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Effect of lactation on the incidence of carcinoma of breast

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THE EFFECT OF LACTATION ON THE INCIDENCE OF CARCINOMA OF THE BREAST

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INTRODUCTION

The Problem of Carcinoma of the Breast

"Cancer" is a word that commands the respect of physicians and the lay population. The remarkable ability of a malignant tumor to extend its claws throughout the body has caused it to be likened to the nature of the crab. Carcinoma is the commonest of all cancers, and it is characterized by its epithelial genesis. It attacks almost all the organs of the body, and in the breast it is the most common type of malignancy occuring in females (1). It has been said that 37 out of every 1000 women who reach maturity will become victims of the disease and that in each year more than 15,000 women in the United States die as a direct result of carcinoma of the breast (2). It is also postulated that there are between 50 and 60,000 women with the disease at this time (3). The condition is rare under 20 years of age and reaches its maximum incidence in the fifth decade (4).

Introduction, Limitation, and Purpose of Paper

In considering a condition so prevalent as this disease and one whose incidence constitutes 25% of

all cancers (5), whose literature is so voluminous and repetitious, a person would be amiss to select more than one factor that relates to the incidence of mammary carcinoma for a single paper. There is much divergency of opinions concerning the association of lactation with breast carcinoma. The effect on the incidence of carcinoma in the maternal breast and the possible transmission of mammary carcinoma from mothers to suckling offsprings, by the so-called milk factor, are both disputed. The aim of this paper will be an attempt to collaborate the statistical and experimental research concerned with the effects of lactation on the incidence of carcinoma of the breast in the mother and her female progeny. No attempt will be made to evaluate the hormonal influence, individual susceptibilities and resistance, or hereditary influences except where they have to be ruled out.

Brief History of Carcinoma of the Breast

Cancer is a disease that is older than present man, older than prehistoric man and even older than the age of mammals. Tumors in the fossil bones of extinct animals were first recognized among the pleistocene mammals, especially the cave mammals of Europe

(6). However, in the Mesozoic era, or the age of reptiles (6-12 million years ago), the bones of the dinosaurs and other reptiles show lesions resembling the modern forms of osteoma, osteosarcoma, and hemangioma of bone (6,7). The evidences of bone cancer increase with each succeeding geological period through the Cenozoic and Holozoic eras until, in the Egyptian, benign and malignant tumors of the soft parts are discovered (6).

Historical records tell us that carcinoma of the breast caused suffering and death more than 2,000 years ago, and it was described with some detail in an inscription from Ninevah (800 B.C.). Democedes (520 B.C.), an accomplished Greek surgeon, describes the cure of Otossa, the daughter of Darius Hystospsis, of breast cancer (6). Hippocrates (460-375 B.C.), the father of medicine, employed the terms cancer and carcinoma and described cancer of the breast and of various organs. What the cause was of this dreadful disease has brought the leaders of medical thought in ages past and up to the present moment to evolve many theories as to the vital etiological agent. Hippocrates and Galen (131-203 A.D.) thought that an excessive accumulation of "black bile" was the cause

of cancer. Paul of Aegina (625-690 A.D.) stated that the thick "black bile" was readily concentrated in the breasts because of their laxity and dependent position. Helmont in the 17th century stated, "Cancer is due to a spiritual being (Archaeus) who resides in the stomach and spleen" (6). The years following the discovery of the microscope (1592), making possible the minute study of tissues, forced imagination and mysticism to give way to more logical theories. Several varieties of bacteria and protozoa have from time to time been assigned as the etiological factor but none were proven to be the exciting cause.

Treatment hasn't varied as much as the etiological theories. Celsus, a contemporary of Christ, practiced excision of cancer of the breast, advising the removal of the underlying pectoral muscles. Galen (131-203 A.D.) also advised the wide surgical removal of cancer. Leonides, who lived in Alexandria in the latter part of the second century, was apparently the first surgeon to carry out a dissection for cancer of the breast. Fabricus Hildamus (1560-1634), the father of German surgery, was the first to practice a complete axillary dissection in the treatment of mammary cancer (6). This method of treatment is not far re-

moved from the present method of treating carcinoma of the breast.

THE EXPERIMENTAL ANIMAL AND ITS RELATIVE VALUE

Mouse as the Suitable Laboratory Animal

By selective breeding over many generations, families of mice have been developed which are either "high cancer strains" or "cancer resistant strains". The females of the high tumor strains exhibit a high tendency to the spontaneous development of mammary cancer, while the females of the low tumor strain have a low incidence of this type of cancer (5). The value of such animals to the experimentalists lies in their short life-span and in the ease with which a large number of tumor-bearing animals can be obtained. By using these mice one can observe the entire life span, not only for one generation, but for many succeeding generations. The experimentalist can isolate peculiar strains by rigid and close inbreeding in a manner entirely impractical for human beings. Little (8) has stated, "Research with inbred stocks of mice is a tool which enables the experimenter to hold genetics constant while other factors such as hormonal levels, chemical, or physiologic states can be varied." To maintain such an inbred stock, Bittner (9) states that the most sensitive physiologic tests now available to demonstrate the

existence of sublimes within a stock due to mutations, isolation of lines before the strain was homozygous (pure), or the occurrence of uncontrolled matings, is obtained by the transplantation of tumors that developed spontaneously in the stock. In mice of the stock line of origin practically 100% of the grafts will grow progressively, while in unrelated stocks or genectically different sublimes of the same strain, nearly all of the mice will be resistant. The genetic theory of transplantation was advanced by Strong and Little (10,11), as well as by Bittner (12,13) and Andervont (14).

