Considerations on the Preneoplastic Lesions of the Mammary Gland

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The general characteristics of the preneoplastic lesions of the human mammary gland, as they are known through histologic description, are outlined, and data obtained from the experimental analysis of mammary gland preneoplasia in five areas of endeavor are discussed. Results obtained with transplantation procedures and aimed at defining the growth potential of hyperplastic outgrowths are reported. Information derived from the study of events able to induce benign hyperplastic outgrowths or their malignant transformation in the murine mammary gland is summarized. Attempts to predict neoplastic transformation in morphologically hyperplastic epithelium of human and rodent glands are discussed. The present status of efforts toward the prophylaxis of preneoplastic lesions of the mammary gland is described. Considerations of the relationship between preneoplasia and tumor dormancy conclude the presentation. (Am J Pathol 89:413–430, 1977)

A WORKING DEFINITION of a preneoplastic lesion of the mammary gland is that of a parenchymal alteration which increases the risk of a carcinoma. Conventionally, these alterations have been described in morphologic terms, since histology has furnished the tools to illustrate cell populations in continuous growth and with metastasizing capacity, the two major symptoms of neoplasia. Convincing evidence of the transition from hyperplastic or dysplastic epithelium to carcinoma cannot be obtained until and, probably, unless the conditions necessary to induce and maintain "malignant" growth are understood. Consequently, morphologic characterization and experimental attempts to define biologically the transition from normal to neoplastic epithelium represent two integral parts of our discussion.

Morphology

From the day-to-day postmortem practice of the Western Infirmary in Glasgow, Sandison [1] examined the breasts of 800 women over age 20. The organs were collected consecutively and were unselected; macroscopic analysis of abnormalities was carried out on repeated sections, and at least one large block of tissue was taken from each gland for histologic examination. This work is one of the most informative we have on the patho-

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physiologic history of the mature human mammary gland, although the sampling does not strictly represent the population at large. A summary of Sandison's data is reported in Table 1. One is struck by the frequency in the asymptomatic gland of proliferative lesions listed under the terms of *adenosis* and *epitheliosis*. In Sandison's terminology, adenosis refers to "a glandular hyperplasia which produces more and larger lobules of normal or exaggerated physiological pattern" and epitheliosis follows Dawson's definition [2] as "the multiplication of epithelial cells within existing gland structures without formation of new gland elements . . . a cellular filling of the lobule, not a branching, which indicates a more or less pathological response. . . ." Sandison's data epitomize the observations of most pathologists. Whether the hyperplastic condition involves branching of the ductal system or only cell proliferation, the key element under both circumstances is the distinction between hyperplasia evolving toward carcinomas and hyperplasia ending in cell degeneration and possibly cyst formation. Morphologists have collected a long list of changes that should anticipate neoplastic transformation:[3-7] enlargement of lobules which persist after the menopause; coalescence of adjacent intralobular ducts; thickening of the epithelial layer with intraluminal, papilla-like projections; loss of lumina by cell-filling; lessening of cohesiveness among epithelial cells; tendency of lobular epithelium to become columnar; formation of macrovilli on the cell surface; appearance of intracytoplasmic ductules; nuclear pleomorphism; increase in nuclear size; hyperchromasia; and small intranuclear vacuoles. Modifications of the stroma often coexist with the hyperplastic epithelium. They are usually classified as sclerosis and elastosis; both occur rather frequently and are also found in breasts without carcinoma (Table 1). The correlation between increased number of epithelial cells in ducts and lobules and sclerosis or elastosis of the stroma is poorly understood. However, the integrity of the basal membrane is generally accepted as an essential condition for distinguishing *in situ* from invasive proliferation. Although cases have been described where lymph node metastases occurred despite the integrity of a basal membrane, observed in serial sections, the event is exceptional.[3,8] Like carcinoma of the cervix, mammary carcinoma has a preinvasive phase in which neoplastic cells remain confined within the basement membrane of ducts and lobules.[9,10]

The pathologist has the responsibility of detecting the preinvasive phase of neoplastic proliferations and the major tool at his disposal is histologic analysis. However, the limitation of this approach has been pointed out, among others, by Geschickter [11] who stated that "histologic study made on human breast tissue cannot be cited as convincing evi-

