Teaching Monograph

Pathobiology of Neoplasia

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PATHOBIOLOGY OF NEOPLASIA

Foreword to Teaching Monographs

This teaching monograph is being published by *The American Journal of Pathology* for Universities Associated for Research and Education in Pathology as a service to medical students and their teachers of pathology. This venture represents a joint effort to make such teaching material available to a wide audience. It is anticipated that from three to four teaching monographs will be published each year. Separately bound copies of these Teaching Monographs can be purchased from Universities Associated for Research and Education in Pathology, Inc., 9650 Rockville Pike, Bethesda, MD 20014. The charge is $1.00 per copy for orders of up to ten, and $.90 per copy for orders of ten or more (prepaid).

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Pathobiology of Neoplasia

A Teaching Monograph

Richmond T. Prehn, MD, and Liisa M. Prehn, MD

MALIGNANT DISEASES collectively comprise the second most common cause of death in the United States. Progress in the early diagnosis and therapy of cancer is dependent on a better understanding of the biological behavior of cancer. This monograph is designed to introduce the student to the general biology of neoplasia; it presents a basic fund of knowledge organized to emphasize the adverse behavior characteristic of malignancy. The bibliography has been selected to include recent critical reviews which can serve as basis for further reading.

Basic Definitions

Hyperplasia is any abnormal or unusual increase in the number of cells of a tissue, or part of a tissue, which results in an increase in tissue mass. There are two types: physiologic and neoplastic.

Physiologic hyperplasia is due to an external stimulus and subsides when the stimulus is removed. *Neoplastic hyperplasia* or *neoplasia* can be defined as that form of hyperplasia which is due, at least in part, to a heritable abnormality within the involved cells. Both physiologic and neoplastic hyperplasia are often present in a tissue simultaneously.

For example, consider an area of skin which has been abraded or wounded. During the wound healing process the epithelium may become markedly thickened due to physiologic hyperplasia. (In the skin this is called *acanthosis*). Once wound healing is completed and the stimulus to hyperplasia removed, the acanthosis subsides.

In contrast, a focal area of skin may be affected by a particular virus and undergo the focal hyperplasia that is recognized as a common wart (verruca vulgaris). This lesion is considered a neoplasm because the virus is thought to alter the genetic mechanisms of the involved cells. Thus, although the virus may originate as an extrinsic infectious agent, the virus, or a part of it, becomes an intrinsic part of the heritable cellular genetic information. To actually prove that verruca vulgaris is a neoplasm according to this definition would be extremely difficult. In point of fact, many le-

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sions of the human cannot be rigorously classified but may be assumed, on the basis of a variety of circumstantial evidence, to be true neoplasms.

That most of the lesions classified as neoplasms actually are associated with a heritable alteration in the involved cells is shown by experiments with typical neoplasms in the mouse. Mouse neoplasms can be transplanted from mouse to mouse of the same inbred strain and maintain their neoplastic characteristics indefinitely. Furthermore, it can be rigorously proven by genetic experiments that in most cases the cells of the transplanted neoplasm are the progeny of the cells of the original lesion—not newly altered cells of the secondary host animals as would be the case if an external stimulus, for example an infectious agent, were being transplanted. Thus, the basic heritable nature, at the somatic cell level, of the neoplastic alteration is firmly established.

Neoplasms are commonly divided into two ill-defined and overlapping categories, benign and malignant. The characteristics of each of these will be discussed at some length at a later point. For the present, it is sufficient to state that *benign neoplasms* are generally associated with a good prognosis, while *malignant neoplasms* generally are associated with a poor prognosis.

Malignant neoplasms are commonly called *cancers*. Those of epithelial tissues, i.e., those of tissues derived from the ectoderm or endoderm, are usually called *carcinomas*, while those of tissues of mesodermal origin are usually called *sarcomas*. The carcinomas and sarcomas are further designated by descriptive terms to indicate the origin or histopathology in greater detail, i.e., leiomyosarcoma, squamous cell carcinoma, or bronchial adenocarcinoma. If the terms *carcinoma* or *sarcoma* are omitted in the name, the lesion is usually, but not always, benign. Thus, a uterine leiomyoma is a benign tumor. However, the terminology of neoplasia grew without benefit of established rules, and many exceptions have been established by long usage. For example, a basal cell carcinoma of the skin is a benign neoplasm; it is also more properly called a basal cell epithelioma. The peculiarities of terminology will become familiar with usage.

A tumor is, technically, any swelling, but it is often used interchangeably with cancer, especially by the laity.

Morphology and Behavior

It has already been mentioned that neoplasms are commonly classified as benign or malignant. The existence of these two discrete terms does not mean that the two categories are discrete. There is a continuum of lesions from the nearly normal to the most abnormal or malignant, and conse-

quently, there are numerous lesions which are not easy to place in one category or the other.

The hallmark of the malignant neoplasm or cancer is the proclivity of its cells to metastasize, i.e., to spread via the blood stream, lymphatics, or serous cavities, to distant sites. However, even this property is not exclusively found in malignancy or even in neoplasia. Endometriosis involves the "metastasis" of apparently quite normal endometrium to distant sites, and the circulating lymphoid cells could also be thought of as metastasizing. Any tissue has a built in probability of metastasizing; this may be so low as to be virtually nonexistent in the cases of most normal tissues and some benign neoplasms; it may be extremely high in the cases of some malignant tumors. When the pathologist states that a lesion is benign or malignant, he is thus expressing his opinion concerning its probability of metastasizing.

An estimate of the probability of metastasis can usually be gained by a study of the histopathology and/or cytology of a biopsy specimen combined with a knowledge of the behavior of similar lesions in similar circumstances in the past. Although the details vary in each type of neoplasm, certain general principles can be extracted that serve to guide the pathologist in his assessment.

If the entire lesion or a sizable part of it is available for study, the gross anatomy may be suggestive of the degree of malignancy. Benign lesions usually tend to grow by expansion from a central point of origin, compressing surrounding structures, and thus producing a well-defined capsule containing the spheroid or ovoid tumor mass. The shape may, of course, be distorted by the degree of compressibility of the adjacent tissues. Malignant lesions, in contrast, tend to infiltrate the surrounding tissues so that the borders of the lesions are not discrete, and no capsule formation occurs.