Mammary Systems of Mice and Humans Compared

Since mice have been selected as the suitable animal for laboratory experimentation, it will be of value to examine the points of simularities and differences of the two mammary systems before extending conclusions based on data derived from experimental findings.

Similarities:

1. A common function is performed by both systems in the formation of milk for the feeding of their young.
2. Both mammary systems follow a series of cyclic

changes which are closely related to hormonal activity.

3. As far as it is known, there is hormonal homology of the ones controlling the cyclic changes in mice and man (15).
4. In both cases the most frequent type of malignant tumor of the mammary system arises from the adenomatous elements of the system (1,15).
5. In both mice and humans, the peak of mammary tumor incidence coincides with the period during which the reproductive activity of the female is diminishing (16,3,17,18,19,15).
6. The normal functional male of both types rarely develops mammary carcinoma.

Differences:

1. Metastasis of mammary tumors is much more frequent in humans than in mice. Little (15) believes that this difference may be due to the absolute size of the lymph ducts and the relative size of tumor surface.
2. Mice more commonly retain recognizable adenomatous structures while human mammary tumors tend to reach the carcinoma simplex degree.
3. Mice tend to show in the tumor mass areas of

columnar epithelial cells, while humans do not. Little (15) believes that this may be due to a more primitive type of tissue reaction in mice.

From this brief comparison it is apparent that the points of similarity in the two types are much more basic in their biologic significance than are their points of difference.

Is Inbred Mice Data Comparable to Human Processes?

The use of inbred mice raises the question; is inbreeding of mice detrimental for obtaining experimental data which may be expanded to the etiology of human mammary carcinoma? As it is known there is no such thing as an inbred human strain, and the strain is thought of being a mixed stock with, as yet, no known incidence of mammary carcinoma. Bonser (20) helped to clarify the question with carefully controlled experiments using highly inbred mice with a known incidence of mammary carcinoma and "outbred" mice (which correspond to the mixed human stock). He found that the results of data received from the inbred mice were only exaggerated processes of that

found in the "outbred", and the differences were of a quantitative rather than of a qualitative order. This is in accord with genetic experience that inbreeding in itself exerts no harmful influence on the stock but tends to exaggerate certain characteristics which, if of a harmful nature, may in the long run adversely affect the stock (20).

The result of Bonser's work suggests that the experimental data obtained by using inbred mice may be applied with limitations to the human stock.

Conclusions

1. Mice are suitable laboratory animals, for they can be highly inbred with a known incidence of mammary carcinoma, their genetics can be held constant, and many successive generations can be followed.
2. The similarities of mammary systems of mice and humans are much more basic in significance than are their differences.
3. Inbreeding mice only exaggerates the processes found in outbreds (similar to human stock), and experimentation suggests that such laboratory

data may be applied with limitations to the mixed human stock.

NURSING AND STAGNATION

Effect of Breeding and Non-breeding on the Incidence of Mammary Carcinoma

Animal Experimentation:

Lothrop and Loeb (21) stated that in female mice which were allowed to breed freely there was a considerable higher incidence of mammary carcinoma than in mice which were prevented from breeding. The findings were the same regardless of the strains used. In conjunction with use of poor breeders, which was a characteristic of that strain, they found that the incidence of mammary carcinoma was lower than in strains of good breeders. They collaborated and confirmed their results in a later article (22), as Loeb did again in 1919 (23). Murray (24) and Cori (25) confirmed the original work and found similar conclusions.

Statistical Analysis:

Statistical studies of the human incidence of carcinoma of the breast appear to confirm the experimental data found in mice. Table I compiles the percentage incidence of carcinoma of the breast cases of single and married women, and also in childbearing and childless women. The material was collected from a number of previously published papers.

Table 1 Distribution of Carcinoma of Breast Patients Concerning Marital and Pregnancy States

Investigators	Martial Distribution			Parity Proportion of Married Cases		
	No.of Cases	Single	Married	No.of Cases	Childless	Borne at least 1 child
Stubenford (17)	108	19.0%	81.0%	--	--	--
Scheiner & Stenstrom (26)	563	15.0%	85.0%	--	--	--
Shepherd (27)	439	17.0%	83.0%	--	--	--
Finney, et al. (16)	298	23.4%	76.6%	--	--	--
Harnett (18)	2529	22.1%	77.9%	1658	16.1%	83.9%
Lane-Claypon (29)	508	22.8%	77.2%	261	18.4%	81.6%
Control (No CA)	509	17.1%	82.9%	280	12.5%	87.5%
Penrose, et al. (30)	510	20.0%	80.0%	408	22.3%	77.7%
Marshall & Forney (4)	---	-----	------	300	43.0%	57.0%
Adair (31)	---	-----	------	200	37.0%	63.0%
Control (No CA)	---	-----	------	100	22.0%	78.0%
Hanna & Postlethwart (32)	---	-----	------	220	26.0%	74.0%

