# Pituitary Prolactin Content and Mammary Development after Chronic Administration of Prolactin* (32948)

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Feedback mechanisms operating between target endocrine glands and the hypothalamo–hypophyseal system regulate secretion of several pituitary hormones (1). But these direct feedback mechanisms apparently do not regulate prolactin secretion (2). Furthermore, in addition to target organ feedback mechanisms, the pituitary adrenocorticotropic (ACTH) (3), luteinizing (LH) (4), and growth hormones (GH) (5,6) when administered exogenously have been shown to control the endogenous levels of the respective hormones in the pituitary, a phenomenon known as autofeedback control. Also, subcutaneous transplantation of pituitary tumors, which secrete large amounts of prolactin and GH, increased hypothalamic prolactin inhibiting factor (PIF) and decreased pituitary prolactin concentration (7). The objectives of this experiment were to determine whether injections of purified ovine prolactin or prolactin secreted from pituitaries transplanted to the kidney capsule (2) would alter pituitary prolactin concentration and mammary nucleic acid content.

*Materials and Methods.* In the first experiment, mature virgin female rats of the Sprague-Dawley strain received either saline or a total daily dose of 1 or 10 mg of prolactin (NIH-P-$S_7$)[2] for 10 days. Prolactin was injected subcutanously in 0.2 ml of 0.85% NaCl three times a day. In a second experiment 0, 2, 5, or 10 anterior pituitary (AP) glands from mature female rats were transplanted under the left kidney capsule of mature virgin recipient rats for a total period of 14 days. Kidneys of sham-transplanted controls were manipulated surgically in a manner similar to that of rats receiving transplants. In a third experiment, to elucidate the relationship of the ovary to the autofeedback control of prolactin, two groups of mature virgin rats were ovariectomized. At the time of ovariectomy one group received 10 AP's under the left kidney capsule whereas the other group served as sham-transplanted controls. The transplant period lasted 14 days. Vaginal smears and body weight changes were recorded daily for rats of experiments 1 and 2.

Rats were killed by decapitation the day following the end of the experimental period, and AP's were removed, weighed and stored at −20°C. The prolactin assay method of Reece and Turner (8) was conducted as previously described (9), except that high and low doses of standard (NIH-P-$S_6$)[2] prolactin were 1 and 3 μg, respectively; unknown pituitary homogenate doses were 1 and 3 mg, respectively. Eight or 10 pigeons were used at each dose level. Lack of sufficient pituitary tissue precluded replicate assays and statistical comparisons of the mean prolactin potencies.

At autopsy the three right abdominal-inguinal mammary glands were removed and placed in 95% ethyl alcohol for at least 24 hours. Extraparenchymal connective tissue, lymph nodes, large nerves, and blood vessels were dissected from the tissue. Parenchymal tissue was further extracted with 95% ethyl alcohol, chloroform:methanol (2:1), and diethyl ether for 24 hours each. The tissue was dried at 37°C for 12 hours, weighed and pulverized to a fine powder in a micro Wiley mill (Arthur H. Thomas Co., Philadelphia, Pa.). Twenty-or 25-mg samples of this powder were extracted further with 10 ml of diethyl ether for 24 hours, and analyzed for nucleic acids as previously described (10).

*Results.* Daily injections of 1 or 10 mg of prolactin for 10 days decreased pituitary

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TABLE I. Pituitary Prolactin Content of Rats Injected with Prolactin or Transplanted with Anterior Pituitary (AP) Grafts.

| Expt. no. | Treatment | No. of rats | AP wt.[a] (mg) | AP prolactin[b] (IU/mg) | AP prolactin[b] (IU/AP per 100 gm of body wt.) |
|---|---|---|---|---|---|
| 1 | Saline control | 5 | 10.3 ± .3 | .016 ± .008 | .067 ± .033 |
| | 1 mg prolactin | 4 | 9.8 ± .2 | .012 ± .003 | .046 ± .011 |
| | Saline control | 5 | 11.3 ± .4 | .013 ± .004 | .056 ± .018 |
| | 10 mg prolactin | 5 | 9.0 ± .3[c] | .008 ± .006 | .027 ± .020 |
| 2 | Sham-transplanted control | 8 | 10.8 ± .4 | .033 ± .008 | .149 ± .035 |
| | 2 AP transplants | 8 | 8.5 ± .8[c] | .011 ± .010 | .037 ± .034 |
| | 5 AP transplants | 8 | 9.0 ± .4[c] | .012 ± .007 | .040 ± .024 |
| | 10 AP transplants | 6 | 9.1 ± .4[c] | .003 ± .031 | .011 ± .108 |
| 3 | Ovariectomized + sham-transplanted control | 6 | 11.3 ± .3 | .010 ± .004 | .044 ± .017 |
| | Ovariectomized + 10 AP transplants | 6 | 10.0 ± .3[d] | .009 ± .004 | .032 ± .015 |

