Progression and Promotion

The several original studies demonstrating that tumors could be induced in animals by combinations of agents or agencies with different properties all involved a careful analysis of the behavior of the lesions induced [Rous and Kidd (1) and Shubik (2)]. In addition to the discovery that some tumors could be induced in two stages, then and now called “initiation” and “promotion,” the concept of tumor progression as enunciated by Foulds (3) was to a great extent developed from some of these experiments.

Since that time the concept of initiation and promotion has seized the imagination of many carcinogenesis workers, and many experiments are regularly reported that deal with the possible mechanism of action of “promoters.” Since most of such experiments are now performed with the use of tissue culture techniques, it is no longer possible to examine the characteristics of the response as seen in vivo in detail. Tissue culture techniques in carcinogenesis with the use of the end point of “transformation” clearly have provided a basis for many rapid studies and have enabled the elucidation of many new facts. However, these techniques have greatly restricted the ability of investigators to examine the phases of neoplasia in depth, and indeed the equating of transformation with all the fundamental characteristics of the initiation and progression of neoplasms has given rise to a narrow view. In effect, a presupposed basis for cancer is assumed before it has been elucidated.

This comment is, of course, not to underplay in any way the immense usefulness of this technique and other in vitro techniques. A cautionary note is made to point out that, at this juncture, there is still much to be learned from the in-depth study of in vivo experiments.

Initiation and promotion, which describe the two stages apparent in skin carcinogenesis, were terms introduced by Friedewald and Rous in 1944 (4); this paper was the culmination of a series of studies undertaken by Rous and his co-workers using rabbit skin tumors induced by coal tar or various hydrocarbon carcinogens combined with wound healing or turpentine. A central theme throughout this detailed series of papers is concerned with the disappearance and reappearance of benign tumors coincident with the cessation and reimposition of treatment. The characteristics of the various benign tumors induced in the rabbit’s skin and their conditional nature take pride of place. Their findings are said to “... emphasize the need for a sharp distinction in thought between tumor inception and tumor formation, and between those factors that render cells neoplastic and those affecting their subsequent behavior” [Mackenzie and Rous, 1940 (5)].

The subsequent studies in which added credence was given to the concept that skin carcinogenesis could be demonstrated to involve two stages were reported in 1947 by Berenblum and Shubik (6, 7). For these studies a “model experiment” was devised as the experimental method to be used throughout. This model, fashioned after an experiment by Mottram in 1945 (8), used a single subeffective dose of a polycyclic aromatic hydrocarbon carcinogen as the “initiator” followed after an interval by repeated application of the noncarcinogen irritant mixture croton oil. This method is now in constant use by many investigators studying the action of the active principle of croton oil, the phorbol ester TPA, as well as potential, new promoting agents.

Unfortunately, the original studies describing the model studies were somewhat misleadingly published in a series of separate papers by the authors individually. The model experiment studies mainly recorded all the lesions as “tumors” with no detailed histologic or life history descriptions (6, 7). Subsequently, the detailed life history of these tumors was published [Shubik, 1950 (2)] and compared with the effects of the polycyclic aromatic hydrocarbon MCA administered repeatedly alone or followed by croton. The original study used for this comparison was one [Berenblum and Shubik, 1949 (9)] in which three concentrations of a single application of the carcinogen DMBA was followed by a standard treatment with croton oil. The tumors were divided into papillomas regressing, those remaining stationary, and those continuing to grow as well as cancers. Approximately 50% of the papillomas occurring with a single dose of DMBA followed by croton oil regressed, and of the...
remainder about 30% remained stationary and 15% grew progressively.

The tumors induced by a single application of MCA and followed by croton oil were similar in their distribution; i.e., MCA alone administered repeatedly resulted in almost 50% of the cancers; there were no true regressions—some tumors disappeared by incorporation into the carcinomas. This study was taken to suggest that the process of initiation should be considered to be a graded affair in which different degrees of growth potential were confirmed upon the altered or latent tumor cells by the initiating agent (2).

In 1974 Berenblum (10) concluded that the high regression rate of these tumors promoted by croton oil might have been due to the presence of an anticarcinogenic substance in croton oil. This hypothesis, he suggested, is likely, since Van Duuren et al. (11) had reported in 1966 that a semipurified fraction of croton oil did not result in so many regressions—a finding now difficult to understand since numerous studies show that the probable active principle of croton oil, TPA, gives rise to exactly the same tumor distribution as seen in the original studies.

