CXCII. THE INFLUENCE OF HORMONES ON THE SECRETORY ACTIVITY OF THE REGRESSING MAMMARY GLAND

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IN a previous communication [Grant, 1936] it was reported that only a limited secretion of milk, of low lactose content (0.4%), was reinstated when the regressing mammary glands of female guinea-pigs were subjected to the influence of the anterior pituitary lactogenic hormone, prolactin, in daily amounts of 45–80 pigeon crop-sac units [Riddle *et al.* 1933]. In the present investigation it has been found that amounts up to 800 Riddle units of prolactin daily are equally ineffective in inducing the secretion of normal amounts of milk possessing a normal lactose concentration.

The acinar tissue of such regressing mammary glands requires to be re-conditioned before it can again enter upon another cycle of secretion. This has been accomplished by the administration of the hormones oestradiol and progesterone. These hormones, given together, stop the limited secretion of the regressing mammarys, which then undergo a development of the nipple and duct regions. This is followed by a pronounced thickening of the gland itself, which occurs after the injection of the sex-hormones has been discontinued, the mammae being enlarged until they are again comparable in size to those of the guinea-pig at parturition.

Prolactin administered at this stage was found to be considerably more effective, in that the milk secretion was remarkably increased, and the milk secreted possessed a lactose content of 1.7–2.7%.

Oestradiol alone was less effective than the oestradiol plus progesterone in this reconditioning of the regressing mammarys for response to prolactin.

EXPERIMENTAL

Normal multiparous guinea-pigs were separated from their litters 1 week after the onset of lactation. The involution of the mammary glands was allowed to proceed for 3 or 4 days until only small amounts of milk were being secreted daily. The mammarys of these animals are in the early stage of involution, most of the acinar tissue being still present though the majority of the cells resemble the non-secreting type described by Loeb & Hesselberg [1917]. Only a few of the cells are undergoing the "solution process". The gland in this state secretes only small amounts of milk or cloudy fluid daily (up to 0.02 ml.) and has been termed the "regressing" gland. In some cases it is necessary to remove the epithelial cap, which forms and closes the opening of the nipple, with the aid of a little alcohol.

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Effect of prolactin on the regressing mammae

The effect of prolactin on the regressing mammary glands of these animals was followed after the injection of the hormone subcutaneously in amounts varying from 200 to 800 Riddle units daily. The mammae were stripped of milk twice daily, morning and night, and the amount of milk secreted measured by collection in a calibrated capillary micro-pipette. As little as 0.005 ml. of milk could be detected in this way. In a few instances the lactose content of the milk was determined, using the differential fermentation method [Grant, 1935].

The response of the different animals to the prolactin injections is shown in Fig. 1. In two of the five animals (curves 1 and 2) the prolactin caused a detectable increase in the daily secretion of milk. The other animals (curves 3, 4

Fig. 1. The effect of prolactin in maintaining or increasing the secretory activity of the regressing mammary glands of female guinea-pigs.

and 5) continued to produce small amounts of milk during the period of the prolactin injections in contrast to non-injected animals, in which milk secretion at this stage was replaced by cloudy or clear serous fluid in 1 or 2 days. In the case of animal 1 the litter was separated on the second day after delivery and the injection of prolactin commenced on the fourth day, at which time the milk secretion had practically ceased (0.005 ml./day). Lactation was reinstated in this animal and maintained for over 6 days, but only to a limited extent notwithstanding the injection daily of 200 Riddle units of prolactin. With such

small amounts of milk the lactose content is very low (No. 1, 0.4 %; No. 2, 0.8 %). The prolactin used was prepared from ox anterior pituitaries by the method of Bates & Riddle [1935].

Reconditioning effect of oestradiol and of oestradiol plus progesterone

The effects of oestradiol alone and of oestradiol plus progesterone in reconditioning the acinar tissue of the regressing mammary glands for another cycle of secretion are shown in Fig. 2. The hormones were injected subcutaneously in solution in sesame oil, in the following amounts daily:

(a) Oestradiol benzoate, 500 γ for 7 days.

(b) Oestradiol benzoate, 500 γ for 3 days, then 900 γ for 4 days.

(c) Oestradiol benzoate, 500 γ for 3 days, then 500 γ plus 400 γ progesterone for 4 days.

In these experiments the small amounts of milk secreted by the regressing mammarys were replaced by clear fluid on the first or second day following the administration of the hormones. During the period of the injections (7 days) a marked development of the nipple and duct regions of the glands occurred. After the injections were discontinued, a gradual thickening of the regressed glands was noticed and this continued for from 11 to 15 days, at which time a slight milk secretion occurred spontaneously, presumably as a result of prolactin liberated from the animal's own pituitaries.

