated. Carcinogenesis was initiated by administration of 0.05% N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in the drinking water for 4 weeks, then Na3.NTA.H2O was given at 1%, 0.5% and 0.3% in the diet for 28 weeks, and rats were killed in week 32. The incidences and numbers of preneoplastic lesions [papillary or nodular hyperplasia (PN hyperplasia)] in rats treated with 0.3% to 1% Na3.NTA.H2O increased progressively with increasing concentration of Na3.NTA.H2O. The incidences of papillomas in rats treated with 1% and 0.5% Na3.NTA.H2O in the diet and the incidence of transitional cell carcinoma (TCC) of the urinary bladder in the rats treated with 1% Na3.NTA.H2O (P less than 0.05) were significantly higher than those in rats treated with BBN only. Administration of various doses of Na3.NTA.H2O without BBN did not cause any histological changes (PN hyperplasia, papilloma or TCC) in the urinary bladder. These findings showed that Na3.NTA.H2O is a potent promoter of urinary bladder carcinogenesis initiated by BBN in rats, and that its effect is dose-dependent.

9
AU - Furihata C
AU - Yoshida S
AU - Matsushima T
TI - Potential initiating and promoting activities of diacetyl and glyoxal in rat stomach mucosa.
SI - TOXBIB/86/033282
SO - Jpn J Cancer Res; VOL 76, ISS 9, 1985, P809-14
AB - The potential initiating and promoting activities in the rat glandular stomach of the dicarbonyl compounds diacetyl (DA) and glyoxal (G), which are found in various heated foods, were studied. Administration of DA at doses of 300 to 1500 mg/kg body weight and of G at doses of 150 to 400 mg/kg body weight by gastric intubation to male F344 rats induced up to 100-fold increase in ornithine decarboxylase activity (formation of 195 pmol CO2/30 min/mg protein by DA and 302 pmol CO2/30 min/mg protein by G) with maxima after 16 hr. These treatments also induced a more than 10-fold increase in DNA synthesis (incorporation of 11,400 dpm of [3H]dThd/microgram DNA by DA and 15,100 dpm of [3H]dThd/microgram DNA by G) with maxima after 16 hr, and induced apparent unscheduled DNA synthesis in the pyloric mucosa of the stomach within 3 hr after administration. These results suggest that DA and G have potential tumor-promoting activities and may also have initiating activities in carcinogenesis in the glandular stomach.

12
AU - LeBoeuf RA
AU - Laishes BA
AU - Hoekstra WG
TI - Effects of dietary selenium concentration on the development of enzyme-altered liver foci and hepatocellular carcinoma induced by diethylnitrosamine or N-acetylaminofluorene in rats.
SI - TOXBIB/86/027850
SO - Cancer Res; VOL 45, ISS 11 Pt 1, 1985, P5489-95
AB - Three protocols were used to determine the effects of dietary selenium concentration on the development of gamma-glutamyl-transpeptidase (GGT)-positive foci and hepatocellular carcinoma induced by either diethylnitrosamine (DEN) or N-acetylaminofluorene in rats. In the first experiment, foci were induced by a carcinogenic dose of DEN (100 mg/kg body weight, p.o.) at 20-22 h after two-thirds partial hepatectomy. One wk after DEN administration, during which time 0.1 ppm (representing a control level), 3.0, or 6.0 ppm selenium as Na2SeO3 was fed for 8 or 16 wk, at which time focal analysis was conducted using quantitative stereology. The results demonstrated that 3.0 and 6.0 ppm dietary selenium, initiated 1 wk following carcinogen administration, decreased focal growth rate without affecting the number of GGT foci compared to 0.1 ppm selenium. Decreased focal growth was temporary and reversible with 6.0 ppm selenium which may be related to chronic selenosis observed after 16 wk of 6.0 ppm selenium feeding. A second experiment involved a noncarcinogenic dose of DEN (25 mg/kg body weight, p.o.), then 0.1 or 6.0 ppm selenium feeding for 8 wk, followed by 0.05% phenobarbital (PB), a liver tumor promoter in a diet containing 0.1 ppm selenium. Analysis of GGT foci at 5 or 8 wk of PB feeding indicated that 6.0 ppm selenium caused a trend towards an increase in the number of foci/cm3 of liver and mean focal volume and a significant increase in GGT focal volume as a percentage of liver volume by 8 wk of PB feeding. Thus, high dietary selenium concentrations prior to PB enhance the tumor-promoting ability of PB. In a third experiment, using male Fischer 344 rats (150 g), 0.1 or 6.0 ppm selenium was fed concurrently with 0.02% AAF which was fed in a cyclic regimen. After 4 cycles, where 1 cycle equalled 4 wk of AAF, followed by 1 wk of control diet (0.1 ppm selenium), 6.0 ppm selenium significantly decreased the mean focal volume and focal volume as a percentage of liver volume, while not affecting the number of foci/cm3 of liver, again indicating a selenium effect on focal growth while not affecting the number of "preneoplastic: lesions in the liver. Six ppm selenium feeding after AAF treatment had no effect on the percentage of incidence of hepatocellular carcinoma (100%) but did cause a significant decrease in the percentage of liver volume occupied by macroscopic subcapsular liver lesions compared to 0.1 ppm selenium.(ABSTRACT TRUNCATED AT 400 WORDS)

14
AU - Shirai T
AU - Hosoda K
AU - Hirose K
AU - Hirose M
AU - Ito N
TI - Promoting effects of phenobarbital and 3'-methyl-4-dimethylaminoazobenzene on the appearance of gamma-glutamyltranspeptidase positive foci in rat liver pretreated with varying doses of diethylnitrosamine.
SI - TOXBIB/86/027771
SO - Cancer Lett; VOL 28, ISS 2, 1985, P127-33
AB - The promotion potential of phenobarbital (PB) and 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) on liver carcinogenesis after initiation with various doses of diethylnitrosamine (DEN) was assessed using an in vivo short term system. Male F344 rats were pretreated with a single intraperitoneal injection of varying doses of DEN (0, 6, 12, 25, 50, 100 or 200 mg/kg body wt), and 2 weeks later were treated with 0.05% PB or 0.06% 3'-Me-DAB for 6 weeks. All animals were subjected to partial hepatectomy 3 weeks after the DEN treatment. Quantitation of gamma-glutamyltranspeptidase-positive (gamma-GT+) foci revealed a DEN dose-dependent response. Magnitude of promotion by PB and more pronounced by 3'-Me-DAB was, in contrast, strongest at the lower doses of DEN. The results suggest that quantitative differences with regard to initiation level may exist, influencing the promotability of initiated cells.