Furthermore, a study by Shubik (2) in 1950 had already demonstrated that croton oil administered in a mixture with MCA resulted in an effect that appeared to represent a combination of the product of each treatment. Thus as many malignant tumors as would be produced by MCA alone as well as many regressions as would be produced by croton oil promotion occurred together. The croton oil therefore seemed to have a capacity for producing lesions with different biologic characteristics from those induced by the carcinogen alone.

The distribution of types of skin neoplasm induced by polycyclic aromatic hydrocarbons of different kinds at different doses and different dose distributions has been carefully studied in a variety of experiments, and a series of entirely consistent results were obtained. Croton oil- or TPA-promoted neoplasms are notable for their propensity to regress most often and, if not, to result in a benign response; the distribution of neoplasms induced by polycyclic hydrocarbons alone has also been found to be varied depending on the distribution of the dose; thus large single doses of various polycyclic aromatic hydrocarbons result in many regressions, but unlike the croton oil-promoted neoplasms those polycyclic hydrocarbon-induced neoplasms that remain often become malignant.

Until recently, these seemingly minor details of these observations have been ignored. The primary issues requiring elucidation in carcinogenesis were and are to most research workers concerned with the biochemical and molecular biologic mechanisms involved. It is accepted doctrine that the basic definition of neoplasia is satisfactorily established and does not require reexamination. There is, as a result, a circular form of reasoning that has become influential. Most definitions of neoplasia suggest that the primary feature of this “condition” is the occurrence of cellular anarchy. In view of Foulds’ (3) eloquent demonstrations of tumor progression, it is somewhat astonishing that such a simplistic and obviously fallacious view could persist. However, these textbook definitions satisfy the requirements of those who believe that neoplasia is a result of a somatic mutation and that no more need be thought about the nature of the various lesions involved and their systemic accompaniments. The relationship of benign tumors to malignant conditions is dealt with in two separate manners. In clinical medicine, experience delineates those benign lesions that eventuate with high frequency in cancer and those that do not. Thus junctional nevi, polyps in the colon, and papillomas of the urinary bladder, for example, require vigorous therapy, whereas lipomas, for example, are only dealt with cosmetically. In the instance of tumors induced in experimental animals, however, there have been a progressive historical development and drastic change of viewpoint. These happenings have coincided with the initial use of experimental tumor induction to confirm human clinical observations [Yamagiwa and Ichikawa (12)] and with the subsequent use of experimental animals in toxicology to establish carcinogenicity as possibly predictive of human hazard [Shubik and Sice (13)]. In the instance of the earlier studies in which confirmation of observations in humans was sought, only unequivocally malignant tumors were considered to have provided proof. Indeed, it was believed that such lesions needed to be clearly invasive and to have metastasized. Numerous efforts to establish experimental carcinogenicity in animals resulting in hyperplastic or benign lesions were dismissed out of hand during this era. This rigid attitude has now undergone an almost complete about face. This change has occurred slowly over a period of almost 20 years, with most attention being paid to the problem in the past decade. The primary reason for the new interest has been the greatly increased number of chronic toxicity tests performed on many chemicals and the regulatory impact of such tests. The legal implications of these tests have resulted in construction of criteria for evaluation that meet the needs of the rules of evidence rather than the requirements of scientific inquiry. All these influences have resulted in a major fractionation of the scientific community, and it is now extremely difficult to find an objective analysis of the role of benign tumors as part of the general concept of neoplasia. The interpretation of the significance of benign tumors in experimental carcinogenesis studies of a practical nature has now gone through a full circle: During the earlier stages benign tumors were entirely ignored as important end points; then it was concluded that their existence should be noted, suggesting that more experiments should be done; later it was believed that benign and malignant tumors could be added together in the assessment of potency; now there are those who believe that benign and malignant experimental tumors should be treated equally for regulatory purposes.

The nature of benign tumors occupied many investi-
gators’ attention during the early days of cancer research when tumor transplantation was a primary concern. The lack of transplantability of most tumors classified as benign on a histologic basis was observed, and to recommendation for transplantation as a method for diagnosing cancer became customary. The ability of malignant tumors and of embryonic tissues to grow in immunologically privileged sites, such as the anterior chamber of the eye, whereas neither benign tumors nor normal tissues would do so, was noted as long ago as the last century by Cohnheim (14). This observation was repeated several times later.

