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The Direct Mammotrophic Action of Lactogenic Hormone*

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In 1937 during a discussion of methods used for assaying the hypophysial lactogenic hormone,[1] the writer spoke of having induced localized, sector lactation in rabbits by intraductal administration of that hormone. It had been shown earlier,[2] that one could compare at least two lactogenic preparations by injecting them locally over the crop sac areas of pigeons. In the New Zealand White rabbits used by us there are usually 8 nipples, each of which has on an average 6 main galactophores emptying to the exterior. Therefore, 48 different sectors in a single animal are available for local assays of the mammotrophic principle. Further study of this problem has revealed that considerable variability in reaction to lactogenic hormone exists between different sectors, undoubtedly due to such variables as (1) the area over which a given volume of hormone is spread (2) the ratio of secretory to duct tissue, (3) the speed and degree of absorption, (4) the blood supply, (5) the amount of reflux, (6) plugs formed, or non-patent areas in some sectors, (7) ruptures occurring in certain sectors with small capacities, (8) cysts or sacculated lobules proximal to the nipple in which the bulk of the injected fluid may accumulate. Because of the large number of sectors in a single rabbit, and because the minimal effective local dose is slightly less than 1 I.U. as compared with the minimal effective systemic dose of about 75 I.U., some of these variables may be controlled by injecting at least one sector in each gland and using for assay purposes only those sectors showing the least difference in capacity, area, uniformity of fluid distribution, etc.

In preliminary experiments it had been possible to cause limited lactation by local introduction of lactogenic hormone even in virgin rabbits with relatively few alveoli on a good duct system. Sector lactation was also obtained in both male and female rabbits pre-treated with estrone-progesterone combinations and in pseudo-pregnant does. In the present series, virgin rabbits, 4 to 5 months old, hysterectomized and oöphorectomized in connection with a separate investigation were used. They were injected subcutaneously 5 days weekly (Monday through Friday) for 4 weeks with 20 μg of estrone and 1 mg of progesterone, separately administered in 0.1 cc of sesame oil. Since the mammary glands of most of these rabbits already showed some duct development at the time of oöphorectomy, 4 weeks' treatment with estrone and progesterone was enough to cause good prolactational growth.

On the third day after the last injections of estrone and progesterone, the rabbits were injected intravenously with 200 mg of nembutal and the hair shaved from the mammary region. With the aid of a binocular-dissecting microscope, test or control solutions were injected into individual sectors using a 1.0 cc tuberculin syringe and a 27 gauge hypodermic needle cut down to about 1.0 cm in length and without a bevel. First, however, the skin and underlying mammary gland were squeezed gently between the thumbs and fingers in such a way as to press out through the nipple pores any accumulated fluid. In the estrone-progesterone treated animals, this was usually of a clear serous nature, but occasionally was slightly turbid. The keratin scale was removed from the nipple with moist cotton and any plugs found occluding the ducts gently massaged to the exterior. Sectors from which a few drops of fluid could be squeezed without trouble were found best

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1 Lyons, W. R., *Cold Spring Harbor Symposia on Quant. Biol.*, 1937, **5**, 197.

2 Lyons, W. R., and Page, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1049.

able to receive and retain the injected hormone. If the needle pierced the duct epithelium, this was revealed by a uniform accumulation of fluid around the base of the nipple before 0.1 cc had been injected. When this happened another duct was tried, preferably in another nipple. If a plug had formed in the main duct or if the duct happened not to be completely canalized, the injection fluid accumulated before 0.1 cc was injected between the nipple and the block. In this event, such a sector was also abandoned. Care in the preliminary emptying of a sector, cleaning the nipple, and especially in introducing the needle to the proper depth, obviates this trouble. In the tests reported herein, only one sector was used for a single dosage of hormone, but in most instances one or more sectors were injected with a control solution of 2% butanol (the concentration used in preserving the hormone). It had been learned that control sectors only lactated when a test sector had received upwards of 1.0 mg of hormone. In such instances, all of the glands lactated, thus indicating a general systemic effect.

TABLE I.

Mammary Sector Reactions in Oöphorectomized Virgin Rabbits Pre-treated with Estrone and Progesterone and Then Injected Intraductally with Varying Amounts of Lactogenic Hormone in 1.0 cc of a 2% Butanol Solution.*

No. of animals	Dosage of hormone		Reactions	
	mg	Approx. I.U.	—	+
6	.012	0.37	6	0
6	.025	0.75	3	3
6	.05	1.5	5	1
6	.1	3.0	0	6
6	.2	6.0	0	6

*At least one other gland of each rabbit was injected with a control solution of butanol and in no instance was lactation induced.

