Multiple Etiologic Factors in Neoplastic Development

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SUMMARY

This discussion is concerned with the multiplicity of factors that influence the initiation of neoplasia and its subsequent course. Factors that are not strictly “carcinogenic” may, nevertheless, affect the ultimate result decisively. Both genetic and nongenetic factors are implicated in the delivery of a carcinogen to the target tissue in an effective form and amount, and others govern the responsiveness of the tissue. Several factors may operate concurrently or consecutively. The cooperative action of genetic and hormonal factors together with a milk-borne virus is ordinarily most effective in producing a high yield of mammary tumors in mice. Epidermal carcinogenesis provides evidence of successive stages differently controlled. The first stage of initiation establishes a persistent region of incipient neoplasia whence tumors of varied kinds emerge at a later time. When the carcinogenic stimulus is optimal and the genetic sensitivity high, the early lesions are multiple, benign, or “imperfect” neoplasms of the kind usually described as “precancerous”; they are important because progression to malignant neoplasia may take place in one or more of them and because they disclose a region of incipient neoplasia within which malignant tumors may emerge later without spatial relationship to the early lesions.

Effective management of neoplastic disease is likely to depend on better knowledge than is yet available of the successive stages of neoplastic development and of the diverse factors operating at each of them. Different forms of neoplasia will probably call for different kinds of management. Initiation by known carcinogenic agents should be minimized. If the initiating agent is unknown, progression may still be controlled effectively, as in neoplasia of the uterine cervix, by treatment at the “precancer” stage of carcinoma in situ. Even at later stages, some control is practicable, as evidenced by the substantial, if limited, benefits of endocrine therapy for carcinoma of the breast and prostate.

Initiation, the crucial event in experimental carcinogenesis, probably entails persistent replicable change in the affected cells, not necessarily of the kind described as mutation. Recent developments in the theory of genetic structure and genetic action will predictably become increasingly important in the study of neoplasia and are discussed here in a preliminary way.

This paper is concerned primarily with the multiplicity of factors, acting simultaneously or consecutively, that are implicated in the development of individual tumors. The most conspicuous primary inductive or causative mechanisms of carcinogenesis, as revealed by experimental analysis, do not necessarily provide the best clues to practicable measures of prophylaxis and therapy in man. The present discussion therefore is not restricted to the actions of proved carcinogens but extended to the varied factors both carcinogenic and noncarcinogenic that to a significant degree influence the initiation of neoplasia and its subsequent course. The title applies most directly to the multiple factors that act cooperatively to induce a particular kind of neoplasia in a single individual. The cooperative action has been analyzed most thoroughly in mammary neoplasia in mice, which will be discussed first to illustrate some general principles of wider applicability.

MAMMARY NEOPLASIA IN MICE

The detailed analysis of mammary neoplasia in inbred mice has disclosed 3 important etiologic factors, comprising, as stated in Bittner’s 3-factor hypothesis, a genetic factor, a hormonal factor, and a milk-borne tumor agent with the properties of a virus and now usually called the milk agent or the Bittner virus. Within certain limits, an excess of one factor can compensate for deficiency in another, but ordinarily, to ensure a high and early incidence of mammary tumors, the 3 factors must act cooperatively. Mammary neoplasia develops spontaneously and can be induced experimentally by administering car-

The abbreviations used are: DNA, deoxyribonucleic acid; RNA, ribonucleic acid.
cinogenic hydrocarbons or by irradiation in certain mice in which no milk agent is demonstrable. The Bittner virus is an important etiologic factor in mice but not an indispensible one, and no comparable agent has been demonstrated in animals other than mice. The genetic factor is complex. Multiple genetic factors are implicated, and they affect several different mechanisms, including the propagation and transmission of the milk agent, the specific reactivity of mammary tissues to particular hormones, and the production of those hormones. It remains to be decided whether or not genetic factors determine in a more direct way the liability of mammary epithelium to neoplastic change.

The hormonal factor also is complex. Several or many hormones contribute to the end result. Formerly the importance of estrogens was exaggerated; if forced to pick one hormone in preference to the others, many investigators would now choose prolactin. In inbred mice that carry the milk agent, the hormonal factor is doubtfully causative or inductive. Its action seems to be mainly permissive or supportive, being restricted to eliciting and maintaining a sufficiency of mammary tissue upon which other factors can operate to initiate neoplasia (4). In other circumstances, when hormones evoke mammary tumors in mice lacking the milk agent or in rats, which rarely develop mammary carcinomas spontaneously, their action is probably inductive and, surely, more than permissive.

