Mouse Mammary Tumors: Alteration of Incidence as Apparent Function of Stress

Abstract. *Eighty to 100 percent of female mice of the C3H/He strain carrying the Bittner oncogenic virus usually develop mammary tumors within 8 to 18 months after birth when studied under the usual housing and experimental conditions. By subjecting various groups of such mice to environmental circumstances providing different degrees of chronic stress, mammary tumor incidence at 400 days was modified, with incidences ranging from 92 percent under stress to 7 percent in a protected environment. The data suggest that moderate chronic or intermittent stress may predispose such mice to an increased risk of mammary carcinoma, possibly through a resultant compromise of their immunological competence or tumor surveillance system, and that adequate protection from physiological stress may reduce mammary tumor occurrence in mice.*

This report describes the influence of certain environmental stress factors that apparently contribute to a shortened latent period for mammary tumors in C3H/He mice (C3H mice) infected with the mammary tumor virus (MTV). Such stress-associated changes in tumor incidence reemphasize the complex etiology of specific neoplasms and the profound effects of subtle modulating factors in the malignant process (*1*).

New facilities designed to protect both the experimental animals and the research staff (*2*) have largely eliminated respiratory infections, infantile diarrhea, and miscellaneous diseases that frequently attack colonies of rodents maintained in conventional communal rooms on open racks (*3*). In addition, these facilities eliminate drafts, minimize thermal fluctuations, reduce noise, and provide protection from cross-contamination, aerosols, chemicals, and odors. Such facilities also eliminate the transfer of pheromones from shelf to shelf. Thus, adequate protective housing reduces fear, excitement, disease, and the chronic physiological stress experienced by animals maintained in the usual facilities.

In addition to changes in housing, stress has been further reduced by eliminating unnecessary handling or other physical disturbances of the animals. Studies by my colleagues and me (*4*) have demonstrated that even the mildest manipulation of mice leads to apprehension and stress, with measurable adverse physiological and biochemical consequences.

For example, the anxiety and fear induced in mice by shipping, hand capturing, bleeding, and routine handling by laboratory personnel produce typical stress alarm reactions that activate the adrenals, with plasma concentrations of corticosterone increasing from a normal of about 40 ng/ml to over 700 ng/ml (*4, 5*). These increases cause an involution of the thymus and a lymphocytopenia involving circulating thymus-dependent T cells (*4, 5*). Since T cells are a vital component of the immunological surveillance system, the physiological effects of stress lead to an impairment of the host defense system, and thus a presumed increase in susceptibility to cancer (*5*).

Mice used in the experiments reported here, and in a separate but relevant experiment (*6*), were initially taken from separate batches of C3H virgin females supplied by the Jackson Laboratory. Groups A, B, and C consisted of weanlings, obtained at age 4 to 8 weeks, nursed by their mothers and presumably bearing the mammary tumor virus. Animals were supplied in shipments of 50 to 100 uniform mice whose birthdays were the same plus or minus about 3 days. From each batch, animals of normal weight, 18 to 22 g, and normal appearance were selected and divided into experimental groups through systematic randomization. Group A-B mice were housed with males in standard stainless steel shoe box style cages and were subsequently categorized as group A (parous) or group B (nonparous) depending upon whether they became pregnant. Group C mice were housed without males in *plastic* shoe box style cages approximately 8 by 28 by 13 cm and held in the protective facilities described. Group D mice were supplied as weanlings that had been cesarean-delivered and foster-nursed. They were randomly assigned to various experimental groups and housed with or without males. Those housed with males were subsequently categorized as to parity. Since all D subgroups had similarly low tumor incidences, data on the individual subgroups are not given separately. Group D mice were housed in plastic cages under protective conditions similar to group C and were thus provided maximum protection from environmental stress.

Since either isolation or crowding can generate stress factors (*5*), four to six mice were housed in each cage. There is a technical conflict between the need to record the initial appearance of mammary tumors and the necessity to avoid stress induction in the mice through frequent capturing and handling. Where minimal stress was desired, the mice were examined visually during their weekly transfer to clean cages; even early tumors were generally detectable by this procedure.