17
UI - 81219003
AU - Moolgavkar SH
AU - Knudson AG Jr
TI - Mutation and cancer: a model for human carcinogenesis.
AB - A model for carcinogenesis is presented that provides a framework for understanding the roles of "spontaneous: events, hereditary factors, and environmental agents in human carcinogenesis and for interpreting experimental carcinogenesis. This model incorporates two features: a) transition of target stem cells into cancer cells via an intermediate stage in two irreversible steps, and b) growth and differentiation of normal target and intermediate cells. Cast in mathematical terms, the model can be fitted to age-specific incidence data on human cancers of both children and adults and can illuminate the relative importance of agents that affect transition rates, tissue growth, and tissue differentiation. This is illustrated by application of the model to a) the epidemiology of lung cancer with emphasis on the role of cigarette smoking and b) the epidemiology of breast cancer with emphasis on the roles of hormones, radiation, and hereditary. The nature of the two events and of the intermediate stage is considered in light of hereditary conditions that predispose to cancer in humans. The modes of action of radiation and chemicals in carcinogenesis are discussed, as are predictions based on the model and amenable to experimental verification.
SO - JNCI 1981 Jun;66(6):1037-52

18
AU - Degen JL
AU - Estensen RD
AU - Nagamine Y
AU - Reich E
TI - Induction and desensitization of plasminogen activator gene expression by tumor promoters.
SI - TOXBIB/86/008324
SO - J Biol Chem; VOL 260, ISS 23, 1985, P12426-33
AB - Tumor promoting phorbol esters and mezerein strongly induced plasminogen activator (urokinase, uPA) synthesis in porcine kidney cell cultures (LLC-PK1). Induction was due to increased uPA-mRNA levels which rose from 10 to 300 molecules/cell within 2 h of exposure to 16 nM phorbol myristate acetate. We have compared the action of tumor promoters with that of 8-bromo-cAMP, another potent inducer of uPA; the similarities between the two kinds of induction were: both involved transcriptional activation of the uPA gene; both were rapid in onset, changes in transcription rate being detectable within 10-20 min; the initial rates of transcription and uPA-mRNA accumulation were substantial and in the same order of magnitude; neither class of inducer required protein synthesis to stimulate uPA transcription. The main contrast between the two types of agents was that the uPA response to tumor promoters was transient whereas that to cAMP compounds was sustained: cultures rapidly lost their response to tumor promoters within 2 h after initial exposure while retaining responsiveness to cAMP-related agents. The cells developed a specific drug-induced desensitization which was slowly reversed after tumor promoters were removed from the culture medium. Since protein kinase C is now well established as the receptor for phorbol-derived and several other tumor promoters it will be of interest to determine whether desensitization occurs at the level of receptor.

19
AU - Mazzoleni G
AU - Ragnotti G
AU - Enomoto T
AU - Yamasaki H
TI - Influence on cell-cell communication (dye-transfer) of the oncogenic beta-blocker DL-ZAMI 1305: possible relation to tumor promotion.
SI - TOXBIB/86/002633
SO - Carcinogenesis; VOL 6, ISS 10, 1985, P1477-82
AB - The effect of the oncogenic beta-blocker DL-1-(2-nitro-3-methyl-phenoxy)-3-tert-butyl-amino-propan-2-ol (DL-ZAMI 1305) on intercellular communication between cultured cells was studied. Intercellular communication of Chinese hamster V79 cells was measured by the dye-transfer method in which the spread of intracellularly microinjected fluorescent probe, Lucifer Yellow CH, through gap-junctions was used as an index of intercellular communication. When V79 cells are cultured with non-toxic doses (1-60 micrograms/ml) of DL-ZAMI 1305, a significant inhibition of dye-transfer is observed after 4 h. The inhibition is dose-related and greater than 90% inhibition is seen at the dose of 50 micrograms/ml. When DL-ZAMI 1305 is added at 0 and 24 h of experiment, its inhibitory effect is maintained for at least 48 h at high doses (50-60 micrograms/ml), whereas for lower doses of DL-ZAMI 1305, some recovery is seen after 24 h incubation. These results are suggestive of a possible tumor-promoting activity of DL-ZAMI 1305; in vivo studies on this carcinogen are in progress.

20
AU - Hurley DJ
AU - Mastro AM
TI - Changes in a T-cell subpopulation marker induced by tumor-promoting phorbol esters.
SI - TOXBIB/86/002627
SO - Carcinogenesis; VOL 6, ISS 10, 1985, P1435-40
AB - We observed a significant reduction in one specific T-cell rosetting marker after treatment of bovine lymph node lymphocytes with 12-O-tetradecanoylphorbol-13-acetate (TPA). There was a dose-dependent reduction in the formation of neuraminidase-treated sheep erythrocyte rosettes (En), but not aminoethylisothiouronium bromide-treated sheep rosettes (Ea) after as little as 10 min of incubation with TPA. A maximum of approximately 50% reduction was reached after 1 h. When several phorbol esters and mezerien were tested, we found that the reduction in En rosetting induced by the compounds correlated with their in vivo tumor-promoting activity. The reduction in En rosetting appears to be approximately 50% reversible for up to 6 h of treatment with TPA when the cells were incubated in TPA-free medium for an additional 24 h, but it was irreversible after at least 12 h of treatment. Addition of the tumor promoters directly to the rosetting assay had no detectable effect on the sheep erythrocytes or the percentage of Ea rosettes. This specific change in En rosetting marker may represent the maturation of a T-cell subpopulation to T-suppressor cells in the presence of tumor-promoting phorbol esters.