Genetic factors severely restrict the inductive action of hormones. Prolonged administration of hormones, especially estrogens, to innumerable guinea pigs and hamsters has not yet induced mammary neoplasia, nor has it done so in the much fewer experiments on monkeys. Only 1 mammary carcinoma seems to have been induced experimentally in guinea pigs and that by radioactive thorium (Thorotrast) injected below a nipple (17). The few mammary tumors induced in hamsters have resulted from intragastric administration of a carcinogenic hydrocarbon (14). In general, the 2 most consistently successful methods for inducing mammary neoplasia in laboratory animals are intragastric administration of chemical carcinogens and irradiation. Nevertheless, the effectiveness of the chemical carcinogens is considerably affected by hormones, which are important at least permissively (36, 37, 43, 51). Moreover, as first shown by Shay et al. (52), hormones influence the type of tumor induced by the carcinogens; when estrogenic stimulation preponderates most of the tumors are carcinomas, whereas a balanced stimulation by androgens and estrogens evokes fibroadenomas. This action of hormones may be described as directive; it does not initiate neoplasia, but it directs its course.

Mammary neoplasia, broadly surveyed in animals of various species, confirms other evidence to the effect that tumors of one general type can be elicited in several different ways; it illustrates how multiple factors can act cooperatively to produce a maximum effect and shows how one factor, such as the hormonal factor, can act in at least 3 different ways, namely, inductively, directly or, merely, permissively. Genetic factors are decisive in some of the inductive mechanisms and are important, at least permissively, in all. In certain circumstances, even with other factors operating optimally, the permissive factors may be the limiting or decisive ones.

Mammary neoplasia in mice serves also to introduce another matter that needs to be taken into account in a general discussion of multiple etiologic factors, namely, the possibility that varied factors operate at different stages of neoplastic development. Over 50 years ago Apolant and Haaland described what are now called hyperplastic nodules in the breasts of mice, and Haaland (33) believed that they were precursors of mammary carcinomas. About a quarter of a century later Gardner, followed by others, confirmed Haaland’s opinion by observations on inbred mice. It has become recognized that most mammary carcinomas in inbred mice develop either in hyperplastic nodules or in bigger lesions called plaques, which have been described more recently (21). These precursor lesions and in particular the plaques, which can be observed clinically, have yielded illuminating information about the development of tumors through qualitatively different stages as a result of irreversible changes in tumor cells. This mode of development has been called progression (19, 20).

Histologic examinations indicate that most mouse mammary carcinomas develop by focal or multifocal progression in nodules or plaques. The nodules are often very numerous, but carcinoma develops in only one or a few of them whereas the others persist indolently or regress. Plaques are usually multiple but not numerous, and progression to carcinoma takes place in only one of them at a time. Progression may be multifocal, but it is restricted to a small fraction of the plaque tissue (20, 21). Clinical observations disclose a remarkable responsiveness of plaques to pregnancy; the plaques grow during the 2nd half of pregnancy and regress quickly after parturition. Plaques are conditional neoplasms that grow only in certain appropriate environments. During intermissions of breeding they remain in abeyance, and in mice killed during this period, fibrotic remnants of plaques containing atrophic tubules lined by ragged epithelium are sometimes present. Often traces of the regressed plaques are hard to find; yet, in mice that live, regressed plaques always grow again, at the same place, during the 2nd half of the next pregnancy, however long it is delayed. Some residual neoplasia (24) with a capacity for neoplastic growth must persist throughout the period of regression, even though it is hard to find until it is stimulated to grow by another pregnancy. Cycles of growth and regression may recur in several successive pregnancies, but often one plaque changes its behavior abruptly and continues to grow after parturition whereas other plaques in the same mouse regress, as all of them had done after former pregnancies. A pregnancy-independent tumor is thus established by progression in a pregnancy-dependent plaque. It is emphasized that progression is not a mere extension of a pre-existing lesion in space and time but a revolutionary change in a portion of the old lesion establishing a tumor with new properties not formerly present.

It is not known whether or not extrinsic factors directly incite progression through nodule or plaque to carcinoma. Hormones are essential for progression through nodules to
carcinoma but only, it seems, in a permissive sense, and the hormonal requirements vary from one stage of development to another (4). Although not demonstrated in mammary neoplasia in mice, there is evidence for the important possibility that the hormonal environment prevailing during the early stages of neoplastic development acts directly to determine the hormone responsiveness of the definitive tumor and, in women, the reaction to endocrine therapy (15, 42).

The hormone responsiveness of mammary plaques in mice has proved convenient as a "marker" for the study of some general principles of tumor progression applicable to varied types of neoplastic development in animals and in man.

**TUMOR PROGRESSION**

Some general principles or rules of progression were proposed in 1949 and have been re-evaluated recently (25). Two of them are especially relevant to the present discussion. The 1st is the rule of independent progression of tumors, which maintains that progression occurs independently in different primary tumors in the same animal. It is valid for the neoplastic diseases of animals and of man that are characterized by multiple primary tumors or "precancerous" or "precursor" lesions. As a rule progression to carcinoma takes place at one time in only one of the considerable number of lesions that may be present on the skin of men exposed to carcinogenic chemicals or of children suffering from xeroderma pigmentosum. Similarly only one or a few of the abundant papillomas of familial intestinal polyposis give origin to carcinoma by progression. Many other examples could be cited but, in general, neither clinical observers nor laboratory investigators have recorded the developmental histories and fates of individual lesions in sufficient detail.