This difference in mode of growth can often be seen during the microscopic examination of the histopathology. The edges of the malignant lesions are usually poorly demarcated, and individual neoplastic cells can be seen infiltrating the surrounding normal tissue (Figure 1). This infiltration, especially of the cells of an epithelial neoplasm through a basement membrane, is termed neoplastic invasion. Invasion into lymphatic channels and blood vessels is a prerequisite for metastasis and therefore is a very important criterion of malignancy.

The biochemical basis of invasion and metastasis is not understood. It is known that the cells of a malignant neoplasm are less tightly adherent, one to another, than are corresponding normal cells; this is correlated with a diminished calcium content in the cell walls of the neoplasm. (Chelating

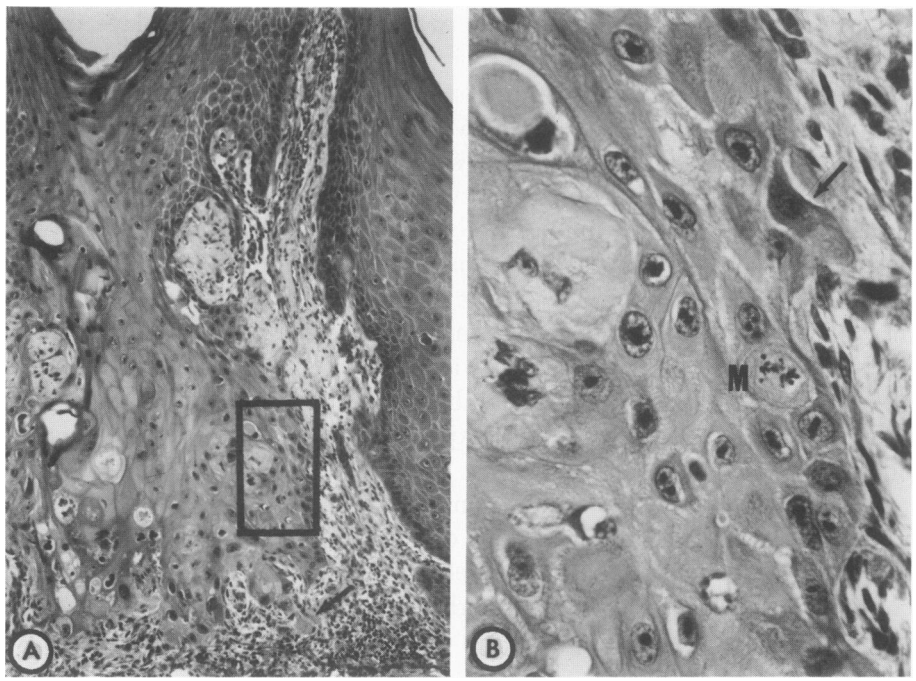

Figure 1A—A focus of early invasive squamous cell carcinoma in the skin of a farmer exposed to intense sunlight for many years. Note the finger-like configuration of malignant cells invading the underlying dermal stroma. The enclosed area is shown at a higher magnification in Figure 2B. (× 75). **Figure 1B**—A malignant cell is shown invading the stroma; note the absence of the basement membrane in this area. A malignant cell in mitosis (*M*) is also shown. (× 450)

agents that remove calcium can be used to separate the cells of tissues in order to form individual cell suspensions.) It is also known that the negative surface charge of malignant cells tends to be high, and the cells might, therefore, tend to repel one another. Ionic communication between adjacent cells of a malignant neoplasm is diminished. Some neoplasms have been shown to produce hyaluronidase, which might facilitate invasion through the tissue ground substance. Malignancies also produce a plasminogen-activator, and the resulting fibrinolysin may aid the process.

Each neoplasm not only has a certain probability of metastasizing, it also tends to have certain sites of predilection. Some of these patterns are determined by purely anatomic considerations, for example, venous metastases are often common in the lung, since the lung capillary bed is the first vascular sieve that intravenous tumor cells encounter. However, some patterns of tumor metastasis seem to be explicable only on the basis of more favorable "soils" in certain sites for the seeding of that particular neoplasm.

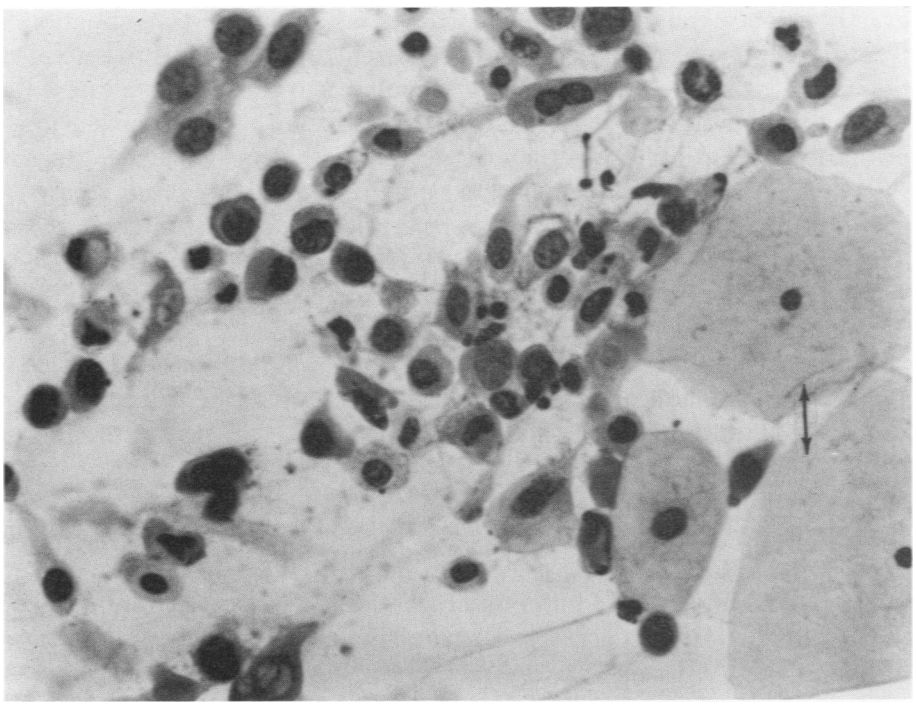

Figure 2—A vaginal smear showing cytologic characteristics of normal and malignant squamous cells. Compare the normal squamous cells (*arrow*) with the adjacent malignant ones; note the marked difference in nuclear size and the nuclear:cytoplasmic ratio. (× 500)

Apart from the microscopic evidence of invasion or lack of it, there are other clues in the microscopic examination of tumor cells that help a pathologist arrive at his assessment of the malignancy of a neoplasm. In general, the cells of malignant lesions tend to be larger and more pleomorphic, the nuclear:cytoplasmic ratio is higher, clumping of chromatin is common, multiple and prominent nucleoli may be present, and abnormal mitoses may also be seen. These cellular changes form the basis for the diagnosis of cancer, especially of the uterine cervix, by the microscopic examination of exfoliated cells—the well-known Papanicolaou smear (Figure 2).

