The Importance of Differential Consideration of the Stages of Carcinogenesis in the Evaluation of Cocarcinogenic and Anticarcinogenic Effects

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This communication is an attempt to clarify and extend the concepts dealing with certain extrinsic or intrinsic factors that augment or retard carcinogenesis. Before discussing carcinogenesis it may be helpful to differentiate again between carcinogenesis and the subsequent growth of a tumor. Carcinogenesis implies the initiation of a neoplasm by a tumor-producing agent acting on normal tissue. Once the tumor is of microscopic size, even before it is perceptible on gross examination, carcinogenesis can be assumed to have been completed; the multiplication of the new tumor cells represents growth. The differentiation between genesis and growth is based both on morphological considerations and on the experimental observation of factors that encourage or inhibit carcinogenesis, while they have little or no influence on the growth of the tumor. Thus it is of experimental and possibly of clinical importance to distinguish between the genesis of a neoplasm from normal tissue, and the growth of such a tumor, or of tumor implants. Nevertheless one still finds references in the literature to the effects of a particular agent or procedure, without regard to the difference between genesis and growth.

A parallel situation, dependent on the recognition of distinct stages in carcinogenesis itself, has developed more recently. On the basis of experimental data and suggestions by Rous and his associates (30, 36), Berenblum (10), Tannenbaum (44), and the earlier work of others (19-21), and for experimental and possibly clinical purposes, it appears desirable to consider differentially the recognizable stages of carcinogenesis.

Stages of carcinogenesis.—In extensive morphological studies many workers have demonstrated that when a chemical carcinogen is applied repeatedly, or even once, to the skin of a susceptible animal a series of changes antecedent to tumor formation takes place. In the main these include epithelial hyperplasia and increase in nuclear and nucleolar size; dermal alterations involving the hair follicles, sebaceous glands, collagen and elastic fibres, and inflammatory cells; and definite chemical changes in the epidermis (13-15, 18, 32, 34, 35, 39, 41, 49). These may be only coincident with, or may themselves actually be, the lesions that have been referred to as initiation or pre-neoplasm. For even though the application of carcinogen is discontinued before tumors are expected, papillomas may develop in the pre-neoplastic site. It is therefore believed that the first stage, either gradually or abruptly, goes over into the second stage of tumor formation or neoplasm.

In a previous communication (44) it was pointed out that biological experiments of diverse nature support this view: That carcinogenesis may be divided into at least two distinct stages: (a) a stage of preparation, latency, initiation, or pre-neoplasm, in which the normal cells become biased toward forming a tumor; and (b) a stage of development, formation, or neoplasm, which eventuates in a perceptible tumor. In this respect our conclusions agree with those of Rous and his associates, Berenblum, and others.

Berenblum (10), in discussing the genesis of skin tumors, also includes the concept of metacarcinogenesis (the conversion of a benign into a malignant tumor). We prefer to omit discussion of this concept, since the relationships of benign to malignant neoplasms are not clear. In the case of the skin, does the malignant cell develop from a papilloma cell or from a normal cell that is converted directly into a malignant cell? Is the carcinogenic agent responsible for this conversion, or is the change produced by the growing papilloma? Often the malignant tumor seems to be formed directly, and not from a benign papilloma. Moreover, other...
malignant neoplasms in animals, such as the spontaneous breast adenocarcinoma and induced sarcoma of the mouse, seem to develop directly and not through an intermediary benign tumor. Consequently, metacarcinogenesis will be omitted from this discussion and carcinogenesis will be regarded as consisting essentially of the two stages: (a) pre-neoplasm (initiation), and (b) neoplasm (development).

**Anticarcinogenesis and cocarcinogenesis.**—In recent years substances have been found that either promote or inhibit the development of skin tumors when they are applied concurrently with a carcinogen. Berenblum (4) applied the term “anticarcinogen” to di-chlorodiethylsulfide (mustard gas), which inhibits the formation of tumors when applied concurrently with the carcinogen, and Shear (38) suggested the term “cocarcinogen” to describe the augmenting effect of the basic fraction of creosote oil. Both stressed the fact that these substances, by themselves, do not initiate tumors, but under optimal conditions either inhibit or promote their induction by carcinogens. Later these two investigators reported further experimental work along these lines (6, 7, 9, 12, 37) and Berenblum adopted Shear’s term “cocarcinogen” to describe the augmenting effect of croton resin.

