The Experimental Study of Tumor Progression: A Review

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The idea that tumors develop by the progressive acquisition of permanent new characters first receives clear expression in Rous and Beard's paper on "The progression to carcinoma of virus-induced rabbit papillomas (Shope)" (86) and in H. S. N. Greene's description of "progressive steps in a graded evolutionary process" in spontaneous mammary cancer in rabbits (53). Increasing interest in progression as a phenomenon of wide occurrence and of probable importance in the management of cancer makes it opportune to review the subject now with the especial purpose of seeking some general principles and working hypotheses to guide investigation of the nature and mechanism of progression, about which little is known. A few examples are chosen, somewhat arbitrarily, to indicate the scope of progression, and most attention is directed to those experimental materials and theoretical principles that seem immediately useful.

DEFINITION AND RECOGNITION OF PROGRESSION

The term "progression" is here used to indicate development of a tumor by way of permanent, irreversible qualitative change in one or more of its characters. Progression is different from mere extension in space and time without qualitative change, and from reversible variation due to environmental factors, for which "modulation," as suggested by Weiss (102, 108), is an appropriate name.

Information about progression comes mainly from three sources: (a) the study by orthodox clinical and pathological methods of the natural history of tumors in animals and in man; (b) observations on the reaction of tumors to extrinsic stimuli, of which the most important are hormones, chemotherapeutic agents, and the chemical and physical agents used in the induction of tumors of the skin in rodents (for the purposes of this Review the ability to react to any extrinsic stimulus is called "responsiveness"); (c) transplantation of tumors which is valuable as a test for reversibility as well as for extending the opportunities for progression to occur. The most detailed information comes from chemically induced tumors of the skin in rodents and from mammary tumors in mice.

CHEMICALLY INDUCED TUMORS OF THE SKIN

Early stages in the genesis of induced tumors.—Rous and his colleagues (87, 88) described the behavior and structure of the tumors induced by repeated applications of tar to the skin of rabbits. Warts develop early, but some regress during the period of tarring, and all regress if tar is withheld. The warts are "conditional tumors" which depend for their growth and survival on continued extraneous stimulation. Few papillomas develop into carcinomas and only after long delay despite continued tarring. Early workers were misled into too-frequent diagnosis of carcinoma by the "facititious malignancy" of carcinomatoids, which despite histological signs of invasive growth depend, like other warts, on continued stimulation and regress when it is withdrawn. Tumors that regress during intermissions of tarring recur at the same site and with the same specific characters when tarring is resumed. Moreover, wound-healing or some nonspecific irritants such as turpentine, which by themselves induce no tumors on normal skin, evoke growths from previously tarred skin at the site of tumors that have regressed. These growths are not fresh tumors but true recurrences. Alternating regression and recurrence can be repeated 2 or 3 times at one site (72). Rous and his colleagues (39) distinguish two separate elements
in carcinogenesis, namely, tumor inception or initiation and tumor promotion. Initiating agents convert normal cells into "latent tumor cells," or cells in a "subthreshold neoplastic state," and promoting agents stimulate the latent tumor cells to proliferate to form a visible tumor. Initiation is irreversible, the subthreshold neoplastic state being demonstrable at least 6 months after withdrawal of tar. Only specific carcinogens, such as tar and the carcinogenic hydrocarbons, initiate tumors. Promotion on the contrary is reversible; when the promoting stimulus is withheld, the tumor that it evoked regresses and tumor cells revert to the subthreshold neoplastic state. Tar and other carcinogens promote as well as initiate tumors; nonspecific stimuli promote but cannot initiate. Promoting agents operate partly on tumor cells and partly on adjacent normal tissues, their main effect, it seems, being to produce conditions favorable to cell proliferation in general.

Friedewald and Rous (40) later describe the steady emergence of new tumors for long periods up to 2 or 3 years after the last application of methylcholanthrene. For several reasons they reject the view that the late tumors develop from cells that have been dormant in the subthreshold neoplastic state, since applications of methylcholanthrene stopped and infer that, in addition to inducing latent tumor cells quickly, methylcholanthrene initiates "latent neoplastic potentialities" whereby cells develop automatically but gradually and at varied rates towards the neoplastic state. It is possible, although not mentioned by Friedewald and Rous, that latent neoplastic potentialities are more apparent in rabbits painted with methylcholanthrene than in tarred rabbits, because methylcholanthrene has much less "promoting" power than tar.

It has long been suspected that the carcinogenesis in mouse skin is divisible into stages which have been variously separated and named (12, 14, 25, 28, 80, 90, 100). Berenblum and Shubik (14) with wide agreement adopted Rous' division into stages of initiation and promotion, but the processes in rabbits and in mice differ in important ways.