Although mitotic figures are usually prominent in a histologic section of a malignancy, it should be noted that the rate of cell division in some malignancies is *less* than in the tissue of origin, a point requiring some discussion. Hyperplasia could theoretically be produced by either an increased rate of mitosis, i.e., increased cell birth, or by a decreased rate of cell death. In a tissue in growth equilibrium, cell births must necessarily

exactly balance cell deaths. Cell deaths are a function of differentiation, a term denoting the acquisition of specialized functions. For example, in the normal skin, the mitotic activity is confined to cells in or very near the basement membrane, the basal layer. The cells of this layer have as their primary function division—they do not make the specialized product of skin cells, keratin. In normal skin, exactly half of the daughter cells produced by mitosis in the basal layer are pushed to more superficial locations. Here they lose the power to divide and concomitantly begin to manufacture keratin—in other words, they differentiate. Ultimately, they are sloughed from the epithelial surface as dead cells.

In neoplastic skin, the rate of mitosis may be altered, but the essence of the neoplastic condition is that now less than 50% of the cells created by mitosis differentiate. Somewhat more than 50% stay in the replicative pool. There is, thus, a geometrical increase in the number of cells. In general, the more malignant the lesion, the smaller the percentage of differentiating cells and, therefore, the faster the growth of the lesion, even if the *rate* of mitosis is, as often happens, *less* than normal.

The percentage of cells differentiating is thus a very important criterion of the growth potential of a neoplasm and is a good indicator of the degree of malignancy. A lesion with a high percentage of undifferentiated cells is said to be *anaplastic.* Broders introduced a method of classifying neoplasms on a scale of I to IV, depending upon the degree of anaplasia. Despite the fact that grading is a rather subjective procedure, many studies have shown that, in a wide variety of different types of tumors, a good prognosis correlates with a low grade, i.e., with relatively little anaplasia.

While many criteria, as have been mentioned, correlate well with the propensity to metastasize, it should be emphasized that no one criterion is absolute. Most of them seem to behave as independently assorting characters, and much experience and a consideration of all available evidence are required to arrive at a good estimate of the prognosis. Of course, all other factors aside, a lesion that has already metastasized, say to the regional lymph nodes, will have a poor prognosis compared to that of a lesion in which metastases are not evident.

One of the most important and characteristic features of the behavior of a neoplasm is called *neoplastic progression.* By this is meant, not an increase in the extent or size of a lesion, but a worsening of its biologic potential. With passage of time, many neoplasms gradually become more malignant. A lesion which might have been assigned a Broders' grade of II at the first biopsy may be found to have a grade of III or IV after the passage of a few months or years.

The biologic basis of neoplastic progression is apparently simple Dar-

winian selection rather than a change in the host or an adaptation of the tumor as a whole. As has been pointed out, mitoses in a neoplasm are often abnormal and replication is imperfect. Hence, there is much variation in properties among the cells of a neoplastic population. In general, those best able to propagate and those least able to differentiate tend to outgrow the rest, so that the nature of the population tends with time to become more and more malignant.

Progression seems usually to be a rather unpredictable phenomenon that proceeds in a stepwise fashion. A lesion may remain biologically quite constant for a prolonged period and then rather suddenly alter in some dramatic way (Figure 3). It seems that the appearance of a new and more malignant race of neoplastic cells often must await the arisal of a mutation-like alteration in one of the neoplastic cells.

It is a debatable question whether or not all malignant neoplasms arise as benign lesions which then undergo progression, or whether some may be highly malignant from the start. Certainly, from a practical clinical

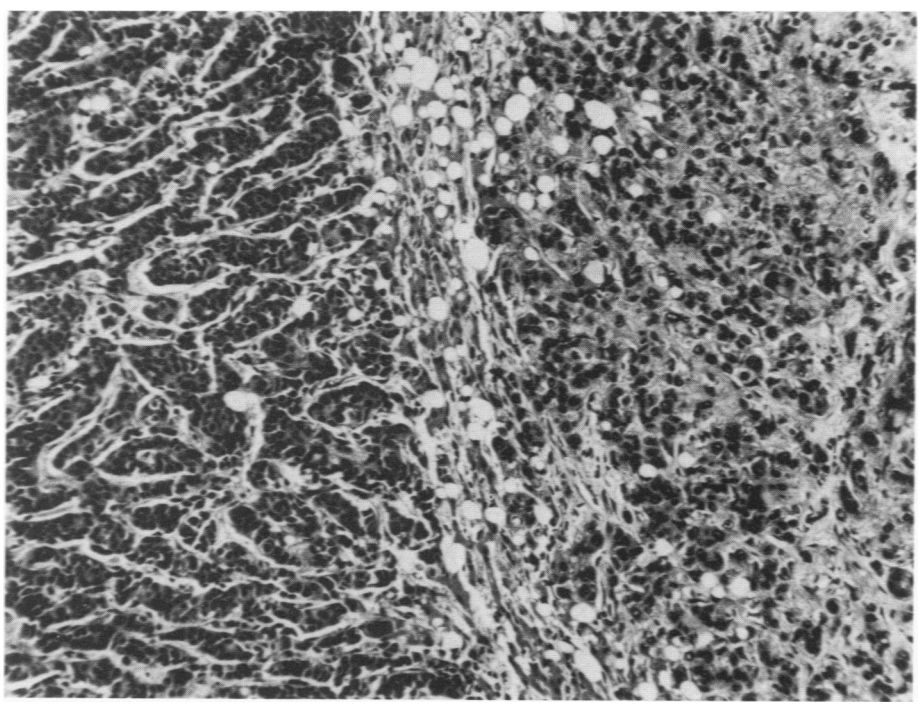

Figure 3—An example of tumor progression from a metastatic nodule of breast cancer in a 42-year-old woman. In the left half of the photograph, well-differentiated cells form tubule-like structures reminiscent of ducts in normal breast; to the right, a nest of less well-differentiated neoplastic cells which have lost their capacity to form duct-like structures are shown (× 135)

standpoint, many quite small neoplasms, for example, those in the breast, are already quite malignant and no benign phase is discernible. However, it seems probable that all neoplasms do go through a benign phase, even if this phase is often not clinically recognized. In cancer of the uterine cervix, the benign phase can apparently last for many years.