Although there is a lower percentage of cases in single and childless women, it is incorrect to assume that there is a higher incidence of carcinoma of the breast in women who are married and have borne children, for the actual percentage of single and childless women in the total population over 25 years of age is probably much lower than those women who are married and have borne children. Wood (33) in a statistical study of death rates in Pennsylvania found that there were 130 deaths of carcinoma of the breast in single women over 35 years of age and 678 deaths among married, widowed and divorced women. He computed the deaths per 100,000 and found that single women had a death rate of 109/100,000 and the other group had a corresponding death rate of 44/100,000. In his series, carcinoma of the breast has an incidence about three times higher in single than in married women. Other investigators are in agreement with his findings but have no statistical evidence to support their beliefs (15,34,3,28). Also, no definite statistics exist which demonstrate a higher incidence in childless than in child bearing women or that the carcinoma appears at an earlier age in that group, but there are several observers that believe

that this is true (35,36,37,29,31).

In the two control series (see Table 1), Lane-Claypon (29) found a 5.9% increase in infertility of the carcinoma of the breast patients over the controls, while Adair (31) found a 15% increase. These figures are believed to be significant, so that infertility may be associated with a higher incidence of carcinoma of the breast. Bogen (35) is in agreement with their findings.

The difference between the experimental data found in mice and the statistics of man is difficult to explain. The following observations may be of some value.

1. Abnormal endocrine unbalance has been shown in certain laboratory material to be a predisposing factor in the formation of tumors (15,38,39,3).
2. It is reasonable to assume that a greater number of married and childbearing women have normal endocrine function.
3. Mice selection for enforced non-breeding is not carried out on a basis which recognizes abnormal endocrine function or animals which are abnormal in being poor breeders.

Effect of Lactation and Stagnation on the Maternal Breast

Many writers, as it was seen, describe the proportion of single and married, as well as, childless and childbearing women with mammary carcinoma, but a much more significant differentiation concerns itself with the history of prior lactation. Cooper (31) in 1845 stated, "Suckling diminishes the disposition to malignant disease of the breast". He believed that the unemployed breasts, such as found in childless women or in those who did not nurse their children, have a greater chance for developing cancer than those who have nursed. Lane-Claypon (29) stated that the connection between cancer of the breast and lactation lied in the absence of function. In 1932, Twart (40) went as far as to state, "Anything contrary to nature such as over-lactation, under-lactation or no-lactation may predispose to cancer." In the years to follow the observers were a little more conservative than Twart, but they stated similar views that there is a higher incidence of carcinoma of the breast in women who have not lactated than in those who have (36,34,35,3,41). However, trustworthy statistics have been lacking.

Much attention has been drawn to chronic mastitis as being one of the main predisposing conditions of mammary carcinoma, but Ewing (42) believes that this theory alone is inadequate to explain the frequent incidence of cancer because many cases, that he has seen, arise without pronounced mastitis and so many arise in the very early stages of mastitis. However, he stated, "Mammary cancer practically never arises in a previously normal breast, but always in an organ altered by involution or inflamation." Many observers have pointed out the prominence of stagnation of secretion in the cancerous breast, and Keynes (43) has emphasized the part played by stagna- in chronic mastitis. Cheatle (36) has pointed out the importance of chronic irritation by retained secretion in the development of cysts and periductal fibrosis in chronic mastitis. Adair and Bagg (44) have intimidated obstruction and stagnation as a factor of prime importance relative to the subsequent development of breast cancer.

Stagnation Theory:

By stagnation of the mammary system, it is meant that certain definite mechanical factors produce partial or complete blockage of the duct at some

place situated between the periphery (acini) and nipple terminus. Some causes of stagnation are localized outgrowth of the lining epithelium, nipple abnormalities such as inverted, puckered, adherent flat nipple, etc., fibious scars across ducts, plugs of epithelial debris, and acute angulations of terminal ducts.

By retention of cellular detritus of the desquamated lining and products of degenerated milk (as in a case of a recent pregnancy or miscarriage), it is assumed that an inflammatory reaction is set up in and about the duct systems (31). Infiltration by lymphocytes and polymorphonuclear leukoytes takes place. Keynes (43) believes the nature of this irritation is chemical. Adair (31) states that the continued presence of the cellular detritus and products of degenerated milk in the ducts and acini causes a stimulation of the epithelium to hyperplastic changes; the lining cells multiply and heap up several rows high giving the appearance of precancerous tissue. Adair gurther believes that the precancerous areas continue their growth and terminate by invasion and infiltration with the ultimate development of true carcinoma.

According to Adair (31,44), the active as well as the inactive breast is concerned with the normal cell regeneration. In the virginal breast the lumen contains degenerated desquamated cells, cell detritus, mucoid material and some crystals, and this material is gradually pushed along from the acini to the terminal duct and extruded at the nipple, providing there is no obstruction in the pathway. This material by itself after a prolonged period in the lumen, as in obstruction, can cause the same hyperplastic changes of the ductal epithelium, and hence the nullipara is subject to cancerous changes of the breast in the same manner as the multipara.

In the physiological active breast the desquamation accumulates in the lumen of the ducts throughout the pregnancy. If the young are not suckled a portion of accumulated cells will remain along with the end products of milk disintegration (i.e. Lactic and butyric acids), and their continued presence over a long period of years will produce hyperplasia of the ductal epithelium and may lead to a true carcinomaous condition (31). However, as yet, the exact proof is lacking which

could demonstrate that the retained material acting as a chemical irritant will cause cellular hyperplasia.

