A Speculative Review: The Probable Nature of Promoting Action and Its Significance in the Understanding of the Mechanism of Carcinogenesis

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The new orientation in our ideas about tumor genesis arose out of the observation, made in 1941 (3, 29, 43), that carcinogenesis—the process leading up to the first appearance of a tumor—is composed of two separate components with independent mechanisms.

Carcinogenesis had previously been looked upon as a single, long drawn-out process, because, under standard experimental conditions where the carcinogen was applied repeatedly for long periods, no discontinuous changes were apparent throughout the latent period prior to the emergence of the papillomatous growth. The first clue of the existence of a kind of “biological chain reaction” during this latent period came from a study of carcinogenesis under suboptimal conditions (reviewed in [4]). After such suboptimal treatment (e.g., even after a single application of a carcinogen to the skin), which normally produces only an occasional growth, tumors could be made to develop in considerable numbers by subjecting the “pre-treated” skin to certain forms of stimulation which were themselves not necessarily carcinogenic (3, 6–9, 16, 17, 29, 33, 34, 43).

The effect of the limited treatment with the carcinogen was interpreted as constituting the conversion of a few normal cells of the epithelium into “dormant tumor cells” (or “latent tumor cells” as they were then called), which remained in a sub-threshold state requiring further “stimulation” for their development into progressively growing tumors.

The change from the normal to the dormant tumor cell has been named “initiating action”; the awakening of the dormant tumor cells into actively growing tumors has been named “promoting action” (7, 16). Initiating action is brought about very quickly, perhaps even instantaneously (6, 33, 34); it is irreversible, persisting for at least 43 weeks in the case of the mouse (7); and it appears to be highly specific. Promoting action, on the other hand, is slowly acting (7, 49); it is, to some degree, reversible (52); and it is less specific than initiating action. The tumors that are produced in this manner develop when promoting action follows a brief period of initiating action, but not when the procedure is reversed (3).

These results have been amply confirmed in other laboratories (1, 12, 26, 46, 49); a similar two-stage mechanism has been demonstrated to operate in other tissues as well as the skin—e.g., in the thyroid (10, 22), the liver (18), and subcutaneous tissues (25); and much useful information has since become available about the conditions under which the two-stage process functions (reviewed in [5]). However, the underlying mechanism of action of the initiating and promoting phases has not yet been elucidated.

I shall only refer very briefly here to the nature of initiating action, which, as already mentioned, represents a specific, sudden, irreversible change in a normal cell. Whether this is brought about by a true somatic mutation (an attractive possibility for which, however, experimental proof is still lacking), or whether it is only a mutation-like process, is immaterial for our present discussion, which is mainly concerned with the nature of promoting action.
Our problem may be posed as follows: If the initiating phase of carcinogenesis represents an irreversible conversion of normal cells into dormant tumor cells, how does the subsequent promoting action bring about the change from dormancy to progressive growth, and what kind of biological process is involved?

The simplest explanation, which was naturally considered first, was that hyperplasia, i.e., a simple, nonspecific stimulation of growth, by encouraging the dormant tumor cells to divide, might be responsible for promoting action.

Some support for this view could be found in the fact that croton oil, the most potent promoting agent known for the mouse’s skin, happened also to be a skin irritant which, in the concentration used for promoting action, caused a pronounced hyperplastic response of the epithelium. No promoting effect at all was observed, however, with most other skin irritants tested in concentrations that elicited a comparable degree of hyperplasia, e.g., with dichlorodiethylsulfide or “sulfur mustard” (2), ultraviolet radiation (47), acridine, castor oil, ricinoleic acid, glyceryl monoricinoleate, silver nitrate (51), acetic acid, cantharidin, podophyllin resin, and allylthioctcyanate or “mustard oil” (21). Even croton oil itself, which is so potent a promoting agent for the mouse’s skin, is ineffective for the skin of the rabbit, rat, or guinea pig, in spite of the resulting hyperplasia (51) and the fact that, in the case of the rabbit, at least, the skin is responsive enough to other promoting influences (29). From these and similar results, it became evident that simple reative hyperplasia, i.e., nonspecific proliferation, was inadequate to account for promoting action.

When experimental facts fail to support a working hypothesis, it is not enough to discard that hypothesis; one must also try to discover the theoretical flaw entailed and thus benefit from the false lead. This is what I shall attempt to do here.

