# THE MAMMARY TUMOR AGENT AND ITS IMPLICATION IN CANCER RESEARCH*

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With the recognition of the honor of presenting this lecture is mingled the pleasure of having known and admired Dr. Allen, a man of broad interests, who had the gift of stimulating younger men with his enthusiasm. On first meeting him, I was struck by his friendliness, the ease with which he could be approached, and his generosity. This last is typified by the amount of time he gave me in helpful suggestions on the occasion of my first appearance on a program with many of the well-known men in clinical and laboratory cancer work. In addition, it is an added pleasure to have the opportunity of discussing with you the problem of cancer, in which he was keenly interested and to which he made a fundamental contribution. With this background, it would be relatively easy to speak on almost any phase of cancer; but the subject was chosen because it may be used to imbue these lectures with the individuality of Dr. Allen, for his interests were not confined to a particular field of science or to one aspect of the cancer problem. Therefore, it seems fitting to examine the problem of mammary carcinoma in mice and the mammary-tumor agent as they relate to the broader side of cancer.

Cancer of the mammary gland in mice has been studied more intensively than has any other tumor of experimental animals. It attained this distinction because it is located externally, is easy to detect, and occurs with exceptional frequency in a common and cheaply maintained laboratory animal. Study of this tumor has pioneered many advances in experimental cancer. It was one of the first to be established as a malignant growth of lower animals at a time when neoplasia was considered an exclusively human disease. It was used to show the importance of hereditary factors in tumor growth long before the production of inbred strains; and when geneticists first established inbred strains of mice in cancer research, susceptibility to this tumor was used as the basis of selection. It was the first tumor used to reveal the relationship between hormonal

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stimulation and the occurrence of cancer in an experimental animal and it was employed extensively in the earlier work on nutrition in cancer.

It taught the importance of long-term projects in cancer research. This came about fortuitously enough, for, as has been mentioned previously, the tumor of the mammary gland was the tumor most generally used to attack any phase of the problem. To begin with, investigators in the Imperial Cancer Research Fund Laboratories and others devoted considerable time to prove that breast tumors in mice, as well as other tumors, were malignant growths and were similar to malignancies arising in man. Here was laid a firm foundation upon which we continue to build. Then geneticists who had used the mouse as a convenient animal for much experimental genetic work developed inbred strains especially selected for high and low tumor incidence. Much of this was done by workers who are or have been connected with the Roscoe B. Jackson Memorial Laboratory. Such work continues there and at widely scattered places. Discovery of the milk agent[5] in mammary cancer of mice and the development of a high gastric-cancer strain of mice[15] are two of the outstanding achievements emerging from this trend of work.

Again, a third trend, developing concurrently and contributing to the foundation of today's knowledge and accomplishments, was the study of the relationship of hormones to mammary cancer in mice, begun by investigators at the Washington University School of Medicine, from which have stemmed fundamental observations for many years. Among these was the demonstration by Allen[1] of the existence of an active ovarian hormone, which, followed by his work on the biologic activities of the hormone, led to his interest in the cancer problem.

Hence, we see that the discovery of the mammary-tumor agent was preceded by years of thoughtful effort on the part of many investigators working in different fields and interested in different phases perhaps, but all concentrated on the study of one type of neoplasm.

The discovery that mother's milk contains an agent responsible for the occurrence of mammary cancer in mice was one of the most important contributions to cancer research. How was this discovery made? As early as 1918 it was noted[11] that the incidence of mammary tumors in hybrid mice was more often dependent upon the tumor rate of their mothers than upon that of their fathers; but this

observation could not be interpreted with confidence until the establishment of inbred strains of mice capable of satisfying the requirements of controlled genetic experiments. At least 20 generations of brother-to-sister matings are essential before a mouse strain is considered homozygous, and careful selection by geneticists produced some strains in which virtually all breeding females developed breast cancer and other strains in which the incidence was less than 1 per cent. Reciprocal breeding between such inbred strains[14] revealed that females born to the high-tumor-strain mothers developed mammary cancer but those born to low-tumor-strain mothers remained relatively free of such tumors. This phenomenon could not be explained by any known genetic theory and was therefore thought to be due to nongenetic influences. It was suggested[10] that this influence could be transmitted (1) by way of the cyptoplasm of the ovum, (2) during intra-uterine life, and (3) by way of the mother's milk.