22
AU - Iversen OH
TI - TPA (12-O-tetradecanoylphorbol 13-acetate) as a carcinogen for mouse skin. A positive dose-response relationship
SI - CA/103/173746W
SO - Virchows Arch., B; VOL 49, ISS 2, 1985,129-35
AB - CBAC COPYRIGHT: CHEM ABS TPA (I) [16561-29-8] was applied on the back skin of hairless mice at different doses and schedules to study a dose-response relationship. There was a significant incidence of papillomas and some carcinomas in the mouse skin. There was a dose-response relationship in the development of skin tumors. TPA is a complete carcinogen and not merely a promoter.

23
AU - Elcombe CR
AU - Rose MS
AU - Pratt IS
TI - Biochemical, histological, and ultrastructural changes in rat and mouse liver following the administration of trichloroethylene: possible relevance to species differences in hepatocarcinogenicity.
SI - TOXBIB/85/301244
SO - Toxicol Appl Pharmacol; VOL 79, ISS 3, 1985, P365-76
AB - Trichloroethylene (TRI), administered by gavage for 10 consecutive days, at doses of 500 to 1500 mg/kg body wt increased liver weight (175% of control), decreased hepatic DNA concentration (66% of control), and increased the synthesis of DNA (500% of control; as measured by [3H]dT incorporation) in B6C3F1 mice and Alderley Park mice. Similar treatment of Osborne-Mendel rats or Alderley Park rats resulted in smaller increases in liver weight (130% of control) and decreases in DNA concentration (83% of control). No effect of TRI on DNA synthesis was seen in rats. The increased DNA synthesis in the mouse was not apparently due to regenerative hyperplasia since no signs of necrosis were seen. Furthermore the increased [3H]dT incorporation probably represented semiconservative replication of DNA and not repair, since a parallel increase of mitotic figures was observed. Hence, the liver growth noted after TRI administration appears to be due to liver cell enlargement (hypertrophy) in the rat, but both hypertrophy and hyperplasia (cell proliferation) in the mouse. An important observation has been that TRI induced the peroxisomal enzyme activities, catalase, and cyanide-insensitive palmitoyl-CoA oxidation (147 and 786% of control, respectively), in mice but not in rats. Furthermore, increases in peroxisome volume density (up to 1110% of control) were observed in mice receiving TRI. These observations lead us to suggest that the species difference in hepatocarcinogenicity of TRI, seen between the rat and mouse, is possibly due to a species difference in peroxisome proliferation and cell proliferation, the peroxisome proliferation leading to increased reactive oxygen species and DNA damage, and the cell proliferation then acting to promote this lesion.

24
AU - Wardlaw GM
TI - Assessing the cancer risk from foods.
SI - TOXBIB/85/290476
SO - J Am Diet Assoc; VOL 85, ISS 9, 1985, P1122-7
AB - Assessing the carcinogenicity of a compound and then determining what dosage is appropriate for human beings is a complex process. Carcinogens act either by altering deoxyribonucleic acid (DNA) or by promoting the growth of already altered cells. Carcinogenicity is evaluated with the use of structural analysis, in vitro mutagenesis assays, epidemiological findings, and dose-response studies in laboratory animals. In the animal studies, high doses are administered. Once a compound is found to be carcinogenic, the dose that will pose an acceptable cancer risk to human beings--one in a million--must be extrapolated from the high-dose data. This virtually safe dose (VSD) will be the allowable dosage for human contact. The extrapolation from high-dose animal studies to a VSD for human beings is based on the models for carcinogenic mechanisms. Debate exists as to how many interactions with DNA the carcinogen must have to initiate neoplastic growth and whether there exists a threshold for carcinogenic action below which there is no risk of cancer. The extrapolation model that is chosen greatly affects the VSD. Knowing how this extrapolation to a VSD is done will help dietitians better understand how allowable levels for carcinogens in foods are determined.

29
AU - Greim H
AU - Deml E
AU - Oesterle D
TI - Drugs and environmental chemicals as promoters.
SI - TOXBIB/85/259928
SO - IARC Sci Publ, ISS 56, 1984, P487-94

30
AU - Frayssinet C
TI - The principle of a threshold dose in chemical carcinogenesis.
SI - TOXBIB/85/258278
SO - Food Addit Contam; VOL 1, ISS 2, 1984, P89-94
AB - The nature of at least three stages in carcinogenesis by chemical agents justifies the existence of a threshold dose: (1) a cytoplasmic stage during which most carcinogenic molecules are eliminated, (2) a nuclear stage during which certain DNA lesions are repaired and therefore cannot help to bring about mutations, and (3) an extracellular stage during which mutations are controlled for a long time by positive or negative epigenetic factors. All these findings are very difficult to collate. Since threshold doses cannot be detected by direct experimentation, the knowledge that a threshold exists is of little practical use and does not much alter the data permitting extrapolation of the results obtained with high doses of carcinogens. At the theoretical level only, this conclusion renders obsolete the opinion that all carcinogenic agents should be completely eliminated from our environment, and also the view that DNA needs only one hit from such an agent for a carcinogenic effect to be produced. The above conclusion also provides a more rational basis for the concept of a 'virtually safe dose' (VSD) or 'allowable daily intake' (ADI).