The conventional tumor latent periods and mammary tumor incidence are shown in Fig. 1 for parous (group A) and non-

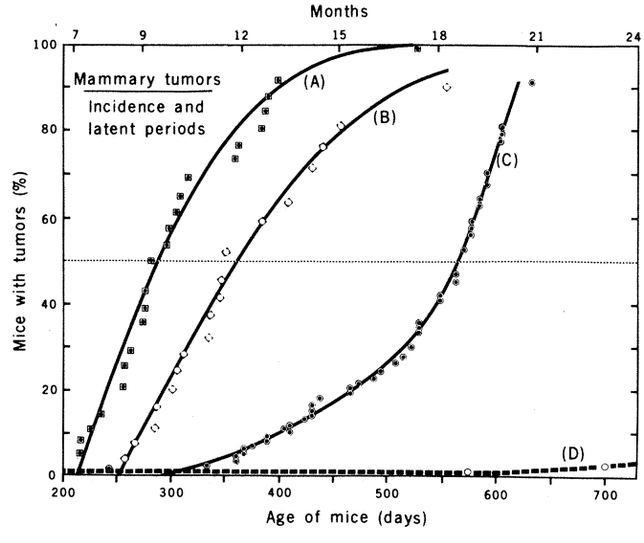

Fig. 1. Incidence and latent periods of mammary tumors in C3H female mice under various experimental and environmental conditions. Group A consisted of parous mice housed under conditions of chronic environmental and manipulative stress; group B, nonparous mice housed under the same conditions of chronic environmental and manipulative stress; group C, nonparous mice housed under protective conditions and subjected only to low or moderate environmental and manipulative stress; and group D, a combination of parous, nonparous, and virgin female C3H mice delivered by cesarean section and foster-nursed to deplete the milk-passaged MTV. However, gamete-transmitted viral genome or NIV is not eliminated by this procedure. Thus, mammary tumor production can occur under proper circumstances. This group was provided the maximum protection from environmental and manipulative stress.

Table 1. Experimental conditions of various groups of C3H/He female mice and the resulting mammary tumor latency and incidence. The median latent period is the time at which 50 percent of mice exhibited mammary tumors.

Group	Tumors at 400 days (%)	Median latent period (days)	Estimated stress	Housing	Handling	Breeding status	Virus status		Mice at 200 days
							MTV	NIV	
A	92	276	High (sustained)	Open racks	Weekly	Parous	+	+	36
B	60	358	High (sustained)	Open racks	Weekly	Nonparous	+	+	27
C	7	566	Moderate (intermittent)	Enclosed ventilated shelves	Weekly	Nonparous	+	+	117
D	0	>800	Low	Enclosed ventilated shelves	Almost none*	Parous, nonparous virgin†	-	+	100

*Mice were transferred to clean cages weekly. Unlike mice in groups A, B, and C, group D mice were not bled periodically. †Groups combined because tumor incidence was uniformly nil.

parous (group B) C3H mice. An additional group of nonparous virgin mice, never exposed to males (unlike group B animals) but maintained in the same animal room with groups A and B, exhibited essentially the same mammary tumor latent period and incidence as mice in group B (*6*). In contrast, extended latent periods were observed in group C, which was provided with the special environmental protection consisting of improved housing and maintenance that reduces exposure to chronic stress (*2, 4, 5*). Such environmental and other protective changes are presumed to have had a beneficial influence on the immunological competence of these mice by maintaining the integrity of their circulating T cell population, as well as a stable tissue T cell concentration (*5*). Group D were foster-nursed C3H females carrying the nodule-inducing virus (NIV) (*7*), but probably not the Bittner milk-transmitted MTV (*8*). These mice were provided maximum protection from chronic and intermittent environmental stress. Table 1 provides additional experimental details.