Progression and lack of differentiation are recognizable in chemical as well as in behavioral or morphologic terms. As a lesion progresses from the normal through the benign to the more and more malignant stages, the chemical attributes of the normal tissue of origin become less and less prominent. For example, keratin production in skin lesions tends to become less and less, until ultimately it may disappear completely. Enzyme systems associated with specialized products of differentiation become less evident. Ultimately, whether the tumor originated in skin or liver, it may be left with only those enzyme systems concerned with primitive processes of replication and with virtually none associated with a tissue-specific differentiation. At this stage of ultimate progression, when almost all of the cells are undifferentiated and are in the replicative pool, it may be very hard, chemically or in any other way, to distinguish a tumor derived from one tissue from that derived from another. This process has been termed *convergence*, biochemical as well as morphologic.

In recent years a biochemical feature of great interest has been recognized. Apparently the change from normal through increasing degrees of malignancy is associated with increasing inflexibility of enzyme patterns. For example, the normal liver is able to alter the levels of many enzyme systems to adapt to environmental stresses such as changes in glucose or the physiologic diurnal rhythm. The hepatoma, in contrast, is much less able to respond in this fashion. Activity of a particular enzyme in a particular tumor may be at any particular level which the normal tissue could reach, but in the tumor it tends to be stabilized at that level, whether it be high or low, regardless of changes in the environment. The reasons for this inflexibility are not known but may be related in some way to the general observation that malignant cells often have many more free ribosomes, that is, ribosomes not attached to the endoplasmic reticulum, than do normal cells.

This biochemical inflexibility of the enzyme systems of a tumor may be an explanation for the success of chemotherapy in some cases, A chemotherapeutic agent is a noxious and toxic chemical and thus represents a profound change in the environment. Normal cells may be better able to adapt to this stress than the neoplastic cells because of the biochemical rigidity of the latter. Chemotherapy usually breaks down because of the emergence, via tumor progression, of variant clones of

neoplastic cells that are resistant to the particular chemical. Thus, although the cells of a neoplasm have little adaptability, the population as a whole is highly adaptable because of the frequency of heritably variant cells.

There is one particular further aspect of tumor progression that requires discussion. It was mentioned earlier that physiologic and neoplastic hyperplasia often occur simultaneously and to varying extents in the same lesion. In particular, consider an endocrine target organ such as the breast. The breast undergoes cyclic hyperplasia (physiologic) in response to the endocrine changes of the estrus cycle. In fact, the breast fails to develop in the unfriendly endocrine environment of the male. Often a tumor of the breast, in the early stages of its progression, will retain this property of the parental tissue—the responsiveness to the female hormonal milieu. At this stage, when regression of the tumor can be produced by altering the endocrine environment, the tumor is said to be *dependent*. Probably all tumors pass through stages when they are still dependent upon physiologic factors required by the tissue of origin, but it is only in the case of the endocrine-dependent tissues that some of these factors are known. Unfortunately, due to tumor progression, a tumor, after a variable period of time, loses its endocrine dependency, and hormonal manipulation ceases to be therapeutically effective.

It has been pointed out that the prime feature of neoplastic progression is the stepwise loss of various characteristics of the differentiated tissue. However, while this is certainly the general tendency, many tumors acquire (at least temporarily during the process) differentiated characteristics that they never possessed before. This is recognized particularly in the so-called *paraendocrine syndromes*. For example, a bronchial carcinoma may be found to be producing insulin! It would seem that the regulation of differentiation tends to be deranged in a variety of bizarre and unexpected ways. However, these changes are not completely random, since some particular types of tumors are more likely to show particular patterns of aberrant differentiation.

It was mentioned that malignancies tend to show abnormal mitoses and large nuclei. Karyotypic analysis shows that most malignant tumors are aneuploid. However, with one striking exception, there has not yet been discerned any pattern of abnormality associated with a particular type of neoplasia. The one exception is the regular presence of an abnormal chromosome, the so-called Philadelphia chromosome, in the cells of chronic myelogenous leukemia. What the relationship of karyotypic changes may be to the etiology of neoplasia is debatable, but the most widely held opinion has been that they are probably a consequence rather

than a cause. It may be that recently discovered methods of banding chromosomes may provide new insights in this area.

What may be one of the most significant and important distinctions between neoplastic and normal cells has come from tissue culture studies. Hayflick has presented evidence to show that the replicative life of normal tissues in culture is finite. Neoplastic cells, in contrast, replicate indefinitely. There is considerable evidence to suggest that this finite life-span of normal cell populations also occurs *in vivo* and that the phenomenon is in some way related to normal aging. It may be that some neoplastic clones occur which are not possessed of immortality; however, such clones might never be seen as clinical cancers because, if they originate from single cells (a problem to be discussed in the next section), they might not reach a clinically recognizable size before exhausting their replicative potential.

Several other attributes of neoplastic, as opposed to normal, cells have been described as a result of tissue culture studies. None of these are absolute, but the correlations are good. For example, neoplastic cells tend, with increasing malignancy, to be less inhibited by population density, i.e., they grow to greater densities in culture before being inhibited. Also, neoplastic cells have a greater tendency to grow in a disorderly fashion and to pile up upon one another (Figure 4). This may be a reflection of a lessened adhesion to a substrate. Most normal cells, with the exception of blood elements, will not grow without a solid substrate; most, but not all, neoplasms will grow in suspension culture or in very soft agar. Recently a further common attribute of malignant cells has been described: a tendency to produce large quantities of a plasminogen activator. Production of a plasminogen activator may be important in the spread of cancer and the ability to lyse a fibrin clot might facilitate invasion.

