SPECIAL ARTICLES

MAMMARY CANCER AND MAMMARY STRUCTURE IN INBRED STOCKS OF MICE AND THEIR HYBRIDS[1, 2]

GARDNER and Strong[3] used the whole mount technique to study the structure of the mammary glands in virgin females of several stocks of mice, including the A and C3H strains, and could not detect any difference in the architecture. Also, the inherited tendency for spontaneous mammary cancer was not found to be associated with any change in the structure of the glands. In a later publication, Gardner, Strong and Smith[4] observed the presence of localized hyperplastic nodules in glands of animals from strains in which mammary cancer frequently resulted. Few, if any, of the nodules were found in the glands of mice from low cancerous stocks.

Taylor and Waltman[5] stated that the essential difference in the structure of the glands from mice of the cancerous dilute brown stock and low cancerous C57 black strains was in the number of acini (hyperplastic nodules) found in glands of mice of the cancerous strain. This difference persisted following the injection of estrogenic hormones. Using the same strains, van Gulik and Korteweg[6] concluded that in virgin females an architectural difference in the structure of the primary ducts and the gland-trees could be found. The F_1 hybrids, produced by reciprocal matings, had glands whose primary ducts were characteristic of the maternal strain, while the gland-trees resembled the maternal type but were modified in the direction of the type found in the paternal strain. They believed the hyperplastic nodules to be associated with the active milk agent.

Loeb and Suntzeff[7] stated that one of the factors determining the difference between mice of various strains toward the development of mammary cancer was the readiness with which the glands gave progressive growth to stimulation by estrogens.

MATERIAL AND METHOD

To determine what the incidence of mammary cancer might be in hybrids, reciprocal matings were made between mice of the cancerous A and C3H stocks. The hybrids have been maintained either as virgin or breeding females, but only the former are considered in this report. In addition to the incidence of mammary cancer, the architecture of the mammary glands was studied by the whole mount technique.

Virgin females of the A stock have a low incidence of spontaneous mammary cancer,[8] whereas non-breeding females of the subline of the C3H or Z stock used in these experiments frequently develop such tumors.[9] Breeding females of each strain have a high incidence of mammary tumors.[10]

The oldest animals have attained the age of 14 months, but because the youngest mouse to become cancerous did so at approximately 9 months of age, only mice which have reached that age will be mentioned.

The animals are receiving Purina Fox Chow and an unlimited amount of water.

RESULTS

In previous experiments, virgin females of the A stock had an incidence of mammary tumors of 4.9 per cent.,[11] whereas virgin females of the C3H or Z stock gave an incidence of 72 per cent.[9] (and unpublished). The number of mice observed was 223 and 51, respectively.

The number of mice being observed at present is given in Table 1, together with the number in each group living to be 9 months or older. The incidence of mammary cancer was determined from the number of mice which had survived for at least 9 months. To date, few animals have died without cancer.

TABLE 1

Stock	Number under observation	Number surviving for 9 months or longer	Cancer incidence for virgins living 9 months or longer
A	101	55	0 per cent.
Z or C3H	104	7	43 " "
AZF_1	121	47	45 " "
ZAF_1	65	10	20 " "

AZF_1 hybrids were produced by mating females of the A stock with males of the Z or C3H strain; the ZAF_1 hybrids were derived from the reciprocal cross. From Table 1 it will be seen that mammary tumors are being recorded in virgin females of the Z or C3H stocks and their reciprocal hybrids; to date none of the virgin females of the A stock has had tumors. (Our earlier studies showed an incidence of 27 per cent.[9] spontaneous mammary tumors in C3H virgins at 9 months.)

The mammary glands of mice from the inbred stocks

[1] Preliminary report.

[2] Assisted by the University of Minnesota Graduate School Cancer Research Fund and The Jane Coffin Childs Memorial Fund for Medical Research.

[3] W. U. Gardner and L. C. Strong, *Am. Jour. Cancer*, 25: 282–290, 1935.

[4] W. U. Gardner, L. C. Strong and G. M. Smith, *Am. Jour. Cancer*, 37: 510–517, 1939.

[5] H. C. Taylor, Jr., and C. A. Waltman, *Arch. Surg.*, 40: 733–820, 1940.

[6] P. J. van Gulik and R. Korteweg, *Proc. Nederl. Akad. van Weterschappen*, 43: 891–900, 1940.