The median tumor latent periods were significantly different in each of the experimental groups. Tumors appeared earliest in group A, which consisted of females that were housed with males, had one or more litters, were handled weekly for tumor inspection, and were frequently bled by the orbital technique (*9*). Both groups A and B were housed in metal shoe box containers on open racks in a communal animal room that was subjected to the daily activities of cage cleaning, bleeding, and experimental manipulation of other mice in the same room. Recent studies show that mice in the vicinity of disturbed mice have elevated corticosterone values, presumably as a consequence of contagious anxiety (*10*). The mice in groups A and B were thus exposed to dust, odor, noise, pheromones, potentially infectious aerosols, and chronic anxiety. All are possible stress factors.

The median tumor latent period for group A was 276 days as compared with 358 days for the companion group B consisting of nonparous mice. In contrast, the nonparous group C had a median tumor latent period of 566 days, a significant extension over both groups A and B ($P < .001$). Unlike groups A and B, group C was housed in plastic cages held within the specially designed protective ventilated shelves (*2*). Although these facilities provided unique protection from general environmental stress factors, it was necessary to disturb the mice at periodic intervals since they were weighed, physically palpated for tumors, and bled occassionally by the orbital route (*9*), which thus subjected them to some moderate intermittent stress.

Group D consisted of mice that were cesarean-delivered and foster-nursed and thus probably do not carry the milk-transmitted mammary tumor virus. However, this procedure does not eliminate the gamete-transmitted viral genome (*8*), which, under favorable circumstances, expresses its presence by mammary tumor induction (*8*). Group D, which had the maximal protection from stress, had such a small tumor incidence that determination of a median latent period was not possible. At 21 months of age, only one mammary tumor was detected out of 75 surviving mice. This is a considerably lower tumor incidence than reported for foster-nursed mice in other laboratories (*8*). These mice thus constituted a special control group depleted of the milk-transmitted MTV but undoubtedly carrying the mammary tumor viral genome, and are thus potentially breast cancer candidates under circumstances that have not yet been adequately characterized.

Separate studies suggest that the social history and parity status, while influential, are not adequate to explain the striking delay in tumor appearance observed in the specially protected group C (*11, 12*). The lactate dehydrogenase elevating virus (LDH-virus), which can influence mammary tumor incidence (*1*), was not a factor in these studies.

Working hypotheses for further examination of this tumor suppression phenomenon include the following observations and analyses: The milk-transmitted oncogenic Bittner mammary tumor virus is present in C3H mice from birth as a lifelong infection, and presumably transforms normal mammary gland adenomatous cells to their malignant form at some unknown rate. However, as long as host immunological competence or other effective antitumor surveillance mechanisms are functioning, the transformed malignant cells are recognized and destroyed before they are capable of reaching a multicellular focus that is beyond the capabilities of the relatively weak immunological or other forces of the surveillance system. Under such continuing favorable defense conditions, a tumor-free state is maintained in the host.

However, under stress, the Selye alarm reaction sequence occurs (*5*). This involves the pituitary, which in turn activates the adrenals through adrenocorticotrophic hormone, producing an increased concentration of circulating adrenal corticosteroids. In the mouse this is corticosterone (*4*). Thus, one of the measurable biochemical alterations following stressful stimuli is plasma corticosterone elevation. Such hormone increases occur within 5 minutes, and are followed by a steady decrease in circulating T cell lymphocytes, and a somewhat slower involution of the thymus (*4*). Other important physiological effects undoubtedly occur; however, the measurement of this rapid hormone elevation and a T cell lymphocytopenia provides a simple dual index of the complex stress syndrome. The data reported here indicate that at 400 days of age, the usual spontaneous mammary tumor incidence in C3H mice was approximately 90 percent in parous females and 60 percent in nonparous and virgin females. This was reduced to less than 10 percent in the specially protected virgin females.

Although these data are consistent with the stress hypothesis, a number of alternate possibilities have been considered.