Ewing (42) in the dissection of many cancerous breasts found stagnation in the ducts leading from cancerous areas, but in many breasts, especially the atrophic organs, gross evidence of stagnation was not demonstrated. According to Adair (31), large amount of inspissated or puriform material can be drawn from many cancerous breasts, and also he has obtained from non-cancerous breasts as much as 10-15 cc of a creamy material by pumping up to ten years after the birth of the last child.

Animal Experimentation:

Artificial stagnation of milk in the ducts and acini has been produced experimentally in mice by the ligation of ducts or by rapid breeding with the prevention of nursing. In a strain of low cancer mice Bagg (45,46,47,48) produced breast cancer in 85% of the young at birth. The tumors developed at an early age after the third or fourth litter. They appeared suddenly at one or more points in the system of ducts, recurred after removal and killed by metastasis. Ligation of the ducts along one side of the animals

was followed by cancers in the breasts of that side but not in the nursed side. Murray (49,50), however, found little difference in the incidence among the females of the dilute brown stock which nursed all or none of their liters. The effects of force breeding (i.e. rapid breeding with prevention of nursing) in mice without the milk agent could not be confirmed by Little and Pearsons (51), Fekete (52), as well as by Bagg (48). Bittner (53) using mice of the low cancer black stock, who obtained the milk agent, found either high or low incidences depending upon the sublimes which presented themselves. These facts make it probable that the foster mothers that Bagg used to raise the mice in the original study and which later developed mammary cancer probably transferred an extrachromosomal factor, the milk agent (53).

Bagg and Hagopian (48) postulated by the stagnation theory that the prevention of nursing caused stasis with retention of milk because of improper drainage causing an irritating chemical effect upon the epithelium of the mammary gland. Fekete and Green (54) reported that more tumors developed in the blocked than in the normal secreting glands of dilute brown mice, but they stated that the secreting

function of the occluded glands was found to be normal and premature regression had occurred. Also they stated that complete blockage of the nipple would have little effect in the development of tumors if the stocks did not inherit the cancer susceptibility.

Bittner (9) in studying the question of force breeding and lactation found that the mice subjected to force breeding showed lower incidences than did those who were permitted to nurse their progeny. He also found that the females which were allowed to nurse for twelve days had the lowest incidence of any group and had approximately the same average incidence of any group of the normal breeders. In selecting the mice for this experimentation he was careful to use mice without the milk agent. He sacrificed some of the females 12 days after giving birth to young in order to determine the relative amount of stagnation in the glands of females which had nursed and those which had their young removed at birth. He found that there was relatively little stagnation of milk in the glands of the force breeders, but those which had nursed for 12 days possessed glands which were engorged with stagnated milk.

The results of Bittner's work indicate that stagnation, per se., does not appear to be an important contributing cause of mammary cancer in mice. However, in light of Bittner's earlier work and others (53,49,54,48), stagnation may increase the incidence of carcinoma of the breast if the animals obtain the milk agent.

Clinical Analysis:

The search for statistical evidence giving an accurate proportion of cancerous parity who have lactated in comparison with those who have not, which would validate experimental data or suggest any relative importance, has not been fruitful. Table 11 gives the percentage of parity who have a history of lactation and those which have not, as found in four series of carcinoma of the breast cases.

Table 11 Distribution of Carcinoma of Breast Cases Among Mothers Who Have Lactated and Those Who Have Not.

Investigators	No. of Cases	History of Lactation	No History of Lactation
Shepherd (27)	226	90.7%	9.3%
Adair (31)	126	84.0%	16.0%
Lee (34)	156	69.0%	31.0%
Lane-Claypon (29)	207	84.6%	15.4%
Controls (No CA)	238	92.5%	7.5%

The greater percentage of cases occur in those women who have lactated, but again it is incorrect to assume that mothers who nurse their babies will have a higher incidence of carcinoma of the breast than those who do not. In Table 11 the Lane-Claypon series show that a greater percentage 7.9% of the healthy controls nursed their progeny. This figure may be significant, but more series with accurate controls are needed before one can assume that there is a higher incidence of carcinoma of the breast in women who don't nurse their babies. If it could

be determined how many women have lactated as to those who didn't nurse in the general population, one could determine the rate of carcinoma cases per 100,000 in both instances and then adequate evidence could be gained from these statistics. As it is, there is only suggestive evidence presented which is obtained from only one series that used a control.

Adair (31) in view of his stagnation theory, broke down his number of carcinoma cases of women who have lactated into the normal and abnormal nursing groups. In 106 cases he found only 8.5% gave a normal nursing history, while in 78 control cases 80% gave a normal lactation history. It is suggested from his figures that women with a history of disordered lactation have a higher incidence of carcinoma of the breast than do healthy controls with normal lactation. Dargent (55) recently confirmed Adairs work.

Conclusion:

1. Statistical data does not bear out the experimental data in mice where there is a higher incidence of carcinoma of the breast in freely breeding females than in non-breeders.
2. There is suggestive data that carcinoma of the

breast has a higher incidence in single and childless than in childbearing women, although there are no definite statistics proving this. Also, it has been shown that infertility in itself appears to be associated with this increase.