One gets the impression from Berenblum’s paper (10) that he considers that anticarcinogenic and cocarcinogenic actions influence the conversion of the pre-neoplastic stage to the neoplastic stage (epicarcinogenic action—Berenblum), though his definition and discussion of cocarcinogenesis also imply that it may exert its effect at other times. However, our experimental results, as given both in the preceding and succeeding communications (44, 45), coupled with a critical evaluation of the literature, indicate that it may be possible to differentiate between the effects of a cocarcinogen (or anticarcinogen) in each of the two distinguishable stages of carcinogenesis.

In brief, it is our thesis that cocarcinogenic and anticarcinogenic agents may involve either the pre-neoplastic or neoplastic stage separately, and that the mechanism and nature of the effect may be entirely different during these two stages. It is possible that a particular means or substance may have one effect, cocarcinogenic, anticarcinogenic, or none, on the development of the pre-neoplastic stage; and another effect, cocarcinogenic, anticarcinogenic, or none, on the conversion to the neoplastic stage.

It has been demonstrated that wound healing (30, 36), croton resin (10), and free caloric intake (44) will all promote the production of skin tumors at sites already rendered pre-neoplastic by the previous application of suboptimal doses of carcinogen for a limited period, evidence that the augmenting effect evokes and/or maintains the neoplastic stage. It is likely that the same agents have little or no effect upon the development of the pre-neoplastic stage; thus these means of promoting carcinogenesis can be considered cocarcinogenic actions that affect principally the development of the second or neoplastic stage of carcinogenesis.

On the other hand, certain substances exert their principal influence when applied during the period of limited carcinogen application. It is well known that the potency of a carcinogen is dependent on the nature of the solvent used and the condition of the skin, as well as on other experimental factors. For example, when 0.5 per cent strength of 1,2,5,6-dibenzanthracene was applied in various solvents it was found that tumor production was highest with chloroform, lower with oleic acid, and lowest with liquid paraffin (46). Again, ether or acetone, for a specific concentration of carcinogen, effects a much higher production of tumors than benzene (16, 42), and the addition of as small an amount of paraffin as 2 per cent increases the effectiveness of both ether and benzene (16). On the other hand, dissolving the carcinogen in mouse fat or lanolin rather than in benzene results in a considerable reduction of tumor formation (31, 40). There are many more examples of the now established fact that the physical and chemical nature of the solvent can greatly influence the response to a given concentration of a carcinogen.

In addition, there is much experimental evidence that application to the treated area of various solvents and agents before or between applications of carcinogen may considerably affect tumor formation. Watson and Mellanby (47) review the early work on this subject, and in their own experimental data clearly establish the accelerating effect of mouse fat and olive oil under these conditions, and an increased tumor incidence due to preliminary application of mouse fat has been confirmed (31). Baumann and his associates have also contributed valuable data (26-28).

The same wide variation occurs in sarcoma production when different solvents are used in the subcutaneous, intramuscular, or intraperitoneal injection of carcinogens. Early reports by Peacock (33), Watson (48), and Berenblum and Kendal (8) emphasize this. For example, a carcinogen in homologous fat produces fewer tumors than when dissolved in lard or sesame oil. There is considerable literature on this subject, which has been adequately surveyed by Burdette and Strong (11) and by Dickens and Weil-Malherbe (22). More recently Leiter and Shear (29) have reviewed certain aspects of this subject, and in extensive experimentation have compared tricaprylin

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2 An agent, administered alone, may have a slight carcinogenic effect, but this does not preclude the possibility that it may possess a strong cocarcinogenic or anticarcinogenic action.
and various lard fractions. They report: "In comparison with tricaprylin as the control vehicle, lard residue produced a striking retardation of tumor production whereas lard filtrate promoted it. Further fractionation indicated which components may be responsible for the promoting action and demonstrated that the retarding influence resides in the glyceride fraction richest in saturated fatty acids of high molecular weight."

The effectiveness of a carcinogen is therefore dependent, among other factors, on the nature of the solvent, and, with regard to skin tumors, can be modified by the addition of certain agents to the skin before or between applications of the carcinogen. In other words, solvents and other agents may exert a profound cocarcinogenic or anticarcinogenic influence during the initiation or pre-neoplastic stage. In contrast, these same solvents and agents may have little or no effect when applied after a limited application of carcinogen, i.e., during the developmental or neoplastic stage.

