particularly lung and liver. This suggests that the participation of hyperergic reactions in other viral infections should be more thoroughly investigated, and that the thymectomized mice may provide a useful approach. The only other virus studied here, vesicular stomatitis, produces severe necrotizing encephalitis, and would not be considered at all likely to involve a hypersensitive reaction.

It is possible that the failure of neonatal thymectomy to protect all mice may be due to ectopic thymic tissue, or to variation between mice of this non-inbred strain with respect to degree of thymic activity at time of operation.

Summary. Mice thymectomized at birth were protected against the lethality of intracerebral and intraperitoneal infection with LCM virus given 3 to 4 weeks later. Although virus multiplication was not prevented, as evidenced by high infectivity titers in the brain 6 to 8 weeks after infection, the meningeal inflammatory reaction was suppressed. These observations provide further evidence for the importance of tissue hyperreactivity and lymphocytic inflammatory response in production of symptomatology.

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Starch Gel Electrophoresis of Rat Hypophysis in Relation to Prolactin Activity.* (28644)

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The starch gel method of electrophoresis (1) is used commonly for the separation of proteins and has been applied to homogenates of the adenohypophysis(2,3). However, the localization of pituitary hormones in the bands revealed by staining of such columns is unknown except that human gonadotropin occurs in more slowly migrating fractions but cannot be associated with any individual protein bands(3). This report deals with (a) modification of protein bands in starch gel columns by ovariectomy, thyroidectomy, and replacement therapy with estrogen and (b) distribution of prolactin activity in these columns.

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Methods. Eleven days after ovariectomy of young adult female Sprague-Dawley rats their thyroid and parathyroid glands were excised. These rats are to be referred to subsequently as being ovariectomized and thyroidectomized. Two weeks later 100 μc of I^{131} were injected subcutaneously to destroy remaining fragments of the thyroid gland. The rats were divided into the following groups: (a) ovariectomized, vehicle-treated, (b) ovariectomized, thyroidectomized, vehicle-treated, (c) ovariectomized, estrogen-treated, and (d) ovariectomized, thyroidectomized, estrogen-treated (Table I). Beginning 64 days after ovariectomy in Experiment I and 6½ months later in Experiment II, 2 μg of α-estradiol dipropionate were ad-

TABLE I. Effect of Experimental Treatment on Body and Organ Weights.

Group	No. of rats	Mean body wt (g) Initial	Start est	Final	Mean hyp wt (mg)	Mean uterine wt (mg)
Exp I						
a. Ov, veh	9	176 ± 3*	282 ± 4	296 ± 4	9.3 ± .5	82 ± 4
b. Ov, thy, veh	9	177 ± 3	280 ± 4	284 ± 3	11.2 ± .5	97 ± 5
c. Ov, est	10	173 ± 2	282 ± 5	265 ± 3	14.1 ± .6	380 ± 19
d. Ov, thy, est	10	174 ± 3	276 ± 6	264 ± 5	14.4 ± 1.0	447 ± 25
Exp II						
a. Ov, veh	8	173 ± 1	319 ± 13	315 ± 6	13.2 ± .8	148 ± 6
b. Ov, thy, veh	8	174 ± 1	252 ± 12	283 ± 9	17.3 ± .4	264 ± 33
c. Ov, est	8	175 ± 2	301 ± 17	296 ± 6	16.7 ± .6	353 ± 29
d. Ov, thy, est	9	175 ± 1	242 ± 13	265 ± 11	24.1 ± .7	509 ± 52

Ov = ovariectomized; veh = vehicle; est = estrogen; thy = thyroidectomized; hyp = hypophysis.

* Standard error of mean.

ministered daily to rats in groups c and d, the treatment being continued for 10 days. Groups a and b received a comparable volume of the vehicle, sesame oil. Completeness of removal of the ovary was checked macroscopically. Serial microscopic sections of the cervical region were examined for thyroid remnants in thyroidectomized rats of Exp. I. None were found, which indicated that the procedures followed effected complete ablation of the thyroid gland.