In mice, tar or the carcinogenic hydrocarbons evoke papillomas a little later than in rabbits. Of these papillomas some regress, some grow for a short time as papillomas and then remain stationary, some grow progressively as papillomas, and some transform into malignant carcinomas (69, 96). In addition, some tumors are malignant carcinomas when first seen. When carcinogen is applied repeatedly throughout the experiment, carcinoma develops in almost every mouse. If the carcinogen is withdrawn about the time when the earliest warts are expected, papillomas and carcinomas develop as if continuously treated mice but more slowly (5, 69). As carcinogenic action is further reduced, the eruption of warts is still more delayed, more of them regress, and fewer become malignant. A single application evokes, depending on the potency of the carcinogen and the susceptibility of the mice, either no tumors at all or a few long-delayed papillomas, almost all of which remain benign (26, 74, 75). When the carcinogenic stimulus is below the optimal, the development of tumors is accentuated by various "co-carcinogenic" agents applied concurrently with the carcinogen and, more instructively, by the application after a limited period of carcinogenic action of agents (of which croton oil is the most potent and widely used) that are unable by themselves to evoke tumors from normal skin (18, 89). If carcinogen is applied repeatedly but withdrawn shortly before tumors erupt, subsequent applications of croton oil substitute almost completely for further applications of carcinogen, eliciting the same yield of tumors of the same kind and at the same time as the carcinogen itself (68). After shorter carcinogenic action, croton oil accelerates the eruption of tumors, but more of them are, and remain, benign; after a single application of carcinogen croton oil elicits warts, but many of them regress, and few or none become malignant (18, 17, 62, 81, 91, 96). The tumors evoked by croton oil, therefore, are of the same kind as those that develop a long time after the restricted application of carcinogen alone. The initiating action of the carcinogen determines the particular nature of the tumors, and the subsequent action of croton oil does not change it (96). The initiating action determines also how many tumors develop; croton oil determines when they develop. Croton oil evokes tumors for at least 48 weeks after the last application of carcinogen, and the tumors appear at a nearly constant time after the first application of croton oil, irrespective of the lapse of time since the withdrawal of carcinogen (14–16).

The action of croton oil is gradual; repeated applications spread over several weeks are needed to elicit tumors (14, 63, 91). The effect is reversible until a short time before tumors appear (64, 91). The tumors evoked by croton oil are not conditional tumors that regress, as do rabbit papillomas, when the promoting stimulus is withdrawn, although it is possible that some conditional tumors develop and escape recognition.

Some other procedures, less adequately studied, seem like croton oil to accelerate tumor development from skin previously subjected to carcinogens; they include freezing (11), scalding (28), ap-
plication of monochloroacetone (24), diet (100), and trauma (reviewed by Linell [70]). Shubik (95), finding turpentine and other irritants ineffective, infers that tumor-evoking power is not related in a simple way to the capacity for inducing epidermal hyperplasia and suspects that the superiority of croton oil may be due to a specific chemical action. Croton oil, however, has no promoting action on rabbits, and wound healing is dubiously or weakly effective in mice. Judging from the published evidence it is probable that agents like croton oil differ fundamentally from the "promoting" agents described by Rous and need a different name, Mottram’s term "developing agent" being adequate. Promoting and developing agents are not by themselves carcinogenic, and they evoke tumors whose individual characteristics are determined by previous carcinogenic (initiating) action. In all other ways they differ; they differ in identity, in the time required to produce results, in the mechanism of action since developing agents merely accelerate a process which occurs slowly in their absence, and fundamentally in the results produced for, whereas promoting agents evoke and maintain conditional tumors, developing agents after long delay elicit tumors which persist and advance without them. The assumption that initiation is necessarily rapid and perhaps instantaneous because carcinogen is not for long demonstrable in the skin is unnecessary and probably wrong. Initiation is a process, not an event. It starts when the carcinogen is first applied. In rabbits it may advance quickly to terminate in a subthreshold neoplastic state from which promoting agents evoke conditional tumors, or it may advance slowly for up to 2 or 3 years, during which "latent neoplastic potentialities" develop gradually and at varied rates, to culminate in the growth of papillomas or carcinomas which are not conditional. Recent misgivings about the adequacy of latent tumor cells to account for the tumors evoked by croton oil are reasonable (63, 73, 92). I can find no convincing evidence of a "subthreshold neoplastic state" in mice; if it occurs it is overshadowed by "latent neoplastic potentialities" which develop and culminate, as in rabbits, in papillomas or carcinomas that are not conditional. Croton oil and the few other known developing agents accelerate this process. The observations on rabbits and on mice are complementary; promoting action and the subthreshold neoplastic state are the more conspicuous in rabbits; developing action and latent neoplastic potentialities predominate in mice.

The development of carcinoma.—Rous and his colleagues (39, 88) believe that tar cancers in rabbits seldom develop suddenly but are the outcome of secondary step-like changes in the cells of benign tar tumors. "What happens is no mere exaggeration of the previous papillomatisis but a wholly new event, the genesis of a neoplasm distinct from its predecessor. The changes do not always stop when a carcinoma is established; as a result of further successive changes the growth may go from bad to worse" (88). These late stages are more clearly, and much more frequently, seen in the development of carcinomas from the papillomas induced by the Shope papilloma virus. Some Shope papillomas tend to malignancy from the first and attain it by a continuous series of alterations; others, benign at first, change into malignant papillomas and thence to squamous carcinoma which further progression may advance to a state of "ultimate malignancy" (86). Experimental interference (e.g., injections of Scharlach R, trauma, infection, or transplantation) apparently precipitates the emergence of carcinoma in papillomas predisposed to the change, and then the growth rate may quicken so abruptly as to suggest that malignant activity has been "touched off" in some way. Extraneous factors, however, are not always effective and are not essential. "The trend of a papilloma towards malignancy evidently brings it at length to a state in which cancer is inevitable. Whether local influences are primarily responsible for this trend is another matter" (86).