Taking skin epithelium as a model, it is easy to demonstrate that reative hyperplasia is merely an enhancement of the normal growth cycle, where the rate of division of the “stem cells” (in the basal layer of the normal mouse’s skin, or in both the basal and lower malpighian layers of a more differentiated epithelium) is balanced by the rate of maturation and death of the cells at the surface. (Were this not the case, normal growth of the skin epithelium would either degenerate through an ultimate insufficiency of stem cells or, alternatively, would lead to progressive multiplication, with ever-increasing numbers of stem cells, as in neoplasia.) The number of stem cells in normal skin epithelium manifestly remains constant, with one-half of the daughter cells persisting as stem cells and the other half embarking on the process of maturation toward their ultimate conversion into dead keratin. In reative hyperplasia, the increased rate of division is attended by a comparable increased number of dead cells, so that a new equilibrium is reached, involving a piling up of maturing and dying cells, but not of stem cells.

Promoting action, on the other hand, presupposes an increase in the number of (neoplastic) stem cells, i.e., of those which, through previous initiating action, have become dormant tumor cells. Since the piling up of stem cells is normally prevented by the process of maturation, it follows that promoting action is essentially a process of delayed maturation.

Delayed maturation alone is insufficient, however, to explain promoting action, as it fails to account for the fact that the resulting tumor, once established, usually continues to grow without the need for further promoting action. An additional factor—some trigger-action mechanism—must therefore be looked for.

A possible clue may be found from an analogy with the growth of isolated cells, which often fail to divide unless a critical number of them is present in close proximity. This has been demonstrated with fibroblasts (18) and monocytes (32) in tissue culture, and also with transplantation of very small inocula of tumor cells in vivo (42). If such a situation also exists during the promoting phase of carcinogenesis, one could then postulate that delayed maturation enables a critical number of dormant tumor cells, before these have begun to mature, to accumulate (see Chart 1), and that once this is reached, the group of (previously dormant) tumor cells would then begin to display the characteristic property of established neoplasia—i.e., independent, progressive growth.

This concept of a critical size colony for progressive growth of tumor foci is not new. It was proposed by Rusch and Kline (45), who attributed to nonspecific proliferation the reaching of the critical size; by Fisher and Hollomon (15), who postulated a consecutive sequence of mutations in adjoining cells; and by Charles and Luce-Clauson (14) and by Nordling (35), who suggested a sequence of mutations in the same cell. These alternative mechanisms of reaching a critical size colony are, however, incompatible with the available experimental data.

The suggestion that nonspecific proliferation is responsible for promoting action has already been discussed above and found to be unacceptable. The other two hypotheses—of successive mutations either in adjoining cells or in the same cell—
are equally unacceptable, since they presuppose that initiating action and promoting action are similar in their effects and interchangeable (an inevitable condition if both processes were mutational), whereas it has been abundantly proved that the two have entirely different modes of action (3, 33, 34, 54) and that tumors arise when promoting action follows initiating action, but not when the promoting action comes first (3).

There is yet one other aspect to be considered, before turning to the evidence in favor of the delayed maturation hypothesis: this is the suggestion that not only promoting action but also initiating action was due to a dermal influence. This would be hard to reconcile with all that is known about carcinogenic action. It should be noted, however, that their experimental conditions (12 weeks of carcinogenic treatment for initiating action) rather complicates the issue, involving a considerable degree of promoting action before the transplantation of the epithelium. The possibility of dormant tumor cells being left behind in the hair follicles is not allowed for. Another difficulty may be that of eliminating the possibility of the death of many of

of Billingham et al. (11), based on earlier work of Orr (36–39), that promoting action may result from an indirect effect on the derma. Irrespective of its possible intrinsic merits, this possibility does not necessarily invalidate the present hypothesis, since such a dermal effect would presumably operate by eventually influencing the behavior of the dormant tumor cells in the epithelium, and it is this latter influence which we are discussing here. However, in a more recent publication (30), involving skin transplantations and subsequent treatment with croton oil, little evidence could be adduced in favor of dormant tumor cells being carried over in the transplants. The interpretation of their negative findings goes further than in their earlier publications and, if accepted, would imply the cells in the grafts and their replacement by ingrowing epithelium from the surrounding skin. The controls included in the experiment do not entirely exclude these possibilities.