32
AU - Stanley MA
AU - Crowcroft NS
AU - Quigley JP
AU - Parkinson EK
TI - Responses of human cervical keratinocytes in vitro to tumour promoters and diethylstilboestrol.
SI - TOXBIB/85/255318
SO - Carcinogenesis; VOL 6, ISS 7, 1985, P1011-5
AB - We have compared the responses of normal human cervical keratinocytes (HCE) to diethylstilboestrol (DES), and the promoting agents, phorbol-12-myristate-13-acetate (PMA) and mezerein using the loss of cloning efficiency as a measure of terminal differentiation in vitro. Dose-response studies showed that normal HCE are growth inhibited by chronic exposure to DES at concentrations greater than or equal to 2.5 X 10(-5) M, to PMA at concentrations greater than 10(-8) M and mezerein at concentrations greater than 10(-9) M. Compared to acetone controls, promoter or DES-treated cells exhibited a 10- to 12-fold increase in cornified-envelope formation. Normal HCE exhibit a heterogeneous response to PMA in that 85-90% of colony-forming cells lose their colony-forming ability after a 24-h exposure to 10(-6) M PMA. The PMA-resistant subpopulation, PMAR, remains constant and is not reduced even after 96 h chronic exposure to PMA. In contrast, the colony-forming ability of normal HCE is almost totally suppressed after 24 h exposure to 10(-6) M mezerein. After 24 h incubation with 5 X 10(-5) M DES, 20% of normal HCE are capable of colony formation but this resistant fraction is eliminated after 96 h chronic exposure. Cornified-envelope formation was negligible in malignant cervical keratinocytes grown in the presence of DES or promotors and these cells were characterised by a very large PMAR fraction - 85 - 90% of cells retained colony-forming ability after exposure to 10(-6) M PMA for 24 h. Furthermore, 90-100% of malignant cervical keratinocytes retained their colony-forming capacity after exposure to 10(-6) M mezerein. However, colony-forming ability declined steadily in the presence of 5 X 10(-5) M DES and after 96 h only a tiny fraction, 1% of malignant cervical keratinocytes could form colonies on replating. The mechanisms by which DES inhibits growth and induces cornified-envelope formation in HCE would appear to be distinct from those activated by PMA and mezerein.

34
AU - Schm:ahl D
TI - Critical remarks on the validity of promoting effects in human carcinogenesis.
SI - TOXBIB/85/234613
SO - J Cancer Res Clin Oncol; VOL 109, ISS 3, 1985, P260-2

35
AU - Kabelitz D
TI - Modulation of natural killing by tumor promoters: the regulatory influence of adherent cells varies with the type of target cell.
SI - TOXBIB/85/233049
SO - Immunobiology; VOL 169, ISS 4, 1985, P436-46
AB - We have analyzed the regulatory effects of two classes of tumor promoters, phorbol diesters and indole alkaloids, on human natural killer (NK) cell activity in vitro. In accordance with previous reports, we found that 12-O-tetradecanoylphorbol-13-acetate (TPA) inhibited natural killing against K 562 targets by unseparated mononuclear cells. Here, suppression of NK required the presence of adherent cells (macrophages). Contrary to the results obtained with K 562, tumor promoter-induced suppression of NK activity tested against U 937, another cell line of known NK susceptibility, was independent of the presence of adherent cells. Thus, NK cytotoxicity of effector cells rigorously depleted of adherent and Ia-positive cells was still inhibited when assayed against U 937, while it was generally enhanced when tested against K 562. Identical results were obtained with teleocidin and dihydroteleocidin B, two members of the recently discovered indole alkaloid class of tumor promoters. Therefore, we demonstrate that the regulatory effect of tumor promoters on human NK activity (suppression or stimulation) is determined not only by macrophages at the effector cell level but also by the type of target cell under study.

36
AU - Edwards AM
AU - Lucas CM
TI - Induction of gamma-glutamyl transpeptidase in primary cultures of normal rat hepatocytes by liver tumor promoters and structurally related compounds.
SI - TOXBIB/85/228558
SO - Carcinogenesis; VOL 6, ISS 5, 1985, P733-9
AB - Rat hepatocytes maintained for up to 6 days in primary culture were used to test a variety of xenobiotics and steroids for effects on the activity of gamma-glutamyltranspeptidase (GGT) in normal cells. In control cultures GGT activity was low and increased slowly with time. When added to cultures for 5 days, a variety of xenobiotics and steroids increased GGT activity to levels 2- to 6-times those of control cultures. Induction of GGT was potentiated for most test compounds by 20-30 nM dexamethasone and diminished by nicotinamide or adenosine-3',5'-monophosphate. Effective non-genotoxic inducers included phenobarbital and some structurally related compounds, p,p'-dichlorodiphenyltrichloroethane,alpha- and gamma-hexachlorocyclohexanes, Aroclor 1254, butyl hydroxytoluene, nafenopin, various estrogens, progesterone, pregnenolone-16 alpha-carbonitrile and cyproterone acetate. A number of compounds including barbituric acid, butyl hydroxyanisole, acetaminophen, saccharin, caffeine, clofibrate and some bile acids failed to induce GGT. Except for 2-acetylaminofluorene and diethylnitrosamine, genotoxic compounds tested did not increase GGT. The results establish that a structurally diverse group of xenobiotics and steroids, many of which are considered to be liver tumour promoters, may directly enhance GGT gene expression in normal hepatocytes. Thus, a variety of compounds used in experimental studies of liver cancer induction as promoters may elevate GGT by mechanism(s) not necessarily related to carcinogenesis.

