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The Natural History of Carcinogenesis: Implications of Experimental Carcinogenesis in the Genesis of Human Cancer

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Although the long latent period after administration of a carcinogen until development of a cancer has been recognized for more than a hundred years, until the last four decades little consideration had been given to the phenomena occurring during the latent period itself. Systems in which to study the molecular mechanisms underlying the phenomenon of latency have not been available to the investigator until relatively recently. Furthermore the importance of taking into account the natural history of neoplasia in whole animal bioassay procedures used for carcinogen testing is still not appreciated. The comparison of tumor-bearing test animals with controls and, in some instances, the time from the initial administration of the test agent until the appearance of the first neoplasm are the principal data from which conclusions about bioassays are drawn.

It is clear that we do not understand all the biological changes that occur during the latent period before the development of any neoplasm. The beginnings of an experimental basis for the biological changes occurring during the latent period were initiated with the studies of Rous and Kidd [1], Mottram [2], and Berenblum and Shubik [3]. However, even these studies told little of the detailed biology and far less of the molecular biology of the earliest changes occurring in cells initiated by carcinogens, since the endpoint of these experiments was the appearance of grossly visible neoplasms. Although "preneoplastic" lesions had been described both in experimental [4, 5] and in human neoplasia [6, 7], not until the last decade was it experimentally feasible to quantitate the number of such lesions. Such quantitation was first successfully carried out with "preneoplastic" lesions during hepatocarcinogenesis following diethylnitrosamine administration [8]. Furthermore, studies from human pathology [9–11] suggested that, with certain neoplasms, the progeny of some initiated cells never developed into neoplastic foci but rather regressed and disappeared into essentially benign, even normal, tissue cells.

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STAGES IN THE NATURAL HISTORY OF CARCINOGENESIS – DEFINITIONS

In order to consider the implications of the natural history of carcinogenesis in relation to our knowledge of human cancer, it is necessary to define and understand the natural history of the development of neoplasia. Since the 1940s carcinogenesis in mouse skin has been divided into the stages of initiation and promotion. Later Foulds, largely on the basis of his studies of mammary carcinogenesis in the mouse [12], proposed the term *progression* for virtually all of the developmental stages following the initial event in the conversion of a normal to a neoplastic cell. While Foulds saw the natural history of carcinogenesis as a continuous event that could be arbitrarily divided into several phases, modern oncologists take the position that the process of promotion is distinct from that of progression even though each of these phases has been divided into several steps by previous investigators [12, 13].

Furthermore it is now apparent that the two-stage concept of carcinogenesis as originally proposed [1–3] and reviewed and extended by Boutwell [14] is applicable to a variety of tissues during their conversion to malignant neoplasms [15]. Therefore we can consider the characteristics and definitions of each of the stages in the natural history of carcinogenesis as applicable to virtually any cell type.

For the purposes of this discussion, we will divide the natural history of carcinogenesis into three stages: initiation, promotion, and progression. A simplified diagram of this process is given in Figure 1 [16]. The definitions of initiation and promotion listed below are excerpted from the same reference.

> *Initiating Agent*—a chemical, physical, or biological agent that is capable of directly altering irreversibly the native molecular structure of the genetic component (DNA) of the cell. Such alteration(s) may be the result of a covalent reaction of DNA with the initiating agent itself or with one of its metabolites, but this alteration may also include a distortion of the structure of DNA without covalent binding of the agent to DNA. Finally, the agent may cause one or more complete scissions of the DNA chain, an elimination of one of its component parts (eg, bases or sugars), or errors in DNA repair. All such capabilities of an initiating agent, however, do not in themselves prove that alteration of DNA is the only or the absolute requirement for the neoplastic transformation.
>
> *Promoting Agent*—an agent that alters the expression of genetic information of the cell. Examples of such agents include hormones, drugs, plant products, etc, which in themselves do not directly react with the genetic material but rather affect its expression by a variety of mechanisms, including their interaction with cell surface receptors or with cytoplasmic and nuclear components and functions.

The definition of an initiating agent is made in reference to molecular species, especially DNA, because of the advances in molecular biology and our understanding of the mechanisms of mutational events. While the definition clearly hedges in stating that initiation may not always be the result of mutation, there is no question that the initiation of the neoplastic transformation and genetic mutation are closely related in the majority of instances of carcinogenesis. Unfortunately, the definition of a promoting agent given here is still relatively

inexact. However, one may hypothesize that promoting agents may be divided into specific and nonspecific classes. Specific promoting agents are those that interact with receptors or receptor-like molecules on or within target cells. Such specific promoting agents have a defined range of tissues susceptible to their promoting action. Examples of this class would be steroid or polypeptide hormones [15], which are known to be effective promoting agents in their target tissues, their metabolic effects being mediated by cellular receptors. Nonspecific promoting agents are those that do not act through receptor mechanisms but alter gene expression by a variety of nonspecific mechanisms. Examples of this class would be iodoacetate or detergents in the case of epidermal carcinogenesis.

