Prolactin Production by Rat Pituitary *in vitro*.*‡ (26961)

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A number of investigators have attempted to cultivate anterior pituitary (AP) *in vitro* and assay for hormone production(1-4). While hormone production by serial propagation of this tissue has met with limited success(5), organ explants of pituitary tissue have produced detectable quantities of ACTH(6) and TSH(7). Prolactin should be an ideal hormone for *in vitro* production by AP tissue, since there are definite indications that removal of CNS influences results in increased prolactin secretion. Thus, (a) transplantation of the AP to the kidney capsule of rats elicits pseudopregnancy and mammary growth(8-10), and (b) induction of a specific hypothalamic lesion in estrogen-primed rabbits promotes lactation(11). The present *in vitro* study demonstrates that AP tissue from rats releases many times more prolactin into culture medium than is normally present in fresh AP, and that pituitary explants from immature, mature and lactating rats show significant differences in prolactin production.

Methods. Female rats of the Carworth CFN and Sprague-Dawley strains provided pituitaries for these experiments. The former consisted of virgin females 3-4 months old, and primiparous lactators 15-17 days postpartum; the latter included 21-30 day old immature females, non-pregnant multiparous females, and primiparous lactators 3-4 days postpartum.

Both the Chen(12) and a modification of the Trowell 1959(13) organ culture method of Fell and Robison(14) were employed. After decapitation, the AP was removed aseptically from each rat and placed on moistened filter paper in a Petri dish. The AP was cut into 6 pieces, each about 2 mm in diameter, placed on rafts of cellulose-acetate or stainless steel mesh, and floated on a nutrient medium in a watch glass inside of a Petri dish. Explants from 2 or 3 AP's were incubated in each dish. Pituitary explants from Carworth rats were cultured on "199" medium containing 2 units Zn-free insulin §, 50 IU penicillin G potassium and .1 mg streptomycin sulfate per ml. Pituitary explants from Sprague-Dawley rats were cultured on Trowell T-8 medium according to the procedure outlined by Merchant et al.(15) Cultures were incubated at 35 ± 1°C in air atmosphere or gassed continuously with 95% O_2-5% CO_2.

Medium cultured in air atmosphere was replaced daily with 1 cc fresh medium, and medium gassed with O_2-CO_2 was replaced every 3 days with 3 cc fresh medium. The collected medium was preserved in a freezer, with the exception of medium from the 1st day of culture from groups 1-4 in experiment 1 (Table 1), which was discarded to remove possible contamination from cellular debris. The medium from each group was lyophilized, dissolved in distilled water, and assayed for prolactin in mature White Carneau pigeons by the intradermal method of Lyons(16) as modified by Reece and Turner(17). Approximately 8 Reece-Turner units are equal to 1 IU prolactin by our method of assay. The 6-day medium from each group in Experiment 1 was combined for assay; in Exp. 2 each 3-day medium was assayed separately and the results were combined for the 9-day period. Some pituitary explants were also assayed at the end of 6 days culture. For controls, the same volumes of incubated "199" medium without pituitary explants were assayed for prolactin activity in 3 separate trials in 13 pigeons, and insulin alone was assayed in 3 birds. At the end of each culture period, most pituitary explants were fixed in Bouin's fluid, sectioned at 6μ, and stained with eosin and hematoxylin. Data were analyzed by the

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TABLE I. Prolactin in "199" Medium from Cultures of Anterior Pituitaries from CFN Rats.

Group	No. of pits	Type of rat	Days of culture	Atmosphere	No. of Birds per assay	Prol. Units per pit/day
1	6	Mature ♀	2-7	Air	3	.1
2	4	Lactators	2-7	"	3	.4*
3	6	Mature ♀	2-7	"	5	.2
4	3	Lactators	2-7	"	5	.4*
4	3	"	8-14	"	5	.2
5	3	Mature ♀	1-6	O_2-CO_2	5	.9†
6	3	"	1-6	"	5	1.0†
7	3	"	1-6	"	5	1.1†

* Groups 2 + 4 *vs* 1 + 3 : P<.05 † Groups 5,6,7 *vs* 1 + 3 : P<.001

Kruskal-Wallis sum of ranks test(18).

