precultured pituitaries. This decrease may be due to either tissue necrosis which occurred during culture, or to removal of the hypothalamic influence. Histological examination, however, indicated that cultured pituitaries were maintained in a highly viable state. It has also been noted that anterior pituitaries homotransplanted to the kidney capsule had ½ to ⅓ the lactogen concentration of non-transplanted pituitaries (Gala and Reece, unpublished). It is believed that the decrease in lactogen concentration was due to removal of the hypothalamic influence.

A culture environment of pure O_2 appeared to be toxic to anterior pituitary tissue. This toxicity was reflected not only in cellular necrosis, but also in lactogen production and in lactogen concentration of the cultured tissue. Addition of only 5% CO_2 to the gas environment was capable of overcoming the toxicity of pure O_2 noted; however, the level of flow of 95% O_2 – 5% CO_2 mixture must also be considered. Levels below 150 cc/min were no better than air, and above 300 cc/min there was a decrease in lactogen production. Lactogen production and cellular viability for AP cultured as primary explants are therefore dependent upon the type of gas environment and level of gas flow.

Summary. Anterior pituitary explants from rats, cultured *in vitro* in the chemically defined Mixture 199 for 3 days, were capable of synthesizing and releasing into the medium 6 to 8 times more lactogen than initially introduced into the culture system. Lactogen production and cellular viability were dependent upon the gas environment and a 95% O_2 – 5% CO_2 gas mixture at a flow of either 150 or 300 cc/min was optimum. A pure O_2 environment did not support lactogen production and appeared to be toxic to anterior pituitary tissue. Culturing AP in air resulted in half the lactogen production noted for AP in 95% O_2 – 5% CO_2 and tissue survival was poor.

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Stimulation of Ovulation by Purified LH-Releasing Factor (LRF) in Animals Rendered Anovulatory by Hypothalamic Lesion.* (28696)

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There is now considerable evidence that extracts of the median eminence region of the hypothalamus contain a substance which stimulates the release of ovulation hormone (LH)(1,2,3,4). We have recently reported partial purification of this substance (LRF, for LH-releasing factor) using the ovarian ascorbic acid depletion method as a test of endogenous release of LH(5). To validate any possible physiological significance of these observations we had to demonstrate that this material (LRF) would stimulate secretion of LH in animals in which the cyclic release of LH was blocked by a suitable hypothalamic lesion, the most specific criteria for LH-activity being used, *i.e.*, ovulation with direct observation of tubal ova and subsequent formation of corpora lutea.

Materials and methods. Experiment I. Forty-two female rats, Wistar origin (Duterme Farms, Condé s/Huisne, France) B. W. 170-200 g were utilized here. In each of them a hypothalamic lesion was produced by a high frequency coagulation between the

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tip of 2 electrodes (1.0 mm apart) descended simultaneously on each side of the sagittal sinus. The stereotaxic coordinates (Krieg-Johnson instrument, de Groot's coordinates) were: A = 6.4 mm, H = −2 mm. Vaginal smears were begun 2 months following placement of the lesion; they were read daily for 4 weeks. Of this series, 26 animals were considered to be in "persistent vaginal estrus" when there was predominance of epithelial or cornified cells in at least 90% of the daily vaginal smears. With randomization tables, they were divided into 5 groups; in the first one, 4 animals were sacrificed with no further treatment; the anterior lobes of the pituitary glands were extracted as a pool in 4.0 ml of ice cold saline, with a few grains of sand and a ground glass rod. This extract, after centrifugation was assayed to determine LH content of the pituitaries (U_1 = 1/32 pituitary, U_2 = 1/8 pituitary) using the ovarian ascorbic acid depletion method (6,7) with the NIH-LH as a standard (S_1 = 0.8 μg LH, S_2 = 3.2 μg LH). The remaining animals were divided into 4 groups which received respectively LH (NIH-S_1) 4.0 μg, oxytocin (1.0 U), lys-vasopressin (1.0 U) and a preparation of LRF (1.2 mg). The preparation of LRF used here was a crude material prepared by gel filtration on Sephadex G 25 of an acetic acid extract of sheep hypothalamic tissues(5). Oxytocin and lys-vasopressin were materials of synthetic origin (gift of Dr. R. Boissonnas, Sandoz S.A., Basel). All injections were made i.v. (jugular vein). Twenty-four hours after the i.v. injections, one ovary was removed with the attached oviduct; the presence of tubal ova was investigated with a Zeiss 16 × binocular loupe; 72 hours after the i.v. injections, the second ovary and attached oviduct were removed and observed for presence of corpora lutea and tubal ova. Both ovaries were saved in Bouin's fluid and eventually were serially sectioned and stained with H and E. The brains of *all* the animals were perfused and fixed with 10% neutral formalin; they were serially sectioned at 100 μ and the sections stained with thionin, for reconstruction of the lesion.

