

THE EFFECT OF EXOGENOUS LUTEINIZING HORMONE AND  
ESTROGEN ON THE CORPORA LUTEA OF THE  
HYPOPHYSECTOMIZED RABBIT

by

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## INTRODUCTION

The ovary has two responsibilities in the intact normally functioning animal:

(1) the production of follicles with fertilizable ova, (2) the production of steroids for normal physiology of the uterus and mammary tissue and feedback mechanisms to the pituitary via the hypothalamus. A malfunction results in aberration of the reproductive cycle and associated behavioral phenomenon.

The graafian follicle ovulates as a result of prolonged action of follicle stimulating hormone (FSH) and 10-12 hours after a surge of release of luteinizing hormone (LH) from the anterior pituitary (Hilliard et al., 1964) which, in the rabbit, is a result of the copulatory stimulus. This stimulus sends impulses to the hypothalamus which releases a luteinizing hormone releasing factor (LRF) into the portal circulation which in turn causes the anterior pituitary to release LH (Sawyer, 1959). Estrogen being produced in the graafian follicle also affects directly the anterior pituitary and the hypothalamus to activate release of LH into the circulation where it is reflected in elevated plasma ovarian ascorbic acid depletion (Kanematsu and Sawyer, 1964).

Hypophysectomy, involving the complete removal of the pituitary gland, is a useful tool in neuroendocrinological research and therapy. By this technique pituitary factors that control ovarian function are removed thus allowing evaluation of known levels of hormone replacement therapy.

The present experiment was designed to determine the effects of (1) hypophysectomy, (2) exogenous LH treatment, and (3) exogenous estrogen alone and combined with LH, for elucidation of the pituitary role in luteal maintenance and regression.

## LITERATURE REVIEW

Nalbandov (1961) suggested that the action of LH on the graafian follicle is that of lysing the papillary region, possibly accompanied by endosmosis resulting from rapid depolymerization of antral mucopolysaccharides by hyaluronidase (Zachariae, 1957), resulting in ovulation. The drastic release in pressure following ovulation allows for a slight contraction of the theca externa effecting increase of the folds of the granulosa. Hyperplasia and hypertrophy of the granulosa and theca interna results in cells filling the antral cavity while the theca externa gives rise to no specialized cells but serve only to encapsulate the forming and formed corpus luteum (CL). It consists of fibrous connective tissue vascularized with arterioles and venules. The granulosa cells become luteinized forming the blossom cells of the mature CL. The theca interna cells, closely associated with the blossom cells, are the companion cells of the formed CL (Gier and Marion, unpublished). These LH initiated postovulatory changes result in the glandular corpus luteum which, under proper stimulation, secretes progesterone for the maintenance of pregnancy or the luteal phase of the cycle. Progesterone causes proliferation of the uterine glands, in an estrogen primed uterus, thus providing a method of assay for the relative amount of progesterone (McPhail, 1934). Progesterone also has a feedback mechanism that suppresses pituitary gonadotrophins (Sawyer and Everett, 1959) possibly by a hypothalamic releasing factor inhibition (Sawyer and Kawakami, 1961; Smith and White, 1931).

Fee and Parkes (1929) and Smith and White (1931) showed that normal ovulation would not occur if the rabbit was hypophysectomized within one hour after copulation. Hypophysectomy later than one hour after copulation had no inhibitory effect, although subsequent development of the corpora lutea appeared to be abnormal (Fee and Parkes, 1929). The corpora lutea developed normally for two days but no further and were regressed by day 8. Spies et al. (1966) showed that the blossom cell count in the pseudopregnant rabbit reached its maximum by day 8 and then persisted until day 16 at

which time it rapidly decreased.

Rennie et al. (1964) and Kilpatrick et al. (1964) showed the pituitary to be necessary through pregnancy and pseudopregnancy for normal maintenance of the CL in rabbits and that replacement therapy with prolactin did not prevent luteal atrophy as it does in the rat.

A drop in pituitary LH of rabbits immediately after mating was reported by Hill (1934). He speculated that this drop represents aftereffects of an increased LH secretion. He further showed that the ovulating potency of the pituitary increased rapidly on day 3 and began to decline on day 10 and interpreted this rise to indicate a decreased rate of release of LH from the pituitary. Presumably a gonadotrophic complex was involved in the assay rather than just LH. Furthermore, actual levels of pituitary gonadotropin have been positively associated with plasma levels which increase concurrently with ovarian progesterin (Hilliard et al., 1964).