Etiology

The etiology of neoplasia is, in most cases, manifestly multifactoral, even though a single underlying mechanism is assumed by some theories.

One of the best examples of the multifactoral nature of the process is to be found in breast cancer in mice—a system which is probably an excellent model of the human disease. By a process of inbreeding and selection, mouse strains have been developed that are characterized by various incidences of breast cancer. This fact suggested that breast cancer is a genetically determined trait. However, when a strain with a high incidence of breast cancer was crossed with one with a low incidence, the F_1 hybrids were found to have a incidence of breast cancer approximating that of the maternal strain. The supposedly genetic trait was thus trans-

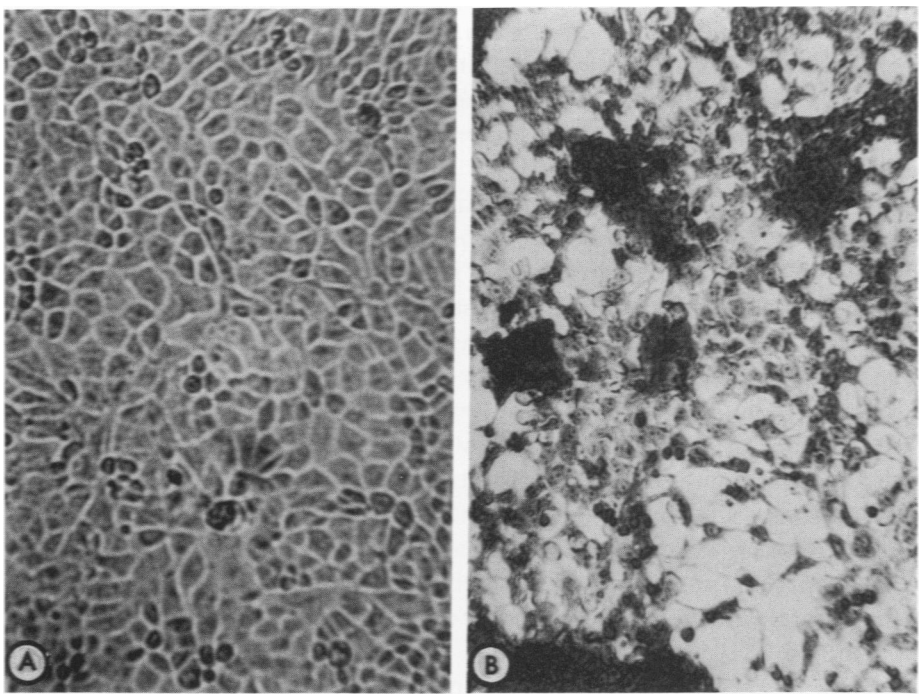

Figure 4A—A confluent (contact-imhibited) culture of green monkey (CV-1) kidney cells (× 165). **Figure 4B**—A culture of transformed (CV-1) cell; note the multilayered foci due to a loss of contact inhibition (× 165).

mitted only by the mother. Further work showed that the maternal influence was actually transmitted by the milk (although in some strains transmission in the egg or *in utero* can be shown instead). However, this milk agent, now known to be a virus, produces cancer to varying degrees in different mouse strains. Furthermore, males are resistant to its action unless they are given female hormones, i.e., estrogen and/or prolactin. Thus, the incidence of breast cancer is indeed determined by the genotype but in a number of ways, i.e., via susceptibility of the animal to infection with the virus, via an influence upon the susceptibility of the mammary gland itself to oncogenesis, and via an influence upon the hormonal environment. Additionally, the presence or absence of the virus (tumors can occur in very susceptible mice in the absence of the virus) and of other influences such as x-radiation or chemical carcinogens all influence the tumor incidence. These facts have led to the concept of a threshold level of etiologic factors required for tumor production.

The genetic influence on tumor incidence is very striking in some human tumors of childhood in which the genotype seems to be the

predominate influence. For example, high susceptibility to most retinoblastomas is clearly inherited in a rather simple Mendelian fashion. In most human tumors, however, the heritability is more complex, being dependent upon many genetic factors or being overlaid with a variety of environmental influences.

From what has already been said about tumor progression, it should be clear that a variable number of steps occur during the change from the normal to the fully malignant cell. The first step apparently occurs in a single cell—cancer is a clonal disease. The change in the first neoplastic cell is a random stochastic process as are the changes during the subsequent tumor progression. Any given etiologic factor simply increases the probability that any particular cell will be "transformed" so that it gives rise to a race of neoplastic cells. However, in some highly artificial tissue culture experiments, it is possible to transform virtually all of a population of target cells by appropriate treatment with a chemical oncogen.

Hundreds of different chemicals are capable of producing neoplastic transformation, and many of these chemicals are probably important environmental oncogens in man. How they produce transformation is still not known.

What is known about their action is that many of them must be metabolically activated by cellular enzymes to a "proximal" form. In the absence of the appropriate enzymes, transformation cannot occur, and it is the presence or absence of the necessary enzymes for the activation of particular chemicals that determines the tissue and species specificities that many of them exhibit. The proximal or active form of the chemical oncogen always seems to be an electrophilic compound capable of binding to DNA, RNA, and protein. Which of these reactions is essential to oncogenesis is undetermined.

Many investigators subscribe to the mutation hypothesis. The arguments in its favor are as follows: a) All oncogens (including chemicals, radiations, and viruses) can be shown, under proper conditions, to be mutagens. b) Defective DNA repair mechanisms, as in xeroderma pigmentosa, are apparently associated with an increased risk of neoplasia. c) Neoplasia occurs as a clonal disease, heritable at the cell level. Somatic mutation could account for this heritability.

Impressive as the above arguments may seem, there are strong counterarguments. Somatic mutation is, of course, not the only means of heritable somatic cell variation. Organogenesis during ontogeny is not due to a mutational process, but to some other, not yet understood, form of somatic cell variation. Perhaps neoplasia is due to aberrations in this mechanism, whatever it may be. The fact that 100% of target cells may,

under some conditions, be transformed by a chemical agent seems rather inconsistent with a mutational mechanism, even if one postulates specific "hot spots" in the genome. Furthermore, most of the characters associated with neoplasia are not of an all-or-none nature, as would be expected in a mutational system, but rather occur in an apparently nearly infinite number of gradations. The processes of gene expression are obviously markedly abnormal in the neoplastic cell (i.e., the lung tumor cell that secretes insulin, previously discussed) so that more than simple somatic mutation is involved. As far as oncogens being mutagens is concerned, even that correlation is not entirely secure. They admittedly can all produce heritable abnormalities. However, it is not clear that all such heritable abnormalities are due to classic gene mutation, since Mendelian segregation cannot usually be used as a test in the bacterial systems employed.