3. The stagnation theory, which is retention of cellular detritus and products of milk degeneration due to obstruction and improper drainage, lacks exact proof that this stagnated material in the ductal system of humans acts as a chemical irritant and causes cellular hyperplasia.
4. Stagnation, per se., does not appear to be an important contributing cause of mammary cancer in mice, but if an extrachromosamal agent be present (the milk factor) rapid breeding with prevention of nursing or ligation of ducts may cause a higher incidence of carcinoma in mice.
5. According to two small series, women having a history of disordered lactation have a higher incidence of carcinoma of the breast than do healthy controls.
6. The greater percentage of cases of carcinoma of

the breast occur in women who have lactated. However, one series which used controls suggests that there is a higher incidence in women who don't nurse their babies, but more statistics are needed before complete and definite evaluations can be made.

THE EXTRACHROMOSOMAL FACTOR

Animal Experimentation

Demonstration of the Presence of the Milk Factor:

Slye (56), in studying neoplasms of white mice through a number of generations pointed out the tendency for the new growths to be transmitted according to the Mendelian law and how it is possible, by inbreeding the offspring of parents having malignant growths, to produce litters in which 100% develop neoplasms. She states that she has been able to breed out any tendency for neoplasms to develop. Furthermore, she believes that the tendency to transmit neoplasms is not a dominant but a recessive characteristic. Meanwhile, Lynch (57,58) surmised from his experiments a dominant characteristic and Little (59) a sex-limited dominant with hymozogous lethal effect. Dobrovolskaga-Zavadskaja (60) after extensive experiments came to the conclusion that there exists a hereditary predisposition to cancer, and that the different tumors are controlled by different, mutually independent genes. However, Bittner and his co-workers (61) disproved the Mendelian genetic nature as being the sole

etiological factor of spontaneous mammary tumors by reciprocal crosses between high tumor and low tumor strains. Females of a high tumor strain were crossed with males of a low tumor strain, and the average incidence of carcinoma in the progeny was 70.1%. However, when low tumor strain females were crossed with high tumor males, the incidence was only 3.23%. According to Bittner and his co-workers, this difference represents an extrachromosomal influence. This influence was transmitted to the following generations, and its presence was indicated in over 1100 second-generation females. However, the workers stated, "This should not be taken as denial of the existence of chromosomal influence, for there is clear evidence that such an influence is also present."

By the use of high tumor offspring, Bittner (62) was able to demonstrate the presence of an extrachromosomal influence. He observed that an even division of mice had developed breast tumors, primary lung tumors and the remaining were non-tumorous. When the progeny were fostered on females that had breast and lung carcinomas, 77% of the fostered females developed breast cancer similar to that of the foster mother as compared with 17% of the fostered

females which developed lung tumors that fostered from a mother which had a primary lung tumor. According to Bittner, this demonstrated that the incidence of mammary carcinoma in mice may be affected by nursing and offers an explanation for the so-called extrachromosomal influence of the development of this neoplasm.

Hoagensen and Randall (63) showed by "fostering experiments" that they could produce an incidence of 76.1% of mammary carcinoma in a low tumor strain by foster nursing on high tumor strain females. In the offspring of the formerly low tumor strain, the incidence persisted. However, the observers noted that although influence of the milk factor is transmitted through successive generations, it must be renewed through the ingestion of milk itself for each generation if it is to exert its full effect. Hoagensen also stated that the mothers were not transfering some agent other than milk, which may play a part in the development of carcinoma, on the basis of finding 42.6% tumor incidence in low tumor strain mice that were fed artificially the milk from high tumor strain females. He accomplished this with no more that 1.0 cc of the artificially obtained milk.

It was found by Miller and Pybus (64) that the frequency of tumors in low tumor mice fostered by high tumor mothers in the first 24 hours was much greater than in animals fostered after 24 hours had passed. They also noted that if low tumor mice had spent from 6 to 12 days with their own mothers before being fostered, they were no longer susceptible to the action of the milk agent, although they were able to transmit it to their young.

Bittner (62) states that he has one line of fostered susceptible mice started in 1934 which has continued for over 30 generations with an incidence of approximately 1%, while in the cancerous unfostered line of the same stock there has been over 50 successive generations of mammary cancer, and it has an incidence in excess of 90%. He found that if mice of the fostered line obtain the agent, either by nursing or the ingestion of extracts of the tissues from mice with the agent, these mice became cancerous and give rise to cancerous lines.

Physical, Chemical, and Biologic Properties of the Milk Agent:

Andervont and Byran (65) claimed to have obtained the agent from filtered extracts of mammary tumors and likened it to a virus in this respect.

Other viral properties ascribed to the milk factor include its ability to produce neutralizing antibodies (65), its survival in cold, and its lack of resistance to heat. The tumor agent when exposed to a temperature of 61 C for 30 minutes is inactivated, and this indicates that the ordinary pasteurization of milk will destroy the agent (66). Barnum and his co-workers (67) showed that the agent is stable at pH values between 5.0 and 10.2, and that it is not inactivated by petroleum, ether, or acetone, nor was it soluble in these solvents. Andervont and Byran (65) claimed that the agent is 160 times as infective by intraperitoneal administration as compared to oral ingestion. Bittner and his co-workers (68,53) showed that the agent persists in association with tumor transplants by carrying it through 10 serial passages in mice that did not themselves carry the milk agent but only had inherited susceptibility for spontaneous mammary cancer. Bittner (69) stated that the transplantability of mammary tumors is not dependent upon the milk agent, for the milk agent is in association with the tumor.