38
AU - Pasquinelli P
AU - Bruschi F
AU - Saviozzi F
AU - Malvaldi G
TI - Immunosupressive effects and promotion of hepatic carcinogenesis by thiobenzamide
SI - CA/103/018151E
SO - Boll. - Soc. Ital. Biol. Sper.; VOL 61, ISS 1, 1985,61-6
AB - CBAC COPYRIGHT: CHEM ABS Subacute administration of thiobenzamide [2227-79-4] (1 g/kg diet for 4 wk) to rats, following a single dose of diethylnitrosamine (100 mg/kg, s.c.) as initiator, promoted the growth of hepatocellular preneoplastic lesions (increase in gamma-glutamyltranspeptidase-pos. hepatocellular foci). When thiobenzamide was given at the above dose without the initiator, it depressed the response of the immune system to a T-cell-dependent antigen (sheep red cells). This immunosuppressant action of thiobenzamide may be part of its tumor-promoting mechanisms.

40
AU - Zeggari M
AU - Susini C
AU - Viguerie N
AU - Esteve JP
AU - Vaysse N
AU - Ribet A
TI - Tumor promoter inhibition of cellular binding of somatostatin.
SI - TOXBIB/85/199140
SO - Biochem Biophys Res Commun; VOL 128, ISS 2, 1985, P850-7
AB - Tumor promoting phorbol esters inhibited the binding of 125I-[Tyr11] somatostatin to isolated acinar cells from guinea-pig pancreas. Maximal inhibition reached 69.7 +/- 5% at 1 microM TPA. Receptor affinity was decreased by 2.5-fold without change in binding capacity. The ability of TPA in inhibiting somatostatin binding was decreased in 30 nM Ca2+ medium, abolished at 4 degrees C or in a membrane preparation. The effect of caerulein, a secretagogue which also caused loss of binding, and that of TPA were not additive. We concluded that TPA inhibits somatostatin binding not by binding directly at the active site of somatostatin receptor. TPA may act at a later point than caerulein via a similar pathway to modulate somatostatin receptor affinity.

44
AU - DiGiovanni J
AU - Decina PC
AU - Prichett WP
AU - Cantor J
AU - Aalfs KK
AU - Coombs MM
TI - Mechanism of mouse skin tumor promotion by chrysarobin.
SI - TOXBIB/85/176685
SO - Cancer Res; VOL 45, ISS 6, 1985, P2584-9
AB - The skin tumor-promoting ability of 1,8-dihydroxy-3-methyl-9-anthrone (chrysarobin) was compared with that of 12-O-tetradecanoylphorbol-13-acetate (TPA) and 1,8-dihydroxy-9-anthrone (anthralin) in SENCAR mice. Although dose-response comparisons indicated that chrysarobin was several orders of magnitude less potent than TPA for promoting papilloma formation, this anthrone was 1.5 to 2 times more potent than anthralin. Maximal papilloma responses were achieved by 15 weeks of promotion with TPA whereas at least 25 weeks of promotion were necessary to achieve maximal papilloma responses with chrysarobin or anthralin indicating marked differences in tumor latency between the two classes of compounds. Interestingly, at optimal promoting doses, chrysarobin gave a carcinoma response (22% with 0.3 carcinomas per mouse at 45 weeks) similar to that of TPA suggesting that this compound may be more efficient at promoting carcinomas than papillomas. In two-stage promotion experiments, chrysarobin was incapable of functioning independently as a Stage I or II promoter despite its complete promoting activity. Chrysarobin and TPA were compared at optimal promoting doses for their ability to induce: (a) skin edema, (b) epidermal hyperplasia, and (c) epidermal ornithine decarboxylase. In each case, distinct differences were noted between the two compounds. When taken together, the data support the hypothesis that anthracene-derived skin tumor promoters work at least in part by a mechanism different from the phorbol esters.

46
AU - Diwan BA
AU - Ward JM
AU - Rice JM
AU - Colburn NH
AU - Spangler EF
TI - Tumor-promoting effects of di(2-ethylhexyl)phthalate in JB6 mouse epidermal cells and mouse skin.
SI - TOXBIB/85/152346
SO - Carcinogenesis; VOL 6, ISS 3, 1985, P343-7
AB - Di(2-ethylhexyl)phthalate (DEHP), a confirmed promoter of hepatocellular carcinogenesis in mice, was studied for tumor-promoting activity on the skin of SENCAR mice in vivo and on JB6 mouse epidermal cells in vitro. DEHP enhanced transformation to anchorage-independent growth of promotable JB6 clonal lines, C141, C121, and R219. DEHP induced colony formation in concentrations from 500 p.p.m. to 20 000 p.p.m./ml culture medium with maximum induction occurring at 10 000 p.p.m./ml. DEHP was inactive as a complete promoter of skin carcinogenesis initiated by 7,12-dimethylbenz[a]anthracene (DMBA) in SENCAR mice. Like the plant-derived natural product mezerein, however, DEHP significantly enhanced skin carcinogenesis in SENCAR mice when initiation by DMBA was followed by short term applications (2x/week, 2 weeks) of 12-O-tetradecanoylphorbol-13-acetate prior to application of DEHP. A random sample of the tumors observed was processed and examined histologically. Most neoplasms were papillomas; a few were squamous cell carcinomas. The kinds of tumors seen were identical with those reported in two-stage carcinogenesis experiments in SENCAR mice in which phorbol ester tumor promoters have been employed. Thus, DEHP promotes transformation in JB6 mouse epidermal cells and acts as a second-stage promoter in mouse skin carcinogenesis. DEHP therefore has promoting capability for at least two distinct kinds of epithelium in mice.