[a] Mean ± SE. Statistical comparisons were made within experiments relative to respective controls.

[b] Prolactin assays were based on pooled homogenates of all rats within a group. Values are mean potency ± SE of assay. Average lambda of assays was .41.

[c] $p < 0.01$.

[d] $p < 0.05$.

weights 5% ($p > 0.05$) and 20% ($p < 0.01$), respectively, relative to saline injected controls (Table I). The concentration of prolactin (IU/mg of AP) decreased 25 and 38% from saline control values in rats receiving 1 or 10 mg of prolactin daily, respectively. Expressed as prolactin content (IU of prolactin/AP per 100 gm of body wt.) these decreases were 31 and 52%, respectively. Transplantation of 2, 5, or 10 AP's to the kidney capsule depressed ($p < 0.01$) pituitary weights 21, 17, and 16%, respectively; prolactin concentration decreased 67, 64, and 91%, respectively; and prolactin content decreased 75, 73, and 93%, respectively.

Ovariectomy alone reduced (21–70%) prolactin concentration and content relative to either saline control rats or the sham-transplanted control group containing intact ovaries (Table I). When 10 AP's were transplanted to the kidney capsule of ovariectomized rats prolactin was further reduced 10% (IU/mg) and 27% (IU/AP per 100 gm of body wt.).

Daily injection of 1 or 10 mg of prolactin increased ($p < 0.01$) mammary DNA/100 gm of body weight 100 and 225%, respectively, over saline control rats (Table II). Two, 5, or 10 AP transplants increased mammary DNA 150 to 212% ($p < 0.01$). Ovariectomized rats with 10 AP transplants contained 114% more ($p < 0.01$) mammary DNA than ovariectomized controls. However, ovariectomized rats with 10 AP transplants contained significantly less ($p < 0.01$) DNA than the intact rats with AP transplants in experiment 2. Increases in mammary RNA content /100 gm of body wt. of rats after receiving prolactin injections or pituitary transplants were even greater than increases in mammary DNA (Table II).

Vaginal smears of rats receiving prolactin injections or pituitary transplants became leukocytic during the experimental period. At autopsy some uteri of rats given prolactin or AP transplants contained deciduoma. When these uteri containing deciduoma were deleted from the data, prolactin or AP transplants consistently reduced average uterine weights compared with respective control

TABLE II. Nucleic Acid Content of Rats Injected with Prolactin or Transplanted with Anterior Pituitary (AP) Grafts.

| Expt. no. | Treatment | Body wt.[a] (gm) | (mg/100 gm of body wt.) DNA[a] | RNA[a] |
|---|---|---|---|---|
| 1 | Saline control | 251 ± 6 | 1.1 ± .12 | 1.8 ± .24 |
| | 1 mg prolactin | 258 ± 4 | 2.2 ± .14[b] | 4.0 ± .16[b] |
| | Saline control | 255 ± 3 | 0.8 ± .05 | 1.2 ± .12 |
| | 10 mg prolactin | 274 ± 5 | 2.6 ± .10[b] | 4.8 ± .14[b] |
| 2 | Sham-transplanted control | 243 ± 3 | 0.8 ± .03 | 1.0 ± .06 |
| | 2 AP transplants | 251 ± 5 | 2.0 ± .16[b] | 2.6 ± .44[b] |
| | 5 AP transplants | 264 ± 6 | 2.0 ± .19[b] | 3.2 ± .46[b] |
| | 10 AP transplants | 262 ± 5 | 2.5 ± .27[b] | 4.2 ± .66[b] |
| 3 | Ovariectomized + sham-transplanted control | 262 ± 8 | 0.7 ± .02 | 1.0 ± .06 |
| | Ovariectomized + 10 AP transplants | 270 ± 5 | 1.5 ± .14[b] | 2.8 ± .36[b] |

[a] Mean ± SE. Statistical comparisons were made within experiments relative to respective controls.