These results imply that the developing corpus luteum requires a luteotrophin for normal development, and that to remain functional, persistent luteotrophic substances must be present. A luteotrophin is any substance which directly or indirectly maintains a functioning corpus luteum. A luteolysin on the contrary causes breakdown or lack of function of the corpus luteum. Both types of response have been reported with LH in the rabbit (Kilpatrick et al., 1964; Stormshak and Casida, 1964; Spies et al., 1966).

The luteotrophic activity of LH is favored by Kilpatrick et al. (1964) in that they consider that LH acts indirectly on the corpus luteum effecting the interstitial tissue to secrete estrogen (Everett, 1961) which in turn maintains the functional corpus luteum. The ability of estrogen to counteract the luteolytic effect of LH would support this hypothesis (Spies et al., 1966). Stormshak and Casida (1964) and Spies et al. (1966) found that the ovary was unresponsive to human chorionic gonadotrophin (HCG) or LH prior to day 5 and that LH or HCG would not regress the original set of CL until after day 5. The first set of CL was maintained until the second set, which was formed after day 5, regressed.

Gurya and Greenwald (1964) showed a mobilization of lipid droplets in the estrual rabbit ovary which were thought to be steroid precursors, two hours after injection of HCG. These droplets disappeared when ovulation had taken place, 10-11 hours after injection of HCG, and by 3 days after ovulation the interstitial tissue was again laden with lipid droplets. Mobilization of these lipids as precursors of preovulatory progesterone may well explain the unresponsive state of the ovary just prior to and immediately after ovulation (Hilliard et al., 1964). Within a few days after ovulation, basal progesterone secretion gradually increases and ovarian responsiveness to exogenous LH appears to be more consistent (Endroczi and Hilliard, 1965). This lack of progesterone secretion after ovulation may in some way be coupled with the inability of LH to be luteolytic until day 5 of pseudopregnancy.

Stormshak and Casida (1964) found FSH on day 9 to cause reduced weight of the CL and the same effect was obtained with relatively small doses of HCG or LH. Contamination of the FSH with LH is inevitable and could explain the FSH luteolytic effect. Spies et al. (1966) noted similarly a reduced blossom cell count, indicative of luteal involution following FSH injection on day 7. LH appeared to be the gonadotrophin which most readily caused reduction in weight and decreased luteal cell counts. Stormshak and Casida (1964) hypothesized that stages of growth of a CL to maturity appears to be paralleled by an increasing susceptibility to the regressive action of LH. If LH does cause regression of CL in normal pseudopregnant rabbits, then it is not released in sufficient quantities to do so until late pseudopregnancy.

Luteal function in pregnancy may be controlled by estrogen, and a decrease in the production of estrogen may be followed by regression of luteal activity and parturition. In the intact pseudopregnant and pregnant rabbit, continual estrogen will maintain the corpus luteum (Allen and Heckel, 1936). Allen (1937), and Robson (1937, 1939) showed that estrogen would maintain the corpora lutea of the hypophysectomized rabbit sufficiently to maintain pregnancy (Robson, 1940).

Microcrystals of estrogen implanted into the corpus luteum maintains parts of the corpus luteum, establishing the concept of local effect of estrogen (Hammond and Robson, 1951). Local luteal cell maintenance was also effected in the hypophysectomized rabbit with a corpus luteum graft in the mammary tissue (Hammond, J. Jr., 1952). Spies et al. (1966) reported localized luteal regression of corpora lutea of the same age following LH and FSH treatment. Westman (1934) showed by isolating the CL from interstitial tissue that the luteal tissue regressed but could be maintained with exogenous estrogen.

Greep (1941) indicated that corpus luteum-uterus relationship was not mediated through the pituitary gland. He reported that corpora lutea, in both intact and hypophysectomized pregnant animals, hysterectomized in the latter half of pregnancy, underwent precipitous decrease in size, and that estrogen prevented this luteal involution.

The uterus, by its hormonal activity, can exert local effects directly on the ovary in some species as well as systemic effects on the ovary directly or via the hypothalamus, to provoke regression of corpora lutea (Anderson et al., 1964).

It has been shown that the life span of the CL is somewhat longer in hysterectomized than in pseudopregnant rabbits, being increased by hysterectomy to 23-29 days which approximates pregnancy maintenance. Chu et al. (1945) postulated a luteal stimulating substance of placental origin of the nature of estrogen. They found that the placenta transplanted to the peritoneal cavity maintained the CL to day 28.7, and postulated that the placenta may provide the stimulus for the secretion of the luteotrophin, or it may synthesize estrogen.