The 2nd important rule is the rule of independent progression of characters; different characters of a particular tumor undergo progression independently of one another. This rule leads to the more general proposition that the structure and behavior of tumors are determined by numerous unit characters that, within wide limits, are independently variable, capable of highly varied combinations, and apt to progress independently. This rule has proved valid and useful in diverse studies of neoplasia in animals and in man. The associations of characters such as growth rate, invasiveness, powers of metastasis, responsiveness to hormones, and morphologic characters in tumors of one general kind are highly varied and include many anomalous associations or "dissociations" of characters, as exemplified by the "locally malignant" and "metastasizing benign" tumors of man. The rule still holds good when the analysis is pushed to the level of enzymes and histocompatibility genes, and investigations at this level confirm earlier suspicions about the individuality of tumors; probably no two tumors are exactly alike in every respect even when they are evoked by similar means from the same tissue and have the same general properties (18, 39, 46, 57). Many wide generalizations about "cancer" have broken down, as most of them eventually do, because they have not taken into account the independent variability of characters and the individuality of tumors. Moreover many characters often prominent in tumors are not essential to the neoplastic disease but are only incidental consequences or accompaniments of it; they may be wholly lacking from certain otherwise typical growths described by Potter (46) as "minimal deviation" tumors. The wider "deviations," although incidental, might be exploited nevertheless in prophylaxis or, as Potter notes, in the therapy of particular kinds of neoplasia.

Progression is an important mechanism in the clinical course of many neoplastic diseases, but it is not applicable, without redefinition, to the critical changes that take place during the preclinical or induction period. Visible lesions, even the microscopically visible ones, do not adequately reveal the whole nature and extent of the neoplastic disease. The experimental analysis of skin carcinogenesis, briefly reviewed in the following paragraphs, has provided most of the available information about the earliest steps.

**SKIN CARCINOGENESIS**

The skin, because it is accessible to direct observation during life, is the ideal site for studying neoplastic development throughout its course. Moreover observations on occupational neoplasias of human skin correspond with, and in some respects extend in an important way, the extensive investigations of experimentally induced neoplasia in the skin of animals. It is possible here to summarize only briefly and somewhat dogmatically the vast amount of information that has been accumulated. The primary aim is to infer broad general principles that are widely applicable to other sites where direct observation is more difficult.

Experiments on rabbits and mice have shown that chemical carcinogens applied to the skin quickly effect a persistent, probably irreversible, change in the skin that is not manifested by enduring histologic or cytologic signs and that, in particular, does not entail proliferation of cells. This first and decisive step of initiation changes the functional reactivity of the skin and establishes a new capacity for neoplastic development that is revealed at a later time when varied lesions emerge focally from the exposed region.

It is convenient to divide the emergent lesions into 3 main groups—A, B, and C. The Group A lesions are trivial ones exemplified by freckles and other familiar effects of overexposure to sunlight. Many, perhaps all, of them are side effects due to nonspecific irritation by the carcinogen. They are not neoplastic by the usual clinical or microscopic criteria and have no capacity for neoplastic development, but they deserve notice because they provide evidence of substantial exposure to a potentially carcinogenic stimulus.

The Group B lesions are the earliest lesions recognizable as neoplastic by the usual criteria. They are mostly benign or imperfect neoplasms, the term imperfect being applied to tumors that do not allow an unequivocal diagnosis of benign or malignant neoplasm because of anomalous associations, or "dissociations," of unit characters. These lesions in animals and in man include the following: (a) conditional papillomas that grow only when stimulated...
by certain extrinsic factors, regress when those factors are withdrawn, and recur when they are restored; (b) spontaneously regressing papillomas; (c) indolent papillomas that endure for a long time with minimal growth; (d) papillomas that grow progressively without changing their characteristics; (e) papillomas in which carcinoma originates by progression; (f) carcinomatoids or keratoacanthomas that resemble invasive carcinomas histologically but regress spontaneously; (g) noninvasive carcinomas in situ, in which invasive carcinoma may, or may not, originate by focal progression.

The Group B lesions include the varied "precancers," "precancerous lesions," and "morphologic precursors of cancer" observed on human and animal skin. The basic objection to these terms is that many of the lesions to which they are applied are not "pre-" anything; they are terminal lesions with no future. Group B lesions are subject to at least 4 different fates—namely, (a) regression; (b) indolent persistence; (c) progressive growth without qualitative change; and (d) progression to true, invasive, carcinoma. Progression to carcinoma is often the least likely outcome. The fate of a particular lesion is rarely predictable. Progression seems to be random in time and site. Even when it is almost certain that true carcinoma will emerge somewhere in a region exposed to optimal carcinogenic stimulation, the time and site of progression are not predictable.