At termination of the experiment the rats were exsanguinated during the morning while under ether anesthesia. In Exp. I the hypophysis was excised, weighed, and the pars nervosa separated from the pars distalis. The pars distalis was homogenized in distilled water and subjected to immediate electrophoresis. In Exp. II, after separation of the pars nervosa, the pars distalis was placed in a test tube and frozen by immersion of the tube in liquid nitrogen. The glands were stored at –20°C. After thawing, each hypophysis was weighed, crushed in an agate mortar and suspended in an equal volume of distilled water. This suspension was absorbed onto a piece of filter paper and placed in a starch gel column.

For electrophoresis, the procedures of Smithies(1,4) were followed, using a 0.03 M borate buffer (pH 8.5). A voltage of 120 volts was placed across each column which measured 20 cm in length. Electrophoresis was carried out for 4 hours at room temperature. The columns were sliced into 2 halves and the cut surface stained with amido black in methanol and acetic acid.

For localization of prolactin activity, hypophyses from 14 190-210 g female rats were used (Table II). They were prepared as for Exp. II except that a suspension of 2 glands was placed in each column. Following completion of electrophoresis each column was divided longitudinally with a corrugated piece of metal. One-half was frozen at –20°C while the other was stained. Even though the stained portion shrank, the presence of corrugations in the stained and frozen columns allowed the matching of similar areas in the 2 halves. On the basis of the identification of bands in the stained half, the frozen half of the column was cut into 8 areas (Fig. 1). Each area was thawed, placed in a piece of cheesecloth which was suspended in a test tube, while held in place by a rubber stopper. The tube was centrifuged for 10 minutes after which most of the liquid had moved from the gel to the bottom of the tube.

The eluate from each area was tested for

TABLE II. Response of Pigeons to the Eluate from Areas of Starch Gel Columns.

Area column	No. pigeons	No. positive responses	Mean rating of response
1	6	0	.00
2	7	0	.00
3	7	2	.11
4	7	0	.00
5	7	1	.11
6	7	5	.43
7	6	4	1.17
8	6	2	.08

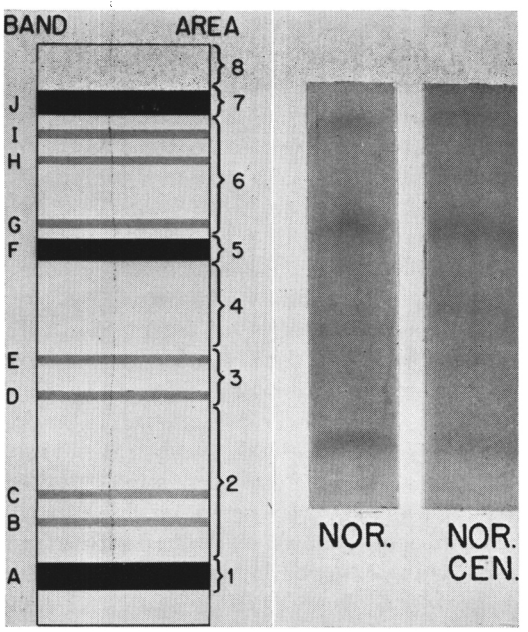

FIG. 1. Bands identified and areas analyzed for prolactin activity. Nor = uncentrifuged suspension of adenohypophysis from an untreated adult female rat. Nor cent = supernate from a centrifuged suspension of adenohypophysis from an untreated adult female rat.

prolactin activity by the method of Lyons as modified by Reece and Turner(5). One-tenth ml of eluate was injected intradermally over the crop sac of pigeons daily for 4 days. The pigeons were killed on the fifth day and crop sac proliferation estimated visually on a scale from 0 to 4 in increments of 0.25. Since 2 hypophyses were run on each column only ½ of which was frozen for elution, the activity obtained per area represents roughly that from one hypophysis. Thus, each pigeon (Table II) represents activity for one rat. The amount of elution secured from each area was sufficient to inject at least one pigeon for each of the 8 areas.

The protein bands were designated by letter, at least 10 bands being identified (Fig 1). A, F and J were most prominent. Centrifugation of the pituitary suspension prior to electrophoresis reduced smearing of the column and made certain bands appear slightly sharper. Others, especially band J, became less distinct following this procedure.