Morphological Studies:

The actual separation and isolation of the

tumor agent was accomplished by sedimentation from extracts of mammary glands (70) and mouse milk (71). Barnum, et al. (146), stated that lactating mammary gland was a most potent source of the milk factor.

Passey, et al. (73), used the electron microscope for studies of milk from high tumor strain mice and described particles 20 millimicrons in diameter that he had separated by ultracentrifugation. Porter and Thompson (74) observed spherical bodies which had an average diameter of 130 millimicrons in cultured mouse carcinoma cells. The uniform morphologic aspect and the association of these bodies in closely packed clumps suggested to them that the bodies were of extraneous origin and probably represented the virus like milk factor. Graff and his associates (75), by using digestive enzymes on milk, claim to have successfully freed the agent and were able to isolate it by differential sedimentation. They described a substance consisting of particles that had the dimensions and properties of a virus. When they inoculated it into low tumor strain mice, carcinoma of the breast was produced.

Mode of Action:

With the vast amount of research done on the

milk agent the question arises, does the milk factor alone provoke the development of carcinoma, or is it one of several factors that exerts a combined influence? Bittner (76) in his original work assigned roles of equal importance of cancer development in mice to three influences which are hormonal stimulation, inherited susceptibility and the milk factor. Bittner's work was substantiated by Andervont (77) who went on to state that some tumors may develop in the absence of or with subthreshold amounts of the milk influence. In such cases he proposed that the deficiency is overcome by an increase in hormonal stimulation. Heston and his workers (78) emphasized the importance of genetic influence. Bittner (79) states that genetic factors are involved, but they are concerned with susceptibility. Murray and Little (80) investigated the matter and stated, "Some extrachromosomal influence, which is ten times as powerful as any possible chromosomal factor, is instrumental in determining whether or not mammary carcinoma appears in the first out-cross generations." Heston (81) concludes his evaluation of the relative importance of factors producing

mammary carcinoma by saying that the genes should be pictured as having a more basic position, with their actions becoming manifest through three gene-action pathways, the first involving the milk agent, the second the hormonal stimulation and the third the susceptibility of the mammary gland. Bittner in later works (9) describes another inherited factor which is involved. He terms it the inherited hormonal influence. He states that it may determine whether or not virgin females of susceptible strains with the agent will give rise to mammary cancer, but its effects are not needed for the development of cancer in breeders because of the increased hormonal stimulation associated with pregnancy. Bittner postulates that the mammary tumor agent may alter hormonal metabolism by the production on "carcinogenic hormones", but more work is needed before an evaluation can be made.

Human Milk Factor

Demonstration of its Presence:

Since no inbred strains of humans exist, one is forced to look at genetical studies to see if they suggest the presence of a milk factor being transmitted from mother to daughter, and generation to

generation. In 1911, Rust (82) described cancer as being transmitted from parents to children, especially from mothers to daughters and persisting through entire generations. Broca (83) published an important record of a pedigree nearly a century ago showing apparent transmission from mother to daughter in which four generations of females had mammary cancer. Paget (84) in dealing with breast cancer found familial occurrence of cancer in 16 out of 80 patients. Another English investigator, Butlin (85), published a material of 183 cases of breast cancer collected by means of a questionnaire. It comprises 68 families in which hereditary disposition to the disease was present. There were 99 cases of malignant tumors in all and cancer of the breast preponderated with 34 cases.

In a more recent invertigation, Williams (86) in a non-selected series of 136 breast cancer probands found the hereditary taint in 33 (24.2%) with 48 cases of cancer in all, 19 of which were cancer of the breast. One hundred and one females with benign tumors served as a basis for comparison; among these, hereditary disposition was found in only 15.8%. Wainwright (87) conducted an investigation which com-

prises of 784 females treated for cancer of the breast, with a control of 576 sound females in ages between 45 and 70 years. He found that the incidence of breast cancer was four times as great among the mothers of the cancer patients as among the mothers of the controls. He also found that cancer of the breast, as a cause of death, was twice as frequent among sisters of breast cancer patients as among sisters of sound females. Wassink (88), in a Dutch study, found when the proband had cancer of the breast, there was a considerable increase of cancer among the female relatives and that this increase was due to a homologous form of tumor. Martynova (89), using 201 cases which had been diagnosed by a physician as sure cases of cancer and which were also examined by herself, compared them with 796 controls which were patients of a dental clinic. She found that cancer of the breast was 18 times as frequent in mothers of the breast cancer patients as in the mothers of non-cancer females. In 28.7% of the female relatives with a history of cancer, the malignancy was cancer of the breast which is much larger than the anticipated figure of 4.7%. Jacobsen (90) in his series of 200 cancer

patients compared with 200 controls found an excess incidence of breast cancer among the female relatives of the patients with the exception of the grandparents. In the study of Penrose, et al. (30), a significant excess of mammary cancer was found among the maternal grandparents as compared with the paternal grandparents, and also the excess was found in the rest of the maternal female relatives.