Stormshak and Casida (1964) evidenced a sparing effect of the placenta and embryo on LH luteolysis in that animals with six embryos versus two maintained corpora lutea more consistently. Thus it was postulated that uteri with six embryos produced more estrogen, or metabolized the LH, thus removing the luteolytic effect described.

The luteolytic effect of LH (HCG) in the pseudopregnant rabbit is not likely via



the uterus, as similar response is observed in both intact and hysterectomized rabbits (Spies et al., 1966).

#### MATERIALS AND METHODS

Thirty-six mature New Zealand White and Dutch Belted crossbred female rabbits were individually caged for at least one pseudopregnant period of 18 days to insure an estrual condition. The animal room had fluorescent lighting regulated for 14 hours light and 10 hours dark. Temperature was maintained at approximately 25 C by means of air conditioners and steam radiators. All animals received a complete pelleted ration and water, ad lib.

Six groups of rabbits, five to seven per group were utilized (Table 1). Group I animals were stimulated to ovulate by mating to vasectomized bucks on day 0 of the experimental period and served as pseudopregnant controls. Group II was treated the same as Group I except that on day 7, the pseudopregnant animals were hypophysectomized. Group III was treated the same as Group II except that on day 7, six hours post hypophysectomy, 25 ug NIH-LH-B<sub>1</sub> was administered subcutaneously, and treatment was continued every 12 hours until sacrifice on day 18. Group IV was hypophysectomized on day 7, given 25 ug NIH-LH-B<sub>1</sub> two times daily, plus 1 mg estrone in sesame oil, administered subcutaneously daily. Group V was hypophysectomized on day 7, and 1 mg estrone in sesame oil administered six hours post hypophysectomy, then daily thereafter. Group VI was treated the same as Group V except the dosage level was 50 ug rather than 1 mg of estrone.

Corpora lutea were induced in all animals either by mating to a vasectomized buck or by injecting 50 ug NIH-LH-B<sub>1</sub> via the posterior marginal ear vein, on day 0 of the treatment period. On day 3 or 4, via paralumbar laparotomy, the corpora lutea were marked. The animals were lightly anaesthetized with sodium pentobarbitol (Dibutol, Haver-Lockhart) diluted 1:2 with sterile saline. The surgical site was shaved and disinfected with tincture of Rocal 1:750 and 2% procaine was infiltrated at the site.



Into the papilla of each corpus luteum, 0.05 ml of India ink was injected through a 30 gauge needle attached to a 0.5 ml syringe operated by a rubber tube and air pressure applied by the operators mouth. This procedure frees both hands for ovarian manipulation, as well as giving better control of pressure. Muscles and peritoneum were sutured with a mattress stitch and the skin with interrupted stitch using 00 Ethicon Merselene.

On day 7 after mating, Groups II-VI were hypophysectomized by a modification of the infrahyoid parapharyngeal method described by Rennie et al. (1964b). The animal was partially anesthetized with 13.6 mg/kg sodium pentobarbital in 3 ml solvent via the marginal ear vein. The animal was placed in a supine position on the operating table (Fig. 3) and intubated by inserting a water moistened rubber tube, 5 mm OD and about 12.5 cm long, which had been cut on one end to form a 1 cm long taper point. The pointed end was inserted so that the point of the tube was ventral in the mouth and would catch on the glottis and be directed into the trachea with the aid of the animal's swallow reflex. It is important that the animal be only lightly anesthetized when intubating or the swallow reflex will be lost. Saliva and mucous secretion will collect in the animal's throat in a very short time so prompt tubation is ideal. Tubation is complete when one can hear an unobstructed air flow through the tube. This may also be checked by listening to the chest cavity with a stethoscope. If the flow is obstructed, the tube must be partially withdrawn and reinserted. The alignment is facilitated by manipulating the trachea externally while pushing the tube into the throat. When a cough reflex occurs, constant pressure should be put on the tube as the cough usually indicates proper alignment. About 75 mm of the tube should slide in easily. Smaller animals may require a smaller canula.