Results. Neither the 3 control media nor insulin showed prolactin activity. By contrast, all media incubated with pituitary explants contained substantial amounts of prolactin. Pituitary explants from lactators (Table 1, Groups 2 and 4) released 2-4 times as much prolactin per day into the medium as explants from mature females (Groups 1 and 3). When explants from Group 4 were cultured for an additional 7 days (days 8-14), about half as much prolactin was produced as in the previous 6 days. Pituitary cultures gassed continuously with 95% O_2 - 5% CO_2 (Groups 5-7) released 5-10 times more prolactin into the medium each day than cultures exposed to air atmosphere (Groups 1 and 3). These differences are highly significant. The limited number of assays of prolactin content in pituitary explants at the end of a 6-day culture showed much less prolactin than fresh AP tissue at the beginning of culture.

In the 2nd experiment (Table 2), about half as much prolactin was released into the medium each day by AP explants from immature rats (Groups 1 and 4) as by explants from mature rats (Groups 2 and 5). This difference was significant at the 2% level. Pituitary explants from lactating rats (Groups 3 and 6) released about twice as much prolactin into medium as explants from mature females (Groups 2 and 5). This difference was significant at the .1% level. Histological examination of AP explants after 6-7 days of culture in Exp. 1, revealed that tissue exposed to air atmosphere was necrotic with only a thin outer rim of living cells (Fig. 1), while explants gassed with 95% O_2 - 5% CO_2 consisted predominantly of viable cells (Fig. 2). The latter compared favorably in appearance with fresh rat AP tissue (Fig. 3).

Discussion. These results show that AP explants from rats can synthesize and release substantial amounts of prolactin into chemically defined media when cultured *in vitro*. Pituitary prolactin content in a mature female Carworth rat shows a range of .3 - .5 RT units when assayed in mature White Carneau pigeons(19). By contrast, gassed explants in Exp. 1 released 2-3 times more prolactin per AP (.9 - 1.0 RT units) into culture medium each day. In 6 days, each pituitary released

TABLE 2. Prolactin in Trowell Medium from Cultures of Anterior Pituitaries from Sprague-Dawley Rats. (95% O_2-5% CO_2 Atmosphere)

Group	No. of pits	Type of rats	Days of culture	No. of birds per assay	Prol. units per pit/day
1	4	Immature ♀	1-9	9	.1
2	4	Mature ♀	"	"	.2*
3	4	Lactators	"	"	.4†
4	3	Immature ♀	"	"	.1
5	3	Mature ♀	"	"	.2*
6	3	Lactators	"	"	.4†

* Groups 2 + 5 *vs* 1 + 4 : P<.02 † Groups 3 + 6 *vs* 2 + 5 : P<.001

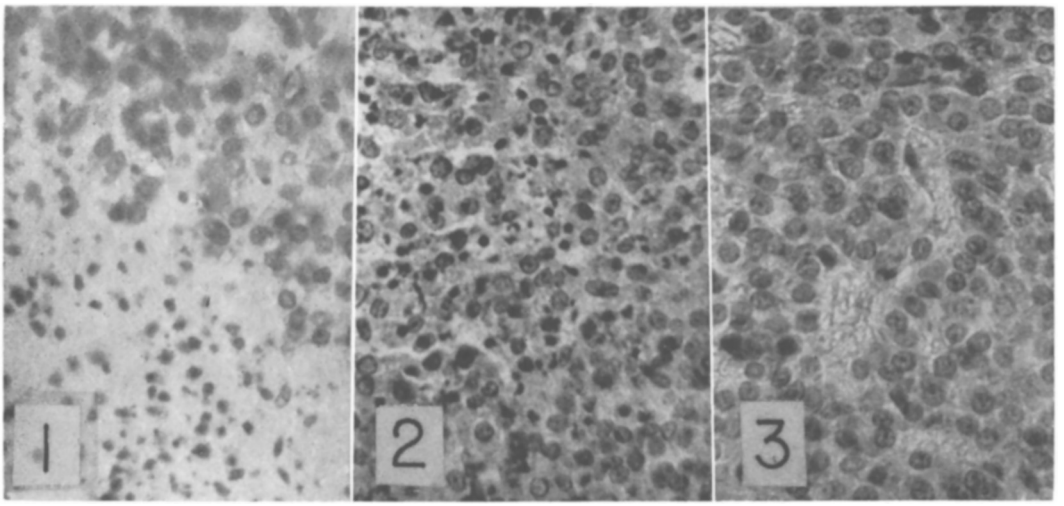

FIG. 1-3. Histological sections from rat anterior pituitary (AP): (1) Explant cultured for 7 days in air atmosphere. Note outer rim of living cells (2) Explant cultured for 6 days in 95% O_2–5% CO_2. Most cells are viable (3) Section from fresh, non-cultured AP. × 440.