Before leaving the question of somatic mutation versus aberrant organogenesis, it should be pointed out that these hypotheses are not mutually exclusive; perhaps some neoplasms are due to one mechanism and some to the other, or perhaps both occur in a single neoplasm. One final comment is in order: there is no character in any neoplasm, be it immortality, invasiveness, growth potential, etc., that cannot be found as a normal character in one or another nonneoplastic cell. Thus, there seems to be no need to postulate new information in the neoplasm, but rather the misprogramming of the information that is normally present in the zygote and in all somatic cells.

Although no new information in the cell need be postulated to account for any single neoplastic cellular trait, there is no doubt that neoplastic transformation can be caused by viruses, either of the DNA or RNA varieties, and that in some systems, the viral genes seem necessary to the maintenance of the transformed state. With the aid of temperature-sensitive viral mutants, it has been possible, in some cases, to show that transformation is reversibly dependent upon the activity of temperature-sensitive viral genes. These experiments imply that the neoplastic change depends upon new genetic information from the virus—a virus whose DNA (or the cellular DNA programmed by viral RNA in the case of RNA viruses) is integrated into the cellular genome. Furthermore, the integration must be at very specific sites in order to produce the neoplastic change. This would seem analogous to mutation.

Even this conclusion may be premature. It may be that the DNA which specifies viral structures and functions is already in the normal cellular genome merely waiting activation by a variety of means. According to the "oncogene" theory and the related protovirus hypothesis, this is actually

the case. In this event, viral oncogenesis may be more akin to aberrant differentiation than to mutation.

In any event, it is clear that many tumors involve a viral etiology. Perhaps all tumors, even those overtly caused by other agents such as chemicals or radiation involve viruses. Certainly, viruses can often be isolated from such tumors. Furthermore, often more than a single virus may be a part of the system. Most viruses do not cause transformation in cells in which they replicate, in part, because replication in some virus systems causes cell lysis. In many systems only defective viruses (i.e., viruses which cannot complete their replication) transform cells. Such defective viruses are able to replicate only in the presence of a complimentary "helper" virus. The implication, furthermore, may be that any virus might be oncogenic if it found itself in a cell in which it could not replicate—perhaps a cell of some foreign species. Human adenoviruses, for example, have been shown capable of producing tumors in rats or hamsters in which they are defective, but there is little to suggest any oncogenic activity for human cells.

At the present time the leading candidates for oncogenesis in man are the Epstein-Barr virus in Burkitt's lymphoma and nasopharyngial carcinoma and herpesviruses in carcinoma of the cervix. Whether there is a virus similar to the mouse virus in human breast cancer is still debated.

From what has already been discussed concerning neoplastic progression, it is clear that many steps are involved in the change from normal to malignant. The question can be asked whether or not an etiologic factor, chemical, virus, etc., influences anything more than the first step. Perhaps the etiologic agent starts the process by some triggering mechanism, and the end result is then the inevitable consequence without further exogeneous stimulus. Some information on this point has been obtained from a study of chemical oncogenesis in mouse skin.

If a subthreshold dosage of a potent chemical oncogen is applied to the skin of the mouse, the initial inflammatory reaction and physiologic hyperplasia soon subsides, leaving the skin overtly normal. If nothing further is done, there is nothing to mark the fact that an important, quite permanent, but subtle change has indeed occurred. If, on the other hand, a second small suboncogenic dose of the same or another oncogenic chemical is applied, even after an interval of a year or more, tumors are induced. Some permanent, or nearly permanent, change initiated by the first dose caused the skin to be, even after a year, more susceptible to a second dose. Since cancer is a clonal disease, it is thought that the initial dose transformed some of the epithelial cells into "latent" tumor cells. The second dose then "promoted" the growth of these latent cells. In this

system, then, the chemical appears to have been necessary for more than just the initial step of the tumorous process. The cumulative nature of the stimulus is an important facet of the oncogenesis in man produced by chronic exposure to weak environmental oncogens, for example, tobacco smoke. Also, with few exceptions, various kinds of oncogens—chemicals of different types, viruses, and radiation—are all additive.

There is some evidence that the two steps described above, initiation and promotion, may be fundamentally different from each other, giving rise to the *two-stage hypothesis*. A variety of irritants (in the classic case, croton oil) can be substituted for the second dose of chemical oncogen. Croton oil is, of itself, only very marginally oncogenic, and it cannot be substituted for the initiating dose. The mechanisms of initiation and promotion may, therefore, be qualitatively distinctive, but these data do not seem conclusive.

Epidemiologic data from insurance statistics and governmental records suggest that a wide variety of human tumors involve five or six steps, if one assumes that progression from initiation to death is due to independent, random events. The incidence of death from most common cancers increases exponentially with age, and from the slope of the mortality curve one can deduce the number of "hits" or events.

The mortality from different kinds of cancer differs markedly in different populations. While some of this geographic and ethnographic variation may be due to genetic differences, much of it is clearly due to differences in "life style." For example, epidemiologic data suggest that the infrequency of carcinoma of the uterine cervix in Catholic nuns is due to the fact that this tumor is a viral venereal disease transmitted during coitus by male carriers. Very important in other cases are the prevalence of environmental chemical oncogens and variations in dietary habits.

Homeostatis

Ever since the publication of Virchow's *Cellular Pathology* in 1863, the emphasis in the study of disease, and in particular in the study of neoplasia, has been on the cell. Although this emphasis is justified, the cell does not exist in a vacuum. Cellular transformation and progression may be the critical events, but the cellular environment can influence both the occurrence of transformation and whether or not the transformed cell will grow into a clinically overt tumor.

That the cellular environment can potentially influence the probability of transformation is self-evident in those cases in which transformation is produced by an exogenous virus. Antiviral immunization can markedly inhibit viral spread and the consequent probability of cell transformation. In

the case of radiation-induced transformation in tissue culture, the incidence per exposed cell is vastly increased in crowded cultures with dead and dying cells. The mechanism in this case is not known.

