Induction of Mammary Growth and Lactation in Rabbits with Epinephrine, Acetylcholine and Serotonin.* (25776)

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Recently we reported that numerous drugs, neurohormones, nonspecific stresses and other factors could induce mammary growth and lactation in estrogen-primed rats, and that some of these could maintain mammary secretion in postpartum rats after litter removal (1-7). These agents are believed to evoke release of prolactin and probably ACTH from the anterior pituitary *via* autonomic and central nervous system. Epinephrine, acetylcholine and serotonin were among the most effective agents used and are of particular interest since they are natural substances in the body, and may normally mediate some effects of ovarian hormones or of other physiological stimuli in producing mammary growth and lactation. It was of interest therefore to see whether these 3 agents could induce milk secretion and mammary growth in the rabbit, since in pseudopregnant rabbits such responses are readily elicited by prolactin injections(8).

Methods. A total of 38 female, predominantly New Zealand White rabbits, ranging from 3.1-4.4 kilos, were used. All rabbits were first injected subcutaneously with 0.2 mg estradiol in corn oil daily for 15 days. For the next 5 days they were each injected subcutaneously as follows: controls (11 rabbits), 0.85% saline once daily; epinephrine in oil‡ (8 rabbits), 0.25 mg/kg BW twice daily; acetylcholine iodide§ (8 rabbits), 12.5 mg/kg BW twice daily; serotonin creatinine sulfate‖ (11 rabbits), 5 mg serotonin base/kg BW once daily. On day after last injection (21st day of experiment), attempts were made to express milk manually from nipples of each rabbit, and results recorded. Rabbits were then killed and mammary glands exposed for gross observation. Ratings of 0-5 were assigned each rabbit for mammary growth and lactation, depending on degree of mammary development and amount of milk present. Mammary tissue was also removed and prepared by standard histological technics for microscopic examination.

Results. Milk could be expressed from nipples of only 1 of 11 saline-injected controls, and this animal also showed slight lobulo-alveolar development. An insignificant volume of thin watery secretion could be expressed from nipples of 3 of the other 10 controls, and only extended ducts and end buds could be seen in the mammary glands. Milk was expressed from nipples of 6 out of 8 epinephrine-treated rabbits, and they were assigned an average lactation rating of 2. Good lobulo-alveolar mammary development was present in these rabbits (avg rating = 2.7). Microscopically, the alveoli were distended with secretion. Acetylcholine-injected rabbits showed a lesser response, with gross lactation in 5 out of 8 (avg rating = 1.5) and only fair lobulo-alveolar growth (av. rating = 2). The best responses were seen in serotonin-injected rabbits, 9 out of 11 showing gross lactation (avg. rating = 2.8) and good to excellent lobulo-alveolar growth (avg. rating = 3.1). In general, microscopic examination of stained sections of mammary tissue from these rabbits confirmed gross observations. Mammary responses to different treatments are shown in Fig. 1-4.

Intense salivation was usually observed in acetylcholine-injected rabbits a few minutes after injection of drug. In preliminary trials with higher doses of acetylcholine, convulsions were frequently seen and often were followed by comatose state or death. No obvious symptoms were noted following epinephrine or serotonin administration.

Discussion. The present results in rabbits demonstrate that epinephrine, acetylcholine

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‡ Parke, Davis and Co.

§ Eastman Organic Chemicals

‖ Nutritional Biochemicals Corp.

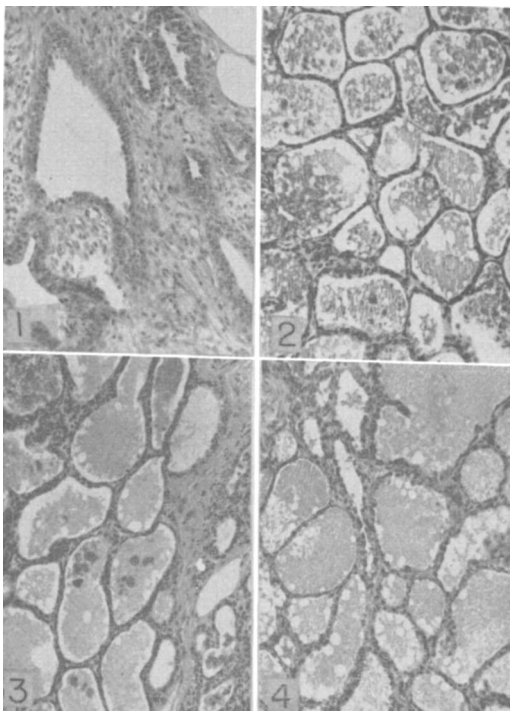

FIG. 1-4. Histological sections from mammary glands of estrogen-primed rabbits treated for 5 days with (1) saline, (2) epinephrine in oil, (3) acetylcholine iodide, (4) serotonin creatine sulfate. × 70.

and serotonin can produce lactation and mammary growth in estrogen-primed rabbits, as previously reported in estrogen-primed rats. Gross lactation was observed in ducts and lobules of these rabbits and milk could readily be expressed from the nipples, whereas only histological evidence of mammary secretion was seen in rats. Although amounts of milk noted in mammary glands of these rabbits were not equal to that usually seen after parturition, it is probable that better initial development of mammary glands, and perhaps longer treatment with drugs, would have produced greater milk production. The 3 agents produced mammary lobulo-alveolar growth as well as lactation, since only ducts and end buds were present in the saline-injected controls.