EVIDENCE SUPPORTING THE DELAYED MATURATION HYPOTHESIS OF PROMOTING ACTION

Morphological considerations.—Though there is plenty of histological evidence of delayed matura-

tion in tumors, the “immaturity” of the cells is probably the result of the rapidity of growth of the tumor rather than a causative factor in its development. Even examples of immaturity in slowly growing tumors, notably of mesenchymal origin, can more readily be accounted for on the assumption that these are derived from undifferentiated
primitive cell types than by supposing that they represent examples of delayed maturation of adult cells.

When we turn to the question of histological evidence of delayed maturation at the earliest stage of development, before the actual appearance of a recognizable tumor, clinical material is unfortunately almost valueless, because of the unreliable criteria of "precanceroses," and because of the inability to recognize the early stages of carcinogenesis in man. Such evidence is only possible from experimentally induced preneoplastic lesions in animals, and preferably derived from quantitative cytological criteria rather than from qualitative histological inferences.

The first quantitative approach to the problem of maturation in preneoplastic skin epithelium was by Glücksmann (19), who evaluated the proportion of "resting cells" (i.e., "stem cells"), in comparison with differentiating, dying, and dead cells, in the skin epithelium of the mouse treated with carcinogenic and noncarcinogenic irritants. He observed an increase both in the relative and absolute numbers of resting cells after carcinogenic, but not after noncarcinogenic, treatment. (Unfortunately, his data are only semiquantitative, since his distinction between "resting cells" and early differentiating cells is based on somewhat arbitrary criteria.) Glücksmann concluded that the hyperplasia resulting from carcinogen treatment involved a delay in maturation of the epithelium, in spite of the contrary indication of apparent differentiation toward a more squamous type than that seen in normal mouse skin.

It was taken for granted at the time (1945) that the whole area of tissue undergoing "preneoplastic" hyperplasia was equally implicated in the carcinogenic process (though suspicion should already have been aroused by the fact that the resulting tumors were invariably focal in origin). The modern concept of dormant tumor cells rejects this "field effect" interpretation and attributes the specific initiating action to a few isolated cells hidden in the hyperplastic tissue. On this basis, the diffuse hyperplasia, in the early stages of carcinogen painting, must be looked upon as a side effect (5), unrelated to the carcinogenic process, except insofar as it also affects the few dormant tumor cells present.

The action of croton oil (and other promoting agents) also involves both a (nonspecific) increased rate of proliferation and a (specific) delay in maturation. The effect of this on the normal parts of the skin epithelium is to produce a type of proliferation which, on analysis by the method of Glücksmann, involves an increase in the number of stem cells. Any stem cells that happen to be dormant tumor cells (resulting from previous initiating action) would, under these conditions, give rise to a little colony of viable neoplastic stem cells in close proximity, which would constitute the beginning of an independent tumor. (According to Salaman and Gwynn [50], the effect of croton oil on skin previously treated with a carcinogen is more complicated, but this awaits confirmation.)

Experimental evidence.—More interesting and convincing support for the delayed maturation hypothesis of promoting action is derived from previous experimental findings which have, till now, appeared anomalous:

a) The fact that croton oil is a potent promoting agent, while most other chemical irritants are not, might suggest, at first sight, that promoting action is a highly specific process. Yet we know, from the work of Rous et al. (16, 17, 29, 43), and others (28, 31), that in the rabbit, wound healing (resulting from a punched hole in the ear) can serve as a highly effective promoting stimulus. This seems all the more surprising, since wound healing in the mouse (following a simple incision) is, at best, only a feeble promoting stimulus (40, 41). There is, finally, the significant observation of Linell (28) that, while deep traumatization in the rabbit's ear, produced by a punched hole, is an effective promoting stimulus, superficial traumatization is not so, though the degree of hyperplasia produced is at least as effective in the latter as in the former.

These anomalies are readily explicable on the basis of the delayed maturation hypothesis. A punched hole removes a large area of tissue, and its replacement, in the process of healing, involves a numerical increase of persistent stem cells. If a dormant tumor cell happens to be present among the peripheral stem cells, it, too, will multiply, and the daughter cell will thus reach the critical number presumably required for progressive independent growth. In the mouse (simple incision) and in Linell's experiments in the rabbit (with superficial traumatization), where only a small amount of destruction or removal of existing stem cells is involved, the healing process mainly induces ordinary hyperplasia which, as explained above, merely speeds up the growth cycle without increasing the number of persistent stem cells.

