Challenging Problems in Cocarcinogenesis

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The role of hormones as sensitizers in mammary carcinogenesis can be surmised on a priori grounds: (a) from general evidence of hormone involvement in the maturation of mammary tissue; (b) from differences in incidence of mammary tumors between the sexes; (c) from dietary influences on the incidence of mammary tumors, a high-fat diet affecting the hormonal status of the body (8); and (d) more specifically, from the role of hormones in the development of hypertrophic alveolar nodules, the precursors of mammary tumors in mice (48).

The induction of mammary tumors by gastric instillation or injection of polycyclic aromatic hydrocarbons (35, 61) provided a reliable means of distinguishing between hormonal effects according to the times of administration, i.e., before, during, or after the carcinogenic action. We are concerned here only with the effects before the start of carcinogenic action.

It was thus possible to show that neoplastic transformation of mammary tissue (the initiating phase of carcinogenesis in that organ) failed to take place in the rat in the absence of ovarian hormonal participation (15). [Dependence on hormonal stimulation had been demonstrated previously under different conditions, specifically, in male mice receiving ovarian grafts prior to chemical carcinogenic action (42).]

Mammary carcinogenesis is the result of a complex series of events, with different hormones operating during the various stages of maturation of the organ. Consequently, apart from involvement in the carcinogenic process (23, 36) and the participation of a virus, at least in some strains of mice, convincing demonstration of hormonal preparative action, as distinct from permissive (see later section) or promoting action, is not as clear-cut or straightforward a process in mammary carcinogenesis as is the case of many other tissues or organs.

In an earlier analysis of modifying influences in chemical carcinogenesis (3), I defined the different forms of cocarcinogenesis as follows: (a) additive action by a modifying agent that itself possesses carcinogenic activity; (b) syngeneic action (in the pharmacological sense) in which the combined action of the 2 agents exceeds the summation of their separate effects; (c) incomplete carcinogenic action by a modifier responsible for only one of the phases of carcinogenesis, i.e., either initiation or promotion; (d) preparative action, rendering the target tissue more responsive to the action of the inciting agent; (e) permissive influence on the action of the carcinogen itself through solvent effects, metabolism, etc.; (f) effects on the action of a virus, e.g., activation of a latent virus or depression of the host's immune response to the virus; and (g) action as a conditional influence on tumor development, e.g., in the case of hormone-dependent tumors or of factors associated with the immunological resistance of the host to the tumor.

Attention should also be directed to anticarcinogenic action that may throw light on the reciprocal process of cocarcinogenic action, e.g., disturbances in systemic control mechanisms whereby inhibition of carcinogenesis brought about by excessive control action can point to augmentation of carcinogenesis by deficient control action.

The prevailing preoccupation with tumor promotion as a major factor in carcinogenesis has tended to minimize the importance of cocarcinogenic determinants which are unrelated to multistage carcinogenesis or which affect it only indirectly. With few exceptions (30, 70), the trend has been to use "cocarcinogenic action" and "promoting action" as synonymous terms without regard to the different forms of cocarcinogenesis, of which promoting action is only one kind.

Of the different kinds of cocarcinogenesis listed above, the 2 most often confused with, or loosely attributed to, promoting action are preparative action and permissive influences. The present article will focus on these 2 factors, including borderline situations in which distinctions are apparent but the mechanisms involved are not yet clearly defined.

The importance of distinguishing between true promoting action, i.e., the completion of the carcinogenic process instigated by initiating action, and the "pseudopromoting" factors to be considered here lies in trying to develop appropriate methods of cancer prevention, whether by exclusion of incriminating agents or interference with the carcinogenic process.

Preparative Action

The theoretical basis for preparative action on the responding tissue rests on the fact that the intensity of carcinogenic action differs somewhat according to the functional state of the cell with respect to proliferation versus differentiation (73). In practical terms, preparative action, rendering tissues more responsive to carcinogenesis, is most pronounced in connection with hormonal influences on hormone-sensitive tissues, notably in the case of mammary carcinogenesis. It is, however, also demonstrable in other tissues following reparative hyperplasia resulting from non-specific mild injury.