In his earlier work Foulds suggested that there are at least two basic characteristics of progression [12]. The first is the independent progression of neoplasms; ie, progression occurs independently in different primary neoplasms within the same host. The second characteristic is the independent progression of specific characteristics of the neoplasm, each of which undergoes progression independently of the others in any single neoplasm. These characteristics include growth rate, invasiveness, metastatic frequency, hormonal responsiveness, morphologic characteristics, etc. As can be seen from Figure 1, another crucial characteristic of progression, as defined herein, is karyotypic change. The following operational definition of the stage of *progression* in neoplastic development will be used here.

> *Progression*—that stage of neoplastic development characterized by visible karyotypic alterations as evidenced by light microscopic techniques within a majority of the neoplastic cells that make up the tumor. These karyotypic alterations in turn are associated with increased growth rate, increased invasiveness, metastases, and alterations in biochemical and morphologic characteristics of the neoplasm.

IMPLICATIONS OF STAGES IN THE NATURAL HISTORY OF CARCINOGENESIS

A variety of implications are derived from the above definitions of initiation, promotion, and progression. The definition of an initiating agent as one capable of covalent interaction with DNA, or any other macromolecule, implies but does not prove that one or more mutational events in the genome result in the conversion of a normal cell to an initiated cell. In contrast, evidence from chimeras produced by transplantation of malignant cells into blastocytes [17], the transplantation of nuclei from neoplastic cells into eggs which then exhibit normal development [18], and the forced terminal differentiation by chemical means of a variety of neoplastic cell lines (19, 20] argue that permanent genetic damage is not necessary for the initiation of neoplastic transformation. Various theories have been proposed that argue for a permanent alteration in the initiated cell resulting from extragenomic changes [21–25]. Despite these latter considerations, the most common working hypothesis is that initiation does involve covalent and/or structural changes in the genome.

The exact molecular mechanism of action of promoting agents has not, however, yet been defined. The definition above clearly suggests a mechanism for promoting agents—that of altering gene expression [14]—but the variety of such

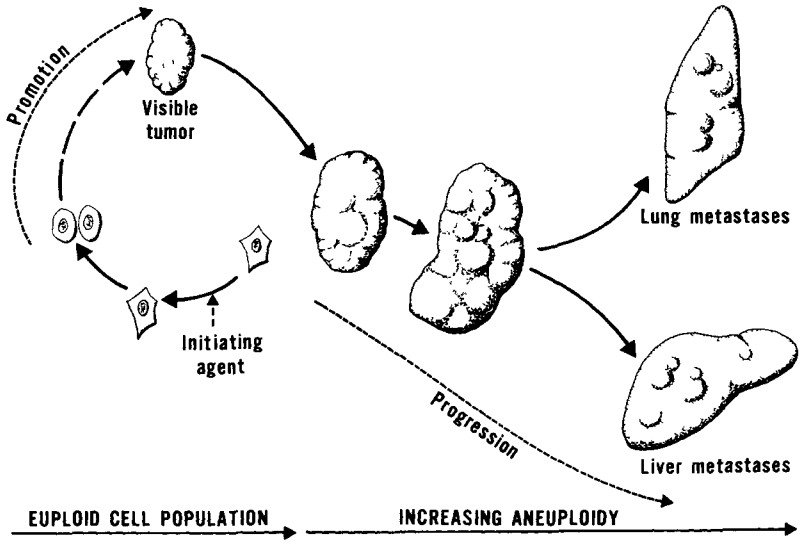

Fig. 1. The natural history of neoplastic development in relation to initiation, promotion, and progression in reference to cell karyotype.

mechanisms is so great as to suggest that this definition is too general to determine the ultimate action of promoting agents in carcinogenesis. Yet many of the characteristics of promoting agents (see below) correlate well with this definition.

That progression is a result of karyotypic abnormalities may be construed as a somewhat arbitrary definition. However, virtually all neoplasms that exhibit characteristics of metastases, high rates of growth and invasiveness, high rates of glycolysis, and anaplastic morphologic characteristics are aneuploid, suggesting that such an operational definition is reasonable. The final common pathway of the natural history of carcinogenesis, the metastatic lesion, is nearly always the result of the growth of aneuploid neoplastic cells. The concept of Goldenberg [26] that progression may be due in part to cell fusion further supports the importance of aneuploidy and chromosomal abnormalities in progression.

CHARACTERISTICS OF THE STAGES IN THE NATURAL HISTORY OF CARCINOGENESIS

Until the last decade the characteristics of the stages of initiation and promotion were based exclusively on experiments carried out with mouse skin as the test tissue. Although it had become apparent from studies of the pathology of human cancer, as well as from several experimental situations [15], that the two-stage process also applies to the genesis of neoplasms other than those in the skin, only in the last decade have experimental systems amenable to study and in some ways superior to the mouse epidermis model been exploited. Also, Foulds' concept of tumor progression was based on studies of yet another experimental

system, mouse mammary carcinogenesis [27]. Therefore, our knowledge of the natural history of neoplasia can now be developed from and applied to a wide variety of tissue systems.