Table 1—Hyperplastic and Dysplastic Lesions of the Human Mammary Gland*

	Percent of all cases†	Percent by decades				
		31–40	41–50	51–60	61–70	71–80
Atrophy Duct ectasia Cysts	48.0	17.4	37.8	52.1	57.3	57.6
Apocrine metaplasia	39.2	23.5	29.1	40.2	46.7	49.1
Adenosis	29.4	49.3	44.4	23.4	11.1	6.8
Sclerosing adenosis	7.0	6.1	5.7	7.2	7.2	9.7
Periductal elastosis	16.1	6.8	6.5	17.3	22.0	21.2
Epitheliosis	22.0	6.4	20.2	20.4	27.0	31.0
Papillomatosis	2.9	3.0	2.0	2.6	3.1	4.8
Fibroadenoma	0.5					

* From Sandison.[1]
† Total of 800 autopsy cases, ages 21–90.

dence of the transition of hyperplasia or involutional dysplasia into malignancy so long as morphologic criteria are lacking to distinguish between benign neoplasia and early malignancy." Thus the efforts to define preneoplasia in the mammary gland originated a good deal of experimental work.

Experimental Analysis of Preneoplasia

Transplantation of Hyperplastic Outgrowths

This approach was one of the first used to establish whether hyperplastic nodules of the mammary gland had growth potential comparable to that of neoplastic cell populations. Mice were used, since hyperplastic nodules had already been described in the mammary gland of this species at the beginning of this century.[12] A first basic observation revealed that transplantation of hyperplastic nodules into the gland-free mammary fat pad of isogenic mice produced mammary tumors sooner and in greater frequency than normal tissues transplanted into the contralateral mammary fat pad.[13,14] The study of the tumor-producing capabilities of hyperplastic nodules revealed that the transplanted tissue proliferates within the fat pad and fills it, partially or completely, but never invades beyond the fat pad limits. The invasion of the fat pad follows morphogenetic rules that are poorly understood. For instance, the newly formed ducts remain

at a distance of about 250 μ from each other and stop growing when they reach normal mammary parenchyma instead of invading it, as does the neoplastic tissue.[15] Normal mouse mammary parenchyma can also be serially transplanted into the fat pad of young syngeneic virgin or hormone-stimulated mice, but the life-span of the transplant is limited. In contrast, nodule outgrowths, like tumors, can be propagated for years, and their growth potential is practically unlimited.[16,17] Indeed, permanent lines have been developed.[18]

These transplantation experiments suggest that continuous growth is a property acquired by the tissue independently from its invasive capacity. The growth potential of nodular outgrowths is unlimited but only within the boundaries of the fat pad; only when a carcinoma develops is invasion of surrounding tissues observed. If this conclusion is valid and transferable to the human mammary gland, then some of the hyperplastic lesions found at biopsy already possess unlimited growth capacity, although they are still limited to the mammary area and are morphologically indistinguishable from others lacking this property.

Serial transplantation of hyperplastic outgrowths has also permitted a characterization of the cell population of these lesions. Using allophenic mice derived from the fusion of blastomers of C3H $\leftrightarrow$ C57BL zygotes, both alveolar and ductal cells were found to coexist in the outgrowths. The allophenic mice from these two strains have a high frequency of both mammary tumors and hyperplastic nodules. When these nodules were transplanted into C3H females, they produced outgrowths of alveolar structure; when transplanted into C57BL females, they produced ducts.[19,20] The coexistence of ductal, alveolar, and myoepithelial cells in the hyperplastic nodes persisted after years of transplantation.[21] The interplay among these cells for the existence and growth potential of the hyperplastic node is unknown. However, the survival of hyperplastic cells seems to be dependent on the presence of normal cells, although this dependency ceases when the hyperplastic epithelium becomes neoplastic.[22-24]

Tumorigenesis in Hyperplastic Outgrowths

By the development of outgrowth lines it was found that appearance of tumors, without any treatment of the host, was a stable characteristic of each established line. Within a stable environment, these properties could be transmitted by transplantation and maintained for generations.[25]