Group C lesions comprise the malignant, mainly epidermoid, carcinomas which can emerge in at least 2 different ways—namely, as a result of progression in a Group B lesion, or directly from a place in the exposed region where no Group lesion is, or ever has been, present. Most carcinomas in mouse skin probably originate by progression in a Group B lesion, but it seems to be a general rule to which no certain exception has been found that, in every tissue in which carcinoma usually originates by progression in a Group B lesion, the same sort of carcinoma sometimes emerges from the exposed region as a carcinoma ab initio with no spatial relationship to past or present Group B lesions. Briefly summarized, not all carcinomas originate by progression in a Group B lesion; not all Group B lesions have the capacity for progression to carcinoma, and of those with the requisite capacity only a fraction, usually a small fraction, manifest it by actual progression.

In certain circumstances the neoplastic lesions are very numerous, and they emerge both concurrently and consecutively. The lesions tend to emerge in an ascending order of gravity, but there is no fixed rule and often the emergence of the various types seems to be random; lesions of varied types may be present contemporaneously and an ulcerating carcinoma may be the first, although more probably it will be the last, type to emerge (53). It is important to note in man, as well as in animals, that the first carcinoma may emerge long after the last known exposure to carcinogen and after all Group B lesions have disappeared and that carcinomas are apt to emerge consecutively throughout remaining life.

The experimental analysis of epidermal neoplasia has led to a 2-stage hypothesis of carcinogenesis, the 2 stages being described as initiation and promotion. Rous and his colleagues studied early conditional tumors in rabbits and inferred that initiation establishes a subthreshold neoplastic state characterized by the presence of latent tumor cells that proliferated to form a visible tumor only as a result of promotion by various extrinsic stimuli not, by themselves, carcinogenic (48). Berenblum and Shubik (3), using suboptimal or subcarcinogenic applications to mice, inferred similarly that the carcinogen initiated latent or dormant tumor cells and that these cells proliferated in response to promotion by certain noncarcinogenic, or very weakly carcinogenic, agents of which croton oil was the most effective. Both groups of investigators maintained that initiation and promotion were different and distinct consecutive processes.

The accuracy of the observations leading to the 2-stage hypothesis is not questioned, but the interpretation is still doubtful. The rabbit tumors studied by Rous were conditional papillomas or carcinomatoids, whereas the tumors evoked by croton oil or other promoting agents were mostly spontaneously regressing papillomas or papillomas with a low capacity for progression to carcinomas. The initiation-promotion experiments seem to apply predominantly to special cases of imperfect neoplasia, and their wider relevance to carcinogenesis in general is questionable. In particular, it is doubtful if promotion substantially increases the eventual yield of carcinomas (13, 45, 50, 54).

The evidence for a crucially important first step of initiation is extremely strong, but the further assumptions that it affects only a few isolated cells scattered through the exposed area and that promotion merely stimulates these cells to proliferate are open to criticism. The origin of tumors from single cells has not been demonstrated and the histology of early lesions, especially carcinoma in situ, does not support it (45). There is good evidence, moreover, that the early visible lesions, whether evoked by promotion or not, do not reveal the full neoplastic potentialities of the exposed region. The latent or dormant tumor cell hypothesis does not account satisfactorily for the variety of the early lesions or for the late tumors that emerge consecutively long after the withdrawal of known initiating or promoting stimuli. To account for the consecutive emergence of tumors for up to 2 years after withholding carcinogens from rabbit skin, Friedewald and Rous (27) proposed that, as well as initiating latent tumor cells, the carcinogen initiates latent neoplastic potentialities in cells, which then advance at varied, often extremely slow, rates towards the neoplastic state.

Berenblum (2) has rightly emphasized that tumors emerge focally and not diffusely, but it is not obligatory to presume that initiating carcinogenic action is limited to the places where tumors emerge or that the time and site of emergence and the qualitative variety of the tumors are determined, once and for all, at the time of initiation. An alternative and more elastic hypothesis is that initiation establishes a region of incipient neoplasia (meaning, literally, "the beginnings of neoplasia") that is coextensive with the area of exposure to carcinogen and has a permanent new capacity for neoplastic development. The term capacity is used here in the special sense in which
Grobstein (31) applies it to embryonic development to refer to “the range and character of the demonstrable and immediate developmental alternatives.” The concept is similar to that of embryonic competence, but it is more generalized. An essential feature of competence and capacity is the element of choice between 2 or more developmental paths available in a region; individual cells must choose one of them. The initiation-promotion experiments disclose 1 or 2 of the available paths of neoplastic development without revealing the total capacity of the region of incipient neoplasia. The concept of incipient neoplasia can accommodate the latent tumor cells as a special case, with the reservation that they are more likely to be cell aggregates from the first, rather than isolated cells (23–25).