Although it is possible that the natural development of neoplasia in each tissues exhibits unique characteristics, it is to be expected that certain characteristics will be common to each stage during the development of all types of neoplasms. These characteristics are reviewed here.

CHARACTERISTICS OF INITIATION AND THE INITIATED CELL

The characteristics of initiating agents in skin and their comparison with liver have been previously reviewed [15]. The accumulated experimental evidence supports the concept that the effects of initiating agents on cells are essentially irreversible. Furthermore, agents capable of initiating the neoplastic transformation in vivo or in vitro can be divided into two general classes. Those agents capable not only of initiating neoplasia but also of causing promotion and progression of the initiated cell are termed "complete carcinogens." Those agents capable only of initiating cells but not of promoting them are termed "incomplete carcinogens" or "pure" initiating agents. Once a cell has been initiated it will remain so throughout its life-span, and the characteristics of initiation will be transmitted to all daughter cells, unless the initiated lesion is repaired or eliminated by some other mechanisms [28]. At least theoretically, and in some instances experimentally [29], initiation can result from a single "hit" of the initiating agent on the target cell. The irreversibility of the effects of an initiating agent, the single "hit" concept, and the corollary—ie, the additive of the effects of initiating agents—are applicable to both incomplete and complete carcinogens. Furthermore, these characteristics are identical with what one would expect for a mutagenic agent.

The apparent efficiency of initiation varies widely, depending on the system employed. Sachs reported that treatment of a mixed cell population of normal hamster embryo cells with carcinogenic hydrocarbons resulted in a 3–20% incidence of transformation in these cells [30]. However, x-irradiation resulted in only a 0.5% transformation rate. In contrast, in the mouse skin system, the average number of tumors produced in any animal is usually less than 25 [13]. On the assumption that each neoplasm arises from a single neoplastic cell [31] and that all epidermal cells are targets for the carcinogen, this means that the incidence of initiation is in the range of 10^{-7} to 10^{-8}. In the liver system, a single dose of diethylnitrosamine will, on the average, initiate 1 in 10^4 to 10^5 hepatocytes [32]. Although it appears that all initiated cells in vitro and in the skin develop into neoplastic foci, this is not so clear in the liver system. Of the foci produced (about 1,000/g liver), less than 1% develop into histologically defined neoplasms. Preliminary investigations from our laboratory have indicated, however, that it is unlikely that all foci are capable of transplantation into syngeneic hosts [33]. If this is true, then, as the term "neoplasia" is defined and characterized [16], it is likely that all such enzyme-altered foci in rat liver are neoplastic or at least potentially neoplastic. Obviously, with low doses of a complete carcinogen or with the administration of incomplete carcinogens (pure initiators), no neoplasms result, although significant numbers of enzyme-altered foci may appear under the conditions of the experiments [32, 33].

The latter point then raises the issue of the ultimate fate of initiated cells. Although the apparent incidence of initiation in mouse skin is extremely low, on the basis of the findings with liver it is quite possible that many more initiated cells occur in mouse skin but, as in rat liver, that these do not become neoplasms, even following promotion, and the initiated cells and/or their progeny remain in the animal for life. In liver such initiated foci can be identified by suitable histochemical means [32]; once such foci are produced they also do not disappear during the life-span of the animal [34].

Incomplete carcinogens (pure initiating agents) have been identified both in skin [35] and in liver [36] carcinogenesis. Agents that are incomplete carcinogens for these tissues are either complete carcinogens in other tissues (eg, urethan for liver and lung, and dimethylbenzanthracene for skin) or are noncarcinogenic in the adult. However, incomplete carcinogens are themselves mutagenic or may be metabolized to a mutagenic form by liver. Urethan induces hepatocellular carcinomas, pulmonary adenomas, and lymphomas in mice but does not by itself cause epidermal carcinoma [37]. Promotion by croton oil of the skin of animals given urethan results in epidermoid carcinoma [35]. Similarly, polycyclic hydrocarbons that effectively induce epidermoid carcinoma do not induce any neoplastic response in the liver of adult rodents. However, if a mitotic stimulus is applied to the liver following parenteral administration of the hydrocarbon, with subsequent promotion by phenobarbital, then heptocellular carcinomas will result [34,38]. The induction of enzyme-altered foci following short-term promotion by carbon tetrachloride has been reported for alkylating agents such as N-methyl-N-nitro-N-nitrosoguanidine and the colon carcinogen 1,2-dimethylhydrazine [39]. Although these latter experiments were not carried to the formation of neoplasms, on the basis of earlier arguments that such foci are initiated hepatocytes [15,33], these agents exhibit the characteristics of incomplete hepatocarcinogens.

It is clear, therefore, that incomplete carcinogens, capable only of initiating cells in specific tissues, do exist and appear to have most, if not all, of the characteristics of mutagenic agents. These compounds appear to differ from complete carcinogens in that they exert virtually no promoting action on the cells of the tissue in which they serve as incomplete carcinogens. The reason for this is not clear, but one possible component is their failure to induce an increase in cell division in the tissue in which their action is incomplete.