[b] $p < 0.01$.

TABLE III. Organ Weights of Rats Injected with Prolactin or Transplanted with Anterior Pituitary (AP) Grafts.

| Expt. no. | Treatment | (mg/100 gm of body wt.) Uterus[a][b] | Ovaries[a] | Adrenals[a] |
|---|---|---|---|---|
| 1 | Saline control | 136 ± 16 | 30.9 ± 2.5 | 25.9 ± 1.0 |
| | 1 mg prolactin | 105 ± 6 | 27.4 ± 1.1 | 22.6 ± 0.9[c] |
| | Saline control | 139 ± 24 | 33.5 ± 1.9 | 26.6 ± 0.8 |
| | 10 mg prolactin | 114 ± 1 | 30.7 ± 0.6 | 22.7 ± 1.3[c] |
| 2 | Sham-transplanted control | 176 ± 15 | 34.8 ± 1.6 | 25.8 ± 1.2 |
| | 2 AP transplants | 149 ± 15 | 31.3 ± 1.2 | 22.3 ± 1.0 |
| | 5 AP transplants | 130 ± 11[c] | 32.0 ± 1.5 | 23.0 ± 1.4 |
| | 10 AP transplants | 123 ± 7[c] | 36.9 ± 2.2 | 23.6 ± 0.4 |
| 3 | Ovariectomized + sham-transplanted control | — | — | 23.6 ± 0.9 |
| | Ovariectomized + 10 AP transplants | — | — | 23.5 ± 0.6 |

[a] Mean ± SE. Statistical comparisons were made within experiments relative to respective controls.

[b] Weights of uteri with decidua were not included.

[c] $p < 0.05$.

groups (Table III). However, statistically significant differences ($p<0.05$) were observed only in the 5 and 10 AP transplant groups which contained the greatest number of rats. Although ovaries of rats treated with prolactin or AP transplants contained numerous corpora lutea, such treatment did not significantly ($p>0.05$) alter ovarian weights. Adrenal weights were reduced ($p<0.05$) in intact animals given prolactin or AP transplants. However, no significant reduction ($p>0.05$) in adrenal weight occurred in ovariectomized rats implanted with 10 AP's. Rats given 10 mg of prolactin or 5 or 10 AP transplants gained significantly more ($p<0.01$) weight than respective control rats during the experimental period.

*Discussion.* Injection of NIH ovine prolactin or transplantation of anterior pituitaries to the kidney capsule decreased prolactin content of pituitaries of virgin rats. These data are in agreement with results of Chen *et al.* (7) who observed a depressed prolactin content and pituitary weight, and increased hypothalamic PIF concentrations in rats bearing pituitary tumors capable of secreting large amounts of prolactin and GH. As expected, ovariectomy alone reduced prolactin content of the pituitary (8, 11), but transplantation of 10 AP's to the kidney capsule further reduced the level of prolactin in the AP of host rats. These results indicated that the autofeedback control of prolactin can function in the absence of the ovary.

The marked increase in total mammary DNA as a result of prolactin injections or AP transplants confirmed histological observations (7,12,13) that exogenous prolactin increased mammary lobulo-alveolar development. However, 10 AP's transplanted into ovariectomized rats did not produce as much total development (DNA) as 10 AP transplants evoked in intact rats. This observation supports the concept of Lyons *et al.* (14) that ovarian hormones synergize with pituitary hormones to produce mammary growth. These data are contrary to results (13) based on histological observations of mammary tissue that pituitary transplants alone produced complete lobulo-alveolar development independent of the ovary. Whether the greater stimulation in mammary RNA than in DNA produced by exogenous prolactin reflects increased milk secretion or increased protein needed for mitotic activity in the mammary gland is unknown.