The concept of a regional type of neoplasia based on a substantial expanse of incipient neoplasia from which multiple tumors of varied types can emerge both currently and also consecutively over long periods of time is applicable to neoplasia in various other tissues.

REGIONAL NEOPLASIA

NEOPLASIA IN THE URETHRAL BLADDER

Neoplasia of the vesical epithelium illustrates several important aspects of the operation of multiple etiologic factors including some not yet mentioned, and there is an industrial regional form that is reproducible in laboratory animals and clinically similar to the sporadic disease in the general population.

Epidemiologic studies of the industrial disease in man and experiments on animals alike indicate that the disease is induced by aromatic amines, notably 2-naphthylamine. These substances are not carcinogenic at their point of entry, but after absorption they act selectively on the bladder in man and in some, but not all, species of laboratory animals. The probable chain of events is as follows: The amines are absorbed through the skin, digestive tract, or lungs and carried to the liver, where they are detoxified by oxidation to orthoaminophenols or arylhydroxylamines. The latter substances are believed to be the effective carcinogens, but they are immediately conjugated in the liver with glucuronic acid and excreted in this noncarcinogenic form by the kidneys. The effective carcinogen is liberated from the glucuronide in the bladder by hydrolysis effected by β-glucuronidase present in the urine. If the effective carcinogen released in the liver is not quickly conjugated, it is likely to induce hepatoma. If it is conjugated but excreted mainly in the bile, it may induce neoplasia in the intestines. Even if conjugated and excreted mainly by the kidneys, it will not induce neoplasia in the bladder unless the appropriate hydrolytic enzyme for liberating the effective carcinogen is present in the urine or, perhaps, in the vesical mucosa (6, 8, 9).

The pattern of induced neoplasia varies from one species of laboratory animal to another as a consequence of genetically determined differences in the biochemical processes involved in the detoxification and excretion of the absorbed amines. Genetically determined differences, if less in degree, may be expected also in man. Furthermore, within the limits imposed by genetic factors, the biochemical processes will be subject to enhancement or repression by a variety of nonspecific factors, such as the pH of the urine and the time it remains in the bladder. A variety of genetic and physiologic factors must operate in sequence before the effective carcinogen begins to act on the mucosa of the bladder. After it has begun to act a long induction period antecedes visible neoplasia, which may not be apparent, in man, until many years after the last exposure to a known carcinogen, and then tumors may emerge consecutively throughout the remaining life.

Neoplasia of the human bladder is of 2 main types. One type is characterized by solid tumors, usually solitary, that are carcinomas ab initio. The other regional type is characterized by multiple papillary tumors. Those that emerge early seem to correspond with the Group B lesions of the skin because they are benign or imperfect tumors with a low capacity for progression to carcinoma. The disease can often be controlled for many years by fulgurating individual tumors, but new primary tumors are apt to emerge consecutively and as time passes to be increasingly dangerous until, eventually, some of them grow invasively, disseminate, and kill the patient. The papillary type of disease in man and in animals is a regional one affecting a wide expanse of vesicle mucosa, and perhaps all of it (7, 47, 49). The origin of carcinoma by progression in a papilloma has been described in the industrial disease in man and in the induced disease in animals, but the more conspicuous phenomenon seems to be the consecutive emergence of primary tumors that, as time passes, are of increasing gravity or malignancy when they first emerge (7, 29, 30, 56).

NEOPLASIA OF THE UTERINE CERVIX IN WOMEN

The etiology of carcinoma of the uterine cervix in women is obscure, but the pattern of neoplastic development is remarkably similar to the regional type observed in the skin. There is substantial evidence for widespread incipient neoplasia affecting much or all of the cervical epithelium and extending into the vagina. The outstanding feature is the origin of carcinoma by progression in Group B lesions comprising the varied dysplasias and carcinomas in situ. The Group B lesions in the cervix are subject to the same fates as those in the skin. It is widely agreed that most invasive carcinomas originate in Group B lesions, especially carcinoma in situ, but there is less information about the proportion of Group B lesions that give origin to invasive carcinomas. Clemmeson (11) estimates that 25–33% of such lesions will give origin to carcinoma within 15 years; the others persist harmlessly or regress.

It must be emphasized that carcinoma in situ does not turn into invasive carcinoma by mere extension in space and time; it gives origin to invasive carcinoma only as a result of a decisive change by progression, whereby neoplastic cells acquire powers of invasion which they did not possess before (22, 34, 35).

REGIONAL NEOPLASIA IN OTHER SITES

The examples of regional neoplasia quoted so far relate to surface epithelia that are accessible to direct observa-
tion during life. It is much more difficult to study this kind of neoplastic development in less accessible sites, but evidence is accumulating to show that it is more common than formerly supposed. The regional pattern is evident in familial intestinal polyposis in man (16). It seems to be the usual type of induced neoplastic development in endocrine tissues of animals (5), and good examples have been described in thyroid neoplasia in man (26). Mammary neoplasia in "high-incidence" strains of inbred mice, already discussed, conforms with the same pattern. Mammary neoplasia in women, also, is probably a regional disease, but the regional pattern is clearly evident only in the development of carcinomas that originate in chronic cystic disease. Cutler (12) believes that about 20% of all breast cancers develop in this way. At the present time, attention is being given to a similar pattern of neoplastic development in the lung (1).