In the recent past no attention has been paid by basic research workers to the distinction between benign and malignant neoplasms. Verma et al. (15) recently reported that papilloma induction by a combination of initiation by DMBA and promotion by TPA can be inhibited by retinoic acid, whereas they observed no inhibition of tumors induced by DMBA given repeatedly. This seems to be the second reason for believing that the papillomas induced by these different treatments are, in fact, different biologic entities. Morphology alone provides only a superficial understanding of the dynamic characteristics of the lesions. In an even more recent paper, Hennings et al. (16) using various carcinogens as the initiator followed by croton oil or TPA confirmed and extended the original observations that few of the papillomas induced by the initiation-promotion sequence become malignant. The rate of malignant transformation was remarkably similar to that reported in the original studies—ranging up to 4.6% of the papillomas. Reddy and Fialkow (17) using DMBA followed by TPA reported that up to 88% of papillomas induced by the initiation-promotion sequence regressed. When DMBA was applied repeatedly, fewer papillomas regressed. Using cell markers they concluded that the difference is explained largely by the numbers of cells changed.

Although the differences between these lesions have not been elucidated, surely the most interesting aspect of the croton oil-TPA experiments in “in vivo” experiments is that the separate actions do not overlap in time; (ii) neither action alone should be carcinogenic to any degree; (iii) augmentation should occur when the two agents are administered in one particular sequence but not in the reverse order; (iv) extending the interval between the two actions should not lead to a significant decrease in tumor yield; and (v) the ultimate tumor yield should be quantitatively related to the dose of initiator while the efficacy and speed of tumor induction should be determined by the promotor.”

Berenblum (10) added that it was more difficult to adduce such rigid proof for systems other than skin, but nevertheless he believed that some evidence favored the possibility that a similar sequence was demonstrable in other organs. At the time of writing—1974—relatively few “promotion” studies in organs other than the skin had been reported. Since then, a series of experiments have been reported, notably using induced liver tumor or induced bladder tumor models that have been equated with the skin initiation-promotion studies. Unfortunately, none of these studies meet Berenblum’s carefully worded criteria. Clearly, many studies are suggestive of similarities to the skin studies, but there are many differences. It is necessary for some of these studies to be considered in detail and together for an overall perspective to be obtained.

The numbers of studies so far reported using the urinary bladder of the rat as the target organ are most clearly illustrative of the oversimplification that has crept into the field; at the same time, however, it seems reasonable to hope that a more objective analysis of these data may yield information of interest.

The first studies using the bladder as a target organ were those of Hicks et al. (18), who devised an interesting system in which the bladder epithelium reaction was “initiated” with a single subeffective intravesical dose of MNU and was “promoted” with continuous feeding with either saccharin or cyclamate. This study was well designed and should have met all the criteria in (10). Unfortunately, the initiating dose of MNU proved to have been in error and repetition resulted in the induction of many tumors with this dose alone. In addition, saccharin alone has since been demonstrated to be carcinogenic to the urinary bladder. Indeed, in the first experiments of Hicks et al. (18) saccharin alone induced 1.6% of bladder tumors, with none observed in the controls. In a repetition of these studies by Hooson et al. (19), 30% of the rats developed tumors with an initiating dose of 1.5 mg MNU alone. There is an unusual debate reported in this paper in which some authors said that although saccharin did not enhance the incidence of cancers in the bladder, it did increase the incidence of hyperplastic lesions. The lesions might be considered to be benign neoplasms by some pathologists. Whether they regressed is not known.

Two other series of bladder carcinogenesis studies have used slightly different techniques and have been interpreted, once again, as having demonstrated the existence of a two-stage mechanism of carcinogenesis.
that can be equated to the findings in the original mouse skin studies. The studies of Nakanishi et al. (20, 21) have used the systemically administered urinary bladder carcinogen BBN as the initiator and, primarily, saccharin as the promotor. Once again, two carcinogens acting on the same target organ have been administered sequentially, but only in one sequence to demonstrate the desired finding. In analyzing these experiments in detail, one sees that they differ significantly from the original skin studies in several ways that render the hypothetical interpretation quite different. In these studies the BBN has been routinely administered in the drinking water for 4 weeks, and it is reported that a tenfold change in the dose of the initiating agent BBN (0.01–0.001%) does not significantly change the incidence of induced tumors.