One of the difficulties in determining the role of hormones as "sensitizers" of tissues for carcinogenic action is to distinguish such preparative action from actual involvement in the carcinogenic process. When the experimental set-up permits the hormonal treatment (or alternatively, interference with hormonal action by ablation of endocrine organs) to continue operating after the start of the carcinogenic process, the distinction between preparative action and actual involvement becomes impossible to draw.

Difficulties also arise in the case of thyroid carcinogenesis, in which hyperplasia, brought about by hormonal imbalance, serves as a sensitizing factor (5); its role would seem, however, to be...
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more complicated than mere preparative action, involving a feedback mechanism via the pituitary gland.

Hormonal involvement in liver carcinogenesis can be assumed from sex differences in incidence of both spontaneous and induced hepatomas (18), although this in itself does not necessarily denote preparative action as distinct from other possible involvements in the carcinogenic process. There is, in fact, experimental evidence of hormonal preparative action in liver carcinogenesis (77).

Stimulation of cellular proliferation of hepatic cells is, however, best brought about by partial hepatectomy (21); when this is performed prior to the administration of a carcinogen, it increases responsiveness of the remaining liver tissue to carcinogenesis whether the latter is caused by 2-acetylaminofluorene (40), urethane (10), thioacetamide (16), or nitrosamines (14, 52). These results must be clearly distinguished from partial hepatectomy after carcinogenic treatment (24, 52), the cellular proliferation acting, in this case, as a tumor-promoting influence.

Increased responsiveness resulting from nonspecific stimulation of cell proliferation was likewise demonstrated in skin by scarification or application of various irritants prior to the initiating phase of 2-stage carcinogenesis (53).

There is also growing awareness of prior proliferative cocarcinogenic influence in urinary bladder carcinogenesis, both from epidemiological evidence in humans in connection with cigarette smoking and various occupational hazards (33) and in relation to schistosomiasis (6), and from more direct evidence from experimental studies (31).

Preparative influences are less easily demonstrable in viral carcinogenesis or in cases where a virus is involved in a complex system of carcinogenesis.

For instance, the dual involvement of infectious viral hepatitis and ingestion of aflatoxin in the development of liver cancer (32) might possibly represent reparative cell proliferation, the liver damage resulting from viral action causing reparative hyperplasia as a preparative influence; however, the situation is more complicated, with part of the viral material being incorporated into the genome of the liver cell. There has recently been a shift in emphasis from aflatoxin action to viral hepatitis as the dominant factor (79).

Modifying influences, partly preparative and partly permissive, in murine T-cell leukemogenesis include: (a) "activation" of a latent AKR-type virus by X-irradiation of the bone marrow; (b) sensitization of stem cells (prothymocytes) still in the bone marrow into proleukemic cells (dormant leukemic cells (?)); and (c) after migration of the latter (facilitated by derepression of the immune response of the animal), some form of preparative action on the thymus, permitting the proleukemic cells to proliferate there and eventually to develop into autonomous leukemic cells (25).

Other types of leukemia and lymphoma induction possessing independent modes of development are no doubt also subject to a variety of modifying influences, although differing from those affecting murine T-cell leukemogenesis. Little is known about the preparative influences involved in such cases, despite the recent advances in our knowledge of the role of viral and cellular oncogenes in neoplasia (76).

Permissive Influences

Carcinogenic action can be influenced in many ways apart from changes induced in cell responsiveness, discussed under "Preparative Action." Such permissive influences include: (a) factors enabling the carcinogen to reach the target tissue; (b) conditions affecting the stability of the carcinogen; (c) factors influencing the metabolism of carcinogenic precursors; (d) various dietary influences; (e) germ-free conditions versus the natural presence of saprophytic microorganisms; and (f) conditions tending to lengthen the life span of the animal, thereby increasing the chances of an induced tumor to manifest itself.

