were representative of the total mast cell population in the organism, then neonatal thymectomy did not significantly reduce the size of this cell population. These results are consistent with the proposal that mast cells constitute an independent, self reproducing population in the postnatal mouse. Since runting disease was not seen in this series, peripheralization of thymocytes may have occurred before thymectomy. However, if the thymus is responsible for starting the mast cell population, rather than maintaining it, this would have to occur in the embryo, since mast cells are present at least as early as 15 days postconception in the mouse(7). That is, seeding of other tissues with lymphocytes by the thymus cannot have the same significance for mast cells as it does for immunological reactions by lymphoid cells. A difference of a day or two in the time of neonatal thymectomy could not influence the initiation of the mast cell population since the latter had already been initiated at least 4 or 5 days earlier than this. Therefore, while this experiment does not provide information on the embryonic origin of mast cells, it does show that the mast cell population in postnatal mice is not dependent on the thymus as a source of new mast cells.

Summary. Neonatal thymectomy did not significantly reduce the size of the mast cell population in young and adult mice. Consequently, the thymus is not necessary for mast cell production during postnatal development.

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Influence of Estrogen on Anterior Pituitary Lactogen Production *in vitro.** (29109)

RICHARD R. GALA AND RALPH P. REECE
Department of Dairy Science, New Jersey Agricultural Experiment Station, New Brunswick

Reece and Turner(1) first reported that estrogen administration to rats increased pituitary lactogen content. A similar increase in pituitary lactogen after estrogen injection has been reported for guinea pigs(2,3) and rabbits(4). The addition of estrogen to anterior pituitaries cultured *in vitro* has been reported to increase oxygen consumption of rat(5) and human(6) adenohypophyses. Recently, Nicoll and Meites(7) observed an increase in lactogen production by rat pituitaries cultured *in vitro* when estrogen was added to the medium. It has also been noted that rat pituitaries estrogenized *in vivo* were capable of increased lactogen production *in vitro*(8). The purpose of this endeavor was to examine the effects of estrogen on anterior pituitary lactogen production *in vitro* by adding estrogen to the medium, by estrogenizing pituitaries *in vivo* and subsequent *in vitro* culture, and by culturing both pituitaries and hypothalami together with estrogen added to the medium.

Materials and methods. Anterior pituitaries (AP) were obtained from female hooded Norway rats approximately 3 months of age (165-175 g body weight) and cultured *in vitro* according to the technique of Meites and co-workers(9). A total of from 6 to 12 AP were cultured per experiment, and each pituitary was divided into either 4 or 6 pieces. In the estradiol *in vitro* and the AP-hypothalamus co-culture studies, a piece from

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each pituitary was placed in each culture group until the equivalent of one AP was present per dish. In the estradiol *in vivo* study, a single fragmentized pituitary from the same animal was placed in each dish. Insulin,† at the level of 0.1 mg/ml of medium, was added only in Experiments 3 and 4 from the estradiol *in vitro* study; and in all experiments 2.0 ml of 2.8% $NaHCO_3$ were added per 25 ml of medium. The antibiotics streptomycin and penicillin were added to the medium at a level of 0.2 mg and 0.068 mg per ml of medium. The culture duration was 3 days. In the estradiol *in vitro* and estradiol *in vivo* studies, the culture temperature was 35°C, whereas in the AP-hypothalamus co-culture study, the temperature was 37°C. In all experiments the gas environment was 95% O_2-5% CO_2 at a flow of 300 cc/min.

Estradiol 17-B was dissolved in absolute ethanol and added to the medium at a concentration of 0.1, 0.5, and 5.0 μg per ml medium in the estradiol *in vitro* study. In the AP-hypothalamus co-study estradiol was added to medium at the level of 0.5 μg/ml. In the estradiol *in vivo* study, estradiol at the level of 1 and 10 μg/day was injected subcutaneously into 3-month-old female rats for 10 days and on the 11th day *in vitro* pituitary culture was initiated. Control cultures were run simultaneously with all estradiol groups and in those experiments in which estradiol was added to the medium, absolute ethanol was added to the control cultures at the same concentration (1%) as that added to the experimental medium.

In the AP-hypothalamus co-culture study, the hypothalami and AP were from the same animals. The hypothalamic region included the area from the optic chiasma to the mammillary bodies and weighed an average of 35 mg. These regions were divided into 2 or 3 mm squares; in Exp. 1 one hypothalamic equivalent and in Exp. 2 two hypothalamic equivalents were placed in the culture dish with the pituitaries.