In the series of studies by Cohen et al. (22), the bladder carcinogen FANFT has been used as the "initiating" agent followed by saccharin in most studies. Again, two carcinogens acting on the same target organ have been administered in one sequence. In this instance, the main deviation in findings from the key observations in the original skin studies has been that a decrease in tumor incidence was observed if the "promoting" treatment was begun 6 weeks after rather than immediately after the administration of FANFT. These studies additionally suffer from the induction of many tumors by the FANFT alone. In the mouse skin studies by Berenblum and Shubik (6, 7), the incidence of tumors bore a direct relationship to the quantity or quality of the initiating agent. Thus changing the concentration of the initiator (with the use of the same compound for this purpose) resulted in a change in incidence; the latent period could be maintained as a constant by use of the same promoting procedure. The tumor incidence could also be changed by varying the kind of carcinogen used for the initiation procedure. In addition, changing the time interval between initiation and promotion was seen not to change the incidence of tumors induced (23), a finding that has been the subject of several more recent studies. If the interval between the two treatments was increased to 60 weeks as compared with the 43 weeks used in the first studies, a decreased incidence was observed [Roe et al. (24) and Van Duuren et al. (25)]; a repetition of experiments under these extreme conditions has demonstrated that the original conclusion, namely, that the initiating effect was irreversible, has been confirmed. The decrease in tumor incidence appears to be related to a decreased sensitivity to skin carcinogenesis of older animals (26, 27). Thus the first initiation-promotion studies were considered to provide a foundation for the hypothesis that the initiation involved the conversion of a certain number of normal cells to irreversibly changed latent tumor cells. The studies using the urinary bladder carcinogenesis models do not conform to this pattern, and no similar deductions can be drawn from these published studies.

Of a series of studies in which the enhancement of hepatoma induction, particularly in the rat, was used to demonstrate the existence of two stages in carcinogenesis, few (28, 29) conformed to the principles in (10). However, one can induce or enhance the development of tumors by combining agents with apparently different mechanisms of action sequentially. In most if not all of these studies, both compounds used have been capable of performing the entire action alone. The studies in which lung adenoma induction in the mouse has been used in the same manner have been confounded by the presence of many similar tumors occurring in the untreated controls. Clearly, an additional factor is present that needs to be understood before such studies can be used as a basis for general hypotheses of mechanisms of carcinogenesis. In summary, it is clear from early studies in skin carcinogenesis in the mouse that tumors can be induced by two factors being combined in a particular sequence. One of the factors may be a noncarcinogen when administered alone. With the use of tumor induction in the urinary bladder, a series of experiments have demonstrated that different factors can be shown to be capable of interacting in different ways to induce tumors; however, no consistent pattern is apparent when a series of experiments using combinations of different carcinogens are compared. The same finding is true of studies using either hepatoma in the rat or lung adenoma induction in the mouse. There are unquestionably a series of different factors that can exert profound modifying effects on chemical carcinogenesis in a variety of organs. However, to equate these differing factors simply with the original skin carcinogenesis studies and to attempt to draw similar conclusions about mechanisms would seem to serve no practical purpose.

Despite the critical nature of this review of studies of promotion in carcinogenesis, the large amount of work that has been undertaken by the molecular biologists and biochemists cannot be ignored. Although no clear-cut established relationship exists between many of the observations to be cited and carcinogenesis, the relevance of some of these investigations to our eventual understanding of carcinogenesis cannot be dismissed. On the basis of information largely derived from the skin model experiments, the promotion phase, unlike the initiation phase, has been suggested to be reversible [Slaga et al., 1978 (30); Weinstein et al., 1982 (31); and Hecker et al., 1982 (32)]; as a consequence, promotion most likely represents an epigenetic phenomenon.

The phorbol esters are exceptionally active biologically and have resulted in the discovery of different effects, including inhibition of the epidermal growth factor binding to cellular receptors and various other effects mediated at the cell membrane [Yamasaki et al., 1981 (33)]. The possibility that some of these effects are mediated by a mechanism involving cell-to-cell communication mechanisms has been suggested by the experiments of Trosko et al., 1982 (34). The fact that TPA as well as other compounds such as saccharin have been found to be clastogenic has suggested to some authors that such effects occur as a result of
changes in the cell membrane on the chromosome and that this phenomenon may play a role in promotion [Emerit and Cerutti, 1981 (35)]. The idea that promotion somehow or other involves a modification of cell differentiation has attracted many researchers; this hypothesis and many of the biochemical hypotheses of promotion are well reviewed by Slaga (36).

In few of the studies of possible two-stage carcinogenesis, in organs other than the skin has any emphasis been accorded to the study of the life histories of the induced lesions. Although the studies of Teetor and Becker (37) and Farber (38) were not concerned with the two-stage mechanism of carcinogenesis, these investigations of the growth potential of induced liver tumors revealed behavior not dissimilar from that observed in the induced skin lesions by Rous and Kidd (1) and Shubik (2) as well as the mammary tumors observed in the work of Foulds, 1949 (39). These lesions regressed upon cessation of treatment or stimulation, only to reappear when the stimulus was reinstituted.