57
AU - Kitagawa T
AU - Hino O
AU - Nomura K
AU - Sugano H
TI - Dose-response studies on promoting anticarcinogenic effects of phenobarbital and DDT in the rat hepatocarcinogenesis
SI - CA/102/107909E
SO - Carcinogenesis (London); VOL 5, ISS 12, 1984,1653-6
AB - CBAC COPYRIGHT: CHEM ABS Possible discrepancy between the dose level required for promoting action, when given after the initiation process, and that needed to exert an anticarcinogenic effect when given simultaneously with a carcinogen, of hepatic promoters were investigated in an attempt to obtain a practical threshold dose for promoters. Phenobarbital (PB)(I) [50-06-6] and DDT [50-29-3] were used as promoters and 3'-methyl-4-(dimethylamino)azobenzene (3'-Me-DAB) [55-80-1] was used as the carcinogen. Male weanling rats were treated with 600 ppm 3'-Me-DAB for 3 wk followed by a diet contg. a promoter at various dose levels (5-500 ppm), or the animals were treated with a low dose (100 ppm) of 3'-Me-DAB plus a promoter at various dose levels (20-500 ppm). The effects of promoters were measured by scoring size and no. of enzyme-altered islands at weeks 12 and 24 of rat age. The promoting effect of PB and DDT was demonstrated in dose-dependent fashion, in the dose range 10-500 ppm and 20-500 ppm, resp. However, promoters given simultaneously with a low dose of carcinogen enhanced the carcinogenesis at all the dose levels tested, quite in contrast with their inhibitory effect on carcinogenesis when given together with relatively high doses of carcinogens.

59
AU - Kopelovich L
AU - Chou TC
TI - The proliferative response of low-density human cell cultures to tumor promoters and its relevance to carcinogenic mechanisms in vitro
SI - CA/102/091178C
SO - Int. J. Cancer; VOL 34, ISS 6, 1984,781-8
AB - CBAC COPYRIGHT: CHEM ABS The effects of the tumor promoter TPA (I) [16561-29-8], its analogs, and other tumor promoters on the proliferation of human skin fibroblasts (SF) were studied. The biphasic dose-response pattern, consisting of an inhibitory phase and a stimulatory phase, was shared by the active analogs of TPA, e.g., phorbol 12,13-dibutyrate [37558-16-0] and phorbol 12,13-dibenzoate [25405-85-0]. This biphasic dose-effect relation, however, was not seen with phorbol 12,13-diacetate [24928-15-2] or the inactive analogs of TPA such as phorbol [17673-25-5] and 4-O-methyl-12-O-tetradecanoylphorbol 13-acetate [57716-89-9], nor was it seen with mezerein [34807-41-5], teleocidin [78474-55-2], cholic acid [81-25-4], deoxycholic acid [83-44-3], lithocholic acid [434-13-9], or chenocholic acid [474-25-9]. An anal. of the cloning efficiency data by the median-effect equation (T. C. Chou and P. Talalay, 1981) showed that in low-d. cultures both the inhibitory phase and the stimulatory phase of the dose-effect relation of TPA, its analogs, mezerein, and teleocidin exhibited a linear median-effect plot and thus closely followed the basic mass-action principle. The median-effect plot of these data allowed quant. detn. of growth curve characteristics such as regression coeff., slope (a measure of sigmoidicity), median-effect concns. such as I50 for the inhibitory effect and A50 for the stimulatory effect (i.e., the relative potency of the analogs), and the transition point of the biphasic phenomenon in the case of the phorbol esters. In addn., a relation was demonstrated between the dose-response effect of TPA on the proliferation of various human cells and tumor progression in vitro.

61
AU - Jones TD
TI - A unifying concept for carcinogenic risk assessments: comparison with radiation-induced leukemia in mice and men.
SI - TOXBIB/85/079234
SO - Health Phys; VOL 47, ISS 4, 1984, P533-56
AB - This paper presents a new, general mathematical dose-response model which can use human, animal and cell culture data to predict the incidence of leukemia as a result of exposure to ionizing radiations. The model is based on simple considerations of fundamental biological processes of carcinogenic initiation, carcinogenic promotion and competing risk due to other toxic or disease reactions. The model can be used to predict the risk of leukemia for either human or animal populations which have been (or will be) treated with any radiation dose-time treatment protocol of interest. The model is both an extension and an outgrowth of earlier work done for the Oak Ridge dosimetry program in support activities for the Atomic Bomb Casualty Commission (formerly) and the Radiation Effects Research Foundation (currently).

82
AU - Boyland E
TI - Some actual aspects of tumor induction and promotion.
SI - TOXBIB/84/264731
SO - J Cancer Res Clin Oncol; VOL 108, ISS 1, 1984, P3-5
AB - Quantitative data on chemical carcinogenesis illustrate how difficult it is to decide whether there are thresholds for complete carcinogens. With tumor promoters there probably are safe levels, but these are difficult to determine. The results recorded in the experiments of Fibiger, in which tumors of the stomach were seen, could have been caused by the tumor-promoting effects of a biological agent.

88
AU - Perera FP
TI - The genotoxic/epigenetic distinction: relevance to cancer policy.
SI - TOXBIB/84/207826
SO - Environ Res; VOL 34, ISS 1, 1984, P175-91 (REF: 88)
AB - Should federal agencies use separate, less stringent guidelines for regulating epigenetic or nongenotoxic carcinogens on the assumption that thresholds are likely to exist for these agents? This article reviews recent initiatives by the Environmental Protection Agency that either propose or informally adopt this approach in light of responses from the scientific community and a review of the recent literature. Relevant background is provided by current research concerning the role of chromosomal damage and oncogene activation in carcinogenesis along with findings that classical promoters or "epigenetic: agents can induce both DNA damage and chromosomal rearrangements. The conclusion is that such a revision of cancer policy is not now supported by available scientific data concerning chemical carcinogenesis.