The mechanisms by which the 3 drugs induced prolactin release from the anterior pituitary remain to be determined. That anterior pituitary is essential is shown by negative mammary responses to these 3 drugs when injected into rats hypophysectomized after estrogen-priming (unpublished observations). They may act directly on the anterior pituitary, on hypothalamus or on other parts of CNS. Rat hypothalamic tissue has recently been shown to induce mammary secretion in intact, estrogen-primed rats(5), which suggests but does not necessarily prove that the hypothalamus contains a prolactin-releasing factor. On the other hand, there is considerable evidence that the hypothalamus normally inhibits prolactin release(9), and the 3 agents may therefore depress the hypothalamic center or centers concerned with prolactin release. Participation of the autonomic nervous system in release of prolactin is suggested by reports that bilateral cervical sympathectomy in the goat leads to marked and prolonged inhibition of milk secretion(10), and interruption of sympathetic pathways prevents induction of pseudopregnancy in rats by electrical stimulation of the uterine cervix(11).

Summary. 1. Thirty-eight mature female, predominantly New Zealand White rabbits, were injected subcutaneously daily with 0.2 mg estradiol for 15 days to develop the mammary duct system. They were then divided into 4 groups and injected subcutaneously as follows for 5 days: 0.85% saline; 0.25 mg epinephrine in oil twice daily; 12.5 mg acetylcholine iodide/kg BW twice daily; 5 mg serotonin base/kg BW once daily. On 21st day the rabbits were killed and mammary glands examined for growth and lactation. 2. Only 1 of 11 saline-injected rabbits showed gross lactation and lobulo-alveolar development, in contrast to 6 of 8 epinephrine-treated rabbits, 5 of 8 acetylcholine-injected rabbits and 9 of 11 rabbits treated with serotonin. The latter drug induced the greatest, and acetylcholine the least degree of mammary growth and lactation. 3. These results demonstrate that the 3 drugs in pharmacological doses can induce prolactin release from the anterior pituitary of rabbits.

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Secretion of Dietary Strontium 90 and Calcium in Human Milk.*† (25777)

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The preferential utilization of calcium over strontium by man and many species of animals has been well studied; the concepts and magnitude of the differential behavior have been generally established(1-7). For the cow and goat under usual management, it has been shown that Sr/Ca ratio in the milk is about 0.1 of that in the diet, with values from various parts of the world falling well within the range 0.09 to 0.16(4,6,7). No direct evidence, to our knowledge, has been reported for strontium-calcium relationships between diet and human milk. It seemed desirable to obtain data on this point (a) to see if the same general relationships hold for man as for animals, since much information is being acquired under carefully controlled conditions in animals and any basis for judicious extrapolation to man might be helpful; (b) to permit estimations or predictions of Sr^{90}/Ca ratios or Sr^{90} levels in human milk from knowledge or predictions of dietary levels; and (c) to permit estimations of relationships between Sr/Ca of diet and Sr/Ca of the body in healthy individuals; this type of information has been difficult to obtain. It became possible to measure these relationships in normal healthy women by making use of Sr^{90} levels that existed in our diet during May 1959. In principle, it appears that calcium and strontium of milk are derived under normal conditions largely from calcium and strontium of the contemporary diet with a small contribution from skeletal reserves, and this most likely from regions that have been labeled recently (4,8). Thus, a study was done in which a known diet was fed to 5 women starting immediately postpartum; after a conditioning period of about 20 days, a 5-day collection of food equivalent to that eaten and a 5-day collection of all milk were obtained for analysis.

Methods. Each of the 5 subjects began 30-day participation immediately postpartum. The program was as indicated in Table I. If necessary, lactation was enhanced by pumping the breasts to assure a good volume of milk secretion and an active lactation for at least 30 days. Breast milk secreted during the last 5 days of the 30-day period was collected for analysis. a) *Dietary control.* The hospital diet was consumed during the first 5 or 6 days postpartum. On leaving the hospital the subjects went to an apartment where they were

* Actual data in this experiment refer essentially to (Sr^{90}/Ca of extracellular fluids)/(Sr^{90}/Ca of diet) during period of observation. The same $OR_{body/diet}$, however, would be expected to hold over most, if not all, of the life of the individual, as shown by previous animal experiments where short-term double tracer studies have been compared with lifetime feeding(1,2).

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