There is substantial evidence that in mice the "trend" recognized by Rous and Beard is determined by the initiating action of the tar or carcinogenic hydrocarbon, in harmony with the early opinion of Leitch (69) that the cells acquire the "bias towards malignancy" during the "preneoplastic" stage. Carcinoma develops in two or three different ways. Some tumors are malignant when first seen (25, 48, 69, 78, 97, 100), but most carcinomas develop from apparently benign papillomas. Some papillomas undergo gradual continuous change into carcinoma (6, 48). Others develop into carcinomas by abrupt, discontinuous change evidenced by abrupt change in growth rate or by histological evidence of focal progression to carcinoma within a papilloma (7, 78, 97). Whether continuous or discontinuous the change from papilloma to carcinoma represents a new step in the evolution of the tumor (7, 48). The frequency of primary carcinomas and of conversions of papilloma into carcinoma depends, according to Shubik's (96) observations, more on the duration than on the intensity of carcinogenic action. In general, the fewer the applications of carcinogen the smaller the proportion of malignant tumors until with a single application there is usually none, although Bielschowsky and Bullough (17)
record carcinomas as well as papillomas after a single massive dose of methylcholanthrene. Croton oil, applied after the carcinogen, accelerates the development of papillomas but does not increase the proportion of malignant tumors or accelerate their appearance (1, 2, 12, 62, 81, 91, 96). Application of croton oil to papillomas does not encourage their conversion to carcinoma and may even prevent it (2, 62, 64, 96). Acceleration of the transition from benign to malignant tumor has been reported as a result of cauterization of the base of papillomas (25), of repeated scalding (28), and of applications of croton resin (19), but needs further experimental study.

**Tumors Induced in the Urinary Bladders of Mice by Acetylaminofluorene**

The induced tumors of the urinary bladder are varied in structure, ranging from localized intravesical papillomas of benign appearance through pleomorphic tumors invading the bladder wall to malignant sarcoma-like growths penetrating the bladder wall and disseminating in the peritoneal cavity. There is, apparently, a graded series of developmental stages. Progression does not always reach an end-point in the original host; it may halt at any stage, or the host may die from hemorrhage or urinary obstruction before progression is far advanced. If progression is incomplete in the original host it advances during serial transplantation in normal mice, demonstrating that progression is not dependent on continued action of the carcinogen that induced the primary tumor. Observed steps in the progression of transplanted tumors correspond approximately with various primary tumors which seem, therefore, to represent consecutive stages in development (37).

Acetylaminofluorene evokes widespread hyperplasia or papillomatosis in the bladder of mice (4), but early gross tumors are solitary and often pedunculated, developing from only a small area of altered epithelium, and during progression epithelial changes occur at many points but not diffusely. In general, progression from microscopic hyperplasia to gross papilloma and thence to carcinoma is essentially focal.

**Hormone-Induced Tumors**

Prolonged, continuous hormonal disturbances evoke tumors of various endocrine and reproductive organs in rodents, the effects being strongly conditioned by the genetic constitutions of the animals. In the pituitary (42, 43, 45), testis (19, 45), and thyroid (18, 76, 77, 85) there is usually a stage of conditional tumor in which growth, although neoplastic and even “malignant” by histological criteria, continues only in a disordered hormonal environment. These tumors are transplantable only in animals having the same hormonal imbalance as the primary host. Most tumors do not advance beyond this stage within the lifetime of the primary host, many do not develop further than benign adenoma, and many are dubiously neoplastic, but progression often goes on during serial transplantation and eventuates in carcinomas which grow independently of unbalanced hormonal stimulation. There is no decisive evidence about the mechanism of this progression. It might be due to prolonged specific hormonal action (77), to continued cell proliferation per se, or even to mere extension of the time available for an automatic process to reach completion.

Many hormone-induced overgrowths seem to begin as hyperplasia and advance thereafter from benign neoplasia to malignant neoplasia without abrupt or distinct separation of successive stages (23, 71). This method of progression is not universal among hormone-induced tumors. Tumors of the breast and uterine cervix evoked by estrogens in rats or mice may develop long after the withdrawal of estrogen and after the initial hyperplasia has waned. Moreover, there is evidence of discontinuous progression through successive stages in which the hormone is possibly not the sole etiological factor (32, 44, 46, 83).

Histological studies disclose step-wise progression of tumors of the interstitial cells of the testis induced in mice by estrogens. Several successive generations of cells are distinguishable, differing in cytological appearance, function, and behavior. At one stage the tumors secrete androgen and are neither invasive nor transplantable. Later, secretion of androgen stops, and the tumors become invasive and transplantable in estrogenized hosts (59). The progression from adenoma to carcinoma in the thyroid of rats is also discontinuous (85).

Goitrogens such as thiourea evoke adenoma and carcinoma of the thyroid gland in rats and, if preceded by short administration of acetylaminofluorene, insufficient by itself to induce thyroid tumors, the goitrogens evoke adenomas much more quickly, but the ultimate result is not changed, and carcinoma develops no earlier. It has been inferred that acetylaminofluorene acts as an initiator accelerating the development of benign adenomas and that the goitrogen is a promoting agent (57, 58); but since goitrogens alone produce the same final result as the two agents in succession the analogy with epidermal carcinogenesis is unconvincing. An alternative interpretation is that neoplastic cells develop “spontaneously” in rat thyroid, acetylaminofluorene speeding up the formation of neoplastic cells and goitrogens stimulat-
aminofluorene corresponds with developing agents. On this view there is no initiating agent, acetylationing the neoplastic cells to develop into tumors (85). The steps in the induction of tumors by hormones need more critical study. There is a dearth of information about the stage at which neoplastic properties are irreversibly determined. It should be noted that regression after withdrawal of hormone does not differentiate hyperplasia from conditional tumor and that a conditional tumor as judged by histological criteria may be either “benign” or “malignant.”