There is no evidence to support genetic drift or alteration as the factor responsible for the dramatic decrease in tumor incidence. The question of possible loss of Bittner mammary tumor virus was studied in group C mice. Both blood and mammary tumor extracts from these mice were active in producing hyperplastic alveolar nodules; this activity is indicative of MTV presence (*7*). This evidence, together with ultimate mammary tumor production, indicated that MTV was present as a persisting infection in these animals. Such assays may not answer conclusively, however, the more quantitative question of MTV attenuation. In respect to the possibility of gaining a new passenger virus that might repress mammary tumor development, serological tests for the spectrum of known murine viruses did not reveal any provocative contamination in these C3H mice (*13*); however, these tests are not conclusive evidence for the absence of latent infections, particularly those of more common strains of mouse encephalomyelitis viruses (*14*).

Additional possibilities for explaining the effect of stress on tumor induction include the enhanced cellular release or synthesis of MTV. Injected cortisol increases the concentration of intracytoplasmic A and extracellular B particles (MTV) in mouse mammary tumors (*15*). A variety of corticosteroids have been reported to stimulate MTV synthesis in cell cultures (*16, 17*). Moore (*17*) has suggested that sociological stress in women may be a factor in the etiology of breast cancer in view of the finding that corticosteroids can increase MTV production in mice and in cell culture. Such in vivo data are consistent with the hypothesis associating the adverse effects of stress with impairment of immunological surveillance and the consequential escape of transformed cells; however, the in vitro observations suggest that the corticoid influences may cause an increase in mammary tumor virus production as well as an impairment in the immunological control of transformed cells.

If physiological stress is assumed to be an auxiliary potentiating factor in the early appearance and high mammary tumor incidence of groups A and B, several mechanisms of action are possible. For example, does the intact surveillance defense mechanism act against the transformed malignant cell, against the oncogenic virus, against the neoplastic transformation process, or against some combination of these? The impaired surveillance hypothesis provides a simplistic but logical rationale to explain the multiple observations if it is assumed that viral transformation of normal cells occurs regularly but is continuously negated as long as there is a constant and effective host surveillance. When immunological competence is compromised, even temporarily, by loss or inactivation of T cells or other vital defense elements following stress-induced corticoid hormone elevation, the host surveillance fails to destroy the transformed malignant cells during their immunologically vulnerable stage. The data further imply that once a cancer cell escapes to an organizational state beyond the limited defensive abilities of immunological surveillance, the production of a lethal tumor may then be inevitable and not reversible by natural host defenses.

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6. Another group, not a part of this report, was maintained under the same conditions employed for groups A and B but without exposure to males. These virgin animals were not initially identical to the other groups of this report; however, they are referred to because of a tumor incidence and latent period (370 days) similar to that observed in the nonparous subgroup B. This similarity may be interpreted to mean that any initial differences in breeding stock and experimental differences due to exposure to males and failure to become pregnant as in the case of group B coincidentally negated one another so as to result in similar tumor incidences. Alternatively, it may be interpreted, as we have done, that none of these factors had a major bearing on the observed tumor incidence.
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Cyclic AMP and Cyclic GMP: Mediators of the Mechanical Effects on Bone Remodeling

Abstract. *Compressive forces of physiological magnitude (60 grams per square centimeter) reduce the adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate content of the epiphyses of tibiae from 16-day-old chick embryos. An equivalent hydrostatic pressure applied directly to cells isolated from this tissue also affects cyclic nucleotide accumulation. The tissue response is uniform throughout the epiphysis, whereas the cell response varies according to the area of origin.*

Living bone responds to mechanical forces by "adaptive" changes in internal architecture (*1*). The mode of conveyance of the mechanical signal to the bone remodeling process is still unknown. In this study we present evidence for the involvement of adenosine 3',5'-monophosphate (cyclic AMP) and guanosine 3',5'-monophosphate (cyclic GMP) in the response of chick-embryo long bone to compressive forces. There were several reasons for considering the cyclic nucleotides as potential messengers in the transduction of the physical stimuli into biochemical signals. Hormones that affect bone remodeling—parathyroid hormone and thyrocalcitonin—modulate the cyclic AMP level in bone cells through inter-