The marked variation in uterine weights of control groups probably reflects changes associated with different stages of the estrous cycle. Nonetheless, in agreement with the data of Bates *et al.* (15) exogenous prolactin reduced weights of uteri that did not contain deciduoma. Whether endogenous prolactin enhances uterine involution following parturition and during lactation, a time when endogenous prolactin secretion is normally high, remains to be tested.

Ovarian weights were not significantly altered by exogenous prolactin but adrenal weights were consistently depressed. Thus, prolactin administration may have interfered with ACTH synthesis and /or release. Alternatively this effect may have been mediated through the ovary since ovariectomy also depressed adrenal weights, and AP transplants to ovariectomized rats caused no further decreases in adrenal weights.

In agreement with the observations of Bates *et al.* (15) 10 mg of purified ovine prolactin of the present study stimulated body weight increases. The contamination of the ovine prolactin preparation with GH was so negligible that the observed body growth effect can not be attributed to GH. The body weight gains of rats bearing 5 or 10 AP transplants were also greater than sham-operated rats.

*Summary*. Daily injection of 1 or 10 mg of prolactin for 10 days or transplantation of anterior pituitaries (AP) to the kidney capsule for 14 days markedly decreased pituitary weight and prolactin content of mature virgin rats. Ovariectomy reduced prolactin content of pituitaries relative to intact controls, but ovariectomy plus transplantation of AP's reduced prolactin content even further. These results indicated that function of the autofeedback control of prolactin can occur without the ovary. Prolactin injections and AP transplants increased total mammary DNA and RNA, but these increases were somewhat reduced in ovariectomized rats compared with intact rats. Exogenous prolactin reduced uterine and adrenal weights, stimulated body weight increases but had no significant effects on ovarian weights.

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# Incorporation of Labeled Palmitate into "Alveolar" and Whole Lung Phospholipids of Fetal and Newborn Lambs* (32949)

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The internal surface of the lung is believed to be lined with a surface-active material that stabilizes the alveoli during respiration(1). This material is presumably a lipoprotein complex(1) containing highly saturated phosphatidylcholine(2–4).

The observations that intact mammalian lung rapidly incorporates labeled acetate(5, 6) or palmitate(6–8) into phospholipids suggests a metabolic pattern that may reflect the synthesis in the lung of the phospholipid portion of surface-active material. Autoradiographic studies have shown that labeled acetate and palmitate appear mainly in the large alveolar cells which are thought to be the cellular site of the formation of surface-active material(6). Previous studies by us (9) have shown that dipalmitoyl phosphatidylcholine appears in the fetal alveolar space late in gestation. This is at a time when the phospholipid has increased markedly in concentration in the lung tissue. With the onset of respiration, dipalmitoyl phosphatidylcholine is discharged in large amounts into the alveolar spaces.

The purpose of this study was to determine if labeled palmitate injected into the circulation is incorporated into the phospholipid components of the pulmonary surface-active material of fetal and newborn lambs.

*Materials and Methods.* Seven fetal and six newborn lambs were studied. All of the fetal lambs were twins, near term, and were delivered by cesarean section under spinal anesthesia. With the placental circulation intact, and the fetus *in utero*, the first of each set of twins (except No. 242A) was given palmitate-1-$^{14}$C complexed to albumin(10)[1] (100 $\mu$C/kg) via coteleydon vein. After 1 hour, the fetus was delivered, the umbilical cord was tied, and the lamb was allowed to breathe normally for 10 min. (One hour was selected because preliminary studies had revealed peak radioactivity of label in lung tissue at 15 min. It was assumed that the label would reach its peak in the "alveolar" phospholipids later.) At the end of 10 min of respiration, the lamb was killed by injection of 1% xylocaine intracisternally.

The second of each set of twins was delivered onto a warm table, taking special precautions to maintain an adequate placental circulation after the mother had first been sedated with intravenous Nembutal and placed on respiratory pump. Labeled palmitate similar to that given its twin was then injected via an isolated jugular vein. After 1 hour, the lamb was killed without allowing it to breathe.

All six of the newborn lambs had delivered spontaneously and ranged in age from 2 hours to 8 days at the time of study. Labeled palmitate was likewise injected into them via

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[1] Palmitic acid-1-$^{14}$C with a specific activity of 50 mC/mmole was obtained from New England Nuclear Corp., Boston, Massachusetts.