The lower jaw and neck region was shaved and more anesthesia administered, sufficient to eliminate the foot pad reflex. A 4 cm midline incision was made 4 cm posterior to the mandibular symphysis, extending posteriorly beyond the base of the tongue. Retractors furnished with the Brookline operating table (Fig. 3) were used

to expose the hyoid bone (Fig. 7). After blunt dissection and retraction of the salivary glands and muscles, the stylohyoid muscle (Fig. 8) was cut (Fig. 9) allowing the hyoid bone and larynx to be retracted lateral to the midline exposing the prevertebral muscles (Fig. 10) which insert on the basisphenoid bone. The sublingual musculature was retracted so that the fascia connecting the posterior wall of the nasopharynx to the basisphenoid could be cut anterior to the point where the branching of the basisphenoid vein may be seen (Fig. 11). The surgery to this point is bloodless. A special silastic covered retractor (Fig. 2) was designed to both retract the musculature and depress the prevertebral vein as it runs anteriorly along the presphenoid bone. After the retractor was in place, the basisphenoid vein was broken and the blood aspirated from the cavity through a blunted 75 mm no. 15 needle. The basisphenoid was exposed anteriorly to the foramen cavernosum by use of a dental wax carving instrument (Fig. 1) and the drill hole was then started with a low speed 2 mm plain excavating round bit 1.5 mm posterior to the foramen of the sinus cavernosis (Fig. 6). The pilot hole was made at a right angle to the plane of the table. Further widening and directing of the hole was accomplished with a 4 mm barrel bit (Fig. 1) aimed in a plane downward and slightly rostral. When the pilot hole was  $\approx 3$  mm deep, a bone wax plug was inserted into the hole. Then a 5 mm pear bit was used to complete the drilling to the dura mater over the pituitary. When the dura had been exposed and the operator ascertained the position of the hole with respect to the pituitary, a bone wax plug was inserted and drilled out; taking care not to drill into the pituitary. This is most successfully accomplished if the bone wax plug is inserted prior to removing the final thin layer of bone, thus eliminating the chance of inserting the wax too far and pushing the pituitary out of the sella tursica into the brain cavity. The wax fills the bone sinuses which otherwise drain blood into the field. The exposed dura mater, was cut with a Wheeler discission knife (Fig. 1) and torn back to the margin of the hole with a Graefe cystome (Fig. 1). A Wilder lens loop (Fig. 1) was passed over the posterior end of the pituitary, under the dura mater,

and worked anteriorly to cut the stalk as close to the dorsal of the sella tursica as possible. The intact pituitary was lifted through the hole with the no. 15 suction needle. If the hole was drilled in the proper position the walls of the pituitary capsule could be inspected for remaining tissue with the aid of the Zeiss operating microscope (Fig. 3). The hole was filled with a bone wax plug inserted with a Green spatula (Fig. 1). The field was cleaned of bone wax chips, an antibiotic powder liberally applied and the skin incision closed with interrupted sutures of Ethicon 000 Merselene. One mg atropine methyl nitrate was administered half intramuscularly and half subcutaneously to inhibit salivation after detubation and to decrease incidence of post operative shock reaction which is exhibited by violent contractions of the legs and abdomen. For further protection against infection and shock, 200,000 units Procaine Penicillin G, 0.25 gm dehydrostreptomycin, and 1 mg cortisone (Sterane-Pfizer) was administered IM. The animal was placed in an incubator maintained at 37 C with a high O<sub>2</sub> concentration until it had recovered from anesthesia, usually about two hours, after which it was returned to its cage at 20 C. Glucose was added to the drinking water at the rate of two tablespoons per liter, and lettuce was given daily.

All animals in each Group I-VI, were unilaterally ovariectomized on day 11, at which time one corpus luteum was cut off the ovary with some surrounding interstitial tissue and fixed in formol-acetic for histological analysis. The remainder of the marked corpora lutea were peeled from the ovarian stroma under a 30X dissecting scope and weighed on a chain-o-matic balance. The animals were sacrificed on day 18 of pseudopregnancy and the other ovary was treated as above. In addition, sections of uterus, thyroid and adrenal gland were fixed for histological analysis. The skull interior and sella tursica were examined under the 40X surgery microscope. If pituitary tissue was found, the animal was eliminated from the experiment.

Corpus luteum function was evaluated in three ways: gross observation in vivo, ascertaining the vascularity and color; histological analysis involving blossom cell

count (Spies, Gier, and Wheat, 1959); and corpus luteum weight at days 11 and 18.

Photographs were taken of the corpus luteum in situ prior to ovariectomy at day 11 and at sacrifice for comparison of vascularity.