THE ROLE OF CELL DIVISION IN THE INITIATION OF NEOPLASIA

Borek and Sachs [40] were the first to demonstrate in a relatively unequivocal manner that cell replication was required for the "fixation" of the transformed state in cell culture. Although their studies were initially based on the time required for fixation rather than an actual demonstration of a requirement for DNA synthesis and mitosis, later studies [41, 42] have supported their interpretation of earlier studies. Both in the liver and in the skin, the process of promotion involved cell division, although, as has been pointed out by Boutwell and others [14], cell division is a necessary but not sufficient characteristic of tumor promotion in the skin. In the liver the 2-stage system of carcinogenesis described by Peraino et al [43], Solt and Farber [44], and our laboratory [32] always involves a higher than normal level of cell division. In

Peraino's experiments weanling animals having a relatively high rate of hepatic cell division were utilized, whereas both Farber and our group include the stimulus of partial hepatectomy in the initiation-promotion sequence. Ying et al have reviewed the necessity for cell proliferation as an obligatory step in the induction of enzyme-altered foci in hepatocarcinogenesis [45].

Thus there is substantial evidence that fixation or cell division is required during a relatively early period following application of the initiating agent for optimal efficiency of initiation. If one views initiation as a mutagenic event, this interpretation is entirely plausible, since one would expect that repair of macromolecular damage, the initiating event, could occur if no cell division intervenes to perpetuate the initial damage produced.

CHARACTERISTICS OF TUMOR PROMOTION AND PROMOTING AGENTS

There is no evidence that promoting agents exert their effects by direct covalent interaction with the genome. The available evidence suggests that the effects of promoting agents are on one or more extragenomic processes, which may in turn influence genetic information or its expression (see above). The early studies of Berenblum and Shubik [3] indicated that administration of the promoting agent alone results in no neoplasms in the mouse skin system. However, later investigations have demonstrated that, following the prolonged administration of croton oil or its active component, tetradecanoylphorbol acetate (TPA), a small number of neoplasms is produced. This has led some investigators [46] to propose that promoting agents are merely weak complete carcinogens. If this were so, one would expect that increasing doses of a promoting agent would lead to increasing numbers of neoplasms. As we shall see below, this is clearly not so in the mouse skin system. Furthermore, substantial evidence has accrued since those earlier studies that the efficiency of promotion is a function of diet and of hormonal, environmental, and other factors in the host [15]. Most recently Van Duuren et al [47] have demonstrated that, with increasing age of the animals, the efficiency of tumor promotion in the mouse skin system is significantly decreased.

DOSE RESPONSE TO PROMOTING AGENTS

The evidence for a "no threshold" level and the irreversibility of complete carcinogens has been well documented [48–50]. One of the earliest examples is that shown in Figure 2, which describes the results of Drückrey [48] with an aromatic amine carcinogen, 4-dimethylaminostilbene, in rats. As can be seen from line 1, there is a linear relation between dose and tumor response, which extends through the origin. At extremely low doses (line 2), however, the time until the first neoplasm appears extends beyond the life-span of the animal.

In the case of promoting agents, earlier studies suggested that, although a dose-response relation occurred, the no-threshold effect did not necessarily apply to these materials. Recently Verma and Boutwell [51] have reported a dose-response curve for TPA in mouse skin carcinogenesis. In their studies a distinct

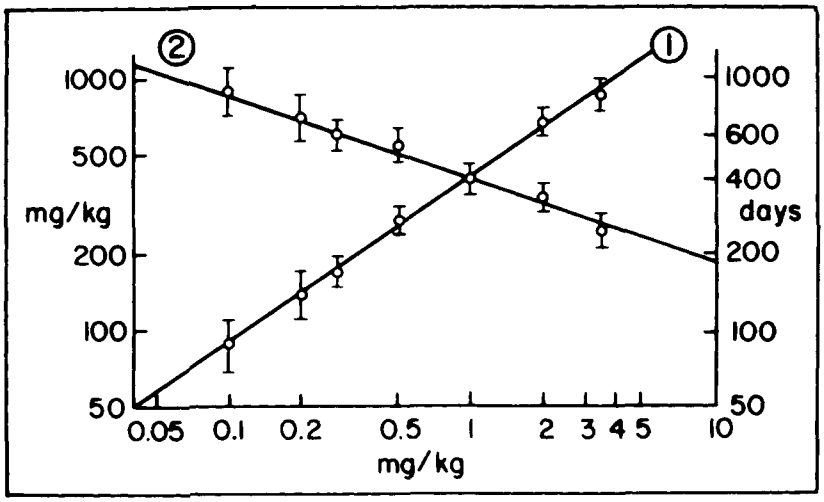

Fig. 2. Dose-response relationships seen in the chronic feeding of 3,4-dimethylaminostilbene to rats. 1. Relationship between the daily dose and the median total dose of animals with carcinoma. 2. Relationship between daily dose and median induction time. The abscissa shows the daily dose, whereas the ordinate on the left is the total dose administered, and that on the right is the time from the beginning of the experiment. All scales are logarithmic. (Modified from Druckrey et al, 1967 [48]).