It is quite probable that most transformants never give rise to overt neoplasms. The transformed cells or their descendant clones are probably subject to a variety of homeostatic mechanisms, of which only a few have been identified. One of the best examples comes from the mouse mammary tumor system.

In the mouse that has received the mammary tumor virus at birth and in which the genotype is receptive, numerous small (1 to 2 mm diameter) incipient breast tumors begin to appear sometime after puberty. These are focal benign lesions scattered throughout the mammary ductal system. They are appropriately called hyperplastic nodules. That they are early stages in the progression of breast cancer is shown by the fact that they have a far greater probability of giving rise to a malignant tumor than does the grossly normal breast tissue. These small benign tumors seldom grow beyond the 1 to 2 mm size because of a growth-controlling homeostatic mechanism exerted by the surrounding normal ductal tree.

This homeostatic mechanism's primary function is a spacing factor in normal gland ontogeny. When the ductal tree begins to grow after puberty, it grows by the proliferation of cells in the terminal buds of the rudimentary ducts. The ducts thus elongate, and from a starting point near the nipple they grow into a branching structure that fills, but never exceeds, the mammary fat pad. Curiously, when viewed stereoscopically it can be seen that the ducts never came in contact with one another. During growth, the ducts are each mutually repellent so that they cannot come into contact. The nature of the repellent principle is unknown.

A hyperplastic nodule is usually a neoplasm derived from alveolar cells of the breast. It can be shown experimentally that normal alveolar cells cannot migrate into and proliferate in the fat pad except in association with ductal cells.

The alveolar cells or the hyperplastic nodule are likewise dependent upon a symbiotic association with normal ductal cells—this property is usually not lost in transformation. However, as we have seen, the ductal cells cannot proliferate in the presence of surrounding normal ducts. Therefore, the neoplastic alveolar cells are also secondarily inhibited. Most of these hyperplastic lesions never do undergo progression to the point at which their dependence on normal ductal cells is lost and they are free to proliferate into large lesions. The inhibitory influence of surrounding normal ducts is thus a major homeostatic defense against nascent breast tumors.

Somewhat similar phenomena have been observed in tissue culture. A

number of investigators have observed that surrounding normal cells can, in some systems, inhibit the growth of transformed clones. (Perhaps this is the mechanism that compels latent tumor cells to remain dormant in mouse skin for so long after *initiation* and prior to *promotion*.)

In recent years, the possible role of immunity against tumor cells has generated much interest and the immunology of cancer, (as distinct from the immunology of oncogenic viruses) is currently one of the most active areas of cancer research.

Immunology

One of the most important defenses against foreign organisms, such as bacteria, protozoa, fungi, etc., is the immune mechanism. This very complex mechanism has the capacity to distinguish "self" from "nonself," i.e., to recognize that which is foreign and react in a manner which is usually more harmful to the invader than to "self." Although a cancer cell is not a foreign invader, most cancer cells can activate the immune mechanism, so it is possible that immunity plays an important role in cancer biology. It is also probable that the importance of immunity in cancer biology is currently being grossly exaggerated.

Inasmuch as the cancer cell is a part of the "self," it was believed for many years that immunity against such cells was theoretically impossible. Now it is realized that autoimmune reactions are common and indeed are the basis of a number of diseases. The absolute immunologic distinction between "self" and "nonself" has been broken. However, the cancer cells' ability to stimulate the immune mechanism is usually quite small and, perhaps, not very consequential in terms of a defense mechanism.

The mechanism by which the immune system distinguishes "self" from "nonself" is still a subject of controversy—and it may be that several different mechanisms exist. There is increasing evidence, however, that "self-tolerance" may be due, not to a lack of "recognition" of a self-antigenic determinant, but to a blockage of the subsequent immune reaction. Lymphoid cells capable of "recognizing" various "self" components can be found, but their usual reaction may be blocked by factors in the serum, either excess antigen or antigen–antibody complexes. A deficiency of these normal blocking factors may be the basis of some autoimmune diseases.

Likewise, in the case of the cancer cell, potentially reactive and cytotoxic lymphoid cells usually exist, but their activity may be blocked to a greater or lesser degree by serum antigen or antigen–antibody complexes. Again, it must be emphasized that this is probably a facet of the normal mechanism that prevents autoimmunity.

The antigens on the surface of the cancer cell are, at least in part, of the

same specificities as exist upon the normal progenitor cells, i.e., a variety of organ-specific antigens, histocompatibility antigens, etc. In addition, most cancer cells appear to possess antigenic specificities difficult to detect or non-existent on the progenitor cells. At least some of these may be the result of derepression of parts of the genome normally functional only in the embryo, thus producing the so-called carcinoembryonic antigens (CEA). When any of these antigens are capable of stimulating a tumor-destructive immune reaction, they are commonly termed *tumor regression antigens* (TRAs) or, especially in animals, *tumor-specific transplantation antigens* (TSTAs), the latter because they are usually demonstrated by tumor transplantation techniques.

The existence of TSTAs in mouse tumors is usually easily demonstrated by using inbred mice. Inbred mice of any given strain and sex are genetically identical, one to another. Consequently, there is no immunologic resistance to the transplantation of normal tissues from one animal to another—the tissues of one animal are not foreign to another. However, when cancer cells from that same inbred strain of mouse are transplanted, the secondary recipients often grow the tumor better if they have been immunologically crippled, perhaps by x-radiation. This suggests that the immune mechanism of the normal animal acts to inhibit the growth of tumor transplants. Dead tumor cells can also be used to immunize the mice against the growth of subsequently inoculated live tumor cells. Induced immunity to the growth of transplantable tumors in inbred mice is the classic method of demonstrating TSTAs, and it was the discovery of this phenomenon that destroyed the concept that immunity to cancer was theoretically impossible.

Virtually all tumors in inbred animals can be shown by the transplantation technique to possess TSTAs. This is true regardless of the etiology of the original tumor, i.e., virus, chemical, or radiation. It is, therefore, presumed that tumors in man also possess TSTAs, but the transplantation test cannot be used. However, indirect evidence, obtained from *in vitro* reactions between patients' lymphoid cells and their tumor cells, may support this presumption.

