A STUDY OF THE SPONTANEOUS MAMMARY GLAND TUMORS OF THE MOUSE IN TISSUE CULTURES

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Investigations on the spontaneous tumors of the mouse begun in June, 1931, in collaboration with Dr. Lionel Strong of the Roscoe B. Jackson Memorial Laboratory were continued and the results prepared for publication.

For this study tissue cultures were made of 55 tumors taken from 49 mice of 7 different strains. In all 2,750 cultures were prepared in various media. Fifty-eight of these failed to grow while the others grew abundantly in chicken plasma but not as satisfactorily in mouse plasma. About 800 cultures showing extensive growths were fixed and stained as permanent records of the behavior of the malignant cells especially of their mitotic figures and chromosomes. In addition to the study of the cultures, pieces of 38 of the tumors used were fixed, sectioned, and stained so that a comparison of the behavior of the cancerous growth in vivo and in vitro could be made.

All of the tumors arose spontaneously in female mice. The tumors were located in the mammary glands and occurred in rather old mice. The growths were characteristic for the mouse, and differed from the spontaneous tumors that appear in the human being or in certain other animals in that they were all encapsulated, extremely haematophorous and of a more or less carcinomatous nature. Some were quite glandular, being almost entirely composed of only slightly atypical tubular epithelium corresponding somewhat to the adenoma of human beings. Others had large or small areas of irregular epithelial cell growth forming adeno-carcinoma and some had more continuous regions of atypical cells forming medullary carcinoma.

In the tissue cultures all of the tumors exhibited the same type of growth when the pieces were explanted into chicken plasma. It took the form of extensive membranes of epithelial cells usually quite free from any growth of stroma cells, although in a few instances where the explants had been selected from an hemorrhagic area of the tumor the cultures contained abundant rapidly growing stroma cells and only small patches of epithelial cells. The membranes grew as thin sheets of cells spread out usually only one cell thick. Where the membranes grew along the coverglass the cells were thinner than a cytological section, so that these large flat cells furnished beautiful material for the study of mitosis.

Abnormal mitotic figures are characteristic of the growth of malignant tissue both in vivo and in vitro. However, they were rather rare in the growths of the spontaneous mouse tumors, except in those from certain strains of mice.

The number of chromosomes exhibited by the dividing cells of the malignant epithelium of the mouse tumors was not definitely

increased in any one type of tumor nor in the tumors of any one strain of mice. Most of the cancerous growths had some mitotic figures with an increased number and some with the normal number of chromosomes.

In all of the cultures it was clearly evident that the chromosomes became split longitudinally early in the division of the malignant cells so that in the late prophase and in the metaphase they appeared as a pair of chromosomes rather than as single ones. This phenomenon took place in the carcinoma cells that exhibited an increased number of chromosomes as well as in those that had the normal number.

ON THE EARLY DEVELOPMENT OF THE MOUSE EGG

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An analysis of the material obtained by W. H. Lewis and E. S. Wright in Baltimore on the early development of the mouse egg was partially completed at Mount Desert Island Biological Laboratory. Nine hundred and one eggs were recovered; 819 were from mice that had copulated and 82 were from non-copulators or from mice without males. Of the 819 eggs recovered after copulation, 641 were normal fertile eggs, 14 probably normal ones were injured, 26 were unfertilized, 7 were distorted but probably fertilized, 58 were fragmented non-fertilized, 29 were immatures, viz. without the first polar body and with the vitellus in contact with the zona, 16 were small opaque browns, 11 were zonas only, the vitellus having entirely disappeared, 12 were unclassified abnormals and 5 were degenerate. Of the 82 eggs from non-copulators and mice without males, 60 were normal unfertilized eggs, 4 probably normal one were injured, 8 were immatures, 5 were fragmented and 5 were degenerate.

Our observations indicate that mating may occur at any time during oestrum which lasts from 1 to 3 days. Sperm were found in the upper end of the tube 15 minutes after copulation. Ovulation may occur during early, mid or late oestrum. Animals killed ¼ to 1½ hours after copulation (in early, mid or late oestrum) had freshly ovulated eggs, hence we conclude that there is probably a definite relation between copulation and ovulation.

The first polar body is given off about the time of ovulation and usually degenrates. The second polar body is not given off until after fertilization and may persist to the blastocyst stage.

The first cleavage into two cells occurs at 24 to 27 hours after copulation or ovulation. About 12 hours later the next cleavage into four cells occurs. Between 50 and 60 hours after copulation most of the eggs have arrived at the 8-cell stage. At 72 hours all the eggs are in the morula stage or beginning blastocyst stage. Some of the morulae are complete and have 32 cells, others have 16 to 32 cells. Cleavage is dichotomous.

The eggs pass rather rapidly to the second loop of the tube and remain there in the one-cell stage for about 24 hours. About 12