threshold was obtained below which no tumors occurred. At the two highest doses of promoting agent employed, maximal incidence of tumors was the same. Thus both a threshold and a maximum effect of the promoting agent were noted. Neither of these characteristics would be expected with a complete carcinogen. More recently Peraino and his associates [52] have established a dose-response relation for phenobarbital administration following initiation of hepatocarcinogenesis by a short feeding of acetylaminofluorene. In those studies a distinct threshold was also noted, as well as a maximum, when the total incidence of hepatic tumors was considered. In our studies on the quantitation of enzyme-altered foci, a maximal number of foci is achieved at doses of phenobarbital in the diet above 0.01%. At extremely low doses (.0001%) no difference in the incidence of foci was noted compared with control animals. Thus it is apparent from three separate studies, using three different endpoints of analysis, that promoting agents exhibit a threshold dose below which no effect is noted, as well as a dose above which no further effect on incidence of tumors or foci is noted. Both of these characteristics clearly distinguish promoting agents from complete carcinogens, whether weak, moderate, or strong.

REVERSIBILITY OF THE PROMOTION STAGE

Boutwell was the first to describe the nonpermanence of the effects of promoting agents [13]. Using the mouse skin system with croton oil as the promoting agent, he demonstrated that changing the format of the dosage regimen could alter the incidence of tumors finally produced. When the promoting agent was applied once every 4 weeks rather than 3 times per week,

but with the experiment extended until the same total dosage of promoting agent was given under both regimens, tumors resulted only in those animals receiving the promoting agent thrice weekly. These studies clearly demonstrated the reversibility and non-additivity of the effects of croton oil in promoting epidermal carcinogenesis.

Preliminary experiments in the hepatocarcinogenesis system employed in our laboratory [33] have supported the findings of Boutwell in that the administration of phenobarbital for 2 days every 2 weeks rather than continuously, as in the control animals, but with the same total dose in both groups, resulted in significantly fewer enzyme-altered foci in the animals that received the promoting agent at 2-week intervals. Thus in these two different systems one can demonstrate the reversibility of the effects of promoting agents, another characteristic clearly distinguishing them from complete carcinogens or pure initiating agents.

MODELS FOR THE STUDY OF MOLECULAR MECHANISMS OF TUMOR PROMOTION

Table I is a list of the model systems exhibiting a 2-stage mechanism of carcinogenesis. Of these systems, those most commonly studied are from the mouse epidermis and rat liver in vivo and mouse and hamster cells in culture. In addition, two other well-studied systems are those of the rat bladder and mammary gland.

In the case of mouse epidermis, mechanistic studies were greatly facilitated by the isolation and purification of the active ingredient of croton oil, tetradecanoylphorbol acetate (TPA), the classical promoter for mouse epidermis [2,3]. Much is now known of the biochemical actions of TPA, the highly active phorbol diester of croton oil. Its action and effects have been reviewed [53] and are the subject of many of the papers at this symposium. The difficulty in studying specific effects of TPA on a variety of cellular systems both in vivo and in vitro is the extrapolation of such results to the phenomenon of promotion in the mouse skin or other tissues in which TPA has been shown to promote tumorigenesis. Since TPA also stimulates a significant inflammatory response in the epidermis, and the role of this in tumor promotion is unknown, the biochemical actions of TPA related to inflammation may or may not be important in the mechanism of promotion. While mouse epidermis is readily accessible to experimentation, the tumor-promoting effects of TPA and other agents used in this model system can only be ascertained through the induction of benign and malignant neoplasms seen grossly on the skin.

Following the report by Berwald and Sachs [54] and later the establishment of the C3H 10 T½ transformable cell line by Heidelberger and his associates [55], cell culture has offered some of the most promising systems for the study of the molecular mechanisms of tumor promotion. Unfortunately, the exact significance of morphologic transformation in cell culture by carcinogens is not fully understood, and in the few epithelial systems available it is not, of itself, sufficient to define the cell as biologically neoplastic [56]. However, the ability to manipulate the cellular environment and the ready access of the system to the investigator make this model one of the most promising now available for the study of the molecular mechanisms of tumor promotion. Unfortunately, because

TABLE I. Initiators, Promoters, and "Preneoplastic" Lesions in Various Organ Systems*

Tissue	Initiating agent	"Preneoplastic" lesions	Promoting agent
Dog bladder	2-Naphthylamine	Alkaline phosphatase-deficient foci	D,L-tryptophan
Rat bladder	Methylnitrosourea		Saccharin
Rat bladder	N-[4]-(5-nitro-2-furyl)-2-thiazolylformamide		Allopurinol
Rat colon	N-methyl-N′-nitrosoguanidine	Proliferative foci	Lithocholic acid
Rat bone marrow (leukemia)	N,N′-2,7-fluorenylbisacetamide		Blood loss
Mouse embryo fibroblasts in culture	3-Methylcholanthrene		Tetradecanoylphorbol acetate
	Ultraviolet radiation		Tetradecanoylphorbol acetate
Mouse epidermis	3-Methylcholanthrene, β-propiolactone, urethan, etc		Croton oil or tetradecanoylphorbol acetate
Mouse forestomach	3-Methylcholanthrene, benzo(a)pyrene, dimethylbenzo(a)anthracene		Croton oil or lime oil
Rat liver	2-Acetylaminofluorene, diethylnitrosamine, azo dyes	Hyperplastic nodules Enzyme-altered foci	Phenobarbital, DDT, PCBs, butylated hydroxytoluene, estrogens
Mouse lung	Urethan		Butylated hydroxytoluene
Rat mammary gland	7,12-Dimethylbenzo(a)anthracene	Ductular hyperplasia Hyperplastic alveolar nodules	Phorbol, prolactin
Rat pancreas	Azaserine N-Methyl-N-nitrosourea	Acinar nodules	
Rat thyroid	2-Acetylaminofluorene	Adenomas	Methylthiouracil