The investigations definitely suggest the presence of the so-called milk factor, but none of the series are without criticisms. Most of the series are criticized for doubtful diagnoses, loose methods of compilation of the materials and the uncertainity of accurate controls (30,90). However, in spite of the lessened values, a suggestion of the presence of the milk factor may be gained from this vast amount of observational material.

Morphological Properties:

After the vast amount of experimental work done in mice, Gessler and Grey (91) demonstrated in human cancer tissue spherical bodies ranging from 80-150 millimicrons by the use of a high speed microtome, which allows cutting of tissue 0.1 micron thick. They stated that there was a close simularity

between these globules and the virus of fowl sarcoma, and they concluded that they were probably dealing with a virus-like causative agent of cancer cells. Hellwig (92) confirmed the observation of the previous workers that large globules are present in cancer tissue but hesitated to accept their interpretation that the particles were of extraneous origin as postulated by the previous investigators. His studies revealed globular bodies in most extracts of tumors, as well as a definite difference in size between cancerous and benign specimens. The particles in the benign tumor extracts seldom exceed 60 millimicions in diameter where as those from cancerous tissue often exceeded 80 millimicrons. Since Hellwig had previously been able to demonstrate similar bodies in cerebrospinal fluid from persons without tumors, he believed that the particles with a diameter of less than 60 millimicrons were apparently normal cell constituents and represented globular proteins rather than viruses. He suggested that the larger particles found in malignant tumors were probable aggrevates of the cytoplasmic globules due to an alteration in the colloidal state of the cancer cell.

Gross, et al., (19) in a recent attempt to find out whether spherical particles, similar to those found in mouse milk would, perhaps, also be found with the aid of an electron microscope in milk obtained from women having a family record of breast cancer. Gross and his co-workers prepared specimens to be examined by centrifuging and digesting with chymotrysin. They found spherical bodies varying in diameter from 10 to 20 millimicrons in 10 samples of milk selected from young healthy nursing women, having sisters, mothers, or grandmothers with carcinoma of the breast. However, 11 samples of 32 healthy control mother's milk, whose history is free of any malignant tumors for two generations, also demonstrate similar spherical particles. They postulated that no definite conclusions could be reached at this time.

Clinical Analysis:

Genetical studies, as it was noted, seem to suggest that some factor could be transmitted from mother to daughter and from generation to generation because:

1. An apparently higher incidence of breast cancer occurs among mothers of cancer patients as among

the mothers of controls.

2. Cancer of the breast appears to have a higher incidence among sisters of cancer patients than among sisters of sound females.
3. Maternal relatives are frequently more afflicted with mammary cancer than are the corresponding paternal relatives.

However, Horne (93) recently investigated the question by comparing incidence of the absence of breast feeding in 88 cases of known cancer as compared with that in 86 control cases. All the women were about the same age. He found that 10.2% of the cancer patients had never received human milk as compared with 8.1% of the controls. He states that it appears that a woman may develop cancer of the breast without ever having human milk. Whether or not the daughters of women with mammary cancer are more likely to develop the same disease, if they are nursed, has not been investigated and from his study he states that this may not be inferred.

Theorectical Considerations

Gross (130) states that there is a practical method of prophylaxis for the prevention of the development of mammary carcinoma in mice. He continues by

saying that isolation of the newly born animals from their tumor-agent carrying mothers and transferring them for the purpose of nursing to females whose milk is free from the agent, will result in mice living their normal life spans, and they won't develop breast tumors. Moreover,their milk is free of the tumor agent, and they can in due time nurse their own litters without transmitting the agent.

However, in the mice which are allowed to nurse from their tumor-agent mothers a long interval occurs between the exposure and the actual appearance of the tumor, and it may even skip generations before some of the female offspring show manifestations of the disease (130). The skipped generations would appear as healthy females nursing their young, but they would be seeding their progeny with the fatal agent. This generation interval, as well as the interval in the host between exposure and actual appearance, is explained on the basis that the invisible agent is transmitted in some inactive form, and it remains inactive during most of the life span of the host. However, when certain conditions develop such as, those relating to aging, or hormonal stimulation and metabolic disturbances, or by exposure to irradiation,

or to certain systemic poisons, the tumor agent becomes activated. At the present time, there is no practical method of determining the presence of the tumor agent in the milk of the nursing female mouse except by checking her family record for the occurrence of tumors.

As yet all observations concerning the mammary tumor agent in mice are insufficiently demonstrated in humans in order to form definite generalized conclusions. If a parallel with human breast carcinoma could be drawn, a history of mammary cancer in a family should indicate to an apparently healthy mother that she may be seeding her female child with the tumor factor even after a few hours of nursing, and it would be justifiable that breast feeding in such a case should be abandoned from birth.

Conclusions:

1. The presence of a transmissible extrachromosomal agent (so-called milk factor) has been demonstrated and thoroughly described in mice. It has been shown that it is transferred through successive generations of females by the milk of mothers carrying the agent.