By foster nursing females from high- and low-tumor strains, it was soon found that mother's milk was responsible. Mice born to a high-tumor-strain mother and suckled by a low-tumor-strain female did not develop mammary cancer, whereas those born to a low-tumor-strain mother and suckled by a high-tumor-strain mouse developed this type of tumor. Many similar experiments in different parts of the world have proved that mother's milk contains an agent which is essential for the production of most breast tumors in the inbred mouse. Investigations leading up to and revealing the presence of the agent were carried out by geneticists, who called it the extrachromosomal influence. It is also referred to as the mammary-tumor inciter, the mammary-tumor influence or agent, the milk influence, the milk agent, the milk factor, and the mouse mammary-tumor virus.

This lecture will not be devoted to a detailed description of the experiments performed to characterize the agent because this has been done at frequent intervals[4, 8] A few of the more interesting properties of the agent will be mentioned, and then we shall proceed to some of its implications in cancer research.

The agent is filterable through Seitz or Berkefeld filters. It can be sedimented by ultracentrifugation, remains active for at least 6 months after lyophilization, does not survive glycerolation for an appreciable length of time, survives and may even become more concentrated in the yolk sac of chicken eggs, survives desiccation at room temperatures, and is not in the ether-soluble fraction of milk or mammary tissue. It is destroyed in mouse milk heated to 61° C.

for 30 minutes and in tissue extracts heated to 56° for 30 minutes, which indicates that the agent is destroyed by the heat of pasteurization. When injected into rabbits, it elicits neutralizing antibodies which are active in vitro and in vivo.

The appearance of any tumor is probably the result of the interaction of a number of influences, and breast cancers in mice are no exception, for at least three factors are important in their production. These are usually referred to as the genetic influence, the hormonal influence, and the milk influence, but other environmental influences are also involved.

The genetic influence determines the susceptibility of the mammary tissue to the mammary tumor agent as well as the ability of the mouse to propagate or transmit the agent. It may also control the susceptibility of the mammary tissue to hormonal stimulation and may modify the mechanism of hormonal stimuli.

Hormones control the development of the breast and thus provide a tissue or substrate for the action of the agent and other influences, for mammary tumors do not arise in mice in the absence of estrogenic activity. It is possible that hormones exert some effect upon the propagation of the agent.

These influences, genetic and hormonal, are mentioned to emphasize that the mammary tumor agent should not be regarded as the cause of breast cancer in mice. All three factors are important, and in certain instances it is impossible to state which is the most important. For example, in one inbred mouse strain only 5 per cent of virgin females develop mammary tumors while over 90 per cent of breeding females do so. Since all are exposed to the same agent, it is clear that their striking differences in tumor rates must be due to breeding and in all probability to hormonal stimuli. Again, the agent disappears or becomes ineffective after a few generations of appropriate matings of inbred mouse strains.[13] Here the genetic influence predominates over the agent. Environmental factors, such as underfeeding and overcrowding, also affect the genesis of mammary cancer in mice. The mammary-tumor agent is no exception to other extrinsic disease-producing agents, for its effects are modified by genetic and environmental factors.

This brings us to the first implication derived from the study of this agent. In the past, the discovery that a transmissible agent was responsible for the production of a given malignant tumor usually occurred shortly after the tumor was transplanted into susceptible animals, and it precipitated considerable discussion whether the

growths were or were not true tumors. However, mammary cancers in mice were established as malignant growths long before the agent was found. They grow progressively, invade surrounding tissues, recur after incomplete removal, metastasize, and are transplantable. A filterable agent is involved in the causation of this tumor. If this view is not accepted, then the definition of a malignant growth must be altered or it must be admitted that such growths cease to be malignant if an extrinsic agent is involved in their origin.

Another implication is derived from the fact that a filterable, disease-producing agent is transferred in mother's milk. Are other diseases of unknown etiology transmitted in this manner? Before discovery of the agent, mammary cancer in the mouse was thought to be controlled by genetic and hormonal influences. In fact, the experiments which led to its discovery[14] were designed to evaluate the genetic factors. We do not know whether similar agents are responsible for breast cancers in other species, and thus far the evidence indicates that the mammary-tumor agent is not involved in the genesis of other tumors of mice. Further, mouse milk is not known to contain any other tumor-inciting agent; but there are experimental results[12] indicating that mouse milk exerts some control over the transmission of susceptibility to certain transplanted tumors, and this influence is not the mammary-tumor agent. In the search for other milk influences, investigators should not be prejudiced by the knowledge that the mammary-tumor agent is present in mouse milk during the entire period of lactation and is even widely distributed throughout the body of the animal. It may be unwise to assume that this is true if other agents are conveyed in the milk of the mouse or other species. The agent opens a new approach to the study of parental influences in the transmission of disease, for the possibility that chronic diseases may be the results of the interaction of agents carried in mother's milk and environmental factors offers a field for investigation.