89

AU - Schwarz M
AU - Pearson D
AU - Port R
AU - Kunz W
TI - Promoting effect of 4-dimethylaminoazobenzene on enzyme altered foci induced in rat liver by N-nitrosodiethanolamine.
SI - TOXBIB/84/205974
SO - Carcinogenesis; VOL 5, ISS 6, 1984, P725-30
AB - Female Wistar rats were treated sequentially with 4-dimethylaminoazobenzene (4-DAB) and N-nitrosodiethanolamine (NDEOL) for periods of 6 weeks. One group received first 4-DAB (0.06% in the diet) and NDEOL (2000 p.p.m. in the drinking water) thereafter, while the second group was treated in the reversed sequence; control groups received the single agent alone. The extent of foci negative for adenosine-triphosphatase (ATPase) or positive for gamma-glutamyl-transpeptidase (gamma-GT)-activity was quantitated in liver as a means to assess carcinogenic efficacy. A very low response was obtained in rats treated first with 4-DAB and then with NDEOL whereas a strong increase in number and especially in size of foci was observed when 4-DAB was given after NDEOL . The response in this latter group was clearly over-additive. Treatment of rats with either carcinogen alone resulted in similar pattern of increases in the volumetric fraction of liver occupied by ATPase-deficient foci. A differential behaviour, nowever, was observed with respect to islet size. NDEOL produced large numbers of small foci whereas with 4-DAB only few foci were obtained which grew rapidly in the presence of the carcinogen. These findings are consistent with the hypothesis that 4-DAB, besides acting as an initiator, has very strong promoting activity as was to be expected from the characteristic relationship between carcinogen dose and time of liver tumour induction.

93
AU - Takada K
AU - Zur Hausen H
TI - Induction of Epstein-Barr virus antigens by tumor promoters for epidermal and nonepidermal tissues.
SI - TOXBIB/84/160766
SO - Int J Cancer; VOL 33, ISS 4, 1984, P491-6
AB - Fifteen established tumor promoters belonging to different chemical groups were tested for their ability to induce Epstein-Barr virus (EBV)-specific early antigens (EA) in EBV-genome-positive nonproducer Raji cells. Saccharin (a promoter in urinary bladder carcinogenesis), DDT (a promoter in liver carcinogenesis), anthralin and iodoacetic acid (promoters in skin carcinogenesis) gave a significant induction with a maximum of induced cells of 20% (8 mg/ml), 0.8% (20 micrograms/ml), 0.8% (100 ng/ml) and 0.7% (0.4 micrograms/ml), respectively. In addition, after combined application with a noninducing dose (0.2 ng/ml) of 12-O-tetradecanoyl-phorbol-13-acetate (TPA), seven additional tumor promoters induced 0.3-2.1% EA-positive cells two days after treatment. The results indicate that in addition to mouse skin tumor promoters such as diterpene esters, several compounds reported to possess tumor-promoting activity in other types of tissue induce EBV. The data suggest that EBV induction is an effect commonly exerted by this group of compounds which should be very useful in screening for environmental tumor promoters.

101
AU - Upton AC
TI - Environmental standards for ionizing radiation: theoretical basis for dose-response curves.
SI - TOXBIB/84/084519
SO - Environ Health Perspect; VOL 52, 1983, P31-9
AB - The types of injury attributable to ionizing radiation are subdivided, for purposes of risk assessment and radiological protection, into two broad categories: stochastic effects and nonstochastic effects. Stochastic effects are viewed as probablistic phenomena, varying in frequency but not severity as a function of the dose, without any threshold; nonstochastic effects are viewed as deterministic phenomena, varying in both frequency and severity as a function of the dose, with clinical thresholds. Included among stochastic effects are heritable effects (mutations and chromosome aberrations) and carcinogenic effects. Both types of effects are envisioned as unicellular phenomena which can result from nonlethal injury of individual cells, without the necessity of damage to other cells. For the induction of mutations and chromosome aberrations in the low-to-intermediate dose range, the dose-response curve with high-linear energy transfer (LET) radiation generally conforms to a linear nonthresnold relationship and varies relatively little with the dose rate. In contrast, the curve with low-LET radiation generally conforms to a linear-quadratic relationship, rising less steeply than the curve with high-LET radiation and increasing in slope with increasing dose and dose rate. The dose-response curve for carcinogenic effects varies widely from one type of neoplasm to another in the intermediate-to-high dose range, in part because of differences in the way large doses of radiation can affect the promotion and progression of different neoplasms. Information about dose-response relations for low-level irradiation is fragmentary but consistent, in general, with the hypothesis that the neoplastic transformation may result from mutation, chromosome aberration or genetic recombination in a single susceptible cell.

113
AU - Tsushimoto G
AU - Chang CC
AU - Trosko JE
AU - Matsumura F
TI - Cytotoxic, mutagenic, and cell-cell communication inhibitory properties of DDT, lindane, and chlordane on Chinese hamster cells in vitro
SI - CA/099/207745M
SO - Arch. Environ. Contam. Toxicol.; VOL 12, ISS 6, 1983,721-9
AB - CBAC COPYRIGHT: CHEM ABS The cytotoxicity, mutagenicity, and in vitro inhibition of metabolic cooperation of DDT [50-29-3], lindane [58-89-9], and chlordane [12789-03-6] were studied with Chinese hamster V-79 cells. The differential cytotoxicity of the pesticides was: lindane DDT chlordane. There was no detectable mutagenic activity of any of these pesticides, using 2 genetic markers (6-thioguanine and diphtheria toxin resistance). DDT and lindane, however, had properties similar to TPA (a powerful mouse skin tumor promoter). Above what appeared to be a threshold level, there was a clear dose response of DDT and lindane in the in vitro cell-cell communication assay. Chlordane, which was the most cytotoxic pesticide tested, did not inhibit metabolic cooperation as significantly as did DDT or lindane. The role of these pesticides in carcinogenesis was speculated as being their tumor promoting properties, either at noncytotoxic levels by mimicing TPA-like membrane alterations or at cytotoxic levels by mimicing partial hepatectomy.