2. By transferring female mice of tumor-agent mothers before they have nursed to foster mothers without the agent, the progeny will not develop mammary carcinoma nor will they transfer such an agent to their offsprings.
3. It has been demonstrated that female mice who are nursed by agent-bearing mothers in the first 24 hours of life are more susceptible for the development of carcinoma of the breast than those who do not nurse the agent until they are a few days old.
4. The physical, chemical and biologic properties likens the agent that is found in mice to a virus, and that the agent is inactivated by ordinary pastuerization methods.
5. The demonstration of the presence of such a factor in humans is suggested from genetical studies because an apparently higher incidence of mammary cancer which occurs;
   (a) Among mothers of cancer patients as compared with mothers of controls;
   (b) Among sisters of cancer patients as compared with sisters of sound and healthy females;

(c) Among maternal relatives as compared with paternal relatives.

6. The factor in human carcinoma of the breast has not been demonstrated by the use of an electron microscope where as it has been demonstrated in mice.

7. In a recent series, it has been shown that some women may develop carcinoma of the breast without ever having human milk, although 89.8% of the cases had received human milk.

SUMMARY

The aim of this paper has been an attempt to collaborate statistical and experimental research concerned with the effects of lactation on the incidence of carcinoma of the maternal breast as well as in the female progeny. It has been shown that mice are a suitable animal for experimentation, for they can be highly inbred with a known incidence of mammary carcinoma, and their genetics can be held constant while many successive generations can be followed by an observer. The similarities of the mammary systems of mice and man are much more of a basic significance than are their differences. It has been demonstrated that inbreeding of mice only exaggerates the processes found in the outbreds (similar to the human stock), and experimentation suggests that laboratory data may by applied with limitations to the mixed human stock.

In mice which are allowed to breed freely there is a higher incidence of carcinoma of the breast than in enforced non-breeders. However, human data does not bear out this experimental finding, for suggestive material has been obtained from several clinical studies that there is a higher incidence

of carcinoma of the breast in single and childless than in child bearing women, although exact statistical proof is lacking. It has been postulated that this difference may be due in part to some endocrine unbalance which is assumed to be more prevalent in single and childless women, and it has been demonstrated experimentally that abnormal endocrine unbalance is a predisposing factor in the formation of tumors in laboratory material.

The stagnation theory, which is the retention of cellular detritus and products of milk degeneration in the ductal systems due to obstruction or improper drainage, lacks exact proof that this material acts like a chemical irritant and causes ductal hyperplasia which may lead to a true cancerous condition. Experimental data in mice shows that stagnation, per se., does not appear to be an important contributing cause of mammary cancer, but if the extrachromosomal agent be present (i.e. the milk factor) rapid breeding with the prevention of nursing or the ligation of ducts, both of which cause stagnation, may cause a higher incidence of carcinoma in mice. According to two small clinical series, women with improper drainage of the ductal system, which is assumed from

their histories of disordered lactation, have a higher incidence of breast carcinoma than a group of healthy controls. Compilation of cases where a nursing history was available revealed that the greater percentage of cases of carcinoma of the breast occur in women who have lactated. There is only one of a series which uses a control, and this one suggests that there is a higher incidence in the women who did not nurse their babies. However, more statistics are needed with accurate controls before this phase can be evaluated. Also more direct observational methods should be used with less emphasis on heresay in the compilation of the material.

The presence of a transmissible extrachromosomal agent (so-called milk factor) has been demonstrated and thoroughly described in mice, and it has been shown to be transferred through successive generations of females by the milk of mothers who possess the agent. By the immediate fostering of the offspring of mothers who have the milk factor on mothers who do not possess it, the mice which would have developed mammary carcinoma do not, and furthermore they are unable to pass the factor to their progeny. The physical, chemical and biological properties of

the milk agent have made it likened to a virus, and it is inactivated by ordinary pasteurization methods. The actual separation and isolation of the tumor agent was accomplished from extracts of mammary glands and mouse milk. With the use of the electron microscope, the agent was described as spherical bodies. When these bodies were injected into low tumor strain mice, carcinoma of the breast was produced.

In man such an agent has not been described nor demonstrated by the use of the electron microscope. Its presence has been suggested from genetical studies because of an apparently higher incidence of mammary cancer occurring in mothers of cancer patients as compared with mothers of controls, in sisters of cancer patients as compared with sisters of healthy females, and in maternal relatives as compared with paternal relatives. In a recent study one investigator indicates that susceptibility to human cancer is not transmitted through milk, for he found that some carcinoma cases appear in women who have not nursed, although 89.8% of his cases had nursed. It has been stated that two, or not even three generations, are sufficient for tracing such a factor, if it were present, because of the ability of the virus to re-

main inactive for several generations and yet be present in the milk. It has been postulated that it will take from fifty to one hundred years to carry out an accurate research program to confirm or deny in humans the vast amount of animal experimentation.

CONCLUSION

1. Experimental data in mice shows that stagnation and improper drainage of milk, per se., does not appear to be an important contributing cause of mammary carcinoma. However, if stagnation is produced experimentally by rapid breeding or the ligation of ducts, and if the extrachromosomal agent is present, a higher incidence may be observed.
2. The presence of a transmissible extrachromosomal factor which causes the development of mammary carcinoma in mice has been demonstrated and thoroughly described, and it has been shown to be transferred through successive generations of females through their milk.
3. There have been no adequate research programs nor accurate statistical studies published on human beings which can be offered as definite confirmation or denial of the application of principles which have been demonstrated in the mouse.

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