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Initiation of Lactation in the Albino Rat with Lactogen and Adrenal Cortical Hormone.

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The lactogenic hormone has been proved capable of initiating lactation in a number of animal species. In the rat, however, either slight or negative results have been reported. The early work has been summarized by Turner[1] and Nelson.[2]

Two possibilities might account for this lack of functional responsiveness: first, that the experimentally stimulated mammary glands of the rat are not developed to a state comparable to that which takes place during pregnancy and, secondly, that the pseudo-pregnant rat is non-responsive to lactogen.

Our previous observations,[3] however, demonstrated that secretory activity could be induced in the mammary glands of pseudo-pregnant rats by the injection of lactogen, pituitary glands from spayed rats, or glands from normal female rats. A fair amount of lactation was observed following the injection of pituitary glands from normal and ovariectomized rats which had been injected previously with estrogen. In not one instance, however, was the induced lactation comparable to that which occurs in the post-partum rat. Copious lactation was induced by injecting 30 mg of an acetone-dried powder made from cattle pituitaries into pseudo-pregnant rats which had been injected daily with 200 i.u. of estrogen during the pseudo-pregnant period.

Further attempts have been made to induce lactation in the pseudo-pregnant rat. Pseudo-pregnancy was induced by injecting 100 r.u. of a gonadotropic principle (Folluteinâ€) into each of a group of multiparous rats.â€¡ The injection was repeated 6 days later. On the 12th day following the induction of pseudo-preg-

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1 Turner, C. W., *Sex and Internal Secretions,* 1932, chapter 12 (E. Allen, ed.), Williams and Wilkins Co., Baltimore.

2 Nelson, W. O., *Physiol. Rev.*, 1936, **16**, 488.

3 Reece, R. P., *J. Dairy Science,* 1938, **21**, 117.

† The Follutein and Prolactin used in this study were kindly supplied by Dr. John F. Anderson of E. R. Squibb and Sons.

‡ We are indebted to Dr. W. C. Russell for the rats employed in this work.

nancy the animals were ovariectomized and a mammary gland removed. Various hormone preparations were injected subcutaneously every 8 hours until 6 injections had been made. The acetone-dried powder (designated hereafter as A.D.P.) employed in this work was made from cattle pituitaries and suspended in distilled water so that 0.1 cc of the suspension contained 5 mg of the powder. The lactogenic hormone (Prolactin†) was in an aqueous solution of a pH 8 and contained 40 bird units (Riddle, Bates, and Dykshorn[4] unit) per cc. The adrenal cortical hormone (Eschatin) contained at least 25 dog units per cc. The dosages stated indicate total dosage. Three or 4 hours following the last injection the animals were sacrificed and their mammary glands observed macro- and microscopically. The mammary glands were rated according to the method used by Gardner and Turner.[5]

Nine control animals received the same treatment as the experimental animals with the exception of the injections following ovariectomy. The mammary glands of most of the control and experimental animals were well developed on the 12th day of pseudopregnancy. Secretory activity had begun in the mammary glands of 2 control animals at the time of ovariectomy. In the other 7 control animals there was no evidence of secretory activity. At the time of sacrifice the mammary glands of the control animals were rated (—). Histologic section showed, however, that secretory activity had been induced in the mammary glands of several of the control animals.

Three animals were injected with 30 mg of A.D.P. The mammary glands were rated (—). Two rats received 100 mg A.D.P. and 2 others 200 mg A.D.P. The mammary glands of both groups were rated (++).

Four animals were injected with 100 mg A.D.P. + 15 dog units of the adrenal cortical hormone and 4 additional animals were injected with 100 mg A.D.P. + 15 d.u. of the adrenal cortical hormone + 3.0 cc of a 20% glucose solution. The average rating of the mammary glands of these animals was (++).

Five rats received 100 bird units of the lactogenic hormone + 15 d.u. of the adrenal cortical hormone + 3.0 cc of a 20% glucose solution. The mammary glands of these animals were rated (++ to +++).

Five animals received 150 b.u. of lactogen + 24 d.u. of the adrenal cortical hormone. In this group the mammary glands were rated

[4] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

[5] Gardner, W. U., and Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bul.*, 1933, 196.

(+++). The mammary glands had the appearance of being in full lactation and on section the alveoli and ducts were found to have been distended with milk.

Conclusions. It is possible to initiate lactation in the pseudo-pregnant rat by injecting lactogen + the adrenal cortical hormone or lactogen + the adrenal cortical hormone + a 20% glucose solution. It would seem, therefore, that the refractoriness of the mammary glands of the pseudo-pregnant rat to lactogen can be explained on the assumption that another hormone becomes the limiting factor in milk secretion before lactogen can fully express itself.

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Effect of Pectin on Bacterial Growth.

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Several years ago we became interested in pectin when it was suggested[1] that this substance appeared to exert a germicidal action on intestinal organisms. We have tried various technics for studying different concentrations of numerous commercial pectins of different grades at various H-ion concentrations. From these studies we have developed a testing technic that appears to be satisfactory.

It is necessary to test different pectins under the same conditions to obtain comparable results. Since the higher the concentration the more difficult it is to dissolve the pectin and since the more insoluble pectins are dissolved only by severe heat treatment, which tends to break down the pectin molecule, we have standardized on a 1% pectin solution in nutrient broth. However, we have tested the more soluble pectins at concentrations up to 5% with similar results.

Each pectin was dissolved with minimum heat treatment in sterile nutrient broth of pH 6.5. These pectin broths had low pH's, ranging from 5.5 to 3.9. At the "natural" pH thus obtained, we studied the growth of *Esch. coli.* Similar studies were conducted on each pectin broth adjusted to pH 6.5, which adjustment was made at room temperature to eliminate degradation of the pectin by heat and alkali. As controls for each pectin, we used nutrient broth adjusted to the corresponding "natural" pH and also nutrient

[1] Personal communication from Dr. C. A. Tompkins.