Little is known about the fundamental cellular changes of carcinogenesis, in either its pre-neoplastic or neoplastic stages. However, it is obvious that solvents or agents that augment or inhibit in the pre-neoplastic stage must do so in the presence of the carcinogen (or its conversion products). On the other hand, cocarcinogenic and anticarcinogenic actions in the neoplastic stage may occur principally in the absence of carcinogen, since it has been shown that this disappears from the skin within a few weeks after the last application (2, 23-25, 39, 43 page 474).

It is likely that a solvent or agent applied during the pre-neoplastic stage exerts its main effect on the rate and amount of carcinogen absorbed into the skin; or, in the case of sarcoma production, the rate of removal or diffusion from a subcutaneous site. This mechanism may be considered a physical or solvent influence on the effective dosage of the carcinogen. On the other hand, some agents, whether applied after a limited or throughout a prolonged application of a carcinogen may act on the developing tumor cell itself, during the neoplastic, or developmental, stage, thereby inhibiting or enhancing carcinogenesis.

Carcinogenesis may thus be augmented or inhibited in either the pre-neoplastic or the neoplastic stage. The means, mechanisms, and significance of the changes brought about in these two periods are worthy of differentiation, for such an analysis should result in better understanding. Techniques for separating carcinogenesis into stages may not be possible with all types of tumors, but it is certainly practical for the induced skin tumor and probably for the induced sarcoma (1).

That a particular noncarcinogenic procedure may promote or inhibit in one stage of carcinogenesis and not in the other has been shown in the preceding communication (44), which introduces experimental evidence suggesting that caloric restriction definitely inhibits carcinogenesis in the developmental (neoplastic) phase and has little effect, if any, on the initiation (pre-neoplastic) phase. It is even conceivable that a particular substance may have an inhibiting effect in one stage, and a promoting action in the other. The results of Berenblum's experiments with carbon dioxide snow (3, 5) and of Crabtree's with monochloracetone (17) suggest this latter possibility, but it would be premature to say more until this point is carefully tested.

The factual and theoretical considerations discussed in this communication indicate the desirability of testing, when feasible, the effect of a particular agent or procedure (solvent, chemical agent, hormone, physical agent, inflammation, trauma, wound healing, or dietary change) during each stage of carcinogenesis as well as during the whole experimental period. It is likely that such practice will improve our concepts of carcinogenesis and our understanding of the mode of action of the experimental agent or procedure. It may be worth while to repeat that the effects of these agents and procedures may be different for the two stages of carcinogenesis, and entirely unrelated to their effects on the subsequent growth of the tumor.

From comparisons of experimental carcinogenesis in animals and occupational tumors in man it may be assumed that the latent period may be as long as 20 years in the latter. Therefore, from a prophylactic viewpoint, there are practical possibilities of thinking in terms of cocarcinogenic and anticarcinogenic effects on the separate stages of tumor genesis. It would be advantageous to find means and methods that (a) prevent and retard the initiatory stage of carcinogenesis, and (b) retard or do not accelerate its formative stage. On the basis of this discussion, the author believes that these are practical goals.

SUMMARY

Promotion and inhibition of carcinogenesis by means of noncarcinogenic agents and procedures have been described as cocarcinogenesis and anticarcinogenesis respectively. In view of certain experimental data and interpretations from our laboratory, coupled with consideration of the pertinent literature, it appears desirable to emphasize the following:

1. The genesis of a tumor proceeds through at least two stages:

(a) A pre-neoplastic stage (preparation, initiation, inception), which under proper conditions leads to
(b) A neoplastic stage (development, formation).

3 See footnote 2.
2. Such procedures as wound healing, application of croton resin, and caloric restriction can affect the neoplastic stage, but have little or no effect on the pre-neoplastic stage.

3. Certain solvents and other procedures can have a definite effect during the pre-neoplastic stage, yet may be without significant effect during the neoplastic stage.

4. A particular agent or procedure may have one effect, cocarcinogenic, anticarcinogenic, or none, on the development of the pre-neoplastic stage, and another effect, cocarcinogenic, anticarcinogenic, or none, on the outcome of the neoplastic stage.

5. The desirability of testing, when feasible, the effect of a particular agent or procedure (solvent, chemical, hormone, inflammation, trauma, dietary change) during each stage of carcinogenesis as well as during the whole experimental period.

6. The likelihood that such practice would increase our knowledge of the mechanism of carcinogenesis and have clinical implications.

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