The formol-acetic fixed tissues were infiltrated with paraffin, sectioned at 8 microns and stained with periodic acid Schiff and Mallory's triple. Normal blossom cells (Fig. 13) were counted, and six 1000X oil immersion fields per corpus luteum were averaged for the representative cell count. Pycnotic nuclei (Fig. 15), vacuolated cytoplasm (Fig. 16), and swollen nuclei (Fig. 17) were not counted. Corpora lutea weights were not obtained for Group III, day 18, to advanced regression, but weights at all other stages and groups were obtained. Group differences in luteal cell number and corpus luteum weights were analyzed via analysis of variance. When significant, individual group means were compared for least significant difference (Snedecor, 1956).

## RESULTS

In comparing the two methods of ovulating the does, there was no significant difference in the LH treated versus mating with the vasectomized buck. In the 21 animals which received 50 ug of NIH-LH-B<sub>1</sub>, there resulted a total of 180 ovulations which were divided 89 and 91 for the left and right ovaries, respectively. This represents 8.57 ovulations per treatment as compared to an average of 9.38 ovulations from 21 animals mated to a vasectomized buck. Six animals which would not accept the buck were given LH, resulting in an average of 8.50 ovulations. This number of ovulations was highly consistent throughout the experiment.

The hypophysectomy technique, once perfected, was a reliable means of removing the pituitary as was evidenced by the low incidence (four in 70 operations) of remnants of pituitary gland in the sella turcica at sacrifice. The routine technique may be performed with aid of an assistant, and without complications, in 30 minutes from the time the animal is removed from the cage to the time it is returned to the

oxygen incubator. Some postoperative trauma of the nasopharynx occurred where the restraining fascia was cut allowing the nasopharynx to vibrate so that the animal sounded like it was snoring. In 3-5 days postoperatively, edema in the nasopharyngeal region frequently obstructed air flow and an occasional tracheostomy was necessary. The 12 animals that died from excessive edema were eliminated from consideration.

It was necessary to do the ovariectomy on day 11 under mild sedation and local anesthesia because of decreased tolerance of the hypophysectomized animals to sodium pentobarbital and inability to react to stress.

The peeled corpora lutea were as devoid of surrounding stromal tissue as was possible under the 30X magnification. Clean dissection of corpora lutea was difficult in Groups II and III in which they were greatly regressed, and in Group III was not accomplished at day 18 because of the breakdown of the connective tissue capsule.

Corpora lutea of the pseudopregnant control group averaged 15.9 mg on day 11 and were highly vascular but did not alter the contour of the ovary significantly (Fig. 4). The day 11 luteal cell count on 5 animals averaged 8.3 per microscope field. By day 18 the corpora lutea were regressing, evidenced by the avascular gross appearance and the reduced average weight of 8.8 mg. PAS positive paranuclear spheres were not noted at either day 11 or 18. Cells showing early changes such as shrinkage of the cytoplasm and nuclear pycnosis (Fig. 15) were not counted, resulting in a count of 4.84.

At day 11 the luteal cell count in Group II had not decreased (8.2) below control values. There was some cytoplasmic shrinkage, although nuclei looked normal. By day 18 fibrocyte increase and hypertrophy associated with cytoplasmic shrinkage and nuclear pycnosis (Fig. 14) resulted in a reduced count of 2.46 which was statistically not significantly different from the control (Table 1, Fig. 18) because of the small number of ovaries counted. By day 18 connective tissue of the theca externa was diminished and the corpus luteum was so shrunken that removal from the ovarian stroma was impossible, thus no weights were obtained. These late regressive corpora lutea exhibit few swollen nuclei and very little highly diffuse PAS<sup>+</sup> material in the



cytoplasm. All blossom cells in this group exhibited nuclear pycnosis by day 18.

Luteal cell count of 5.1 on day 11 in the hypox-LH treated group (III) was not significantly different from the count in the controls, although the corpus luteum weight was significantly reduced, to 3.5 mg. Most of the nuclei of the blossom cells were either swollen or pycnotic. The cell size was reduced and there was a high incidence of paranuclear spheres. At day 18 neither the luteal cell count (3.36) or the weight (3.3 mg) significantly differed from the control or the hypophysectomized group (Table 1), probably because of small numbers involved. Histologically, the hypox-LH treated group showed more drastic luteal involution in that the blossom cells were one fourth the size of those in the controls and half the size of those in the hypox group. There was also a higher concentration of connective tissue present at day 18 than in the hypox group. There was a high degree of nuclear pycnosis but there were a few normal looking nuclei in shrunken cells (Fig. 15). The PAS positive material was highly diffuse and paranuclear spheres were few.