The rapid absorption of the injected water of control and test solutions was readily observed. The gland could be seen to become distended when 0.5-1.0 cc was injected and within a few minutes, the sector appeared normal again. Milk has been expressed from a test sector as early as 24 hr after the injection. It was found, however, that the amount of milk formed after a single injection, increased until about the fifth day and that sometimes, the accumulated milk remained until the tenth day or longer. Thus, although a shorter test could be used it was thought better in this study to judge the reaction on the fifth day when the positive effect of border-line doses was more easily determined. The accompanying table contains the data from a typical experiment in which 30 rabbits were used. One sector of a single mammary gland in each rabbit was injected with 1.0 cc of a 2% butanol solution containing a given amount of lactogenic hormone containing approximately 30 I.U. per milligram. Five different levels ranging from 0.012 to 0.2 mg were tested in each case in 6 different rabbits. Each rabbit provided its own control glands, at least one of which was injected with 2% butanol. The lactogenic hormone was given in a single injection, 3 days after the last estrone-progesterone treatment. Attempts were made to express milk from the test and control glands every 24 hr, beginning 48 hr after the hormone was injected. A reaction was considered positive if by the fifth day, milk could be expressed from a sector. In this experiment, confirmation was made in some cases by cutting back a skin flap and judging directly the reaction in the gland with the help of a binocular dissecting microscope. This provided an opportunity for taking specimens for histologic study and also permitted comparison of the injected sector with adjacent uninjected ones.

Results and Discussion. As shown in Table I, all of the sectors injected with 6 and 3 I.U. of lactogenic hormone lactated. Five of 6 on the 1.5 I.U. level, 3 of 6 on the 0.75 I.U. level and none of the 6 on the 0.37 I.U. level gave a positive response. None of the control sectors lactated. It was always possible to express milk from the positive sectors within 48 hr of the injection of the higher doses. With the border-line dose of 25 μg the secretion was turbid at the 48th hr, but changed to milk between the third and fifth day in half of the animals. It was possible to observe through the skin the gradual accumulation of milk in the injected sectors only. That the hormonal effect lasted for several days was shown by expressing the

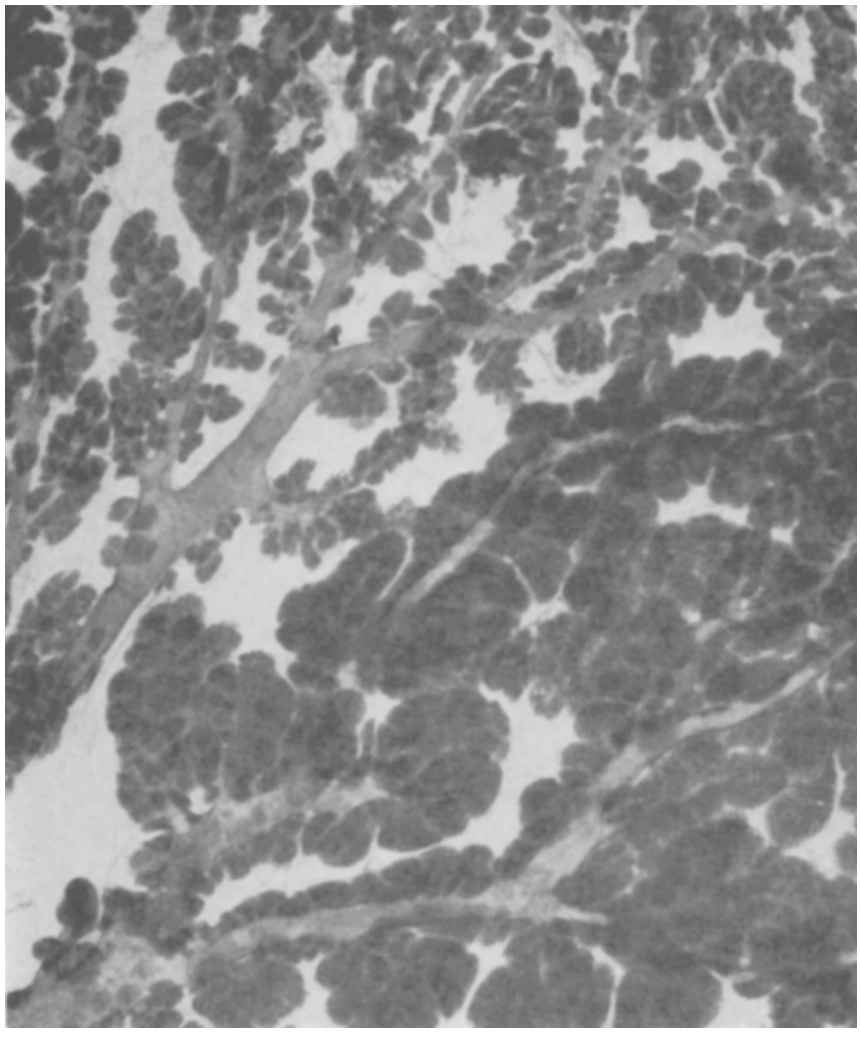

FIG. 1.