[7] L. Loeb and V. Suntzeff, *Arch. Path.*, 32: 739–759, 1941.

[8] J. J. Bittner, *Pub. Health Rept.*, 54: 380–392, 1939.

[9] M. B. Visscher, Z. B. Ball, R. H. Barnes and I. Sivertsen, *Surgery*, 11: 48–55, 1942.

[10] J. J. Bittner, *Cancer Research*, 2: 710–721, 1942.

[11] J. J. Bittner, *Pub. Health Rept.*, 54: 1113–1118, 1939.

and their hybrids have shown minor variations in their architecture when studied by the whole mount technique. These findings will be presented in another report when more data are available.

Hyperplastic ("precancerous") nodules were found in the glands of virgin females of the C3H stock and the AZF_1 and ZAF_1 hybrids which had reached the age when mammary cancer might be expected to develop. The nodules were more numerous in the glands of the hybrid mice than from the C3H animals. They were also found in the glands of mice of these groups which had not developed cancer but had survived to the cancerous age.

The glands from a few virgin females of the A stock of the same age did not have any hyperplastic nodules.

In addition to the virgin females, the glands of fostered breeding females of the A and C3H stocks were examined. All the females had given birth to at least 3 litters and ranged in age from 11 to 16 months. Only occasionally a nodule was found and never more than one to a gland.

Discussion

Spontaneous mammary cancer in mice will develop only in glands which have been stimulated to growth by estrogenic hormones. Although the virgin females of the A stock develop very few mammary tumors,[8] their mammary glands appear to be as well developed —except for the absence of hyperplastic nodules—as are the glands of the virgin females of the C3H stock in which mammary tumors frequently are found.[9]

The differences producing these incidences of cancer between virgin females of the A and C3H stocks appear to be explicable most satisfactorily on the supposition that they are due to characteristic differences in hormonal metabolism, in a broad sense, in mice of the two strains. This deduction finds its strongest support in the fact that repeated pregnancies in females of the A strain brings the incidence of mammary cancer in the latter to that in the C3H mice.[10,12] If this supposition is correct it would be deduced that a difference in the virgin state in (a) the amounts of estrogen produced or available for action on the mammary glands, or (b) the sensitivity of those structures to estrogens, or a combination of both, would be responsible for the strain difference observed.[11] Whatever the cause (or causes) may be, these data suggest that the effect is controlled by intrinsic factors since the first generation hybrids with C3H fathers and mothers from the A stock have developed spontaneous mammary tumors when maintained as virgin females. It can further be inferred that this characteristic difference in the production of mammary tumors in virgin females of the A and C3H stocks is inherited as a dominant character.

The entire problem of the nature of "inherited susceptibility" is brought to the fore by these observations. It is not possible to analyze all the factors involved in detail at this time, but the observations reported indicate that at least one physiological character determined genetically operates through a hormonal mechanism. It is doubtful whether this inherited hormonal character corresponds completely with the "inherited susceptibility for spontaneous mammary tumors"[13] as previously described.

No correlation could be detected between the presence of the hyperplastic nodules or precancerous lesions and the active milk agent alone. The virgin females of the A stock have this influence and nodules were not found in the small number of glands which were examined. However, the nodules were found in virgin females which had the active milk agent and the inherited estrogenic factor, as the virgin C3H females and of the reciprocal hybrid generations. That the nodules usually do not result from the estrogenic stimulus alone was suggested by the small number of them found in the glands of fostered breeding females of the A and C3H stocks. These mice did not have the milk agent, but the estrogenic influence would be greater, because of the production of young, than one would expect in virgin females. In these mice the inherited estrogenic stimulus would be supplemented by the extrinsic (breeding) stimulus. The fostered breeding females of these stocks have a low incidence of mammary cancer.[10]

Thus, the tentative theory may be advanced that the hyperplastic nodules result from the inciting influence of both the active milk agent and the estrogenic hormones[14] and not solely from the action of the milk agent, as suggested by van Gulik and Korteweg.[6]

Conclusions

Characteristic differences in hormonal metabolism in virgin females of inbred strains of mice may result from: (a), the amounts of estrogen produced or available for the stimulation of the mammary glands, and/or (b), the sensitivity of the mammary glands to estrogenic hormones.