Medium from each experiment was pooled and the lactogen content determined by the crop gland proliferation method of Reece and Turner(2). Pooled medium (0.05 ml/day) was injected intradermally over the right crop gland of mature pigeons, and 1.25 μg of lactogen‡ (0.01875 IU), in the same volume, was injected over the left crop gland for 4 days. On the fifth day, the birds were sacrificed and the degree of crop gland proliferation rated visually. Cultured AP tissue was assayed for lactogen content by injecting an aqueous suspension of 1.25 mg AP/0.05 ml/day over the right crop gland, and the lactogen standard over the left crop gland. Lactogen content in medium was estimated in 6 to 10 pigeons per experiment, and lactogen content in cultured pituitary tissue was estimated in 3 to 7 pigeons per experiment.

Representative AP pieces from each culture dish in each experiment were examined histologically and the extent of viability rated as previously described(10). Statistical analysis of the data was accomplished using Student's *t* test.

Pooled medium from control cultures, no insulin, estradiol *in vitro* culture, no insulin, and estradiol *in vivo* cultures were dialyzed and lyophilized and the protein components separated by electrophoresis. Electrophoretic separation was accomplished using cellulose acetate strips in a tris buffer that had an ionic strength of 0.05 and a pH of 8.8. To each strip was added 25 μl of lyophilized medium at a concentration of 5 mg/ml, and electrophoretic separation was at 150 volts for 5 hours at 3°C.

Results. Addition of estradiol to the medium had no effect on anterior pituitary weight. Lactogen production by pituitaries cultured with 0.1, 0.5, and 5.0, μg of estradiol per ml medium was, respectively, 16.7, 23.9, and 27.3% lower than that of the controls. Lactogen content of cultured AP tissue and pituitary viability ratings of estradiol groups did not differ from those of the controls. Addition of insulin to the medium did not influence the estrogen effect on lactogen production (Table I).

† Zinc-free insulin (20 units/mg) kindly supplied by Eli Lilly and Co., Indianapolis, Ind., through the courtesy of Dr. Otto K. Behrens.

‡ Ovine prolactin (15 IU/mg) received as a gift from the Endocrinology Study Section, N.I.H.

TABLE I. Influence of Estradiol on Anterior Pituitaries Cultured *in vitro*.

Estradiol per ml medium	No. of exp*	Avg AP wt post-culture (mg) †	Lactogen/ 100 ml medium (IU) †	Lactogen in medium/100 mg AP (IU) †	Lactogen in 100 mg cultured AP (IU) †	AP viability rating
Control	3	4.47 ± .18	43.7 ± 7.2	26.9 ± 4.3	2.11 ± .34	2.00
.1 μg	3	4.51 ± .20‡	36.4 ± 5.4	22.4 ± 2.8‡	2.77 ± .39‡	2.33
Control	5	4.41 ± .12	47.1 ± 4.2	29.3 ± 2.5	1.89 ± .19	2.46
.5 μg	5	4.31 ± .10‡	34.5 ± 3.8	22.3 ± 1.4§	1.87 ± .26‡	2.28
Control	4	4.40 ± .16	43.5 ± 5.3	27.1 ± 3.2	1.83 ± .31	2.25
5.0 μg	4	4.35 ± .14‡	31.6 ± 4.4	19.7 ± 1.9‖	2.02 ± .50‡	2.35

* Six to twelve AP cultured per experiment.
† Mean and standard error of mean.
‡ Not significantly different ($P > .10$) from control.
§ Significantly lower ($P < .01$) than control.
‖ Significantly lower ($P < .10$) than control.

TABLE II. Influence of Administration of Estradiol *in vivo* on Anterior Pituitaries Cultured *in vitro*.

Estradiol injected per day	Avg AP wt post-culture (mg)*†	Lactogen/ 100 ml medium (IU)*	Lactogen in medium/100 mg AP (IU)*	Lactogen in 100 mg cultured AP (IU)*	AP viability rating
Control	4.50 ± .20	29.0 ± 2.5	17.4 ± 1.5	1.78 ± .20	2.50
1 μg	4.78 ± .12‡	40.4 ± 3.2‖	22.9 ± 1.8‖	2.70 ± .44‡	2.50
10 "	5.42 ± .19¶	44.5 ± 2.6¶	22.2 ± 1.3‖	2.59 ± .30§	2.25

* Mean and standard error of mean.
† Ten AP cultured per group.
‡ Not significantly different ($P > .10$) from control.
§ Significantly higher ($P < .10$) than control.
‖ Significantly higher ($P < .05$) than control.
¶ Significantly higher ($P < .01$) than control.