Factors permitting carcinogens to reach the target tissue play an important part in lung carcinogenesis by inhalation. Normally, the ciliated epithelium in the upper respiratory tract prevents, to a large extent, particulate matter from reaching the smaller bronchi, bronchioles, and lung alveoli. Interference with this protective mechanism by damaging the ciliated epithelium thus allows the carcinogen to penetrate deeper along the respiratory tract. The particle size of air pollutants is, of course, also a limiting factor in determining the degree of penetration along the respiratory tract (20, 58). There is, furthermore, the possibility that nonparticulate pollutants such as SO_2, NO_2, ozone, etc., are capable of damaging ciliated epithelium (38). Of special interest to human lung carcinogenesis, it has been determined that the time for long-term clearance of dust from the lungs is greatly delayed among cigarette smokers (12).

For other organs or tissues, the choice of solvent can also influence the effectiveness of carcinogenic action, especially with carcinogens that are insoluble in aqueous fluids. An early study (66) dealt with the coefficients of distribution of benzopyrene between lipid solvents and serum, indicating a close correlation with effective sarcoma induction by s.c. injection.

The influence of solvents on skin carcinogenesis (see Ref. 63) is complicated by the fact that a volatile solvent evaporating from the surface leads to a change in concentration of the carcinogen, as opposed to the maintenance of the initial concentration within lipoid solvents. The apparent anticarcinogenic effect of lanolin (62) could thus be explained as being due to the need for higher concentrations of the carcinogen when dissolved in this solvent to produce an effect comparable to that resulting from the use of a volatile solvent (4).

The introduction of lipophilic-hydrophilic agents of the Tween and Span group as promoters for skin carcinogenesis (60) drew attention, in a more general sense, to the likelihood of dipolypeptide reagents being especially suitable for skin carcinogenesis (57). Strangely enough, dimethyl sulfide, which might have been expected to be a very effective solvent for skin carcinogens, was actually found to decrease tumor induction by dimethylbenzanthracene in skin and, when tested in connection with the 2-stage technique, failed to influence the initiating phase either way (65). (See also Ref. 1 for recent reviews on skin testing techniques.)

The chemical stability of carcinogens varies greatly, from the very short half-life of nitrogen and sulfur mustards (both weak carcinogens) at one extreme to the very stable polycyclic aromatic hydrocarbons at the other, and is not capable of being artificially influenced in vivo except by transformation into different compounds (see below).

The same inability exists in trying to prevent alkylating agents from combining with substances in transit, resulting in only traces reaching the target tissue (thus explaining why alkylating agents are generally weak carcinogens).
All tissues are not equally susceptible to carcinogenic action, and this varies with the type of compound. Carcinogens can, in fact, be broadly divided into 3 major categories: (a) totipotential carcinogens (e.g., the poly cyclic aromatic hydrocarbons); (b) limited multipotential carcinogens (e.g., 2-acetylaminofluorene, urethan and the nitrosamines); and (c) essentially unipotential carcinogens (e.g., β-naphthylamine, ethionine, and aflatoxin).

While these differences in organ responsiveness are based on species and strain specificities (19, 63), as well as on sex (18) and, to some degree, age of the animal (17, 27, 29, 45), cocarcinogenic influences can nevertheless play a modifying role by altering the organ distribution of the induced tumors, e.g., in the case of 2-acetylaminofluorene (22, 64), urethan (41), and the nitrosamines (67, 69, 72).

With so much already known about the metabolic activation of procarcinogens into proximate and ultimate carcinogens, as well as about the competing enzymic detoxication of procarcinogens, it is surprising how little is known yet about possible cocarcinogenic influences favoring activation. More is known about the opposite process of inducing changes favoring detoxication, largely from independent studies of anticarcinogenic action (75), although the effect is, in most cases, inhibition of tumor promotion rather than antagonism of the activation of procarcinogens (71).

Another aspect of cocarcinogenic action concerned with metabolic processes involves the synthesis of carcinogens in vivo, e.g., the formation of nitrosamines from inorganic nitrates and nitrites plus secondary amines (43, 59). Although the agents involved are precursors of carcinogens rather than cocarcinogens, the process of nitrosation in vivo is capable of being influenced artificially (44).

The role of microorganisms in carcinogenesis has been investigated by different approaches. In earlier studies (with a main interest, at the time, in possible latent viral involvement), the development of spontaneous and induced tumors in animals kept under strict germ-free conditions was compared with that in animals kept under normal conditions (50, 51). The results were ambiguous and largely negative, thus failing to implicate not only viruses but also bacteria normally present in the intestine.