The increase of tumorigenesis in nodular outgrowth lines was obtained in a variety of ways (Table 2). In general, the presence of murine mammary tumor virus (MuMTV) induced a sharp increase in tumorigenesis,

Table 2—Tumor Induction in D-Lines (BALB/c Transplants)*

D1		D2	
Treatment	Percent	Treatment	Percent
No treatment	4	No treatment	45
Treatment +		Treatment +	
MTV	75	MTV	81
Pituitary	7	Pituitary	77
Irradiation	23	Irradiation	61
MTV + MCA	79	MTV + MCA	94
MTV + pituitary	71	MTV + pituitary	94
DMBA + pituitary	92		
Urethane + pituitary	81		
Irradiation + pituitary	9		

MTV = mammary tumor virus, MCA = 3-methylcholanthrene, DMBA = 7,12-dimethylbenz[α]anthracene.
* From Medina,[18] pp 34, 36, 38.

regardless of the frequency shown before infection. This, however, was not the rule. The D5 line of Medina maintained a 6 to 7% tumor-producing capacity regardless of MuMTV infection and despite the presence of B particles in the transplants.[26] The reasons for this exception are unknown; however, Squartini *et al.*[27] showed that superinfection with murine leukemia virus may reduce the oncogenic effect of MuMTV.

Chemical carcinogens such as 3-methylcholanthrene (MCA), 7,12-dimethylbenz[α]anthracene (DMBA), and urethane increased the tumor-producing capability of the hyperplastic outgrowths to a degree, dependent on the carcinogen, the host, and the outgrowth line.[18] Three observations in this area are important for our analysis of mammary preneoplastic lesions. In the urethane-treated or 7,12-dimethylbenz[α]anthracene-treated host bearing a D1 line, the tumorigenic effect was higher when the dose of carcinogen was increased. Moreover, outgrowths exposed to urethane between 3 to 5 weeks after transplantation produced tumors earlier and at a higher incidence than outgrowths exposed to urethane at later periods.[28] The effect of the carcinogen on the hyperplastic cells was also evident at the second transplant generation (Table 3). Of particular interest is the fact that pituitary action of the D1 line does not increase appreciably tumor incidence (Table 2), but the same treatment of second generation methylcholanthrene-treated cells increased tumor incidence dramatically (Table 3). This is a good example of "altered" responsiveness of a hyperplastic epithelium to hormonal influence.

The last observation concerns the interaction between fat pad and hyperplastic epithelium in tumorigenesis. Transplantation in untreated

Table 3—Tumor Incidence in Carcinogen-Treated Outgrowths Transplanted Into Untreated BALB/c Mice*

Treatment†	Generation 1 (%)	Generation 2 (%)
D1 + MCA	60	18†
D1 + DMBA	68	23
D1 + irradiation	23	0
D2 + MCA	82	75
D2 + irradiation	61	39

MCA = 3-methylcholanthrene, DMBA = 7,12-dimethylbenz[α]anthracene.
* From Medina,[18] p 40.
† D1 + MCA second generation + pituitary = 95%.

animals of carcinogen-treated D1 outgrowths produced a high number of tumors only when the whole fat pad containing the D1 implants was transplanted. Fragments of epithelium without fat pad did not take. The role of the fat pad under these circumstances is unknown.[29]

Irradiation (γ -rays) also induced an increase of neoplastic transformation in the D series of BALB/c outgrowth lines: from 4 to 23% in the D1 line and from 45 to 61% in the D2 line (Tables 2 and 3). However, a comprehensive analysis of the effect of radiation on hyperplastic outgrowths of mammary epithelium is not available despite the importance that it might have today in relation to the increase in the use of mammographic analysis in humans.