Experiment 2. Sixteen female rats (170-200 g B.W.) with a hypothalamic lesion as in Exp. 1 were considered in persistent vaginal estrus according to the criteria outlined above. They were all subjected to laparotomy to further ensure absence of corpora lutea. Twenty-four hours later they were divided into 3 groups which received respectively 2.0 μg and 4.0 μg LH (NIH-S_1) and 50.0 μg of a preparation of LRF purified by carboxymethylcellulose chromatography following gel filtration(5). All injections i.v. (intra jugular); 72 hours after injection, both ovaries were removed and kept in Bouin's fluid for subsequent histological examination after H and E staining. The brains of all the animals were fixed and serially sectioned as above.

TABLE I. Presence of LH in Anterior Pituitary of Acyclic Rats with Hypothalamic Lesions. Ovarian ascorbic acid depletion test.

Materials injected	No. of animals	Ovarian ascorbic acid content
NaCl	6	107.92
LH S_1	6	100.02
LH S_2	6	74.69*
Pituitary extract U_1	6	106.93
" " U_2	6	71.08*

Analysis of covariance(7). Variance ratio F for *Treatment* = 3.52.*
Levels of significance *vs* NaCl (multiple comparison test of Dunnett): * = p: 0.01.

Results. Pituitary LH in rats with hypothalamic lesion. The figures presented in Table I show that the anterior pituitary of the rats rendered acyclic after placement of the hypothalamic lesion contain LH in demonstrable quantity; in this experiment, however, the 2 low doses (standard and unknown) gave responses in the ovarian ascorbic acid test that were not statistically different from the control levels (t test of Dunnett); thus, the mathematics of the classical 4-point assay could not be applied here; no attempt was made to repeat this experiment to obtain quantitative figures on LH content or concentration of these pituitaries.

Stimulation of endogenous LH secretion. In Table II, it can be seen that in both experiments injection of LH or of LRF was followed by appearance of tubal ova and subsequent formation of corpora lutea. Injection of lys-vasopressin or oxytocin had no similar effect. Fig. 1 A shows the histological

TABLE II. Ovulation and Formation of Corpora Lutea in Animals with Hypothalamic Lesion upon Injection of LRF. Control studies with LH (NIH-S_1), vasopressin and oxytocin.

Treatments	No.†	Tubal ova at 24 hr	Tubal ova at 72 hr	Corpora lutea at 72 hr
Exp I				
lys-vasopressin (1 U)	5	0	0	0
oxytocin (1 U)	5	0	0	1
LH (4 μg)	6	1	4	6
LRF (1.2 mg)	5	0	2	5
Exp II				
LH (2 μg)	5			2
LH (4 μg)	4			4
LRF* (50 μg)	7			6

* This material was purified by chromatography on CMC following gel filtration(5).
† No. of animals per group.

appearance of one ovary from one of the animals having received vasopressin 72 hours previously: it is the typical picture of the "permanent estrus," with presence of large follicles and absence of corpora lutea. Fig. 1 B shows that 72 hours after injection of LRF the histological picture is radically altered: ovulation has taken place and the follicles have been luteinized. The same picture is seen after injection of LH (2 or 4 μg).

Hypothalamic lesion. Reconstruction of all the brains showed the presence of a large lesion somewhat variable in size but consistent in its location in the anterior-medial hypothalamus.

Discussion. We have previously reported (2) that crude acid hypothalamic extract (sheep origin) will stimulate ovulation and induce subsequent formation of corpora lutea when injected in female rats rendered anovulatory by injection of testosterone within the first 5 days *post natum.* This observation has recently been confirmed by Johnson (8) using similarly prepared hypothalamic extracts, also of sheep origin. It can always be argued, however, that in the androgen-induced anovulatory animal(2,8), as well as in the nembutalized animal(2,4) LH-secretion upon injection of a substance could still be a transhypothalamic phenomenon rather than a direct (hypophysial) effect of the substance tested. In the series of experiments reported here the results observed are best interpreted by proposing that the preparation of LRF injected has stimulated directly the anterior pituitary gland to release LH thus producing ovulation and subsequent formation of corpora lutea. We have shown previously(2,5) with the highly sensitive ovarian ascorbic acid depletion test for LH that the