117
AU - BOYLAND E
TI - SIGNIFICANCE OF PROMOTERS IN DOSE RESPONSE AND EVALUATION OF CARCINOGENIC RISKS
SI - EMIC/81/002989
SO - HEALTH RISK ANAL,PROC LIFE SCI SYMP 3RD(1980); 181-193,1981
AB - EMIC/ORNL

AU - Stott WT
AU - Watanabe PG
TI - Differentiation of genetic versus epigenetic mechanisms of toxicity and its application to risk assessment.
SI - TOXBIB/83/052799
SO - Drug Metab Rev; VOL 13, ISS 5, 1982, P853-73 (REF: 87)

123
AU - Murray AW
AU - Fitzgerald DJ
AU - Guy GR
TI - Inhibition of intercellular communication by tumor promoters.
SI - TOXBIB/82/162591
SO - Carcinog Compr Surv; VOL 7, 1982, P587-91

126
AU - Kopelovich L
TI - Genetic forms of neoplasia in man: a model for the study of tumor promotion in vitro.
SI - TOXBIB/82/162552
SO - Carcinog Compr Surv; VOL 7, 1982, P259-71
AB - In the studies described here, we demonstrated a unique dose-response (concaved upward) to TPA in sparse human cell cultures and a dose-dependent stimulation of cell proliferation in confluent cultures, suggesting a functional difference in putative TPA receptors between these two conditions. This bimodal dose-response to TPA was not seen in sparse cultures of established normal rodent cell lines. The phenotypic profile of ACR cells chronically exposed to TPA, although effecting a change toward a more transformed phenotype (e.g., growth in agar), was in large measure neither stable nor uniform during consecutive passages or for a given cell strain during different periods of TPA application. ACR cells when exposed to TPA alone appear to grow in the anterior chamber of the eye of a nude mouse. We speculated that TPA-induced aneuploidy in these cells, coupled with DNA instability and aberrant chromosomal segregation, may conceivably be consistent with neoplasia in initiated ACR cells. Finally, the apparent susceptibility of ACR cells to further transformation by TPA and N-methyl-N1-nitro-N-nitrosoguanidine (MNNG) (34,48) and by ocongenic viruses (37,45) indicates that genetic information residing within these cells, probably in the form of an ACR mutation, renders them more sensitive to these two distinct classes of carcinogens.

127
AU - Ito N
AU - Tatematsu M
AU - Nakanishi K
AU - Tsuda H
TI - Analysis of the effects of promoting agents on liver and urinary bladder carcinogenesis in rats.
SI - TOXBIB/82/162540
SO - Carcinog Compr Surv; VOL 7, 1982, P133-7

128
AU - Kunz W
AU - Schaude G
AU - Schwarz M
AU - Tennekes H
TI - Quantitative aspects of drug-mediated tumour promotion in liver and its toxicological implications.
SI - TOXBIB/82/162537
SO - Carcinog Compr Surv; VOL 7, 1982, P111-25 (REF: 51)

150
AU - WILLIAMS GM
TI - Liver carcinogenesis: The role for some chemicals of an epigenetic mechanism of liver-tumor promotion involving modification of the cell membrane.
SI - HEEP/82/10561
SO - FOOD COSMET TOXICOL; 19 (5). 1981. 577-584.
AB - HEEP COPYRIGHT: BIOL ABS. Chemicals that produce tumors exclusively or primarily in the liver of rodents following prolonged administration at high dose levels, that show no capacity to induce genetic damage, but that enhance the carcinogenic effect of previously administered genotoxic carcinogens are identified as epigenetic carcinogens of the promoter class. Recent findings suggest that these chemicals may affect the state or function of the cell membrane in such a way as to interfere with the transmission of regulatory factors from normal to spontaneously altered cells, thus releasing the latter for progressive neoplastic growth. This hypothesis is consistent with the dose-response characteristics of carcinogens of this type.

151
AU - SCHULTE-HERMANN R
AU - PARZEFALL W
TI - Failure to discriminate initiation from promotion of liver tumors in a long-term study with the phenobarbital-type induced alpha-BHC and the role of sustained stimulation of hepatic growth and monooxygenases.
SI - HEEP/82/05052
SO - CANCER RES; 41 (10). 1981. 4140-4146.
AB - HEEP COPYRIGHT: BIOL ABS. alpha-HCH (BHC) was administered orally to female Wistar rats for periods of up to 33 mo.; doses were 20 mg/kg per day, 200 mg/kg every 2nd wk or 420 mg/kg every 3rd wk. Increases of liver size, DNA, RNA and protein (by 50-100%) and of drug-metabolizing enzyme activities (up to 300%) persisted after approximately 1/3, 1 and 2 yr of treatment. At 1 and 2 yr, DNA synthesis was measured by (3H)thymidine uptake and was no higher than in controls. All changes regressed upon withdrawal of alpha-HCH after 1 yr of treatment. There was no evidence of protracted development of toxicity or of growth autonomy in the majority of liver cells. Foci of altered cells and neoplastic nodules were detected histologically in the livers of 24 or 34 treated rats. Hepatocellular carcinoma was observed in 2 animals. In livers of 10 of 22 untreated control rats, foci of altered cells developed spontaneously between 12-34.5 mo. If neoplastic lesions were induced by a single dose of diethylnitrosamine, 75 or 150 mg/kg, subsequent treatment with alpha-HCH led to the appearance of hepatocellular carcinoma within 7 mo. Hepatocellular carcinomas were found in 18 of 21 rats treated with both agents but in only 3 of 26 animals treated with diethylnitrosamine alone. It is unclear from determination of tumor numbers alone whether alpha-HCH and similar xenobiotic inducers are initiating carcinogens or merely promote tumorigenesis from spontaneous lesions. The latter possibility is supported by the failure to detect evidence suggesting initiating potential of alpha-HCH, the enhanced mitotic response to alpha-HCH in foci of altered cells as reported elsewhere and the observation of a permanent stimulatory action on liver growth during prolonged exposure to alpha-HCH.