Under natural conditions the mammary tissue is indeed exposed to several carcinogens which may interact and produce additive or synergistic effects. Moreover, the hormonal milieu of the host during carcinogenic action plays a determining role in influencing tumorigenicity, and carcinogens per se can influence the hormonal milieu of the host.[30] The sophistication of the experiments analyzing this set of parameters in hyperplastic mammary outgrowths is far from satisfactory and is insufficient to permit generalizations.[18,31,32] On the whole, however, the data concerning the tumorigenic effects of several carcinogens, acting singly or in combination, on the hyperplastic outgrowths of mouse mammary gland, can sustain the generalization that this tissue is indeed at a higher risk of becoming neoplastically transformed by carcinogens more frequently and earlier than nonhyperplastic tissue.

Induction of Hyperplastic Outgrowths

The presence of hyperplastic outgrowths in the mammary parenchyma has been described not only in humans and rodents but also in dogs and nonhuman primates.[33,34] A major concern of experimentalists is to discover

ways of inducing these outgrowths as an approach to understanding their pathogenesis during the natural aging of the gland. Rodents were mostly used in these experiments. It was soon observed that the carcinogens capable of increasing the frequency of neoplastic transformation in naturally occurring outgrowths were also able to induce the hyperplastic outgrowths themselves. Strains of mice such as C3H and GR that carry MuMTV and have a high incidence of mammary tumors also show a large number of hyperplastic nodules in their mammary glands. Strains supposedly not infected by MuMTV have very few hyperplastic outgrowths; this number, however, is increased by a MuMTV infection via milk or an intraperitoneal injection of infected tissues.[35,36] Adenovirus type 9 was found able to induce, in rats, proliferative lesions defined as fibroadenomas.[37] The mechanism of MuMTV infectivity is poorly understood and, therefore, the role of the virus in the induction of preneoplastic lesions is unclear. There are indications suggesting that MuMTV infection goes through a cycle whereby Type B particles function primarily in the transmission of the viral genome from mother to young while a variant of MuMTV (B-MTV) should be the transforming component active after the transmission has occurred.[38] The kind of lesion that the cell-associated form of MuMTV produces is also not known. Morphologically, a hyperplastic outgrowth often appears as a pregnancy-related structure surviving in a nonpregnancy hormonal milieu. Whether viral infection alters the physiologic cycle of proliferation, secretion, and regression due to hormonal modulation of the mammary tissue remains an open question. Indeed, in the nodulogenic assay of Nandi,[35,36] MuMTV is revealed only in glands which are under strong pituitary stimulation.

New appearance of hyperplastic outgrowths was also obtained with a variety of chemical carcinogens; however, their induction was greatly facilitated by hormonal stimulation.[39,40] Strong hormonal stimulation appears to be an essential component of the outgrowth induction process. Genesis of the outgrowth is controlled by the ovaries and the pituitary gland. After the hyperplastic outgrowth is induced, its maintenance is supported by pituitary hormones.[41,42] Estrogen is not necessary for outgrowth maintenance, but its absence limits the alveolar proliferation. Nodules transplanted into an ovariectomized host show a prevalence of ductal tissue as compared to transplants in normal females.[25] The hormonal milieu not only influences the onset of a hyperplastic outgrowth but probably also controls its cellular constitution.

Modulation of tissue composition was also observed during MuMTV infection. MuMTV-positive and nodule-inducing virus (NIV)–positive C3H mammary glands show more lobuloalveolar development than synge-

neic MuMTV-free, NIV-positive C3Hf mice.[43,44] Exogenous hormone stimulation enhances formation of adenocarcinomas both in the presence and absence of MuMTV, and the most effective way of reaching this objective is transplantation of a pituitary gland under the kidney capsule.[45,46] Nodulogenesis is also enhanced by the mammotropins secreted by pituitary isografts, and the question arises whether hyperplastic outgrowths are an obligatory step in the morphogenesis of carcinomas. The problem has been extensively studied by Sinha and Dao [47-49] in the rat, and their answer is negative. In the mouse, much more frequently than in rats, one sees a carcinoma initiated within a hyperplastic outgrowth. The experts, however, concede that the interdependence is not obligatory.[50] In the human there is the well-proved observation that glands with carcinomas are usually richer in hyperplastic lesions with various degrees of morphologic alteration to make them atypical.[3,7,51,52] Neoplastic transformation of these lesions is also a nonobligatory event, although the correlation between morphologic appearance and risk of carcinoma is still a subjective assessment.

