

of serum as abscissae. Equally active antigens simultaneously titrated in this way with the same serum and the same pool of complement should give identical serum-complement curves; any significant difference in slope indicates a difference in the complement-fixing activity of the antigens under the conditions of the experiment.

The figure presents a titration in which 2 samples of the purified phosphatide are compared with the standard antigen W35, which was prepared for routine diagnostic use.² Of the 2 preparations, P-1c(A) was the more highly purified; it was obtained from P-1a by repeating the purification of the cadmium salt. The serologic activity was evidently not altered by the final purification, since the 2 preparations gave identical titration curves.

The figure shows that the activity of the purified substance is closely comparable to that of the routine antigen, and it may be concluded that the new phosphatide is responsible for the complement-fixing activity of beef heart extracts with syphilitic sera. Chemical and serologic studies of the substance are being continued.

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Effect of Pseudopregnancy on Mammary Carcinoma Incidence in Mice of the A Stock.

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The inbred A stock of mice has a high incidence of spontaneous mammary carcinoma when there occurs a hormonal stimulation of the mammary tissue resulting from breeding. Females kept as virgins, however, have a relatively low incidence of breast cancer. The incidence of breeding females of a selected subline (F38-F58 generations) was found to be 96.3% and for virgin females, 4.9%.^{1, 2}

Fifty pedigreed females, 2 months of age, of a selected subline (F38-F58 generations) obtained from Dr. J. J. Bittner were placed 4 in a cage with a vasectomized A stock male. Oestrus smears were made on mice after discovery of vaginal plugs. All experimental mice were known to be in a state of pseudopregnancy at least once.

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¹ Bittner, J. J., *Pub. Health Rep.*, 1939, **54**, 1642.

² Bittner, J. J., *Cancer Research*, 1941, **1**, 113.

The average length of the pseudopregnant condition was found to be 9.9 days with a range of from 5 to 12 days. This is similar to the length of the pseudopregnant state observed in mice by Turner and Gomez.⁸ There occurred in these animals a distinct hyperplasia of the duct system and some growth of the gland lobules.

Of 43 mice which were living after the appearance of the first tumor, 11 or 25.6% developed mammary carcinoma. The first tumor appeared at 245 days of age, the last at 622 days. The average age of appearance was 14.6 months. The average age of life in months of cancerous mice was 15.7 which is approximately midway between the cancer age of virgin and breeding mice of this selected subline. The average age of non-cancerous mice was 17.5 months, which likewise is between the non-cancer age of breeding and virgin females of this strain.

All tumors were of the adenocarcinoma type. Nine of these were adenocarcinoma simplex and 2 papillary cyst adenocarcinomas.

Any attempt at this time to explain the factors operating in increasing the incidence of mammary carcinoma in mice subjected to pseudopregnancy would be premature. Certain conclusions, however, can be drawn. Production of young *per se* is not a necessary antecedent for the development of breast cancer in the A stock. Given a certain amount of mammary gland growth, produced by the pseudopregnant condition (probably comparable to the growth of the mammary gland during the first half of pregnancy) a significant increase in the breast cancer incidence occurs. Other factors accompanying pregnancy must be acting to produce the physiological state of the mammary tissue cells necessary for complete cancerous transformation within the stock.

Summary. A definite increase in the mammary carcinoma incidence occurred in females of the A stock following induced pseudopregnancy by mating with vasectomized males. Eleven or 25.6% of 43 experimental mice developed breast adenocarcinomas at an average age of 14.6 months.

⁸ Turner, C. W., and Gomez, E. T., *Missouri Agric. Exp. Sta. Research Bull.*, 1933, 182.