about 6 RT units (.8 IU), or 12-18 times as much prolactin as was originally present in the AP. Since some destruction of pituitary cells must have occurred during initial handling and cutting of pituitary explants, potential production of prolactin was probably even greater than noted in these experiments. These data clearly denote active synthesis and release of prolactin by AP tissue, and not mere "leaching" of residual hormone.

Smaller quantities of prolactin were recovered from Trowell as compared to "199" medium when each was gassed with 95% O_2 - 5% CO_2. This is believed to be due to employment of chloramphenicol in Trowell medium which may have interfered with hormone synthesis. The greater viability of gassed as compared to non-gassed AP tissue at the end of 6 days culture was clearly reflected in greater prolactin production. It is of interest however, that we have successfully cultured rat AP tissue for 21 days in air atmosphere with continuous production of prolactin, despite the small area of viable tissue remaining at the end of culture (Nicoll and Meites, unpublished). Although only preliminary assays have been made of AP explants at the end of culture, their relatively low prolactin content suggests very rapid synthesis and release of hormone. Differential staining of AP tissue cultured on Trowell medium for 7 days by one of us (Kahn) indicates that only few atrophic basophils are present and that acidophils predominate. The latter cells are usually associated with prolactin secretion(20).

The present *in vitro* findings corroborate the conclusion that differences in pituitary prolactin content previously reported in immature, mature and postpartum rats(17,21), represent actual differences in secretion rate. Estrogen is believed to be largely responsible for greater prolactin secretion by pituitaries of mature as compared to immature rats, and for the rise in prolactin production at about the time of parturition(21). Administration of estrogen increases pituitary prolactin content *in vivo*(17,21), and we have recently shown that addition of estradiol to "199" medium significantly increases prolactin release by AP explants *in vitro*(22).

These *in vitro* findings can be considered as perhaps the most cogent evidence that prolactin synthesis and release do not depend on hypothalamic or other nervous influences. Other AP hormones show a profound reduction in secretion rate when the pituitary is removed from its normal cranial site and transplanted elsewhere in the body(23). The few *in vitro* studies on AP tissue previously reported indicate that minute amounts of other hormones are produced for a few days only. It remains to be established whether specific factors are elaborated by hypothalamic or other CNS regions which inhibit or otherwise

influence prolactin production. Further *in vitro* studies are in progress to determine the direct effects of neurohormones and other agents on prolactin secretion by AP explants.

Summary. Anterior pituitary explants from female immature, mature and postpartum lactating rats were cultured *in vitro* on "199" or Trowell T-8 medium at 35 ± 1°C for periods of 6-14 days. Medium was assayed for prolactin by the intradermal pigeon crop method. Pituitary explants from mature female rats, gassed continuously with 95% O_2 - 5% CO_2 released 5-10 times more prolactin into medium each day than explants exposed only to air atmosphere. The amount of prolactin per AP recovered each day from the culture medium, under continuous gassing, was 2-3 times greater than that present in a fresh AP prior to culture. This proves that prolactin was actively synthesized and released and not merely "leached" out from AP explants. Explants from immature rats elaborated about half as much prolactin per AP per day as explants from mature rats, and explants from lactating rats about twice as much as explants from mature rats. Assays of some explants at the end of culture revealed a low prolactin content, indicating rapid synthesis and release. Histological examination of explants at termination of culture showed that tissue exposed to continuous gassing was almost entirely viable, whereas tissue exposed to air atmosphere had only a thin outer rim of living cells. These results demonstrate that prolactin can be produced in large amounts when AP tissue is cultured *in vitro*.

ADDENDUM

Improvements in our tissue culture techniques have resulted in release into the medium of 10 times as much prolactin per AP per day as was initially introduced into the medium by the fresh tissue. This constitutes dramatic evidence of the ability of the AP to produce prolactin when freed of hypothalamic inhibition.

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