Administration of estradiol *in vivo*, and the subsequent culturing of AP from these animals, resulted in an increase in pituitary weight and lactogen production. Lactogen production by AP estrogenized with 1 and 10 μg estradiol/day on a pituitary basis was 39.3 and 53.5% higher, respectively, than that of the control. When adjusted for the difference in pituitary weight, lactogen production for the 1 and 10 μg groups was 31.6 and 27.6% higher, respectively, than that of the controls. The differences in each case were statistically significant. Lactogen content of estrogenized cultured AP was higher than that of controls, but only the higher level of estradiol (10 μg/day) was statistically significant. Anterior pituitary viability ratings for the groups studied were similar (Table II).

The co-culturing of anterior pituitaries and hypothalamic fragments resulted in a slight but not significant decrease in lactogen production. Addition of estradiol to the medium of co-cultures did not alter lactogen production when compared with that of either co-cultures alone or control AP cultures. Lactogen content of cultured pituitary tissue and AP viability ratings were similar for the groups studied (Table III).

Electrophoretic separation of lyophilized medium from control cultures revealed the presence of 4 protein components (Fig. 1). The first component, the one that was closest to the origin, migrated the least in the electrical field. The second component migrated some distance from the first and was the most prominent of the four. The third component was light in intensity and was located in close proximity to the second. The fourth component was diffuse in nature and light in intensity.

Electrophoretic separation of lyophilized medium from cultures to which estradiol was added to the medium revealed a somewhat different electrophoretic pattern (Fig. 2). The 4 components observed in the control

TABLE III. Influence of Estradiol (E) plus Hypothalamic Fragments on Anterior Pituitaries Cultured *in vitro*.

Experimental condition	Exp No.	Avg AP wt post-culture (mg)*†	Lactogen/ 100 ml medium (IU)*	Lactogen in medium/100 mg AP (IU)*	Lactogen in 100 mg cultured AP (IU)*	AP viability rating
Control	1	4.13 ± .20	49.8 ± 5.5	32.1 ± 4.0	2.25 ± .78	3.00
	2	4.14 ± .13	43.3 ± 6.2	26.6 ± 3.8	1.44 ± .26	2.00
	Avg	4.14	46.6	29.4	1.85	2.50
Estrous hypothal	1	3.93 ± .15	39.6 ± 5.7	28.1 ± 4.3	2.33 ± .39	3.00
	2	3.89 ± .20	43.0 ± 6.6	27.6 ± 4.3	3.38 ± .71	2.75
	Avg	3.91‡	41.3	27.9‡	2.86‡	2.90
.5 μ E/ml medium	2	3.95 ± .17‡	47.9 ± 5.5	31.4 ± 3.6‡	2.11 ± .34‡	2.50
Estrous hypothal	1	4.06 ± .24	53.9 ± 4.8	35.5 ± 3.1	1.90 ± .32	2.75
plus .5 μg E/ml	2	3.95 ± .23	38.8 ± 6.2	24.8 ± 4.0	1.91 ± .47	2.75
	Avg	4.04‡	46.4	30.2‡	1.91‡	2.75

* Mean and standard error of mean.
† Eight AP cultured per experiment.
‡ Not significantly different ($P > .10$) from control.