In the case of the animals treated with oestradiol, followed by oestradiol plus progesterone, the mammarys had enlarged to the degree found in the preparturient guinea-pig at term. The development was much less extensive for those animals treated with oestradiol alone, even when the amount of this hormone was increased to compensate for the amount of progesterone administered. The higher amount of oestradiol appeared more toxic, and one of the animals died at the end of the injection period.

Prolactin (80 Riddle units daily) was remarkably effective in stimulating milk secretion from this reconditioned acinar tissue of the mammarys in those animals which had received the oestradiol plus progesterone (Fig. 2, curves 1

Fig. 2. Effect of prolactin in increasing the secretory activity of the regressing mammary glands of female guinea-pigs which have previously been injected with oestradiol benzoate (500 γ daily for seven days) and progesterone (400 γ daily the last four days).

and 2), in contrast with its limited effect on the mammary tissue in the regressing state (Fig. 1, especially curves 1 and 2).

A much less extensive response to the lactogenic hormone was obtained from the mammary glands of the animals which had received treatment only with the oestradiol (Fig. 3, curves 1-5). The simultaneous administration of a high dose of progesterone did not have any effect in augmenting the response to prolactin (Fig. 3, curve 4).

Fig. 3. Effect of prolactin in increasing the secretory activity of the regressing glands of female guinea-pigs which have previously been injected with 500 γ oestradiol benzoate daily for a period of seven days.

* In this instance the animal was given oestradiol benzoate 500 γ for the first three days, then 900 γ for the next four days (compare Fig. 2).

There was only a negligible amount of reconditioning of the acinar tissue of the regressing gland by the injection of the gonadotropic hormone (Antex, Leo, 80 units daily for 3 days) alone, or together with prolactin.

DISCUSSION

The results obtained in the present investigation confirm and extend those reported previously, in that prolactin in such large amounts as 800 Riddle pigeon crop-sac units daily caused only a limited milk secretion in the regressing

mammary glands of female guinea-pigs. It has been possible to recondition these inactive acinar cells of the regressing gland for another cycle of secretion by the administration of oestradiol plus progesterone to these animals. This effect was greater with the two hormones than with the oestradiol alone. The glands became enlarged to an extent comparable to that seen in the guinea-pig at parturition, and prolactin given at this stage initiated a considerable secretion of milk, of a more normal lactose content (1.7–2.8 %). The amount of milk obtained varied considerably, even with different glands from the same animal. For example, on the day of maximum response from one of the animals previously conditioned with oestradiol plus progesterone (Fig. 2, curve 1), 0.60 ml. of milk was secreted, 0.45 ml. from the right mamma, compared with 0.15 ml. from the left gland. The second animal in this experiment gave a maximum response of 0.20 ml. milk, of which 0.035 ml. was secreted by the right mamma, and 0.165 ml. by the left mamma. In still other cases one gland secreted only cloudy fluid at a time when the remaining gland of the same animal was secreting milk.

The tissue did not long remain in this responsive state, and after 2 or 3 days the milk secretion rapidly decreased to a low daily level, notwithstanding the continued administration of large amounts of prolactin. It is possible that both here, and in the case of the regressing glands, the resumption of ovarian activity may be the factor responsible for the lack of continued response to prolactin [v. Selye *et al.* 1934]. Experiments with ovariectomized animals are necessary to answer this question.

The recent results of Selye & Collip [1936], and of Gomez *et al.* [1937], seem to indicate that the route of action of the sex-hormones on the mammary gland is through the pituitary, since the oestrogens have little power to effect mammary differentiation in its absence. It is significant in this regard that the reconditioning of the acinar tissue of the regressing gland takes place after the injection of oestradiol plus progesterone has been discontinued. The present findings suggest that progesterone augments the effect of oestradiol, and presumably through its action on the pituitary. It is apparently still undecided as to how far the effect of the oestradiol plus progesterone on the mammary tissue is due entirely to the action of these hormones through the pituitary, and how far to a synergistic effect of these two sex-hormones acting on the regressing mammarys in conjunction with the hypophyseal secretion produced by their action on the pituitary.

SUMMARY

1. Prolactin in amounts up to 800 Riddle units daily only reinstates a limited milk secretion in the regressing mammarys of female guinea-pigs.

2. The acinar tissue of such regressing glands requires to be reconditioned before it can again enter upon another cycle of secretion. This has been best accomplished by the administration of oestradiol plus progesterone. Mammary tissue so prepared is for a short time remarkably responsive to prolactin.

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