The latency of the agent presents other implications in the study of cancer and other diseases. Despite the appearance of tumors long after the administration of the agent, it could be assumed that the agent infects the mammary tissue soon after it enters the body of the mouse but that the appearance of a tumor must await the action of hormonal or environmental stimuli. When thus considered, the agent is comparable to the causative agent of tuberculosis, which can maintain a focus of infection capable of producing the clinically recognizable disease if environmental factors are favorable. Up to

the present time, no immediate changes in the organism of the mouse have been described following introduction of the agent, but there is some evidence that the architecture of the mammary gland is altered. It could also be assumed that the agent increases slowly and induces tumors when a critical amount is present.

If we define latency as the period elapsing between administration of the agent and the appearance of a tumor, then the agent has an extremely long latent period. A susceptible newborn mouse need remain with its mother for only a few hours after birth, and the small amount of milk ingested during that time is sufficient to permit a tumor to develop in middle or later life. When as little as 0.1 cc. of milk is injected into young mice subcutaneously or intraperitoneally or fed by means of a stomach tube, virtually all will develop tumors months later, a fact that indicates that this amount of milk is far above the smallest dose necessary. It is also known that tumor development is not essential for transmission of the agent from mother to offspring, in fact the agent is seldom transferred by way of the milk in the presence of recognized tumors. In certain high-tumor strains of mice if every mother were killed after she had weaned her first litter, the agent could be carried from generation to generation without any manifestation of disease. Mammary cancer in the mouse may be looked upon as an excellent example of a latent virus infection.

What can be inferred from the latency of the agent? First, it is obvious that an event takes place in the first few hours of life which leads to the appearance of cancer months later. This suggests the possibility that other diseases, especially the chronic diseases, may be caused by transmissible agents and have their roots far in the past. Second, an epidemiologist who attempts to evaluate a mammary-tumor agent in the milk of other species must know that only a small amount of milk in early life is necessary to produce a tumor in mice. Ingestion of milk suspected of containing an agent must be ruled out completely. An epidemiologist must also realize that the agent survives and is transmitted in the absence of recognized neoplasia, so this could also be true for other diseases. The detection of agents comparable to the mammary-tumor agent may demand the application of experimental epidemiologic techniques.

Perhaps the most interesting implication drawn from the latency of the agent is the realization of the danger of attempting to draw lines of distinction between tumors on the basis of their etiologic

agents. Filtration and ultracentrifugation experiments show that the agent can be transmitted by cell-free material, and its transference through many generations of mice implies propagation. Now there are other tumors of experimental animals, which are also caused by filterable agents and are often called virus-induced tumors. The tendency has been to place these tumors in a special category by describing certain common properties, among which is the short incubation period between application of the agents and the occurrence of tumors. Indeed, it has been emphasized that this short latent period is one of the outstanding differences between these growths and those induced with chemical carcinogens. While the mammary-tumor agent possesses certain properties in common with the agents of these virus-induced tumors, such as filterability and resistance to heat, as well as tissue and species specificity, it differs remarkably in its relatively long latent period, and in fact resembles the chemical carcinogens. In other words, the agent exhibits some properties in common with the agents of virus-induced tumors and some in common with the carcinogenic chemicals. It is a sort of hybrid. Should we attempt to place cancers in separate groups according to their etiology, or should we treat each tumor as a separate disease? Certainly the study of mammary cancers in mice teaches us to forego generalizations, to consider each type of tumor as a disease entity, and above all to keep an open mind regarding etiology.

The soundness of this approach is shown by the results achieved in the control of breast cancer in the mouse. Appreciation of the importance of hereditary factors led to the virtual elimination of the disease in some mice by the process of inbreeding and selection. In the highly susceptible inbred strains control was another story. Here the disease was prevented by ovariectomy; but this method was impractical because the ovaries had to be removed before the animals reached the age of reproduction, and control of the disease by this procedure would lead to the disappearance of the strain. Nevertheless, the individual could be spared by acknowledging the importance of estrogenic stimulation. Finally, recognition of the mammary-tumor agent as the etiologic agent of this disease contributed to its prevention in the high-tumor strains by the simple expedient of eliminating the agent from the milk. This was accomplished by appropriate foster nursing of mice from susceptible strains before they ingested any of their mother's milk. Breast cancer has not occurred in many generations of descendants of these mice, although they still retain their genetic susceptibility to the agent.