*Taken from Pitot and Sirica [15]; the reader is referred to this source for details.

of the limitation of the use of mesenchymal cells in the most commonly employed systems, the cell culture transformation systems are relatively limited to the number of specific promoting agents that may be studied. Trosko and his associates [57] have recently demonstrated, however, that promoting agents inhibit metabolic cooperativity in cells in culture. Whether this will be an ubiquitous mechanism of all promoting agents and how such a mechanism can account for the biological action of promoting agents remains to be seen.

The liver system in vivo offers the possibility of monitoring transformed cells shortly after initiation more so than is seen in other systems, even those in culture. The ease with which liver cells may be manipulated in vivo and in vitro, together with the extensive biochemical knowledge of this tissue, offers distinct advantages. However, the transformation system occurs only in vivo, and thus far it has not been possible to isolate in pure form the population of initiated cells for studies in culture. Furthermore, despite some reports, it has not been possible to transform adult or even fetal hepatocytes in cell culture into neoplastic cells.

Thus each system has both advantages and disadvantages for the study of the molecular mechanism of tumor promotion. In the hepatocyte system, chemical agents such as phenobarbital, halogenated aromatics, and antioxidants are all effective as tumor promoters, and all act to regulate xenobiotic metabolism. The relationship between this effect and the actions of these compounds as promoters of hepatocarcinogenesis is not yet clear. Since this system has distinct advantages and disadvantages, the molecular biologist interested in studying the mechanism of tumor promotion must decide which aspect of tumor promotion to study—eg, the action of TPA, phenobarbital, or other promoters and the cell biology of tumor promotion in vivo. It is only through a concerted effort of studying aspects of all these systems that we will ultimately understand the molecular mechanisms of tumor promotion.

IMPLICATIONS OF THE CHARACTERISTICS OF THE STAGES OF CARCINOGENESIS

There are two principal implications of our knowledge of the characteristics of the stages of carcinogenesis. The first is the importance of determining the molecular mechanism of action of promoting agents. There is now overwhelming evidence that the initiation of neoplasia involves, in most instances, a direct alteration in the genetic material of the cell. Whether this alteration is ultimately repairable so that a neoplasm may revert to the normal state or whether the genetic alteration invariably results in malignant neoplasia is not critical to our understanding of the mechanism of initiation. However, tumor promotion appears to be regulated by environmental factors, even to the point of a reversal of the effects of such agents during the process of carcinogenesis. Promoting agents differ in their effects on different tissues and in different species, just as do initiating agents and complete carcinogens [15]. Such tissue and species specificities for complete carcinogens can be understood on the basis of the required metabolism to the active carcinogenic form in a specific tissue and the ability of the agent to induce cell replication and tumor promotion in target

tissues. Promoting agents, however, are not readily metabolized but usually must be present in substantial amounts over prolonged periods to exert their promoting activities.

The specificity of promoting agents for tissues may be related to their interaction with specific receptors in the target tissue. Substantial evidence now exists for surface receptors for the active phorbol esters, promoting agents for mouse epidermis and other tissues (58,59), and studies from our laboratory (60) have shown that tetrachlorodibenzodioxin (TCDD) is an excellent promoting agent in liver. This latter compound interacts with a specific receptor molecule in liver and other tissues (61), an interaction that is necessary for the expression of its toxicity and, possibly, its promoting activity. These studies indicate that unless a receptor for a specific promoting agent is present, that tissue will not be promoted to a tumor by the agent. As pointed out earlier (62), virtually all hormones become promoting agents by this concept. Estrogens are effective promoting agents in liver (63), as is prolactin in mammary tissue [64]. However, some promoting agents, such as iodoacetamide, act in a nonspecific manner to alter gene expression or exert whatever other mechanistic effects are required of promoting agents for their action in carcinogenesis (vide supra).

The second major implication is in relation to human carcinogenesis. Probably most important in this area is the question of testing environmental agents to determine their carcinogenicity. At present all such testing methodologies do not distinguish among initiators, promoters, or complete carcinogens, so that all agents are treated in a similar manner. This approach is not reasonable in considering promoting agents, which on prolonged feeding may be expected to induce a significant number of neoplasms in test animals as compared with control animals. However, because of the reversibility of the effects of promoting agents during carcinogenesis and the existence of threshold levels of these agents, the risk of such agents for the human being is significantly different from the risk of mutagenic agents and complete carcinogens. More important is the fact that recognition of promoting agents important in the human environment will allow a rational control of such agents. Specifically, it may not be necessary to completely eliminate promoting agents from the environment but to control their level and the period of exposure of humans to such agents.