**Conditions Favoring Regional Neoplasia**

The regional pattern of neoplasia, of which there are many variants, is conspicuous in animal tissues that have been subjected to optimal carcinogenic action and in human tissues that, by reason of occupation or environment, have been exceptionally exposed to chemical or physical carcinogens. The duration or repetition of the carcinogenic stimulus is probably more important than its intensity, and a strong element of promoting action may help significantly to evoke a multiplicity of varied Group B lesions. The same regional pattern is evident, even when the carcinogenic stimulus is minimal or unrecognized, where an exceptional, genetically determined liability to a particular kind of neoplasia has been demonstrated, as in xeroderma pigmentosum and familial intestinal polyposis. In many experiments on animals a known carcinogen is applied optimally to tissues whose genetic sensitivity is exceptionally high as a consequence of inbreeding, so that the extrinsic carcinogenic factor and the intrinsic genetic factor act cooperatively to produce a maximal effect. This combination of circumstances favorable to the manifestation of regional neoplasia may come about, by chance, in man but probably not often to a comparable degree.

When carcinogenic action is suboptimal or genetic sensitivity is low, neoplasia is more likely to be localized. Applications of the potent carcinogen 9,10-dimethyl-1,2-benzanthracene to the skins of rats and guinea pigs, 2 species notoriously refractory to chemical induction of epidermal neoplasia, evoke mainly solitary carcinomas that emerge as such after long delay. Solitary tumors do not necessarily indicate narrowly localized carcinogenic action; they are interpretable with equal or greater probability as localized manifestations of the neoplastic capacity of a region of incipient neoplasia established by widespread carcinogenic action. Both localized and regional types of neoplasia of the urinary bladder are encountered in workmen exposed to dangerous aromatic amines and apparently with the same relative frequency as in the sporadic disease in the general population. These empirical facts justify 2 reasonable inferences: 1st, that the localized and regional types of neoplasia in the urinary bladder, although clinically distinct, may be initiated by the same or similar carci-ogens acting diffusely on the mucosa of the bladder; and 2nd, that the sporadic disease, of unknown etiology, may be attributed to the action of a chemical carcinogen, possibly of endogenous origin, excreted in the urine (6, 9).

**Implications of Regional Neoplasia**

The finding of an overtly regional type of neoplasia implies that a region of incipient neoplasia has been established coexistent with the action of a known or, as in the uterine cervix, unknown carcinogenic stimulus of substantial duration and intensity. The early emerging lesions of Group B are significant in 2 ways: some of them are potential sites of progression to malignant neoplasia; and, together, they reveal the existence of a region of incipient neoplasia within which malignant tumors may emerge with no spatial relationship to a precursor lesion. Furthermore the Group B lesions provide information about the extent of the incipient neoplasia and, to some degree, about its capacity, which determines the range and character of the emerging lesions. It is probable, but not yet proved, that solitary tumors as well emerge from a region of incipient neoplasia whose neoplastic capacity is manifested at only one place.

**Initiation**

The crucial event in carcinogenesis is initiation; factors that operate before or after this event are different in nature although they may be decisive. As exemplified in neoplasia induced in the bladder by remotely administered carcinogens, numerous physiologic and genetic factors influence the access of administered materials to the target tissues in an effectively carcinogenic form. Numerous factors also affect the consequences of direct application of effective carcinogens to sensitive tissues (53), and genetic factors control the sensitivity of the tissues.

Initiation is effected quickly. It establishes a region of incipient neoplasia with a permanent new capacity for neoplastic development, but it is not manifested by durable characteristic morphologic changes. Empiric observations show that initiation is not an all-or-none phenomenon. The quality or capacity of the incipient neoplasia, and consequently the quality of the emergent lesions, is directly related to the intensity and, probably to a greater degree, to the duration or repetition of the initiating stimulus. Repeated stimuli summate to augment the capacity for malignant neoplasia (41). The course of xeroderma pigmentosum can be retarded to an important extent by using barrier creams to reduce continued exposure of the skin to ultraviolet irradiation (55). There is an important possibility that subeffective carcinogenic stimuli, not necessarily identical with the initiating stimulus, might summate with that stimulus dangerously to increase the capacity of the incipient neoplasia. If this is true, it would be logical and prudent to eliminate, so far as possible, exposure of the tissue to all kinds of potentially carcinogenic stimuli that might summate with the initiating stimulus.