The implications here are twofold: (1) Although discovery of the agent added to the complexity of the problem of breast cancers in mice, it also gave us a method of prevention; the more we learned of this disease, the better became our opportunities for control. (2) The prevention of a tumor was accomplished without the acquisition of knowledge concerning the fundamental changes involved when cells acquire malignant properties. This knowledge may be necessary to prevent other cancers, but here is one tumor in a single species where it was not essential. This may be a ray of hope to those who hold that the prevention and cure of cancer may not require a complete understanding of the differences between the normal and the malignant cell.

Other properties of the agent could be used to present other implications which it has in cancer research and other diseases, but it may be of more interest to leave these and turn to some of the contributions that we may expect from further studies of the agent.

One problem of much interest to cancer investigators is whether a transmissible agent, such as the mammary-tumor agent, is essential for the propagation of malignant cells or whether after initiating a chain of events it can disappear or become inactive while the tumor cells continue to multiply.

Those who suggest a virus theory of the origin of cancer maintain that inability to detect a virus by known techniques does not prove that one is not involved, and in support of this assertion they can point to the study of breast cancer in the mouse. We know now that the agent is present in tumors of high-cancer-strain mice and that it can be detected by the injection of filtrates of these tumors into susceptible mice. The techniques employed are similar to those used to reveal the presence of agents in the virus-induced tumors except the possibility that young animals should receive the filtrates from the mouse cancers. But the mouse mammary-tumor agent was not discovered by the administration of tumor filtrates. One reason for the failure of this kind of experiment was probably caused by too much dependence upon experience with the known virus-induced tumors, for the inoculation of a filtrate prepared from one of them usually produces a tumor at the site of administration in a short period of time. This is not the case with the mammary-tumor agent. While the virus-induced tumors usually contain the virus, is it not possible that we may again be too dependent upon previous experience if we assume that the mammary-tumor agent must be present for the propagation of malignant cells? While there is no proof

that malignant cells fail to multiply in the absence of tumor-producing viruses, and although it may not be possible to separate the virus from the cells in other tumors, should we insist that this is true for all tumors caused by transmissible agents?

The mammary-tumor agent presents a new approach to the problem of the relationship of the etiologic agent to the propagation of tumor cells. This is based upon the varying degrees of susceptibility to the agent exhibited by different inbred strains of mice. Thus far the agent has been found in the milk of all high-tumor strains of inbred mice and in none of the well-known low-tumor strains. When the agent was introduced by foster nursing into one low-tumor strain, it transformed the strain into a high-tumor strain and the strain has remained high-tumor for many generations; but when introduced into another low-tumor strain, the agent disappeared from the milk or became inactive after one generation of inbreeding.[2] Yet the agent will evoke tumors in the latter resistant strain if appropriate techniques are used. One experimental approach would, therefore, consist of the transplantation of an agent-induced tumor into other individuals of the resistant strain. If the presence of the agent is necessary for the propagation of tumor cells, the tumor may not survive many passages. If the tumor undergoes a series of transplant generations, it can then be tested for the presence of the agent. Should this test fail to detect the agent, the results would suggest that the agent had disappeared from the tumor and the cells had continued to multiply. Such findings would certainly stimulate discussion and further investigations.

In case this sounds too hypothetical, it is important to note that the literature contains experimental observations suggesting the disappearance of the agent from transplanted mammary carcinomas of mice[4] and, more important, statements to the effect that the agent disappeared from the milk or became inactive[6] in subsequent generations of a high-tumor strain of mice. Also, it has been shown[16] that growths produced in plants following inoculation of an organism will continue after removal of the inciting agent.

There is another problem in which the mammary-tumor agent may be used advantageously, namely, the problem concerning activation of a latent infection and the origin of viruses. Recently mammary cancers were induced in some inbred mice and their hybrids by subcutaneous injection or skin painting of a chemical carcinogen, and some of these animals were known to be free of the mammary-

tumor agent. Strangely enough, there is no record that any of these tumors were tested for the presence of the agent although the results of such tests would be of considerable interest. Detection of the agent in these tumors would suggest that it was present in the animals as a latent infection or was produced in the animal. If the test failed to reveal the presence of the agent, then the tumors may have a different etiology than those in which the agent is involved.