**Spontaneous Mammary and Uterine Tumors in Rabbits**

Greene investigated the course of spontaneous tumors of the mammary gland and of the uterine fundus in rabbits by means of repeated biopsies and transplantation experiments which included, in particular, implantations in the anterior chamber of the eye.

Following Greene’s descriptions, mammary tumors of rabbits begin either as adenomas in otherwise normal breasts or, more commonly, in breasts with pre-existing cystic disease resembling Schimmelbusch’s disease in women. The disease grades by successive stages from cystic disease through noninvasive neoplasia to invasive neoplasia and to cancer with metastasis. Transplantability increases concurrently (50-53).

The uterine tumors, also, advance through a sequence of structural changes. Repeated biopsies usually disclose progression from early to later histological stages. The progression is not sudden, gradual transitional stages being evident, and it occurs relatively late in the clinical course. Histological structure imperfectly reflects the biological properties as assessed by transplantation experiments. Behavior and structure can change independently of each other, although changes in structure usually accompany extreme changes in behavior (54).

Both investigations indicate that cancer is the final stage in a developmental process. The early neoplastic focus is not a cancer in structure or behavior. Initiation of a neoplastic focus and development of the focus to cancer are, in Greene’s view, distinct processes due to different etiological factors (54). The assertion that progression in biological properties advances only in tumor-bearing animals possibly applies to the mammary and uterine tumors because they are hormone-induced tumors which for a long time remain dependent on an abnormal hormonal stimulus, but it is not applicable to tumors in general as witnessed, for example, by the progression of bladder tumors induced by acetyaminofluorene during transplantation in normal mice.

**Spontaneous Mammary Tumors in Mice**

Certain hybrid mice and their inbred descendants develop mammary tumors that are especially favorable for the study of progression (54-56). The majority of mice have multiple mammary tumors before death, a circumstance which allows comparison of the behaviors of different tumors exposed to the same systemic environment. Some of the tumors grow progressively from their first appearance to the death of their host; their course is represented graphically by an approximately straight line, and they are designated “unresponsive” to distinguish them from the more numerous tumors that are “responsive” to reproductive activity in the host. The responsive tumors grow during pregnancy, reach a peak just before the time of parturition, and thereafter regress partially or completely. The responsive tumors are conditional like the tar warts of rabbits; the systemic changes that accompany pregnancy are promoting factors upon which the tumors depend for their growth and, often, for mere survival. There is no regularity in the proportion or order of appearance of responsive and unresponsive tumors in individual mice. Responsiveness is not proportional to the size or duration of a tumor; it persists sometimes through repeated pregnancies covering a large fraction of the life span of a mouse and in relatively large tumors. Often, however, there is progression from the responsive to the unresponsive type. The change, as a rule, seems abrupt, occurs unpredictably, sometimes early and sometimes late in the clinical course of a tumor, and is permanent. When similar multiple tumors are present, only one of them undergoes progression at a time. The independent progression of multiple tumors indicates that progression depends more on intrinsic properties of individual tumors than on the environment to which all are exposed. Abrupt change from responsive to unresponsive growth is the most conspicuous and most frequent manifestation of progression. More rarely growth rate accelerates abruptly without alteration in responsiveness. An unresponsive tumor grows no more quickly than the responsive tumor whence it derived grew during pregnancy; the result of progression is that growth continues after parturition. Different characteristics of the same tumor, as exemplified by growth rate and responsiveness, thus undergo progression independently of each other. Progression often becomes evident at the end of a pregnancy when the expected regression of a previously re-
cancer does not occur. Pregnancy seems to exert a "trigger-action," making apparent a change that occurred earlier; it is not an indispensable stimulus to progression, because some responsive tumors recur as unresponsive ones during intermissions of breeding. Progression can occur, therefore, when growth is suppressed. When a tumor does not attain the fully unresponsive state in its original host, it undergoes progression towards that state early or late in the course of serial transplantation. Responsiveness of some transplanted tumors is shown by their ability to grow in female or estrogenized male hosts but not in normal males, by their capacity for milky secretion under appropriate hormonal stimulation, and by accelerated growth during pregnancy. In all adequately studied tumors responsiveness has been irreversibly lost after a shorter or longer period, and it seems that progression to the unresponsive type occurs inevitably if the life of the tumor is sufficiently prolonged by transplantation (35). The progression nevertheless does not necessarily occur within the life span of the primary host and may halt for a lifetime at an early stage of responsiveness.