The classic transplantation test in inbred mice for tumor TSTAs reveals some features of considerable theoretic importance. Firstly, most tumors induced by chemicals or radiation are shown to be individually specific. For example, if two separate and distinct tumors, A and B, are induced with a single chemical oncogen in exactly the same manner in a single mouse, A will be found capable of immunizing other mice of the same inbred strain against the growth of implants of A, but not against the growth of implants of B. If, however, the tumors had been induced by an oncogenic virus, the general rule is that they will cross-react, i.e., any tumor

induced by a particular species of virus can be used to immunize against any other tumor induced by that same virus. It should also be noted that subsequent infection of chemically induced tumors by a virus may impart to them a cross-reacting antigenic specificity, even though in this case the virus had nothing to do with the etiology of the tumors.

A second noteworthy feature revealed by the classic transplantation test is that the strength of the induced immunity varies widely from tumor to tumor, perhaps in part due to differing levels of the serum-blocking factors already discussed. In general, the larger the interval or latent period between application of an oncogenic chemical or virus and the gross appearance of tumor, the less immunity that tumor will arouse in the transplantation test.

The explanation of the general inverse correlation between the latent period of tumor formation and immunogenicity, as described above, is not yet known. It might be thought that lesser immunogenicity is the result of immunoselection—tumors exposed to the immune response over a longer period of time during their latency might be under strong selective pressure. However, studies with so-called spontaneous tumors suggest that this explanation is not correct.

Spontaneous tumors, i.e., tumors that arise without deliberate exposure to an etiologic agent, usually have, by the classic transplantation test, very little if any capacity to arouse immune resistance to their growth. (In some cases this may be due to the blocking factors previously described, but this has not been established.)

If the failure of spontaneous tumors to arouse an immune resistance to their growth were due to immunologic selection, similar tumors arising in an immunologically inert environment, where no immune selection is possible, should be highly immunogenic in the transplantation test. The experiment has been done with tumors that arise in tissue culture.

Mouse cells grown *in vitro* tend with time to become transformed and will then grow as tumors when transplanted into the proper, i.e., syngeneic, strain of mouse. If the cells *in vitro* were exposed to a chemical oncogen, the transformation would be accelerated. When the tumors derived from the growth of mouse cells in culture are tested by the transplantation test, it is found that those not deliberately exposed to a chemical oncogen behave just like spontaneous tumors obtained *in vivo*, i.e., they arouse little or no immune resistance to their growth. Those that had been induced *in vitro* by a chemical oncogen are, in contrast, highly immunogenic. Thus, it appears that the immunogenicity of a tumor depends primarily upon the eitologic agent and not upon immunoselection.

The most reasonable explanation of the correlation between short latency and high immunogenicity may relate to the fact, already alluded

to, that tumorigenesis involves a number of steps. If the oncogenic agent is capable of accelerating several of these steps, including antigenic change, it would follow that those tumors that went through their evolution rapidly, because of a large flux of oncogen, would also be most likely to become immunogenic. Tumors induced by low levels of oncogen would have long latencies and little chance of simultaneously acquiring the immunogenic change. This hypothesis thus relates the latent period and immunogenicity to the concentration of oncogen, with spontaneous tumors being at the very low end of the scale, both in regard to latency and immunogenicity. This hypothesis has not yet been rigorously tested.

The fact that highly immunogenic tumors, i.e., tumors induced in the laboratory by oncogenic chemicals and viruses, are inhibited by the immune reaction when they are transplanted, does not necessarily imply that immune resistance to tumor growth exists in the primary host against the *de novo* untransplanted tumor. However, a large number of studies suggest a small but significant increase in ease of tumor induction by chemicals or viruses in animals that have been immunocrippled by a variety of techniques. This result may imply that the immune mechanism is to some extent inhibiting tumor production in the intact normal animal. This postulated function of the immunemechanism against *de novo* tumor formation has been termed immunologic surveillance.

From what has already been said about the intrinsic lack of immunogenicity of spontaneous tumors, one would not expect any significant immunologic survellance in relation to these tumors, and indeed, all of the available data suggest that it does not exist. Lifetime immunodepression with antilymphocyte serum does not increase the incidence of spontaneous tumors in the mouse, nor do congenitally athymic (and thus immunologically incompetent) mice develop many spontaneous tumors even when these animals are enabled to live out a normal life-span by being kept in germ-free isolators. The only tumor type the congenitally athymic mice develop to any significant extent are lymphoreticular tumors, i.e., tumors of the very organ system that is defective. Immunocrippled humans develop similar lymphoreticular tumors rather than the excess of all tumor types that one would expect if the tumorigenesis were due to an absence of the hypothetic surveillance function. Thus, it can be concluded that immunologic surveillance, if it exists at all, is a weak and ineffective homeostatic mechanism perhaps demonstrable to a small degree with tumors of unusual immunogenicity, i.e., those tumors induced in the laboratory by potent chemical oncogens or high multiples of laboratory-selected oncogenic viruses. Immunity to an *in situ* tumor, even when that tumor is potentially

highly immunogenic, is very weak and ineffective compared with that induced by transplantation.

It should be emphasized that, although immunologic surveillance may be weak to the point of nonexistence, immunotherapy of cancer has much promise. Since most and perhaps all tumors may have TSTAs, it may be possible to activate the impotent surveillance mechanism to greater effectiveness. Some success has already been achieved in this direction by that nonspecific immunologic potentiator, BCG (Bacillus Calmette-Guérin). However, the complexity of the immune mechanism—blocking factors and the still to be discussed immunostimulation phenomenon make immunologic intervention hazardous; the potential for doing harm rather than good is very real.

Immunostimulation of tumor growth is a recently described phenomenon. Briefly, it appears that the early immune response may stimulate rather than inhibit tumor cell growth. Titration of the immune response, i.e., dilution of cytotoxic sera or reduction in numbers of reactive lymphoid cells may also result in stimulation of tumor growth. It thus appears that immunity, early in a tumor's evolution or whenever the reaction is very weak, may actually do the patient more harm than good. Whether the tumor stimulation is due merely to a quantitative effect or to different species of cells or antibodies is not yet known. It is obvious that, until this phenomenon is much better understood, manipulation of a patient's immune response will be a very uncertain venture.

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