There is no reason to assume that all spontaneous mammary tumors in mice are caused by the agent. It is true that all the present high-tumor strains possess the agent, but these strains were established before the agent was found. The production of the inbred strains demanded brother-to-sister matings and selection toward or away from tumor development, and in all probability the selection resulting in the present high- or low-tumor strains was toward susceptibility to or resistance to the agent. As stated previously, the establishment of these strains was even necessary before the existence of the agent could be proved. It is of interest to speculate that the geneticists not only selected toward susceptibility to the agent but also may have selected toward a genetic constitution that permitted the activation of a latent infection. This conjecture is open to experimentation, for if mice develop spontaneous breast cancers that are etiologically distinct from those caused by the agent, then it should be possible to produce a high-breast-tumor strain that is free of the agent.

There are some experimental observations reported which indicate that the milk agent may be endogenous. One investigator[7] freed high-tumor females of the agent and found that their descendants for seven generations did not develop tumor; but one mouse of the eighth generation became cancerous, and her descendants for four generations gave a high incidence of breast tumors. Others[9] transferred fertilized ova from high-tumor-strain females into the uteri of low-tumor-strain females; and when the mice from the transferred ova were born, they were suckled by the low-tumor-strain females. None of these mice developed tumor, but in the first three generations of their descendants the incidence of breast cancer was 11 per cent. A test for the presence of the agent in any of these tumor-bearing mice was not recorded; but if the agent was responsible for the tumors, it is important to ascertain how it entered the animals. Since the agent is an extrinsic disease-producing one, it is conceivable that it may be transmitted by routes other than the

mother's milk, but there is little evidence to support this idea. Mice with or without the agent are kept in close contact in many laboratories, and no evidence of contagion has been recorded. Furthermore, when newborn mice were kept with their high-tumor-strain mothers but were not permitted to obtain any of their mother's milk, they did not develop tumor.

Another approach to the problem of the endogenous origin of the agent has been opened recently. Investigators in several laboratories found that when the offspring of certain matings between low-tumor-strain females and high-tumor-strain males were subjected to intensive hormonal stimulation they revealed a high incidence of breast tumors. In one experiment,[3] the milk of the low-tumor-strain females was tested for the agent by permitting them to suckle highly susceptible mice. This test was essentially negative. Tumors arising in the hybrids were also examined for the agent, and these tests also proved negative. These results suggest that the agent was not implicated in the origin of the tumors found in the hybrids.

These hybrids may be used in attempts to ascertain whether the agent occurs endogenously in them, whether it is in the latent or inactive state in their mothers and is activated in the hybrids, or whether the agent is not necessary for the tumors in the hybrids. One method of attack could be as follows: The hybrids are bred before and after they develop tumors, and their offspring are kept to see whether they develop breast cancers. Should tumors appear in those offspring born before and after the hybrids become cancerous, it would suggest that the hybrids obtained the agent in early life. This would indicate that the low-tumor-strain females harbored the agent without developing tumors or became infected through contact with the high-tumor-strain male. Should tumors occur only in those offspring born after the hybrids developed tumors, it would indicate that the agent was produced in the tissues of the hybrids or in their tumors. Should offspring or an occasional one grow a tumor, it would suggest that the tumors appearing in the hybrids were etiologically distinct from those induced with the agent.

There are aspects of this kind of experiment which need not be considered now. It is hoped that this brief outline demonstrates how studies with the agent can be used in attempts to throw some light on problems of general interest to those working with infectious agents.

Viewed as an infectious agent, the mammary-tumor agent has the unique characteristics of a long period of incubation and the ease with which it is detectable. In some respects, it is comparable to the virus of herpes simplex in man. Both agents incite diseases that usually arise spontaneously and are influenced by environmental factors. Both diseases are relatively easy to transmit by reinoculation of the individual in which they appear but difficult to transmit to other individuals of the same species which possess a different genetic constitution. However, the mammary-tumor agent does not possess any characteristic distinctive from all other known infectious agents.

It is not the purpose of this discussion to imply that all tumors are caused by agents similar to the mouse mammary-tumor agent. The discovery of this agent has, however, given added impetus to a theory of cancer origin which only a few years ago had few friends in cancer research. Now, there is hardly an investigator who will not listen with interest. Finally, the study of the agent should instill enthusiasm and teach the value of an open-minded approach to the cancer problem. Since such enthusiasm and open-mindedness were characteristic qualities of Dr. Allen, the subject chosen seems appropriate for the first Edgar Allen Memorial Lecture.

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