b) An analogous example may be found in the experiments on liver carcinogenesis by Glinos et al. (18). Animals receiving suboptimal doses of azo dye carcinogens yielded a higher tumor incidence in the remaining portions of liver of partially hepatectomized animals than in the comparable portions of unoperated controls. The compensa-
undifferentiated daughter-cells of that altered cell potentiality of a normal cell; promoting action, by delaying maturation, allows a sufficient number of results in a sudden, permanent change in the potentiality of a normal cell, resulting in a tumor. Vitamin C deficiency is known to delay the maturation of fibroblasts (24), and, according to the present hypothesis, this would be expected to serve as a promoting stimulus and thus augment carcinogenesis, despite the fact that cell division is inhibited. Such augmentation of carcinogenesis was indeed found to occur, even though the effect was not very pronounced. According to other theories, the effect of vitamin C deficiency should have been actually inhibitory.

d) We have so far been dealing with adult tissues, in which an equilibrium is maintained—i.e., where the number of stem cells remains constant under both normal and reparative growth conditions. In embryonic development, such an equilibrium does not exist, and it might, therefore, be expected, on the basis of the present hypothesis, that the increase in the number of stem cells, as a normal process of embryonic growth, would be sufficient to serve as a promoting stimulus under such conditions. In other words, embryonic tissue which responds to carcinogenic action at all, should do so very rapidly, without the need for prolonged carcinogenic treatment or additional stimulation (or promoting action) of any sort.

It may be noted, in this connection, that tumor production of the lung in newborn mice, resulting from injections of urethan into the mother during the late stages of pregnancy, is exceptionally rapid, tumors becoming recognizable within 3 days after birth (53). Similarly, with the method of carcinogenesis whereby embryonic tissue is implanted subcutaneously, together with a crystal of methyl-cholanthrene, into an adult animal (20, 44), the resulting tumors appear much more rapidly than by the more conventional methods of carcinogenic action combined with a process of delayed maturation uncomplicated by any increased proliferation of tissue. Vitamin C deficiency is known to delay the maturation of fibroblasts (24), and, according to the present hypothesis, this would be expected to serve as a promoting stimulus and thus augment carcinogenesis, despite the fact that cell division is inhibited. Such augmentation of carcinogenesis was indeed found to occur, even though the effect was not very pronounced. According to other theories, the effect of vitamin C deficiency should have been actually inhibitory.

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DISCUSSION

Perhaps the most far-reaching implication of the delayed maturation hypothesis is that the evolution of a tumor may have little or nothing to do with the problem of growth: initiating action results in a sudden, permanent change in the potentiality of a normal cell; promoting action, by delaying maturation, allows a sufficient number of undifferentiated daughter-cells of that altered cell to accumulate, and thus reach a critical size colony. Neither process is dependent on an increase in growth rate, the latter being able only to speed up or exaggerate the manifestation of the carcinogenic effect, but not bring it into being. The fact that some tumors continue to grow expansively, though their mitotic index may be lower than that of the parent, normal tissue, thus ceases to be a mystery.

Normally, in the embryo and in the very young, tumors are rare because of the low probability of initiating action occurring so early in life. (If initiating action is indeed mutational, then the incidence of spontaneous initiating action taking place would naturally increase with age.) When a tumor does arise at so early an age, its evolution is rapid, because the normal expansive growth, characteristic of that stage of development, itself serves as the promoting stimulus. In the adult, on the other hand, initiating action probably arises spontaneously with increasing frequency as the subject ages; however, since the normal growth processes have by then reached an equilibrium, the conversion of the resulting dormant tumor cells can no longer arise spontaneously. Additional promoting stimuli are then required to convert the dormant tumor cells into progressively growing tumors; and, since this may not occur in every case, many dormant tumor cells must remain unrecognized.

Certain phenomena, previously unexplained, thus become clarified. For instance, the fact that dormant tumor cells below a critical size colony remain in a dormant state may explain why metastases sometimes appear in man several decades after an apparently successful radical operation for cancer. It might also account for those rare, but well authenticated cases of spontaneous “cures” of malignant growths. This would occur if, for some reason, the rate of maturation of the tumor cells were to catch up with the rate of division, until the number of undifferentiated tumor cells fell below the critical size required for progressive growth. It might also explain certain kinds of therapeutic arrests of cancer, e.g., the regression of prostatic tumors following estrogen administration (23), and thus serve as a lead for other therapeutic measures.

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