The distinctive histological feature of this material is the manifestation of early neoplasia and the persistence of partially regressed responsive tumors in the form of discs or plaques of characteristic pattern in which the sequence of changes in the growth, regression, and recurrence of responsive tumors is traceable. During regression the discs become sclerotic, and unequivocal evidence of progression is recognizable in the form of foci of proliferating tissue, often distinctly carcinomatous in structure, within the sclerotic remnants of regressed tumors. Similar carcinoma-like areas are present too, although less conspicuously, within growing responsive tumors during pregnancy. Another manifestation of progression is the persistent growth after parturition of one portion of a disc of responsive tumor, while the remainder is undergoing regression and sclerosis. Here there is focal change in responsiveness but no change in histological structure, and similar change instead of being focal may be diffuse, affecting the greater part or the whole of the disc. This form of progression involves only one character, namely the responsiveness to pregnancy; there is no immediate change in histological structure or, as already mentioned, in growth rate. Secondary histological changes usually follow later as a result of progressive growth, but the structural characteristic of a responsive plaque may persist in an unresponsive tumor even after several generations of serial transplantation. During serial transplantation of some tumors, progression is evidenced by foci of more rapidly proliferating cells conspicuously different from the surrounding growth (Foulds).1

**PROGRESSION IN TRANSPLANTED TUMORS**

The earliest workers in experimental cancer research described frequent, conspicuous, and permanent increases in the frequency of "takes" and the rate of growth of transplanted tumors during the first few serial passages. The British called the change an adaptation to a new environment, and the Germans called it an increase in virulence. Later, adherents of the genetic theory of transplantation, led by Strong, ascribed the changes to mutation. The genetic theory, however, does not cover all the vagaries of transplantation (3, 8, 49). Changes are not restricted to increases in transplantability or growth rate, nor to the early transplanted generations. Biochemical properties alter during transplantation, and special functions tend to disappear (36). Old-established transplantable tumors like the Ehrlich mouse carcinoma transform into "ascites tumors" as the result, it is suggested, of a process "akin to spontaneous mutation" (60, 61). Particular cases already cited show that transplantation often continues and completes a progression whose course is already set in the primary tumor. Transplantation gives extra time for the action of etiological agents, hastens by selection the dominance of the most resistant or most aggressive members of a mixture of varied cells, and extends the opportunity for new changes, some of which are, in effect, adaptive (8-10). It is not proved that transplantation specifically induces or stimulates progression.

**DRUG RESISTANCE**

The eventual failure of hormonal or chemotherapeutic control of certain neoplasms in man, despite favorable or even dramatic early response, is reasonably attributable to progression of the tumor cells to an unresponsive state (36, 38). So far, experimental investigation has been restricted to transplantable mouse leukemias which, like human acute lymphocytic leukemia, are sensitive to certain chemotherapeutic agents, notably folic acid antagonists. Repeated serial passage of mouse leukemic cells through mice treated with these compounds often establishes substrains which resist many times the dose of folic acid antagonist that prevents growth of the parent strain. The resistance is permanent and irreversible; it persists after numerous serial passages in untreated mice (20, 22, 66-68, 84, 98). Resistant strains, moreover, sometimes change further to become partial-

1 Unpublished observations.
ly dependent on the folic acid antagonist, growing optimally only in hosts receiving an amount of drug lethal to unchanged leukemia cells (66, 67). Law thinks that the cells acquire the ability to use the folic acid antagonists in a mechanism of nucleic acid synthesis different from that normally used. The transformation from the sensitive to the drug-dependent form advances by discrete stepwise changes. It closely resembles the development of drug-resistant strains of bacteria. Law’s interpretation is that resistant strains develop as a result of spontaneous mutation in leukemia cells. The drug does not induce or determine the mutation; its action is merely selective, eliminating the sensitive cells and thus leading to predominance of the resistant mutants (66, 67).

**GENERAL PRINCIPLES OF PROGRESSION**

In previous publications (36, 38) several empirical principles or rules of progression were derived mainly from the observations on mouse mammary tumors. Shubik, Baserga, and Ritchie (97) find that most of the rules apply to chemically induced skin tumors of mice. The following paragraphs discuss the wider validity and significance of these principles.

1. **Independent progression of tumors.**—This rule is based on the observation, repeatedly confirmed, that only one of the multiple mammary tumors in a mouse undergoes progression at the same time. The rule applies to chemically induced tumors of mouse skin (95, 97) and probably to the tumors of the bladder induced by acetylaminofluorene. It is rare to find more than one carcinoma among the numerous skin lesions in shale oil workers (94), and in familial intestinal polyposis in man only one or a few of the large numbers of papillomas develops into carcinoma (29).

That the progression of one tumor inhibits the progression of others is neither proved nor disproved. Greene and Strauss (55) infer from their study of multiple tumors in rabbits that the onset of malignancy in one tumor inhibits its onset in others, although it does not inhibit the emergence of new benign tumors. Experimental evidence is conflicting partly, perhaps, because of an inclination to test the effect of one tumor upon the development of other tumors of a different kind. The rule is more probably applicable only to the progression of individual tumors of the same kind within one “field” in the sense of Willis (104).

2. **Independent progression of characters.**—In the spontaneous mammary tumors of mice responsiveness and growth rate are independently variable and undergo progression independently of each other (96). In transplanted tumors responsiveness of growth rate to pregnancy and the ability to produce milky secretion in response to estrogenic stimulation are independent of each other and of the ability to grow in female but not in male hosts; the different characters undergo independent progression (35). It is notoriously difficult to classify mammary tumors of mice histologically and to correlate structure with behavior, probably because both structure and behavior depend on numerous different characters that, within wide limits, are independently variable. Invasiveness and ability to disseminate join with responsiveness and growth rate to determine the behavior of a tumor. The two former characters are not directly proportional to each other, and neither is directly proportional to growth rate or responsiveness. At least four characters thus vary within wide limits independently of one another. Furthermore, the characters which determine structure and those which determine behavior, over a considerable range of variation, are independent of each other. The outcome is a wide variety of patterns of structure variously combined with widely varied patterns of behavior.