It is clear from the studies discussed that the current concept that neoplasia represents a change in cellular behavior best described as anarchic cannot be correct. Perhaps croton oil- or TPA-promoted lesions are merely biologic curiosities; or the skin with its special normal life cycle might be an inappropriate organ from which to generalize. However, the overt occurrences in these skin studies have merely drawn the attention of the research worker to other instances in other organs with the use of other agents as inciting factors that reveal identical patterns. A salient fact that cannot be contravened is the observation that the quality of the neoplastic response can be determined by the nature of the treatment employed; i.e., the proportion of neoplastic lesions regressing, remaining localized and benign, or becoming invasive and overtly malignant can be predetermined experimentally by either a quantitative or a qualitative change in the treatment. These major differences do not require careful statistical analysis to establish their significance. A single application of DMBA followed by promotion with croton oil or TPA can result in the induction of lesions, of which as many as 80% may regress and the remainder will be largely benign; this occurrence can be compared with the effects of repeated applications of DMBA that will result in almost no regressing lesions, with most being malignant; a single large dose of DMBA will result in an intermediate picture with many regressions but also many malignant tumors. This is only one overt example of a general pattern to be seen in many experimental situations.

What does this imply? The current philosophy invokes the occurrence of meaningless generalities such as "growth abnormality" or "cellular anarchy" as the basis of cancer. However, it would be not more than a simple description of the observed effects of experimental carcinogens to say that these carcinogens induce intracellular injury that incites a cellular response with many end points, including localized lesions, regressing lesions, and malignant lesions. The in vivo response to carcinogens does not result simply in transformation of normal cells to malignant cells; it is, rather, a graded response following a delineated pathway with many stages and end points; and the final outcome can be determined by the nature, either qualitative or quantitative, of the inciting stimulus.

Chemical, physical, viral, and unknown carcinogens abound in the human environment, and obviously only few of these carcinogens cause cancer. A human lucky enough to survive into old age possesses numerous neoplastic lesions in the form of polyps of the colon and benign lesions of the skin, the breast, the prostate gland, and other organs. There has been little questioning of the meaning of these lesions or their pathogenetic relationship to clinical cancer. Their clinical diagnostic significance alone has been the subject of study. The immunologist continues to search for explanations for resistance to carcinogens in terms of systemic immunologic reactions or cellular immunologic reactions applicable to infectious diseases or transplantation reactions. These analogies are, in all probability, inappropriate, and a reasonable assumption is that we need a better understanding of the nature of neoplasia before we are likely to find solutions to problems of possible resistance.

Is it possible that in neoplasia we are merely dealing with a general cellular mechanism that exists to cope with a variety of intracellular injuries? Is, perhaps, the case that the regressing lesions and the benign lesions are, in fact, the end points for which the immunologist is searching? A great stretch of the imagination is not required to visualize the oncogene as part of a memory mechanism that activates some unknown reaction to intracellular injury.

In terms of general biology, the multicellular organisms have evolved a complex series of defensive responses to extracellular injury involving various inflammatory reactions and their systemic concomitants. Needless to say, these reactions are by no means always successful from the standpoint of the host and indeed may well be the immediate cause of the obvious ill effects noted.

The unicellular organisms, in contrast, react to injury by dividing and moving. In the search for the features that may link chemical, physical, and viral carcinogens apart from their ability to induce neoplasia, only one characteristic in common is obvious, namely, their ability to produce intracellular change or injury while leaving the cell viable. Perhaps the initial and fundamental characteristic of neoplasia is a reversion of the cell to unicellular behavior. Division and invasiveness are the characteristics of the neoplastic cell, and increased motility certainly seems to be the most likely mechanism for invasion.

The suggestions are made once again (40, 41) in the hope that they may stimulate discussion of the nature of the neoplastic response. It is realized that these are broad suggestions that require much in-depth extension, if they are right.
However, if nothing else, the view proposed is as cogent as any current view of the nature of neoplasia and, clearly in the view of this author, more in accord with the facts.

The availability of carcinogens in the laboratory made it possible for the experimentalist to observe neoplasms from their first inception through various stages to their different end points. The detailed picture seen differs considerably from that seen by the clinician who, in most instances, only observes the end point of a process that has taken many years to develop. In this paper I have tried to record the salient observations made by some of the few investigators struck by the different life histories of their carefully observed experimentally induced tumors as compared with the sequence they would have expected from the textbook descriptions. Frequent regression of any lesion, by definition, would be considered to have rendered the lesions in these older in vivo studies is to remind those now working on animal models of carcinogenesis in mice, rats, rabbits and guinea pigs. Cancer Res 1956; 16:728-742.

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