Prognostic Evaluation of Hyperplastic Outgrowths

A basic objective in any study of mammary preneoplasia is to predict which hyperplastic epithelium will become a carcinoma. In our laboratory, we utilized as a marker the angiogenic capacity of the tissue studied by the iris transplantation assay.[53,54] The initial hypothesis sustaining this approach was based on the observation that a fragment of tumor transplanted at the center of the cornea or the vitreous body survived for many days without producing a tumor mass. Cornea and vitreous are not vascularized, but when the transplant was placed close enough to the limbus or retina, a rich vascular network developed, the new formed vessels penetrated the transplant, and rapid growth of a solid tissue ensued.[53-59] The ability to stimulate new vessel formation was considered a property that a hyperplastic tissue might acquire on its way to becoming neoplastic. Thus, the questions to answer were whether the hyperplastic outgrowths did induce angiogenesis and whether a correlation existed between frequency of angiogenic response and frequency of carcinomas. The C3H/A^{vy} mouse was used as a model because of the high frequency of mammary tumors and hyperplastic outgrowths. The answer to both questions was positive (Table 4). About 30% of the hyperplastic nodules taken at random were able to induce angiogenesis. When the D1 and D2 lines of transplantable hyperplastic nodules were compared, the highest frequency of angiogenic response was found in the D2 line that produces carcinomas with the highest frequency (Table 4). When human hyper-

Table 4—Angiogenic Response of Tissue Transplants*

Mouse (C3H/A^{vy})			Human		
	Pos/Total	Percent		Pos/Total	Percent
Primary HAN	7/23	30	Hyperplastic lobules	14/50	28
HAN Outgrowths			Fibroadenomas	0/18	—
D1	19/59	32	Cystic fibrosis	0/96	—
D2	83/109	76			
Papillomas	305/309	100	Papillomas	6/6	100
Carcinomas	89/98	90	Carcinomas†	49/75	65

HAN = hyperplastic alveolar nodule.
* From Brem *et al.*[53,54] and Gimbrone and Gullino.[54]
† Six to eight fragments from eleven carcinomas; each case with a few positive fragments.

plastic lesions were tested, about 28% of them were found to produce angiogenesis, a result remarkably similar to the mouse model.[53,54]

The second question concerning frequency of carcinomas in glands rich in hyperplastic outgrowths able to induce angiogenesis has not been answered in the human. A systematic analysis by the iris assay of human hyperplastic outgrowths detected in biopsy material from cases with benign lesions should probably be rewarding for sorting out a high-risk population.

Prognostic assessment of hyperplastic outgrowths was also attempted by Jensen and Wellings [60] following the transplantation approach in the nude-athymic mouse. Human hyperplastic lobules with varying degrees of morphologic atypia were transplanted into the inguinal fat pad of males and females. After several weeks the morphology of the transplant was examined and the degrees of hyperplasia and architectural disorder were recorded. The greatest morphologic modifications were observed in normal-appearing lobules obtained from cancer-associated breasts of women over age 50.

The end-point evaluation of this assay is dedifferentiation, and it is less precise than the angiogenesis test where presence or absence of a halo of newly formed vessels around the transplant is observed. Both tests, however, point to changes in the biologic properties of hyperplastic lobules which make them closer to overt neoplasia than other lobules, despite identical morphology.

Analysis of nipple secretion has been focused mostly on a search for morphologically atypical cells.[61,62] It is possible that a broader evaluation of the biologic characteristics of both cells and fluid might open new approaches to the assessment of breast preneoplasia.

There is also a variety of biochemical observations that remain anecdotal since their prognostic potential has never been tested. For instance,

a) the lack of estradiol receptors in the rat hyperplastic nodules;[63] b) the capability of the D1 line transplanted into BALB/c mice to synthesize casein versus the inability of the D2 line under identical hormonal treatment;[64] c) the presence of glucose-6-phosphate dehydrogenase isoenzyme-1 in hyperplastic nodules and carcinomas but not in normal BALB/c mammary gland;[65,66] and d) the diversity of hybridizable nuclear RNA species with thermally denatured DNA in mammary tissue with morphologically changing patterns from normal to hyperplastic to neoplastic, suggesting a gradual alteration of gene expression as the lesion progresses.[67]

It seems evident that efforts in the area of prognostic assessment of hyperplastic outgrowths of the mammary gland should be intensified and that the search for biologic properties more than morphologic characteristics appears to offer better chances of identifying morphologically hyperplastic lesions on their way of becoming clinically neoplastic.