However, bacteria were subsequently found to possess cocarcinogenic properties, at least under special conditions: e.g., they were shown to be capable of catalyzing nitrosation of amines (28); to be responsible for hydrolyzing cycasin with the liberation of the carcinogenic product, methylazoxymethane (39); and, in a more complicated manner in connection with experimental colon carcinogenesis, to be responsible for converting endogenous bile acids (54) and cholesterol from the diet (55) into tumor promoters, operating after administration of tumor initiators specific for the colon.

In mammary carcinogenesis with the Bittner virus as the dominant factor, the permissive cocarcinogenic influence is hormonal; this influence was first attributed solely to estrone but subsequently was shown to involve a more complex interplay of hormones, including pituitary hormones, prolactin, and progesterone (11, 46).

Apart from the complicated sequence of preparative cocarcinogenic influences, in the case of murine T-cell leukemogenesis with a virus vertically transmitted (see "Preparative Action"), a permissive influence by whole-body X-irradiation (26, 37) also plays a critical role, causing a breakdown of immunological resistance in low-leukemia strains of mice harboring the virus.

In the case of Burkitt's lymphoma in humans, permissive influences appear to be responsible for the geographical distribution of the disease, which is largely influenced by a particular range of latitude, altitude, temperature, and humidity in East Africa (6). These environmental conditions supposedly determined the likelihood of individuals becoming infected by a possible insect harboring the causative virus.

Cocarcinogens in the diet are now recognized as major determinants in human cancer, notably that of the breast and gastrointestinal tract. The subject has been extensively reviewed (1, 7, 8, 13, 56) and need not be discussed here in detail, although attention should be drawn to some of the known permissive influences involved.

Of the various nutritional factors favoring tumor induction, a high fat content in the diet seems to be the most striking and significant (although a high protein content has also been found, in certain situations, to be implicated).

The effectiveness of dietary fat cocarcinogenesis varies according to the organ involved, being most pronounced in mammary carcinogenesis, somewhat less so in colon and gastric carcinogenesis, and still less so in other organs. Moreover, the mechanisms involved are quite different in these various situations.

In mammary carcinogenesis, the cocarcinogenic (permissive) influence of a high-fat diet is generally assumed to operate by its effects on the hormonal balance in the body (Ref. 8, but cf. Ref. 78). In the case of colon carcinogenesis, the effect of a high-fat diet would seem to operate in a 2-fold manner: (a) by increasing the concentration of bile acids in the intestine, and (b) by causing a change in the bacterial flora in the intestine, thus favoring the conversion of dietary cholesterol into promoting agents (54). [A somewhat simpler mode of action has been suggested based on the irritating effects of free ionized fatty acids (47) and of bile acids (49), which cause cellular proliferation of the epithelial cells of the colon and thus act as a direct promoting stimulus.] In the case of gastric carcinogenesis, the role of fats (or possibly other dietary constituents) seems to involve different mechanisms. One likely influence is the facilitation of nitrosation of amides with the formation of nitrosamides, which act specifically on the stomach mucosa (44). [A high-fat diet has also been shown long ago to be capable of enhancing skin carcinogenesis under experimental conditions (2, 68, 74). The mechanism of action, in this case, is unknown.]

**General Conclusions**

An analysis of the different forms of cocarcinogenesis must, as its primary purpose, be able to explain their mechanisms of action as an aid to a proper understanding of the overall carcinogenesis process. To brand all modifying factors that enhance carcinogenesis as promoting influences is clearly inadmissible, even when the modes of action are not yet properly understood or involve multiple mechanisms. However, there is also a more practical objective, namely, to serve as a guide for rational methods of cancer prevention by interfering specifically with each of the different cocarcinogenic action.

Many of the gaps in our knowledge of the different forms of cocarcinogenesis and uncertainties about their separate modes...
of action are due to insufficient attention being paid to appropriate planning of the experiments performed. The mere recognition of cocarcinogenic effects is no longer so critical an issue. What is now needed is to understand how the different forms of cocarcinogenesis operate. For this, a more sophisticated methodology is called for.

References


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