The concept of incipient neoplasia, implying that initiation effects a diffuse regional change, justifies the investigation of exposed tissues in bulk by chemical or other methods; according to the dormant tumor cell...
hypothesis, such investigations are unlikely to be rewarding (2). The information obtained from chemical studies is not yet decisive, but it is not inconsistent with the widely held opinion that carcinogens bring about in a direct way a permanent heritable change in the structure or utilization of genetic materials. It is questionable, nevertheless, if all forms of neoplasia are attributable to a direct specific action of carcinogens, chemical, physical, or viral, on the genetic material of sensitive cells. The action of some carcinogenic agents may be indirect, neoplasia being a secondary consequence of, for example, “frustrated repair” of injuries caused by the agents (28, 38, 44). It has been questioned, moreover, if carcinogens truly initiate neoplasia or merely accelerate or enhance a process that starts and advances slowly without them (40). The most reasonable view, as expressed by Shubik (53), is that some carcinogens truly initiate neoplasia but that often the basic action of a carcinogen is to enhance a pre-existing latent predisposition. The basic question is whether or not genetic constitution per se can lead to neoplasia without an extrinsic carcinogenic stimulus; it can certainly determine to an important or decisive extent the response to such a stimulus. The interplay of multiple genetic and extrinsic factors is complex and needs re-evaluation in the light of the newer knowledge about the structure of the genetic material and the effects of extrinsic or extragenetic factors on its utilization. Only brief mention can be made here of matters that will surely become increasingly important in future discussions of multiple etiologic factors.

**GENETIC ACTION IN NORMAL AND NEOPLASTIC DEVELOPMENT**

Evidence has been accumulating to show that only a portion of the genetic information coded in the nuclear DNA is in effective use at a particular time, other portions being available for use in different circumstances. The “expression” of the selected portion to yield specific phenotypic characters requires 2 main steps: in the 1st step, called transcription, the genetic information is recorded in RNA, and in the 2nd step, called translation, the coded RNA directs the synthesis of a specific protein. Genetic action and the expression of genetic potentialities thus depend on 3 consecutive processes namely selection, transcription, and translation of a portion of the total DNA code, and these processes are differently and elaborately regulated.

To facilitate discussion, distinctions have been drawn between (a) the total genome carrying the whole of the genetic information; (b) the effective genome, the portion of the total genome that is in effective use in a particular cell at a particular time; and (c) the facultative genome, comprising those portions of the total genome that are available for use as effective genomes in various circumstances (24, 25). The facultative genome provides for the choices of alternative utilizations of the total genome that are implied by competence and capacity in embryonic development and by metaplasia in both embryonic and adult tissues. It is an important empiric fact that the available choices are discrete, mutually exclusive, and limited in number, being few in comparison with the number that random sampling of the total genome could provide. The term “facultative genome” acknowledges these limitations without specifying the mechanisms underlying them, which are obscure. It remains to be seen whether or not they are attributable to a sort of nuclear “differentiation” or chromosomal organization such as Brink (10), for example, has proposed, but the facultative genome seems to be an intrinsically stable and replicable entity. Another important characteristic is that the choice of an effective genome is often biased or heavily weighted in favor of one of those available in the facultative genome. Many embryologists now recognize that the apparent specificity of embryonic induction depends as much on the competence of the reacting tissue as on the quality of the inducing stimulus. In some forms of induction the inducing stimulus is qualitatively specific in that it selects one of the effective genomes available in the facultative genome of the reacting cells. Nevertheless autonomous changes in competence may lead to a state of developmental imminence in which a new differentiation takes place without the customary inducing stimulus, either spontaneously, as it seems, or in response to a wide variety of nonspecific stimuli (31, 32).

The fundamental differences between neoplastic and normal development are probably established at the outset by the process of initiation. In some forms of carcinogenesis, at least, potent carcinogens may be supposed to alter the total genome drastically, perhaps by direct action on DNA or indirectly by interfering with its accurate replication. The alteration, it is reasonable to suppose, is not a mere selection of genetic patterns already present in the total genome of cells of the reacting tissue. This interpretation does not necessarily apply to enhancement by carcinogens, which is especially conspicuous in inbred strains of animals. The total genome may be altered by summation of ordinarily ineffective carcinogenic stimuli, but there is a strong suggestion that developmental imminence is involved; initiation either happens “spontaneously” or, as in xeroderma pigmentosum, is effected by extrinsic stimulation no greater than every member of the population receives. Initiation having been accomplished, neoplastic development reveals several phenomena that have been recognised in normal development. The cells of a region of incipient neoplasia are characterized by a new facultative genome, which provides the basis for the diversity of Group B lesions. The factors that determine the localization and particular characters of individual lesions are not well defined. Promoting agents seem to act selectively to favor the proliferation of cells that form certain kinds of benign and imperfect neoplasms. Promoting stimuli may also summate, as Nakahara (41) believes, to increase the capacity of the incipient neoplasia. A comparable selective action may account for the directive action of certain agents, notably hormones, on neoplastic development. These agents act specifically in the same sense as certain inducers act specifically in embryonic development by selecting one of a number of possible paths of development. Nevertheless there is a possibility with some evidence to support it that the capacity increases autonomously with time as embryonic competence is believed to do and that the incipient neoplasia reaches a state of developmental imminence, when lesions emerge.
without extrinsic stimulation or in response to minor and nonspecific stimuli which may account for the localization. The emergence of tumors at sites of recent trauma recognized in animals as the “Deelman phenomenon” and reported from time to time in man may be a special case of nonspecific stimulation of tissue in a state of developmental imminence. In general the localization and particular characters of emergent tumors seem to be random in time and site except in the special cases already mentioned, but this may reflect only ignorance of the significant, although perhaps nonspecific, stimuli that commonly evoke neoplastic lesions from tissue in a state of developmental imminence.