Table II lists promoting agents known to occur in the human environment. Not all of these agents have been associated with neoplasms in the human. In fact, phenobarbital and saccharin appear to exert little if any effect on the incidence of human liver and bladder tumors, as judged by published epidemiologic studies [65–67]. In contrast, the importance of dietary fat and calories, cigarette smoke, asbestos, and alcohol as promoting agents in the environment has been documented (Table II). In fact, one may conjecture that the production of clinical cancer in the human is largely a result of the action of continued exposure to promoting agents rather than exposure to minute amounts of complete and incomplete carcinogens in the environment. Recently Weber and Hecker [76] have reported that chemicals structurally related to TPA and having a similar promoting action are frequently ingested by people living in areas of Curacao where there is a high rate of esophageal cancer. In addition, Kopelovich et al [77] demonstrated that TPA will promote the transformation of fibroblasts from patients with hereditary adenomatosis of the colon and rectum, an autosomal dominant trait in which all affected individuals eventually develop adenocarcinoma of the colon or rectum.

TABLE II. Promoting Agents in the Human Environment and the Neoplasms Associated With Prolonged Contact With Those Agents

Agent	Resultant neoplasm	References
Dietary fat (calories)	Increased cancer incidence in general with excess caloric intake	[69]
	Mammary adenocarcinoma	[68]
Cigarette smoke	Bronchogenic carcinoma	[70]
	Esophageal and bladder cancer	[71]
Asbestos	Bronchogenic carcinoma and mesothelioma	[72]
Halogenated hydrocarbons (TCDD, PCBs)	Liver[a]	[60,73]
Saccharin	Bladder[a]	[74]
Phenobarbital	Liver[a]	[32]
Prolactin	Mammary adenocarcinoma[a]	[64]
Synthetic estrogens	Liver adenomas	[63]
Alcoholic beverages	Oral cancer	[75]
	Liver and esophageal cancer	

[a]Promotion demonstrated in experimental animals but not yet in humans.

Our knowledge of the action of promoting agents and complete carcinogens thus becomes extremely important in relation to human cancer and the human environment. The demonstration of promoting agents as distinct from complete carcinogens will be necessary in order for rational and valid decisions to be made concerning the regulation of these agents in our environment. Furthermore, the onus and fear that go with the labeling of a compound as a "cancer-causing agent" can be removed or modified in many instances when we understand better what that compound contributes to the natural history of carcinogenesis in the human, as well as in lower animals.

REFERENCES

1. Rous P, Kidd JG: J Exp Med 73:365, 1941.
2. Mottram JC: J Pathol Bacteriol 56:181, 1944.
3. Berenblum I, Shubik P: Br J Cancer 1:379, 1947.
4. Friedrich-Freksa H, Gössner W, Börner P: Z Krebsforsch 72:226, 1969.
5. Farber E: Methods Cancer Res 7:345, 1973.
6. Bannasch P, Clining O: Virchows Arch Abt A Pathol Anat 352:157, 1971.
7. Anthony PP: Cancer Res 36:2579, 1976.
8. Scherer E, Hoffmann M, Emmelot P, Friedrich-Freksa M: J Natl Cancer Inst 49:93, 1972.
9. Berger BW, Hori Y: Arch Dermatol 114:1698, 1978.
10. Johnson LD, Nickerson RJ, Easterday CL, Stuart RS, Hertig AT: Cancer 22:901, 1968.
11. Evans AE, Gerson J, Schnaufer L: Natl Cancer Inst Monogr 44:49, 1976.
12. Foulds L: In Mühlbock O, Emmelot P (eds): "Cellular Control Mechanisms and Cancer." Amsterdam: Elsevier, 1964, pp 242-295.
13. Boutwell RK: Progr Exp Tumor Res 4:207, 1964.
14. Boutwell RK: Crit Rev Toxicol 2:419, 1974.
15. Pitot HC, Sirica AE: Biochim Biophys Acta 605:191, 1980.
16. Pitot HC: "Fundamentals of Oncology." New York: Marcel Dekker, 2nd Edition, 1981.
17. Mintz B, Illmensee K: Proc Natl Acad Sci USA 72:3585, 1975.
18. King TJ, DiBerardino MA: Ann New York Acad Sci 126:115, 1965.
19. Friend C, Scher W, Holland JG, Sato T: Proc Natl Acad Sci USA 68:378, 1971.
20. Sachs L: Nature 274:535, 1978.
21. Markert CL: Cancer Res 28:1908, 1968.
22. Finckh ES: Med J Aust 1:438, 1974.