Prophylaxis of Hyperplastic Outgrowths

The best results in this area have been obtained by suppression of prolactin secretion using two ergot alkaloids, 2-bromo-α-ergocryptine (CB-154) and 6-methyl-8-β-ergoline-acetonitrile (MEA).[68-71] The effectiveness of CB-154 seems superior to MEA (Table 5), and the destruction of the mammary epithelium appears to be the mechanism of action for both compounds. Body weight gain and death rate (non-tumor-related) are not altered by the drugs; thus, medication seems feasible for long periods of time. Estrogen enhances nodulogenesis and also tumor incidence, as has been well documented, but prolactin suppression, while

Table 5—Mature Multiparous C3H/HeJ Females*

Treatment	Inguinal gland development at 19 mo†	Mean No. of HAN at 19 mo	Percent tumors
Control	4.1	15.9 ± 1.5	51
CB-154	1.8	10.0 ± 1.4	1.3
MEA	2.0	7.0 ± 1.0	33
Ovariectomy at 5 mons			
Estradiol‡	4.3	19.3 ± 2.0	46
Estradiol + CB-154	1.6	3.5 ± 0.6	32

CB-154 = 2-bromo-α-ergocryptine, MEA = 6-methyl-8-β-ergoline-acetonitrile, HAN = hyperplastic alveolar nodule.

* From Welsch,[71] p 2623. Eight months at start; daily subcutaneous injections at alternate months for 1 year.

† Wilcoxon rank procedure test.

‡ Estradiol-17β in drinking water for 1 year.

reducing nodulogenesis, was unable to substantially depress tumorigenesis in the ergot-estrogen–treated mice when treatment was started at adult age (Table 5). When young, nulliparous mice were treated with the ergot drugs, practically no mammary tumors were observed, but at the expense of a severe atrophy of mammary epithelium.[71]

Manipulation of the hormonal environment to control fertility has now gained acceptance in the human population. Intensification of efforts in keeping low levels of prolactin in women may produce appreciable results in counteracting mammary hyperdysplasias and, possibly, reducing the frequency of mammary carcinomas.

A phenylalanine-deficient diet has been utilized in attempts to reduce transformation of hyperplastic outgrowths into carcinomas.[72,73] These efforts were expanded by attempting to reduce the level of circulating phenylalanine by ammonia-lyase, an enzymatic preparation derived from *Ustilago hordei* or *Rhodotorula glutinis*.[74,75] The results obtained thus far can be explained in part as being due to the effects of the treatment on the hormonal milieu, atrophy of the ovaries in particular, and depression of food intake. No clear indication of a specific effect on the hyperplastic outgrowths has emerged.

Destruction of hyperplastic outgrowths has been attempted by immunologic means. There is no method that I know of to immunize against primary lesions *in situ*, probably because each of them expresses unique antigenicity. Therefore, outgrowths transplanted into the mammary fat pad are normally utilized for testing the host response. In one of these experiments, Slemmer [76] transplanted the hyperplastic tissue 8 to 12 weeks before immunizing the host by intradermal implantation with two outgrowth lines, one highly and one less antigenic. He showed that the premalignant lesions failed to immunize the host but remained highly antigenic and susceptible to immune destruction. This destruction was mediated by lymphocytic infiltration and was effective only when the original implant was small (about 1 mg). Carcinomas originating from one hyperplastic outgrowth tended to exhibit equal antigenic specificity; however, the carcinomas continued to grow in the immunized host more effectively than the hyperplastic outgrowth.[77]