Luteal cells in the hypox-LH-estrogen treated group (IV), both at 11 and 18 days, exhibited vacuolated cytoplasm (Fig. 16) but relatively normal nuclei. The cell count (6.8) and weight per CL (18.1 mg) did not differ significantly from the control on day 11 (Table 1, Fig. 18). At day 18 the luteal cell count was not significantly different from control and there was no detectable cellular shrinkage or nuclear pycnosis. The weight of the corpora lutea was significantly increased over control weights at day 18, demonstrating the sustaining effect of the estrogen on the corpora lutea. The most obvious abnormality at day 18 was the vacuolated cytoplasm (Fig. 16).

Estrogen alone (Group V) in the hypophysectomized rabbit causes vacuolation of the luteal cell cytoplasm as early as day 11 and persists through day 18 with no apparent harm to the nuclei. Paranuclear spheres were more numerous on day 18 than day 11. Few differences were noted between this treatment group and the control, the

Table 1. AVERAGE CORPUS LUTEUM WEIGHTS AND LUTEAL CELL NUMBERS

Group	Treatment	Corpora lutea weights (mg)				Luteal cell numbers			
		Day 11		Day 18		Day 11		Day 18	
		Means*	S.D.	Means*	S.D.	Means*	S.D.	Means*	S.D.
I	Control	15.9 <sup>a</sup>	2.1	8.8 <sup>c</sup>	2.4	8.3 <sup>e</sup>	1.2	4.5 <sup>fg</sup>	1.5
II	Hypox.	2.9 <sup>b</sup>	.3	-	-	8.2 <sup>e</sup>	2.6	2.5 <sup>g</sup>	.8
III	Hypox. + LH	3.5 <sup>b</sup>	.9	2.7 <sup>c</sup>		5.9 <sup>e</sup>	1.7	3.4 <sup>g</sup>	1.3
IV	Hypox. + LH + E**	18.1 <sup>a</sup>	1.7	16.1 <sup>d</sup>	3.0	6.7 <sup>e</sup>	1.0	6.0 <sup>f</sup>	.2
V	Hypox. + E**	15.5 <sup>a</sup>	3.4	16.3 <sup>d</sup>	1.0	5.2 <sup>e</sup>	.9	3.4 <sup>g</sup>	1.6
VI	Hypox. + E***	17.2 <sup>a</sup>	4.1	18.9 <sup>d</sup>	4.8	7.3 <sup>e</sup>	.4	6.5 <sup>f</sup>	1.3

\* Means with the same superscript letter do not differ ( $P < .05$ )

\*\* 1 mg estrone daily, \*\*\* 50 ug estrone daily, LH=25 ug NIH-LH-B<sub>1</sub> 2X daily

#### Analysis of Variance

Source of Variance	Corpus luteum weight (mg.)				Luteal cell count			
	Day 11		Day 18		Day 11		Day 18	
	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.
Treatment	5	231.56**	4	89.13**	5	4.25	5	8.00**
Within	25	40.91	17	10.48	20	2.85	19	1.46

$P < .01 = **$



exception being the vacuolation of cytoplasm and a higher concentration of connective tissue at day 18. The luteal cell count was 5.2 and 3.4 and the weight was 15.5 and 16.3 mg, day 11 and 18 respectively (Table 1).

The 50 ug dosage of estrone, thought to be more nearly the physiological dosage, maintained papilliform corpora lutea (Fig. 5) through day 18 in such a way that histologically they looked like control CL at day 11. The blossom cells were somewhat larger and as densely arranged as in the control at day 11, lacking the infiltration of the connective tissue that occurred with the higher estrogen level. The corpus luteum weight was well maintained (17.3 mg) at day 11 and increased to 18.8 mg at day 18. The luteal cell count was 7.3 at day 11 and was maintained to 6.5 at day 18 (Table 1).

Both the endometrium and myometrium became highly edemateous in those animals treated with 1 mg estrone, and in all cases the endometrium sloughed severely by day 18. The 50 ug level caused edema of the endometrium but not of the myometrium and caused only occasional endometrial sloughing.