The inherited estrogenic influence is transmitted as a dominant and plays a role in the genesis of spontaneous mammary tumors in virgin females of inbred strains and their hybrids.

The inherited estrogenic influence is probably not identical with the "inherited susceptibility for spontaneous mammary cancer."

Hyperplastic or precancerous nodules in the mam-

[12] J. J. Bittner, *Cancer Research*, 3: 441–447, 1943.

[13] Females of the A stock transmit the inherited susceptibility for spontaneous mammary tumors to their hybrids, but tumors do not result unless the females are used as breeders; unless an extrinsic source of esterogens is obtained.[11]

[14] Nodules have been found in breeding females of the non-susceptible C57 black stock which had received the active milk agent by foster nursing.

mary glands of mice probably result from the inciting influence of the mammary tumor milk agent and the estrogenic hormones.

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AEROSOL, A NEW METHOD OF APPLYING GROWTH REGULATORS TO PLANTS[1]

L. D. GOODHUE[2] found the aerosol method to be an excellent and efficient one for dispersing certain insecticides. One method of producing aerosol involves the use of a highly volatile liquid carrier in which the insecticide is dissolved either directly or after it has been dissolved in some other solvent. The solution of carrier, solvent and insecticide is contained under pressure in a suitable receptacle from which it can be released as a mist. The carrier immediately volatilizes, leaving the insecticide suspended in the air in an exceedingly finely divided liquid or solid state. This method of applying insecticides suggests a new means of applying growth substances to plants for the purpose of modifying development, such as delaying opening of buds, preventing abscission of flowers and fruit and aiding fruit setting. Preliminary results indicate that the method may prove of much value for such applications.

An experiment was designed to determine the effectiveness of a growth substance in setting seedless fruit on tomatoes, when dispersed as an aerosol form. For this purpose three grams of naphthoxyacetic acid were dissolved in 27 grams of cyclohexanone. This solution was placed in a steel cylinder into which 270 grams of di-methyl ether was then forced under pressure.

Ninety-six Pan America tomato plants were grown under greenhouse conditions until the first blossoms of the first cluster had opened. One half of the plants were kept in the greenhouse as controls and the remainder were held for 16 hours in an air-tight room into which aerosol containing naphthoxyacetic acid was released. The naphthoxyacetic acid was dispersed at 240 mg per 1,000 cubic feet. The plants were then taken back to the greenhouse and allowed to grow under the same conditions as the control plants. Three days later fruit enlargement was observed upon the treated plants and none upon the controls. Nine days after treatment the average number of fruit set per plant for the first cluster was 3 for the treated plants and 0.5 for the controls. The average diameter of fruits after 36 days was 2.9 inches for the treated plants and 2.1 for the controls. Ten fruits collected at random from the treated plants were all seedless.

Thirty-two additional tomato plants treated in the open air have also set fruit. In this experiment the cylinder was held at a distance of one foot from the plant and the valve was opened for about one second. The mist covered the flower cluster but was quickly carried away from it by air currents. The plants were then returned to the greenhouse. The number of fruits set per plant in the treated lot was comparable to that obtained in the first experiment. The controls in this instance failed to set fruit.

Further studies are under way to test field applicability of this method and to test various other growth substances. Tests will also be made to determine quality of the fruit developed.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SPRING-PRESSURE-CONTACT ELECTRODE FOR USE IN ELECTRO-ENCEPHALOGRAPHIC RECORDING[1]

WITH the widespread use of the electroencephalograph to survey large populations in both military and civilian medicine and with an increased difficulty in obtaining technicians for this work, it has seemed necessary to devise a fast, simple technique of electrode application.

Since Berger reported the recording of electrical potentials from the human brain by means of silver wires inserted into the anesthetized scalp, electroencephalographers have sought more efficient ways of electrode application.[2, 3, 4, 5, 6, 7, 8] Concerning the

[1] From the Department of Anatomy, University of Oregon Medical School. The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Oregon Medical School.

[1] C. L. Hamner, assistant physiologist, and H. A. Schomer, associate physiologist, Bureau of Plant Industry, Soils and Agricultural Engineering, and L. D. Goodhue, senior chemist, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, Beltsville, Md.

[2] L. D. Goodhue, *Ind. and Eng. Chem.*, 34: 1456–1459, December, 1942.