Progression requires certain permissive factors, as do all stages of neoplastic development, but attempts to incite progression have given erratic results. The few positive results may be attributed to nonspecific stimulation of neoplastic tissue in a state of developmental imminence. More generally, progression seems to be random in time and site although its consequences are circumscribed by the capacity of the tissue in which it takes place (24, 25).

CONCLUSIONS

Overt neoplasia depends on multiple factors which act simultaneously or consecutively and which vary at different stages of the neoplastic disease. At each stage there is a complex interplay of genetic and nongenetic factors. The action of the recognized carcinogenic, inductive or initiating agents is conditioned by varied permissive factors, and the quality of the resulting neoplasia may be determined in part by directive factors. Varied genetic and physiologic factors are involved in delivering an initiating carcinogen to the target tissue in effective form and amount, and others control the responsiveness of the target tissue.

Initiation is effected quickly but is not manifested by persistent characteristic signs or by proliferation of cells. Overt neoplasia is first evident after a substantial latent or induction period. Initiation establishes a new reactivity and capacity for neoplastic development herein referred to as incipient neoplasia. When the initiating stimulus is optimal in quality, intensity, and duration, or when genetic factors are especially favorable, multiple discrete neoplastic lesions emerge concurrently or consecutively from a region of incipient neoplasia coextensive with the exposure to the initiating agent. Many of the early lesions are benign or imperfect neoplasms; they are significant because progression to malignant neoplasia may take place in one or more of them and because they disclose a region of incipient neoplasia within which malignant neoplasms may develop without spatial relationship to precursor lesions. Solitary tumors do not necessarily result from strictly localized carcinogenic action; with equal or greater probability they are localized manifestations of an extensive region of incipient neoplasia.

Initiation almost certainly brings about a permanent replicable change in the genetic material, or at least in the pattern of its utilization; it establishes a new capacity for neoplastic development based on a new facultative genome which circumscribes the range and character of emergent neoplastic lesions. The capacity, and consequently the type, of overt neoplasia depends on the nature and intensity and, probably most of all, on the duration or repetition of the initiating stimulus. Initiation is apparently irreversible, but the capacity of the incipient neoplasia is not static; it can be enhanced by summation of initiating stimuli, and it probably increases autonomously with time so that eventually a state of developmental imminence may be reached. A similar state may be established by genetic factors as in xeroderma pigmentosum, in certain tissues including mammary tissue in selectively inbred mice, and perhaps in the subcutaneous tissues of rats.

Overt neoplasia, which is nearly always focal or multifocal depends basically on 3 consecutive processes—namely selection, transcription, and translation of one of the effective genomes available in the facultative genome established by initiation. The 3 steps are differently and elaborately regulated by genetic and extragenetic mechanisms, most of which are as yet undefined. The directive factors presumably operate at the level of selection, as may some promoting factors. Other promoting factors may favor transcription and translation of certain effective genomes that have been selected already. Some promoting factors, which are also weak carcinogens, may act by summating with the initiating carcinogen to increase the capacity of the incipient neoplasia to a state of developmental imminence. The material basis of this state is unknown but the phenomenon seems to be a real one and important or, often, decisive in neoplastic development. It may account for the erratic emergence of primary tumors at sites of recent trauma and for the equally erratic progression induced by trauma in established tumors. As a rule progression seems to be random in time and site although its consequences are circumscribed qualitatively by the capacity of the tissue in which it occurs.

The aim of prophylaxis must clearly be to protect from initiating carcinogens and from other initiating agents possibly of a different kind that might summate to enhance the capacity of the incipient neoplasia. At present there seems to be little chance of rectifying the genetic change effected by initiation but, as witnessed most encouragingly by the limited success of endocrine therapy in cancer of the human breast and prostate, the effects of the genetic change might be mitigated by controlling permissive and directive factors and factors regulating the selection, transcription, and translation of genetic patterns. The most effective way of interfering with neoplastic development is likely to differ from one type of neoplasia to another and to depend on a more exact knowledge of multiple factors than is yet available.

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Multiple Etiologic Factors in Neoplastic Development

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