#### DISCUSSION

The state of functioning of a corpus luteum can be approximated by several methods. The most obvious, of course, is direct measurement of the progesterone in the corpus luteum, which can be done by the thin layer chromatographic method of Armstrong (1965). Unfortunately, the tissues frozen for this purpose were ruined and no progesterone determinations were possible. Another measure is the weight of the corpora lutea, determined from the excised corpora. Obviously, this method can not be a true measure of function because each corpus luteum is comprised of several components; blood vessels, connective tissue, companion cells, and blossom cells, only the last of which is directly involved in the production of progesterone. Nevertheless, it is a measure, and was used as far as possible (Table 1). The third measure of function is the actual number of blossom cells that are present, the "luteal cell count" described by Spies et al. (1964) and a direct correlation between

cell count and progesterone was shown to exist. The problem of luteal cell count arises in making the determination of what cells to count. In this study, as was recommended by Spies et al. (1964), only full, nonvacuolated cells with normal appearing, plump nuclei were counted. Shriveled, pycnotic or swollen nuclei (Figs. 15, 17) were not considered to constitute normal, functional units. Luteal cell counts, in general, are directly correlated to the weight of the corpora, but early functional failure can be detected by cell counts more quickly than by corpora lutea weights.

The variability in cell size resulting from cytoplasmic shrinkage has converse relationships in the estrogen treated groups versus the control and non-estrogen treated groups. The lack of the luteotrophic effect of LH on the CL or the direct luteolytic effect is exhibited in cellular shrinkage, yet the nucleus maintains its configuration, resulting in higher luteal cell counts. There is a uniformly larger cell under estrogen stimulation, which gives lower cell count per unit area and an increased weight of the corpus luteum.

Failure to maintain corpora lutea in the hypophysectomized rabbit with or without exogenous LH and the more rapid regression with exogenous LH than in the hypox alone indicate that the pituitary is not involved directly in the pathway of the luteolytic response of exogenous gonadotrophins. This observation further verifies the work of Robson (1937) who prolonged the life of functional corpora lutea in hypophysectomized rabbits by treatment with HCG, and with horse and rabbit pituitary extract, the major active constituent being LH, but undoubtedly a gonadotrophin complex.

Early endocrinologists ascribed prolactin as the luteotrophin, however, more recently it has been recognized that luteotrophin may be estrogen, LH, FSH or a combination of these, depending on the species. Furthermore, LH presumably has both luteotrophic (Kilpatrick et al., 1964) and luteolytic (Stormshak and Casida, 1964;

Coon, 1964) properties in the rabbit, possibly depending on whether it is administered to a hypophysectomized or an intact rabbit, and at what stage of luteal development it is administered. The influence of stage of LH administration in the hypophysectomized rabbit and LH effect is seen in the differences Kilpatrick et al. (1964) found between their 2 and 12 hour post-hypox-LH treatment groups. When LH is administered two hours post-hypox, ovulation occurs and the older, original corpora regress in contrast to the luteotrophic effect and no ovulation when LH is administered at 12 hours post-hypox. These differences may be associated with the refractory period the ovary exhibits to steroidogenesis for three hours after ovulation (Hilliard et al., 1964). The presence of steroid precursor droplets (Gurya and Greenwald, 1964) in the interstitial cells at two hours post LH which are depleted by 10-11 hours appears to be associated with steroid production. If the observed steroid droplet depletion indicates an interruption in the production of estrogen, which is luteotrophic, it may inadvertently be responsible for the regression of the corpus luteum. Here again, the level of LH administration may be important in determining the level of steroidogenesis, as an excessive amount of LH may cause metabolic changes with rapid mobilization of steroid precursors yielding a sudden drop of this steroid upon systemic metabolism of the trophic hormone. A few days are required to re-establish the normal integrity of the cells and steroid production. This refractory period of the ovary is probably associated with the failure of HCG to initiate its luteolytic effect (Coon, 1964) until day 5 of pseudopregnancy.

Stormshak and Casida (1964) showed that gonadotrophins containing LH when injected on day 9 caused luteal regression and that the level required to have this effect decreased with increasing age of the CL in the intact rabbit. This suggests possibly a build-up of a pituitary luteolysin which, when released, effects corpora lutea regression. This stimulus may be a steroid mediated feedback mechanism which is moderated by the uterus since, in the hysterectomized animal, luteal lifespan is

extended. In one portion of this experiment, the endometrium was destroyed by edema resulting from treatment with 1 mg estrone, in which case, luteal maintenance was less positive than with the lower estrogen level. The lesser estrogen dosage did not destroy the endometrium and maintained the corpus luteum better. Thus, it is improbable that any of the results of the present series can be interpreted on the basis of uterine interactions.