23. Gronow M: Chem-Biol Interact 29:1, 1980.
24. Pitot HC, Heidelberger C: Cancer Res 23:1694, 1963.
25. Pitot HC: Perspect Biol Med 8:50, 1964.
26. Goldenberg DM, Pavia RA, Tsao MC: Nature 250:649, 1974.
27. Foulds L: J Natl Cancer Inst 17:701, 1956.
28. Cairns J: Nature 255:197, 1975.
29. Smets LA: Biochim Biophys Acta 605:93, 1980.
30. Sachs L: Colloq Gesellschaft Physiol Chem 17:242, 1966.
31. Iannaccone PM, Gardner RL, Harris H: J Cell Sci 29:249, 1978.
32. Pitot HC, Barsness L, Goldsworthy T, Kitagawa T: Nature 271:456, 1978.
33. Pitot HC, Goldsworthy T, Moran S, Sirica AE, Weeks J: In Hecker E, Fusenig NE, Marks F (eds): "Cocarcinogenesis and Biological Effects of Tumor Promoters." New York: Raven Press (in press).
34. Pitot HC: In Estabrook RW, Lindenlaub E (eds): "The Induction of Drug Metabolism." New York: FK Schattauer-Verlag, 1979, pp 471–483.
35. Salaman MH, Roe FJC: Br J Cancer 7:472, 1953.
36. Kitagawa T, Pitot HC, Miller EC, Miller JA: Cancer Res 39:112, 1979.
37. Mirvish SS: Adv Cancer Res 11:1, 1968.
38. Kitagawa T, Harakawa T, Ishikawa T, Neomoto N, Takayama S: Toxicol Lett 6:167, 1980.
39. Tsuda H, Lee G, Farber E: Cancer Res 40:1157, 1980.
40. Borek C, Sachs L: Nature 210:276, 1966.
41. Kakunaga, T: Cancer Res 35:1637, 1975.
42. Mironescu S, Love R: Cancer Res 34:2562, 1974.
43. Peraino C, Fry RJM, Staffeldt E, Kisieleski WE: Cancer Res 33:2701, 1973.
44. Solt D, Farber E: Nature 263:701, 1976.
45. Ying TS, Sarma DSR, Farber E: Proc 11th Intern Congress Biochem 1979, p 664.
46. Roe FJC, Clack J: Br J Cancer 17:596, 1963.
47. Van Duuren BL, Smith AC, Melchionne SM: Cancer Res 38:865, 1978.
48. Drückrey H: In Truhaut R (ed): "Potential Carcinogenic Hazards from Drugs. Evaluation of Risks. UICC Monograph Series, Vol. 7." Berlin: Springer-Verlag, 1967, pp 60–77.
49. Brown CC: Oncology 33:62, 1976.
50. Schneiderman MA, Decoufle P, Brown CC: Ann NY Acad Sci 329:92, 1979.
51. Verma AK, Boutwell RK: Carcinogenesis 1:271, 1980.
52. Peraino C, Staffeldt EF, Haugen DA, Lombard LS, Stevens FJ, Fry RJM: Cancer Res 40:3268, 1980.
53. Diamond L, O'Brien TG, Baird WM: Adv Cancer Res 32:1, 1980.
54. Berwald Y, Sachs L: Nature 200:1182, 1963.
55. Reznikoff CA, Brankow DW, Heidelberger C: Cancer Res 33:3231, 1973.
56. Borek C: Radiat Res 79:209, 1979.
57. Yotti LP, Chang CC, Trosko JE: Science 206:1089, 1979.
58. Driedger PE, Blumberg PM: Proc Natl Acad Sci USA 77:567, 1980.
59. Horowitz AD, Greenbaum E, Weinstein IB: Proc Natl Acad Sci USA (in press).
60. Pitot HC, Goldsworthy T, Campbell HA, Poland A: Cancer Res 40:3616, 1980.
61. Poland A, Glover E: Mol Pharmacol 11:389, 1975.
62. Berenblum I: J Natl Cancer Inst 60:723, 1978.
63. Taper HS: Cancer 42:462, 1978.
64. Medina D: Cancer Res 36:2589, 1976.
65. Clemmenson J: Acta Pathol Microbiol Scand Suppl 261:38, 1977.
66. Hoover RN, Strasser PH: Lancet 2:837–840, 1980.
67. Kessler II, Clark JP: JAMA 240:349, 1978.
68. Tannenbaum A, Silverstone H: Adv Cancer Res 1:451, 1953.
69. Wynder EL: Cancer 43:1955, 1979.
70. Doll R, Peto P: Br Med J 2:1525, 1976.
71. Howe GR, Burch JD, Miller AB, Cook GM, Esteve J, Morrison B, Gordon P, Chambers LW, Fodor G, Winsor GM: J Natl Cancer Inst 64:701, 1980.
72. Selikoff I, Hammond C, Churg J: JAMA 204:104, 1968.
73. Nishizumi M: Gann 70:835, 1979.
74. Cohen SM, Arai M, Jacobs JB, Friedell GH: Cancer Res 39:1207, 1979.
75. Tuyns AJ: Cancer Res 39:2840, 1979.
76. Weber J, Hecker E: Experientia 34:679, 1978.
77. Kopelovich L, Bias NE, Helson L: Nature 282:619, 1979.