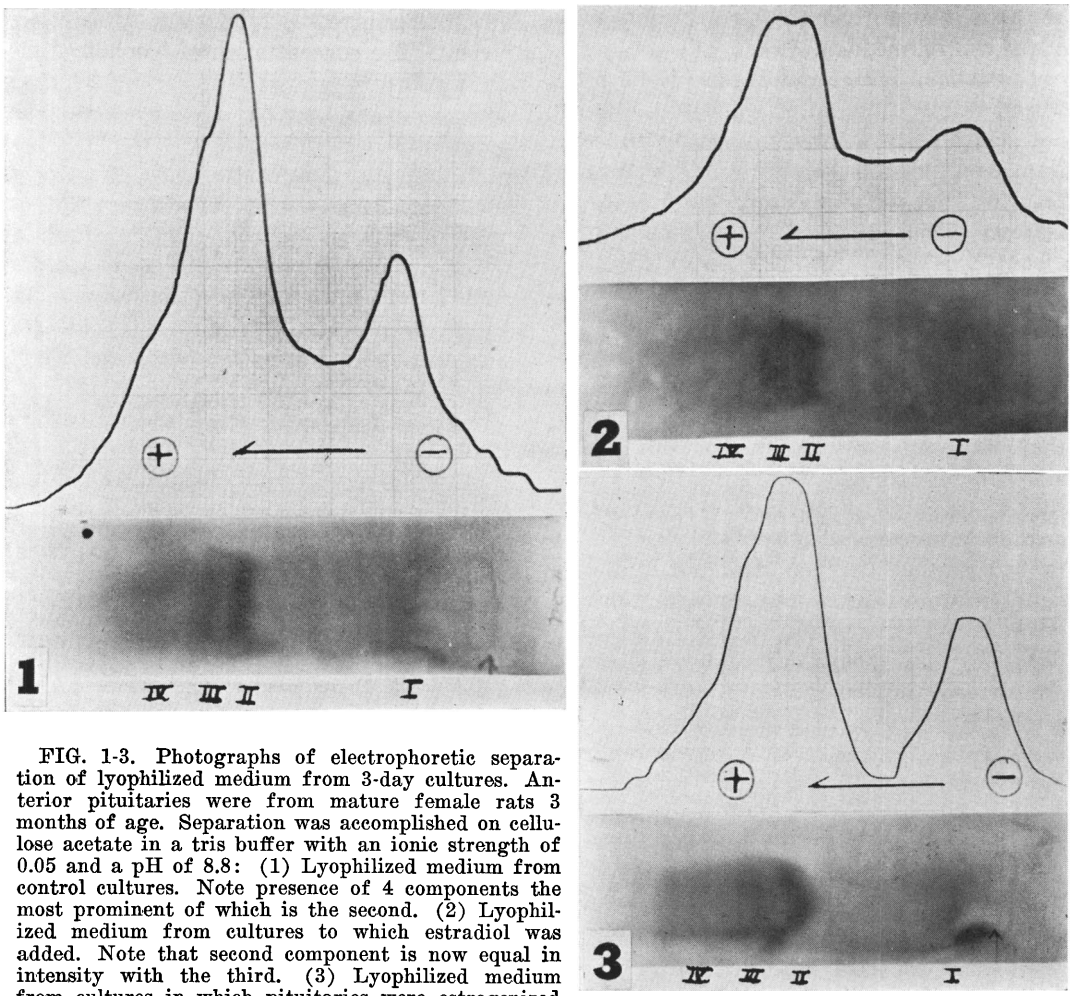

FIG. 1-3. Photographs of electrophoretic separation of lyophilized medium from 3-day cultures. Anterior pituitaries were from mature female rats 3 months of age. Separation was accomplished on cellulose acetate in a tris buffer with an ionic strength of 0.05 and a pH of 8.8: (1) Lyophilized medium from control cultures. Note presence of 4 components the most prominent of which is the second. (2) Lyophilized medium from cultures to which estradiol was added. Note that second component is now equal in intensity with the third. (3) Lyophilized medium from cultures in which pituitaries were estrogenized *in vivo*. Note intensity of second component appears to be greater than second component of control medium.

medium were also evident in estradiol medium, but the relationship of the intensities of these components changed. The second component, which was the most prominent in the control medium, in the estradiol medium was of equal intensity to that of the third. The first and second components, however, presented much the same pattern as in the control medium. When pituitaries were estrogenized *in vivo* and cultured *in vitro,* the electrophoretic separation of medium from these cultures revealed a pattern similar to that of the control medium (Fig. 3). Four protein components were present and the relationship of these components to each other was similar to that of the control medium. The second component, however, appeared to be more intense than the second component of the control medium.

Discussion. Nicoll and Meites(7) have reported that addition of estradiol to the medium significantly increased anterior pituitary lactogen production *in vitro.* They suggest that the lactogen stimulatory effect noted of estrogen when administered to animals *in vivo*(1,2,3,4) was due to a direct effect on the anterior pituitary. Our results do not support this suggestion. Addition of estradiol to AP cultures decreased, rather than increased, lactogen production. The levels of estradiol added to the medium were lower than, the same as, and higher than those added by Nicoll and Meites(7). The inability, in our hands, of estrogen to stimulate increased lactogen production *in vitro,* leads us to believe that estrogen does not stimulate lactogen production *in vivo* by acting directly on the anterior pituitary. As further evidence for this conviction, we have observed an increase in the lactogen production of pituitaries cultured *in vitro* that were previously estrogenized *in vivo.* A similar response has been reported by other workers(8). Kanematsu and Sawyer(11) have reported that intrahypothalamic and intrahypophyseal implants of estradiol resulted in an increase in pituitary lactogen only when estrogen was deposited in the hypothalamus. The co-culture study reported here did not give any indication that estrogen could stimulate lactogen production by acting upon the hypothalamus. Histological examination of the hypothalamic fragments after culture, however, indicated complete necrosis, and it is apparent that additional study is required to determine culture conditions that will maintain hypothalamic tissue viability *in vitro.*