This rule seems widely applicable. Morphology and growth rate of induced skin tumors of mice often undergo progression independently, progression in morphology without change in growth rate being more frequent than the converse (97). The rate of cell proliferation and the rate of growth are not necessarily different in malignant tumors and benign warts, respectively (48). Some induced tumors of the testis in mice have the histological stigmata of malignancy but lack the power to grow without estrogenic stimulation (19), and some transplanted thyroid tumors in mice lose their dependence on hormonal stimulation without change of histological structure (77). Spontaneous uterine tumors in rabbits, carcinosomatoids of rabbits’ skin, and conditional tumors in general show a comparable dissociation between histological signs of “malignancy” and the capacity for independent growth. The acquisition of drug resistance or the ability to grow as ascites tumors entails no change in other properties of the tumors. The enzymatic pattern of transplanted tumors of similar origin is independent of the growth rate (56).

The rule of independent progression of characters leads to a more general proposition that the structure and behavior of tumors are determined by numerous “unit characters” which within wide limits are independently variable and capable of independent progression and which can be assorted and combined in a variety of ways. There are undoubted “linkages” between characters. In mammary tumors, for example, the structure of
cystadenoma is probably the direct consequence of responsiveness to lactogenic hormones. With this reservation it is profitable to apply the rule of independent progression of unit characters to the analysis of complex phenomena such as "malignancy." Malignancy is essentially a clinical concept and invaluable in clinical practice; in research it becomes a menace if it implies an indivisible, invariable entity or quality. A tumor may have one or more of the recognized criteria of malignancy; it may have all of them and kill the patient and yet be responsive and "conditional." It is not enough to label a tumor malignant; it is necessary to specify which characteristics, and the degree of each of them, that make it malignant. The classical textbook descriptions of tumors apply to certain common associations of clinical and pathological characters, but the associations are often "atypical" or "anomalous," the result, as I have suggested previously (38), of disproportionate or "out-of-step" progression of characters. Out-of-step progression is exemplified by "locally malignant" tumors that are highly invasive but do not disseminate, by metastasizing "benign" tumors which disseminate without notable local invasion, and by the undoubtedly "malignant" tumors that are nevertheless responsive and conditional. Wide generalizations about cancer need repeated checking by the detailed study of particular cases. No doubt there is a "highest common factor" in all neoplasia but it operates at all stages of progression and in benign tumors which, having all the fundamentals of neoplasia "without the frills," may yield the more crucial information about its essential nature.

3. Progression is independent of growth.—Some responsive mammary tumors which have regressed after parturition recur as unresponsive tumors during intermissions of breeding after long periods of dormancy and without the stimulus of pregnancy. Progression in morphological characters advances likewise in stationary skin tumors of mice (97). Progression it seems must occur in quiescent tumor cells. It is possible to interpret in the same sense the delayed progression of "latent neoplastic potentialities" in skin carcinogenesis and the lack of proportionality between the developing action of croton oil and the extent of the hyperplasia it induces. On the other hand, observations on some hormone-induced tumors and transplanted tumors suggest that continued growth favors progression and may be essential for it. The importance of the rule depends mainly on its apparent operation in those tumors of man that undergo progression to the unresponsive uncontrollable state while their growth is checked by hormones or chemotherapy.

It is noteworthy that chemotherapy often provides a long period of remission of symptoms and control of growth without substantially lengthening life, the rapidity of the terminal phase counterbalancing the remission due to treatment (81). Progression advances, it seems, independently of the treatment and of growth of the tumor.

Two corollaries of this rule are consistent with observations on skin tumors of mice (97), and with clinical observations on tumors in man, namely (a) at its first clinical appearance a tumor may be at any stage of progression, and (b) progression is independent of the size or clinical duration of a tumor. A tumor may be "early" as judged by size and duration but far advanced in the progression of aggressive characters. Conversely a large tumor of long standing may be at an early stage of progression.

4. Progression is continuous or discontinuous by gradual change or by abrupt steps.

It is convenient to discuss these two rules together and to divide the alternative pathways into (a) different paths leading to different end-points, and (b) different paths leading to similar end-points.

a) Different paths leading to different end-points. —The existence of different paths leading to different end-points follows from the rule of independent progression of characters and from the histological analysis of tumors which shows that widely different tumors develop by "divergent differentiation" of cells of one kind (38). Tumors varied in behavior and histological structure develop from the same parent tissue. Divergent differentiation occurs also in clinically evident tumors. Sessile papillomas of mouse skin, for example, can develop in at least eight different ways (97).