Results. Many changes were observed in protein bands as a result of ovariectomy and thyroidectomy or estrogen treatment. However, only in the case of the more prominent bands A and J were changes detected which seemed significant. Thyroidectomy caused a great reduction or disappearance of band A which was not restored by treatment with estrogen (Fig. 2). As compared with ovariectomized rats, estrogen therapy of ovariectomized rats (Fig. 2) accentuated band J. Combined thyroidectomy and ovariectomy practically eliminated band J. Treatment of ovariectomized, thyroidectomized rats with

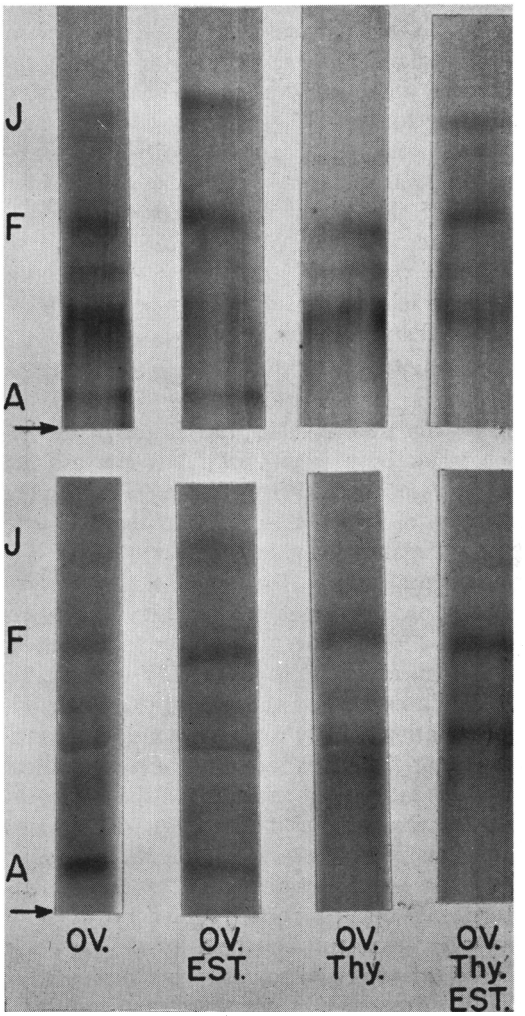

FIG. 2. Columns stained with amido black. The upper series is of glandular suspensions which were not centrifuged before electrophoresis. The lower series is from glands which were centrifuged with electrophoresis being carried out on the supernate. Ov = ovariectomy; est = estrogen; thy = thyroidectomy.

estrogen, restored band J although it was sometimes not as prominent as in the ovariectomized, estrogen-treated rats.

No prolactin activity was detected in any pigeons for areas 1, 2 and 4. Only 2 pigeons reacted to each of areas 3 and 8 and one to area 5. However, 5 of 7 reacted to area 6 and 4 of 6 to area 7. The mean scores for areas 6 and 7 were 0.43 and 1.17, respectively. Thus, 69% of the pigeons used for study of areas 6 and 7 gave a positive response while only 13% of those used for all other areas reacted. Because of many technical problems associated with electrophoresis of the gland and recovery of fluid from the column, quantification of activity has thus far proven impossible and considerable variation in regularity and intensity of the response was observed with the eluate from some of the areas. In several early procedures for bioassay of prolactin the unit of activity was based on a percentage of the birds which responded to the substance tested rather than on intensity of response(6).

Discussion. The disappearance of band A following thyroidectomy was reported by Levey and Roberts(2). The nature of its biological activity is unknown but appears not to be associated with prolactin. The demonstration of prolactin activity in the region of band J makes possible correlation of its biological nature with the known action of hormones on the prolactin content of the hypophysis. Thyroidectomy(7) or treatment of rats with antithyroid drugs(8,9) reduces the prolactin content of the hypophysis while administration of thyroid hormone either restores or increases activity beyond normal (9). Treatment of rats(10) or the hypophysis *in vitro*(11) with low doses of estrogen increases prolactin production. High doses of estrogen are less effective(12). In the experiments being reported the effectiveness of estrogen treatment is shown by the increases in weight of the hypophysis and uterus (Table I). However, the treatment was sufficiently intense to cause a variable loss of body weight in Exp. I and in Group c of Exp. II.

