WHOLE MOUNT PREPARATIONS OF RAT AND MOUSE MAMMARY GLANDS

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Whole mount preparations of rat or mouse mammary glands are widely used to study changes in the structure of these glands developing in the pretumor period [1,2]. However a great deal of time is involved in making such preparations. Usually several days are required for fixation, washing free from the fixation fluid, staining, differentiation, mounting etc.

The epithelial structures of the mammary glands may be demonstrated in the unfixed condition by means of a saturated aqueous solution of the vital nuclear stain thionine.

In an attempt to accelerate the procedure for making total preparations of mammary glands we have attempted to carry out a simultaneous staining and fixation. Having experimented with various concentrations of formalin from 11 to 33%, and of thionine (from 1 to 10 ml of a saturated aqueous solution of dye to 100 ml of aqueous formalin solution) we found conditions at which staining of epithelial structures of the mammary glands occurs rapidly during the time that the preparation is being fixed.

Whole mount preparations of mammary glands of mice and rats were prepared as follows.

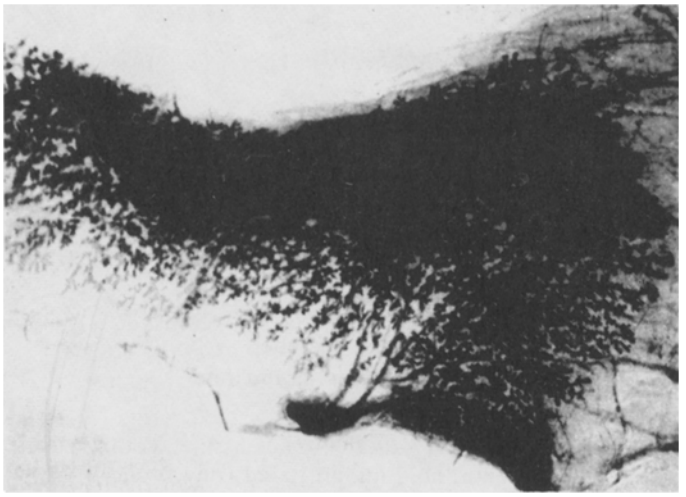
By blunt dissection the fat "cushion" containing the first and second inguinal mammary glands were dissected free from the muscles of the anterior abdominal wall and skin, and were spread out on an object glass. The preparation obtained in this way was dried in air for 2-5 min during which time it became firmly fixed to the glass. Then the preparation was immersed for 24 h in a solution of thionine in 20% formalin (6 ml of a saturated solution of the dye per 100 ml of fixative). After the preparation had been washed free from the dye and fixative for 1 h it was mounted in glycerine-gelatine by Edinger's method, and examined without a cover slip.

The quality of the preparations showed no appreciable change if they were kept in the same solution for 1-10 days before immersion in glycerol-gelatine. The best staining occurrred in the first few days. The epithelial structures of the mammary glands were quite clearly and selectively stained blue after only 5-6 h. In contrast to the epithelium of the mammary glands the connective tissue films and fatty tissue remained practically unstained. Under the microscope such whole mount preparations showed metachromatically stained mast cells lying along the vessels and nerves, and along the ducts and alveoli of the mammary glands.

Thus we obtained whole-mount preparations of the mammary glands of young, sexually mature, or pregnant rats and mice, and also of rats submitted to the action of various hormones (see figure).

In these preparations besides the epithelial structures the tissue of the inguinal lymphatic nodes could also be seen. In such cases when these structures interfered with a thorough examination of the preparation, the connective tissue layer above them could be dissected out by means of a razor, and the lymphatic nodes carefully peeled off. The fact that the lymphatic nodes were stained may possibly turn out to be useful in a study of the condition of lymphoid tissue.

Any part of the stained preparation before it is finally immersed in glycerol-gelatine may be cut off, treated histologically and stained by hematoxylin-eosin.



Total preparation of inguinal mammary gland of female rat; injection of 50 μg estriol and 100 mouse units of chorionic gonadotropin was injected daily between the 10th and 30th day of life. The following structures are stained: the nipple, the branching ducts, alveoli, and the terminations which are characteristic of the growing gland; the darkly stained mass in the center of the gland is a group of inguinal lymph nodes. Micrograph. Stained with thionine, and simultaneously fixed. Magnification 4.2.

Our experiment shows that the use of this method considerably shortens the period elapsing between the moment the tissue is taken and the time the whole mount preparation of the rat or mouse mammary gland is made. The incidental staining of the lymphatic nodes and mast cells leads us to suppose that the method of simultaneous staining and fixation may be useful also in connection with other morphological studies.

LITERATURE CITED

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2. E. E. Pogosyants, Arkh. pat., 9, No. 2 (1947), p. 64.