Progression does not necessarily lead to dominance of the tumor over its host. Rous and Kidd (88) discuss the "liabilities of the neoplastic state" and note that only the "successful" tumors attract attention; the "unsuccessful" ones escape notice. Chemically induced tumors of the skin often regress completely and permanently; even undoubtedly malignant tumors sometimes regress (40). Other tumors reach "dead-ends" of development, never progressing beyond sluggish papillomas. Tumors in man are subject to similar limitations (104) imposed, as it seems from the experimental evidence, by curtailed initiating action. Transplantable tumors not infrequently "die out" unaccountably. Tissue cultures of sarcoma cells, originated by the transformation of normal cells in vitro, and of leukemia cells lose the ability to grow
in presumably suitable animals (27, 31), but tumor
cells in tissue culture do not revert to normal cells
(47). There is no evidence in any of these examples
that the neoplastic process is reversible in the
sense that neoplastic cells revert to normal cells,
but tumors do undergo changes that lead to their
extinction. In general, progression can carry a
tumor to “ultimate malignancy,” as Rous ex-
presses it, to extinction, or to any intermediate
stage.

b) Different paths leading to similar end-points.
—It is possible to sort the various mammary tu-
mors of mice into a series ranging from wholly
conditional, responsive, slow-growing tumors to
rapidly growing, unresponsive tumors with all de-
grees and combinations of responsiveness and
growth rate between the extremes. The bladder
tumors induced in mice by acetylaminofluorene
permit similar “grading” into an ascending series.
These stages do not necessarily correspond with
successive steps in the development of individual
tumors. On the contrary, most mammary tumors
of mice seem to advance by long jumps, evading
many intermediate steps. A tumor may be unre-
sponsive when first seen; it acquires its definitive
properties early without traversing the numerous
intermediate steps which are theoretically possible
and which, in fact, are observed on the alternative
path or “responsive detour.” The detour leads ul-
timately to unresponsive growth, but progression
along it may be slow and gradual or delayed at any
stage, the end-point being reached, perhaps, only
after serial transplantation. Slow gradual progres-
sion seems to lead usually to sluggish irregular or
dubiously unresponsive growth. Progression, seem-
ingly abrupt, often switches tumors from the re-
sponsive to the unresponsive path, vaulting or by-
passing intervening steps. The unequivocal rapid-
ly growing, unresponsive tumors, as a rule, are
either unresponsive from the first or develop by
abrupt progression from a responsive and often
strictly conditional tumor (36).

Chemically induced carcinoma of rodent skin
may originate as such or it may develop from a be-
nign papilloma. Similar “direct” or “indirect” de-
velopment of carcinoma occurs in the skin of work-
men in the shale oil industry (94). Intestinal car-
cinoma in man, again, may originate as carcinoma
or develop from long standing papillary adenoma
or familial intestinal polyposis (29, 99).

The direct and indirect routes of development
possibly depend on two different types of pro-
gression:

1) Progression advances along a predetermined
path to a predictable end-point: The “direct” tu-
mors already mentioned follow this path, as do
those “precancerous” lesions which almost always
terminate in cancer. Development entails the
progressive expression of characters that are
already determined at an early stage. The
characters themselves are not necessarily present
early; a precancerous lesion which is destined to
become cancer is not itself a cancer; the characters
of cancer develop later by progression through in-
termediate stages. This type of progression is char-
acteristically gradual and inevitable. It probably
operates in many hormone-induced tumors, in
some transplanted tumors, and in many tumors of
man. It is possible to recognize those papillomas of
the human bladder that are destined to become
carcinomas (30, 93); their course is evidently de-
termined early, but it may nevertheless involve a
true progression and development of new charac-
ters during that course.

2) Progression involves a change into a new
path as in progression from responsiveness to un-
responsiveness in the mammary tumors of mice. It
occurs also in the induced skin tumors in mice (97)
and in the thyroid tumors of rats (88). Characteris-
tically it is abrupt and unpredictable. The new
course is the result of a change of path rather than
an acceleration along the previous path. It in-
volves “a wholly new event, the genesis of a neo-
plasm distinct from its predecessor.” (88). In man
progression from intestinal papillary adenoma or
familial polyposis to carcinoma occurs in this way.
Sunderland and Binkley (99) remark on the “ex-
treme unpredictability” of the change and on its
abruptness. Histological evidence, so far as it goes,
indicates that the change depends on focal progres-
sion as already described for the mammary tumors
(37, 78, 79, 85, 99).

Two dissimilar investigations have led to suspi-
cions that abrupt change is due to the sudden
manifestation, as a result of an extrinsic stimulus,
of a process already far advanced; carcinoma ap-
pears on rabbit skin as though “touched off” by an
external irritant (86), and pregnancy seems to
exert a “trigger-action” revealing progression
which has already occurred in mammary tumors of
mice (36). On the other hand, an apparently grad-
ual change might result from abrupt progression
(97). The difficulty is to distinguish between pro-
gression itself and the manifestation of its conse-
quences. At present it is not possible to decide
whether the apparently abrupt and continuous
types of progression are fundamentally different,
and the distinction between growth along a pre-
determined path and growth involving a switch of
path is probably more important.

6. Progression does not always reach an end-point
within the life-span of the host.—This rule is illus-
Progressions often follow automatically one after another; progression is "canalized" at initiation and advances along a predetermined path to a predictable end-point, in the manner of normal development. Alternatively, the sequence is discontinuous, and progression switches abruptly and unpredictably to a new path. The idea of progression thus generalized seems comprehensively applicable to the development of tumors from beginning to end.