In view of the above reports, diminution in band J, which represents prolactin activity, after combined ovariectomy and thyroidectomy, is to be expected. Furthermore, its return following therapy of these animals with estrogen agrees with the observation of Meites and Turner(8) that estrogen will increase pituitary prolactin content in hypothyroid rats.

The secretion of prolactin is attributed to the acidophil class of pituitary cells(13,14). These cells are reduced in number by ovariectomy and practically eliminated by thyroidectomy. Treatment of intact rats with estrogen increases their number and activity as indicated by cytological change(15). Furthermore, these effects are obtainable in the absence of the thyroid glands(16). Thus, there is a satisfactory correlation between the known effects of estrogen on the cytology and prolactin content of the hypophysis and the changes observed in band J in this study.

Summary. As revealed by starch gel electrophoresis of hypophyseal suspensions, a slowly-moving protein band (A) was eliminated by combined ovariectomy, thyroidectomy and parathyroidectomy. It was not restored by estrogen therapy. These operations similarly reduced the prominence of a rapidly moving band (J) which was restored by estrogen therapy. Prolactin activity was concentrated in band J and the adjacent portion (area 6) of the column.

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Estradiol-17β, Estrone, and Progesterone in the Ovaries of Lamprey (*Petromyzon marinus*).* (28645)

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Present knowledge of the sex hormones in the lower vertebrates and the invertebrates is limited for the most part to inferences based entirely on what is known about the mammals. This tendency to project such knowledge down through the animal kingdom has led to some rather peculiar conclusions. How far down the phylogenetic series these mammalian endocrine situations actually do extend ultimately depends upon a knowledge of the presence and identity of the gonadal hormones. Recent analyses have confirmed the presence and identity of estrogens and progesterone in a representative series of invertebrates and vertebrates. Such steroids were hitherto assumed on the basis of indirect evidence(1). The series includes echinoderms, mollusks, arthropods, fish and birds(2-10). This report presents results of an effort to isolate and identify estrogens and progesterone from the ovarian tissue of cyclostomes.

Methods and results. Lamprey ovaries (568 g) were collected and stored in approximately twice their volume of acetone. At time of extraction, the acetone was poured off, the ovarian tissue was homogenized and reextracted with acetone. The combined acetone extracts were flash evaporated and finally taken to near dryness under nitrogen. The oily residue was dissolved in sufficient 70% ethanol and extracted 3 times with ligroin (B.P. 30-60°C). The ethanolic fraction was diluted with water to contain 35% alcohol and subsequently washed with ligroin (3 ×). The alcoholic solution, bioassayed by the Astwood method(11), showed the presence of estrogenic material. The ethanolic fraction was then dried *in vacuo* and the residue partitioned countercurrently between 70% methanol and a 50:50 mixture of chloroform and carbon tetrachloride with 29 transfers. In brief, those tubes corresponding to authentic estradiol, estrone, and estriol were pooled and bioassayed.

The results (Table I) indicated estrogenic activity in tubes 3-9 (estrone) and 10-19 (estradiol). The estrone and estradiol fractions were divided into 2 aliquot portions, and each portion was subjected to the following 2 paper chromatographic systems, at room temperature; (1) benzene-formamide 1:1 for 22-24 hours; (2) toluene-propylene glycol 1:1 for 6 hours.

In each of the solvent systems the unknown was applied to one of 4 strips, estrone to another, estradiol to a third, and a mixture of estrone and estradiol to the fourth. The 4 strips in each system were run simultaneously. The 3 strips treated with the au-

TABLE I. Results of Bioassay (Astwood Method) from Countercurrent Separation.

Fraction	Uterine wet wt (mg)	% increase over control
Controls*	21.5	0
0-2	21.13	0
3-9	25.2	17.2
10-19	27.7	28.8
20-29	21.7	0

* Avg uterine wet wt for 20 untreated animals.

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