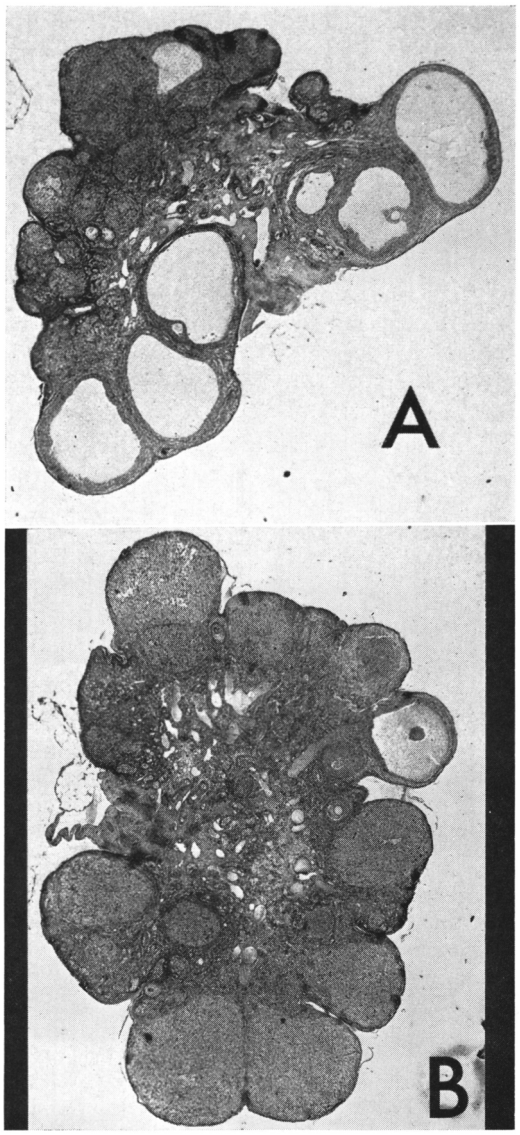

FIG. 1. A. One ovary from one of the acyclic rats following hypothalamic lesion, 72 hr after injection of 1.0 U of vasopressin: large follicles, no corpora lutea, "persistent estrus." B. One ovary from one of the acyclic rats following hypothalamic lesion, 72 hr after injection of LRF fraction of hypothalamic origin: evidence of ovulation and luteinization.

substance LRF is not active in hypophysectomized animals in conditions in which the sensitivity to LH is known to be adequate (9). Similarly we have shown earlier that acid extracts of the brain cortex or of the cerebellum, *i.e.*, control tissues not containing hypothalamus, do not stimulate endogenous secretion of LH(2). Thus the results observed here with the most specific criteria for evidence of secretion of LH (*i.e.*, ovulation and formation of corpora lutea) and in animals with a hypothalamic lesion blocking spontaneous release of LH, confirm our earlier conclusions based on the ovarian ascorbic acid depletion test, which had led us to conclude that we had purified in hypothalamic extracts a substance stimulating the secretion of luteinizing hormone.

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Failure of Cortisol to Reduce Glucose Uptake in Granuloma Tissue.* (28697)

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Inhibitory effects of corticoids on glucose uptake have been reported for several tissues, including rat granuloma pouch(1), rat epididymal fat pad(1,2), lymph nodes, thymus, lymphosarcoma(3), rat diaphragm muscle(4, 5,6), mouse ear skin(7), and human white blood cells(8). These studies have served to renew interest in the view that corticoid action at a cellular level may involve inhibition of glucose metabolism(9,10).

Since formation of granuloma tissue, as stimulated by foreign body implants, is inhibited by adrenocortical hormones(11), this tissue is especially suitable for examination of this hypothesis. Granuloma tissue, as used here, provides a conveniently excised sample of connective tissue elements, consisting of two principal components: a thick, well-organized, fibrillar outer zone, containing fibroblasts, monocytes, and giant cells; and a central zone of acute inflammation(12). Growth of the tissue, *in vivo*, is influenced by insulin (14), and growth hormone(15), as well as by corticoids(11,12,15,16). Accordingly, studies were undertaken to determine the effects of cortisol upon glucose uptake in granuloma tissue formed by subcutaneous implantation of cotton pellets. The results indicate that cortisol, whether administered *in vivo* or *in vitro*, does not decrease glucose uptake.

Methods. Male rats of the Sprague-Dawley strain (Charles River) weighing 135 to 170 g were adrenalectomized, at which time cotton pellets were inserted into subcutaneous tissue at sites about 1 inch lateral to the incision. Two pellets of unbleached cotton (Pride, Lockport Mills) weighing 6 mg each were implanted per rat. Saline (0.9%) was supplied for drinking water. Groups of rats were injected with medium or cortisol on the 6th day or 7th day after implantation as noted below and in Table I. Granulomas were excised on the 7th day after implantation and incubated in 2 ml of one of the following media: (a) Krebs-Ringer bicarbonate (KRB); (b) Krebs-Ringer phosphate ($KRPO_4$); (c) Eagles' L-amino acid medium supplemented with glutamine, and bicarbon-

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