Two sectors of a mammary gland of an oöphorectomized rabbit, pre-treated with estrone and progesterone, and then given 1.0 cc (3 I.U.) of lactogenic hormone into the main duct (lower left) of the bottom sector. Milk could be expressed from this sector 48 hours after the injection, and the gland was removed 24 hours later, fixed in formol stained in alum-carmine and cleared in methyl salicylate. No other sector of this or other glands lactated. The prolactational growth seen in the upper control sector is in striking contrast to the additional, lactational growth induced by the local administration of lactogenic hormone. × 6.

milk from these sectors daily and observing its re-accumulation for at least a week. Whether this be due to an initial "trigger-action" of the hormone, or to effective residual amounts of it continuing to bathe the secretory units cannot be decided at this time.

Fig. 1 shows representative areas of 2 separate sectors in a mammary gland of a rabbit pre-treated with estrone and progesterone as specified above and then given 0.1 mg (approximately 3 I.U.) of lactogenic hormone in 1.0 cc. The large duct in the lower left corner is the main galactophore of the injected sector. As may be seen, the lower or injected sector was in full lactation when this gland was removed 3 days after the injection was made. The upper or control

sector shows only the prolactational growth induced by the estrone-progesterone treatment. Before sacrificing the animal it was possible, by pressing the area of the gland shown in the figure, to observe milk exude from the injected pore in contrast to a clear serous fluid from the upper sector. However, this manipulation was quite unnecessary since the contrast between the positive and negative sectors was even more striking as seen through the skin than in the accompanying figure.

Histologic study of the glands used in these experiments was especially interesting because it was possible to compare in a single section lactating and non-lactating lobules from adjoining sectors. Such sections were found valuable in contrasting the prolactational growth induced by the estrone and progesterone treatment, and the additional, lactational growth brought about by lactogenic hormone. That the latter should be considered a mammary growth-promoting hormone is maintained for the following reasons: (1) the number of epithelial cells forming the circumference of alveoli growing under the influence of lactogenic hormone is several-fold that of the control alveoli; (2) a large number of the alveolar cells are cast off in the formation of the first milk and are replaced by newly-proliferating cells; (3) mitotic figures may be observed in the alveolar epithelium during the lactational growth phase, and the probability of amitotic proliferation must also be entertained; (4) there are not only more cells per alveolus during this growth phase, but they are, at certain stages of their cycle, larger than the cells of the resting alveoli; (5) when full lactation has set in, the secretory cells have to be constantly renewed either in their entirety or—as is more usually the case—in their supra-nuclear cytoplasm only. The directly-stimulated sectors were also studied in their regressive stages and the decrease in the number and size of the secretory cells after the withdrawal of the hormonal influence was just as striking as their increase when hormone was added.

Summary. Thirty oöphorectomized, virgin rabbits, injected subcutaneously 5 days weekly for 4 weeks with 200 I.U. of estrone and 1 I.U. of progesterone showed prolactational mammary growth approximating that of a 3-weeks' pregnant rabbit. Three days after the last of these injections, 5 different levels of lactogenic hormone were tested on groups of 6 rabbits. The hormone was given intraductally in such a way that an individual sector in each animal received a constant volume of fluid (1.0 cc). Of the different doses of hormone tested, 6 and 3 I.U. caused localized sector lactation in all 6 animals; 1.5 I.U. in 5 of 6; and 0.75 I.U. in 3 of 6 animals. None of the 6 animals injected with 0.37 I.U. lactated; nor did the uninjected or control-injected sectors in any animal lactate. This experiment demonstrates for the first time that the hypophysial lactogenic hormone has a direct mammotrophic effect just as it was previously shown to have a direct crop sac stimulating action. With all other factors essentially equal in any 2 adjoining sectors in a mammary gland, the mere bathing of the parenchyma of one with 25 μg of this purified protein brought about the complex cell-growth and cell-renewal processes of lactation, whereas the introduction of a control solution or no treatment whatsoever, caused no detectable change in the other.

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