The electrophoretic separation of the protein components present in lyophilized medium gave some indication that estrogen does not have a direct stimulatory effect on pituitary lactogen. The decrease in intensity of the second protein component of lyophilized medium from cultures to which estradiol was added to the medium parallels the lactogen content of the medium. Although the third component of this medium was equal in intensity with the second, it was not believed due to the increased production of this component. The concentration of lyophilized medium added to the cellulose acetate strip was always the same, and if amount of one component was decreased, then it would be expected that amount of the other components would be increased. The intensity of the second protein component from medium in which AP were estrogenized *in vivo* also paralleled the lactogen content in the medium. In a study where the protein components were eluted from the strip (Gala and Reece, unpublished), the area in the region of the second component possessed the majority of the lactogenic activity.

When estrogen was administered to animals *in vivo* there was an increase not only in lactogen concentration but also in anterior pituitary size(1,2,3). The addition of estradiol to the medium of pituitaries cultured *in vitro* had no effect on anterior pituitary weight, but when pituitaries were estrogenized *in vivo* and cultured *in vitro* there was a significant increase in AP weight. Nicoll and Meites(7) noted no effect of estradiol *in vitro* on cultured pituitary weight. Lactogen content of cultured pituitaries was not altered when estradiol was added to the medium, but when pituitaries were estrogenized *in vivo* and cultured *in vitro,* lactogen content was significantly higher than that of the control. The effect of estrogen on lactogen content of cultured pituitary tissue and on pituitary weight may be offered as additional

evidence against the direct action of estrogen on the anterior pituitary and suggests an indirect effect, probably through the hypothalamus.

Summary. Addition of estradiol 17-B to the medium of anterior pituitaries cultured *in vitro* for 3 days resulted in a decrease in lactogen production. Estradiol levels of 0.1, 0.5, and 5.0 μg/ml of medium lowered lactogen production 16.7, 23.9, and 27.3%, respectively, below that noted for controls. The administration of 1 and 10 μg of estradiol/day for 10 days *in vivo,* and subsequent culturing of pituitaries from these animals *in vitro,* resulted in a lactogen production 31.6 and 27.6% higher, respectively, than that of the control. Co-cultures of anterior pituitaries and hypothalami with estradiol in the medium (0.5 μg/ml) did not significantly alter lactogen production from that noted for the control. Electrophoretic separation of the proteins present in lyophilized control medium revealed the presence of 4 components, the most prominent of which was the second. In cultures where estradiol was added to the medium, electrophoretic separation indicated that the second component was now equal in intensity to that of the third. Lyophilized medium from cultures in which pituitaries were estrogenized *in vivo* resulted, when separated electrophoretically, in the second component being more intense than the second component of control medium. The intensity of the second protein component appeared to be related to the lactogen content of the medium. It is believed that lactogen stimulating effect of estrogen *in vivo* is not directly on the anterior pituitary but acts indirectly, probably through the hypothalamus.

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Experimental Hypertensions Treated with CCl_4: Measurements of Adrenal Function, Vascular Responsiveness, Angiotensinase and Converting Enzyme.* (29110)

HUBERT F. LOYKE (Introduced by A. C. Corcoran)
Department of Clinical Investigation, Research Division, St. Vincent Charity Hospital, Cleveland, Ohio

Elevated blood pressure of experimental renal (ER)(1) and desoxycorticosterone (DCA) hypertensions(2) has been lowered to normal by chronic administration of CCl_4 in rats. A likely cause of this is deficiency of renin-substrate (hepatogenous angiotensinogen (ATgen)(3), but serum concentrations of this protein were not altered by CCl_4 in ER hypertensive rats(4). Other possible causes of the blood pressure reduction considered in this report are: 1, adrenal insufficiency, 2, decreased vascular responsiveness, 3, increased "angiotensinase" (ATase) and 4, impairment of "converting enzyme" (CE). This last transforms inactive deca-peptide, angiotensin I (AT I), to the active octa-peptide, angiotensin II (AT II).

Methods. Male rats weighing 120 to 140 g were divided into groups. Renal hypertension was produced in 42 animals by ligating both

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