According to this hypothesis the primary and fundamental change in neoplasia is a qualitative determination; it does not inevitably entail any growth at all. If growth ensues, with or without an extrinsic stimulus, it is of the peculiar neoplastic type. The "conditional," "conditioned," or "dependent" tumors, reviewed recently by Furth (41), do not grow without an extrinsic stimulus, but neoplastic properties are irreversibly determined and are expressed, in response to appropriate stimulation, by growth with the characteristics of neoplasia and even of malignant neoplasia. Dependence, a special case of responsiveness, is a unit character liable to independent progression and can join with any assortment of neoplastic characters. It often concurs, as in cancer of the prostate in man, with all the orthodox criteria of a malignant tumor.

The capacity for progression is probably inherent in the nature of tumors. There is no more evidence for a persisting "cause" of progression than for a persisting "cause" of embryonic development and no greater or less reason to expect one. Initiation of a tumor, like fertilization of an ovum, starts a process which, once under way, continues of its own accord. Some permanent changes detected in late stages of tumor development have been ascribed to "mutation," but, in view of the close affinities with embryonic differentiation, it is advisable to attribute all progression to mutation which, in the general view, is not responsible for the comparable changes that lead to the permanent distinctions between, say, normal liver cells and normal nerve cells. A mechanism other than mutation probably effects differentiation (103). Progression, as here defined, may include processes of more than one kind with different mechanisms which, later, will need separate designation.

All kinds of treatment of human cancer that fall short of rapid total eradication are liable to ultimate frustration by progression to states of unresponsiveness to stimuli which previously controlled growth (98), and major advances in therapy may depend on learning how to regulate progression. Under experimental conditions giving an extended time allowance, progression seems almost inevitable; but under natural conditions it is not

In view of the observations on drug resistance, change to "ascites tumor," and other modifications in old strains of transplanted tumors it is perhaps wrong to speak of an "end-point"; new methods may disclose more and more examples of progression in long established transplantable tumors like the Ehrlich carcinoma, which, it might have been supposed, reached its limit long ago.

The Nature and Mechanism of Progression

The basic idea of progression is the same as that of epigenetic development in embryology. Both presume a consecutive development of characters, not a progressive unfolding of characters present from the start. It is immaterial to the present argument whether the characters be interpreted as losses or gains; they are new, permanent, and irreversible. Progression involves a series of determinations in the embryological sense. The choices of path, the "canalizations," and the switches of path in progression correspond with the embryological phenomena represented graphically in Waddington's "epigenetic landscape" (82, 101). The rule of independent progression of characters has its counterpart in the "dissociability" or "disengagement" of characters described by Needham (82). Progression resembles embryonic differentiation at so many points, not because tumor cells are "embryonic," which they are not (33), but because similar biological laws operate in normal and neoplastic cells at all stages of development. The hypothesis suggested here is that initiation of a tumor is a process of determination which lasts a long or a short time. When this process is completed, neoplastic properties are irreversibly determined but are not necessarily expressed quickly or even at all without an additional stimulus. A sequence of new determinations or progressions carries the tumor forward towards its definitive stage. The progressions often follow automatically one upon another; progression is "canalized" at initiation and advances along a predetermined path to a predictable end-point, in the manner of normal development. Alternatively, the sequence is discontinuous, and progression switches abruptly and unpredictably to a new path. The idea of progression thus generalized seems comprehensively applicable to the development of tumors from beginning to end.

According to this hypothesis the primary and fundamental change in neoplasia is a qualitative determination; it does not inevitably entail any growth at all. If growth ensues, with or without an extrinsic stimulus, it is of the peculiar neoplastic type. The "conditional," "conditioned," or "dependent" tumors, reviewed recently by Furth (41), do not grow without an extrinsic stimulus, but neoplastic properties are irreversibly determined and are expressed, in response to appropriate stimulation, by growth with the characteristics of neoplasia and even of malignant neoplasia. Dependence, a special case of responsiveness, is a unit character liable to independent progression and can join with any assortment of neoplastic characters. It often concurs, as in cancer of the prostate in man, with all the orthodox criteria of a malignant tumor.

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All kinds of treatment of human cancer that fall short of rapid total eradication are liable to ultimate frustration by progression to states of unresponsiveness to stimuli which previously controlled growth (98), and major advances in therapy may depend on learning how to regulate progression. Under experimental conditions giving an extended time allowance, progression seems almost inevitable; but under natural conditions it is not
inevitable. In animals and in man progression may halt for a long time, and tumors do not always approach an end-point within their primary hosts. In all likelihood progression is liable to acceleration or retardation by extrinsic stimuli. Initiating agents seem to determine the course and extent of progression, and their continued action beyond the minimum needed to initiate a tumor is liable to advance the end-point of progression; but once the initiating process is sufficiently advanced, progression is independent of specific carcinogenic stimuli. Developing agents accelerate the initiating process without changing its direction or extent, and similar agents can probably accelerate or retard subsequent steps in progression in a comparable way. Promoting agents evoke and maintain conditional tumors, but their influence on progression is uncertain. Possibly these agents or similar ones exert a trigger-action whereby a progression already determined is abruptly expressed. Extrinsic factors also hasten the manifestation of progression by selective action on mixed populations of cells; how the mixture comes about is obscure. It is likely, a priori, that extrinsic stimuli induce abrupt progression by change of path, but the experimental analysis of this phenomenon is unsatisfactory.

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