The neonatal infection of the host by MuMTV modifies the immunologic resistance to growth of transplanted preneoplastic nodes and carcinomas. C3Hf mice free of biologically identifiable MuMTV accept grafts of a C3Hf nodular outgrowth but react strongly against implants of outgrowths derived from isogenic C3H donors infected by MuMTV. Immunogenicity of hyperplastic outgrowths in mice resides, at least partly, in antigens induced or possessed by MuMTV.[78-80] In fact, MuMTV

infection, either neonatal or later in life, depresses the humoral as well as the cell-associated immune response to unrelated antigens and the deficiency in response increases with age.[81] The immunogenicity of outgrowths induced by chemical carcinogens such as methylcholanthrene has a component related to the carcinogen per se, which confers to the tissue a distinct antigenic specificity.[76,82,83]

Preneoplasia Versus Tumor Dormancy

Histologic evidence that clinically normal persons carry tumor cells in a "dormant" state has been obtained in several instances, e.g., foci of neuroblastoma cells in the adrenals of infants,[84] unsuspected small carcinomas of the prostate,[85] and occult primary carcinomas in thyroids without clinical evidence of disease.[86] Whether histology can actually define the neoplastic potential of cell populations is debatable, but for practical purposes, histology is the only available tool for detecting "early" neoplasia. Accepting this premise, Ashley [87] observed that frequency of prostatic carcinoma, histologically detected in routine necropsies, rose with the cube of age, while clinically manifested carcinomas, as represented by death rates, rose to the seventh power of the age. If one accepts the hypothesis that neoplasia is the end result of a series of events determining a progression of tissue changes,[88,89] then the inference can be drawn that clinical carcinoma is the result of additional events which affected the cells after a first series of events had made them *preneoplastic.* Without the additional events, the cell population would have remained hyperplastic and without clinical manifestations of neoplastic transformation. A majority of researchers involved in the study of preneoplasia accept, more or less, this working hypothesis. However, there is another possibility.

Clinical cancer is defined by one symptom, unrestrained growth of cell populations, *in situ* or at a distance from the origin. In either case, death occurs because the host is or becomes unable to control the proliferating cells. This control may occur in different ways, two of which are known to me.

The erythroleukemia produced by Friend leukemia virus can be arrested by injections of statolon, an extract of *Penicillium stoloniferum.* The suppression of the disease in 90% of animals is due to the production of interferon and cytotoxic antibodies, in particular against virion p12. The infection, however, remains latent, as shown by the reappearance of leukemia either late in the life of treated mice or by the transfer of cells in normal mice.[90,91] Infection and leukemia are, in effect, dormant because the organism can keep them in check.

The second example of tumor dormancy is found in the lack of angio-

genic response, as reported in the previous section. If the host tissue fails to provide neovascularization, the neoplastic cell population does not form a clinically evident tumor. This has been shown by transplants in avascular organs.[92] Theoretically one can foresee that a tumor can be kept dormant if angiogenesis could be blocked. Indeed, it seems that cartilage, a tissue normally deprived of vessels, does have the capacity to block angiogenesis.[93-99] If this is correct, then the effectiveness of the basal membrane in containing proliferating cells within the boundaries of the lobule or duct might, in effect, depend on its antiangiogenic activity. The 28% of human hyperplastic lobules found, in the iris assay, already able to induce angiogenesis could in effect be dormant tumors not growing since their ability to induce new formation of vessels is counteracted.

In conclusion: The study of preneoplasia can be approached either as an analysis of the events necessary to transform hyperplastic into neoplastic cells or as an analysis of conditions necessary to keep neoplastic cells in a state of dormancy, without the clinical manifestations of a growing tumor. Regardless of the approach, however, one must remember that the focus of our efforts remains concentrated on a symptom—unrestrained growth of cell populations. Our colleagues of 200 years ago or more had infections as a major medical problem. They also focused their attention on a major symptom of these syndromes—fever. They described it carefully and found among other things "mental obnubilation and delirium" in the majority of their patients with fever and congestion of cerebral veins with edema of the meninges at autopsy. Many of them believed that the cause of fever was in the brain, and they bled their patients to relieve brain congestion.[100] Today we know better, but one has the impression sometimes that for neoplastic diseases we are in the same kind of bind as our colleagues of the past years were about fever in infectious diseases.

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