It appears that source of the LH, time or stage of luteal development, and level of dosage are factors determining whether a forming or formed corpus luteum will function or regress in the hypophysectomized or in the intact rabbit.

#### CONCLUSIONS

Several points are directly indicated by the combined evidence from the six groups of rabbits used in these experiments.

- 1) Hypophysectomy, properly done, is not seriously detrimental to the well being of the animals, and provides a useful procedure for study of pituitary gonadotrophins.
- 2) Bovine pituitary LH (NIH-LH-B<sub>1</sub>) is not effective in maintaining corpora lutea in hypophysectomized pseudopregnant rabbits when given at 12 hour intervals beginning 6 hours after hypophysectomy.
- 3) Estrone, injected IM in oil, is effective in maintaining the corpus luteum, either with or without LH.
- 4) An estimated physiological estrone level (50 ug) is more effective in maintaining corpora lutea and has less adverse uterine side effects than are present with higher levels (1.0 mg).

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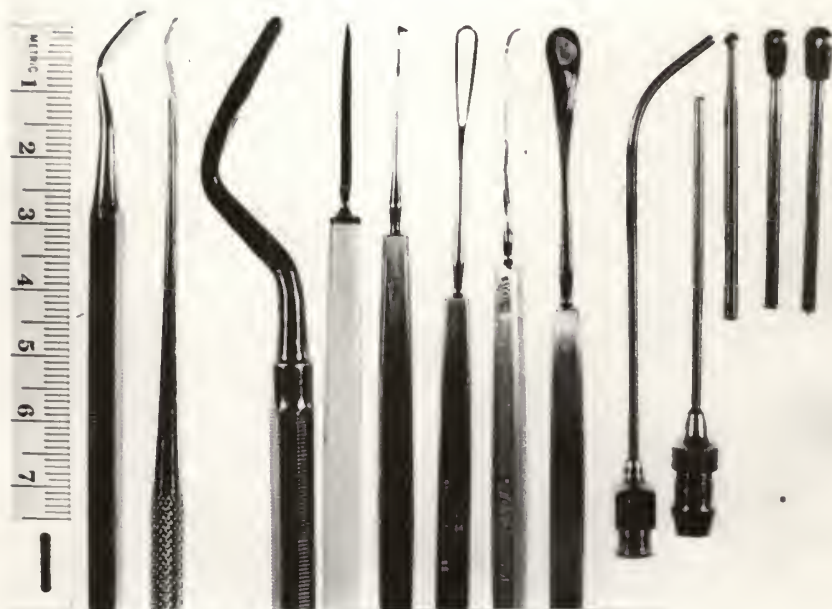
## EXPLANATION OF PLATE I

Fig. 1. Surgical instruments used in the hypophysectomy technique in the rabbit are, from left to right:

- Dental spatula
- Dental wax carver
- Special retractor
- Wheeler discission knife
- Graefe cystom
- Wilder lens loop
- Malable sterling spoon
- Bone wax spatula
- Blunted aspirating needles, curved and straight
- 2 mm plain excavating bit
- 4 mm pear bit
- 5 mm pear bit

Fig. 2. Retractor especially designed to retract nasopharynx musculature and depress the branching prevertebral vein as it runs over the presphenoid bone. A dental wax carving spoon formed with stainless steel filling material was covered with a thin layer of silastic and several layers of inert tough plastic. The tip was contoured to fit firmly in the wedge formed by the pterygoid bones and their covering facia and musculature.

## PLATE I



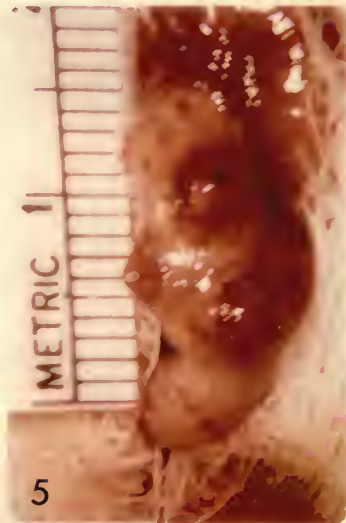
## EXPLANATION OF PLATE II

Fig. 3. Brookline operating table with retractors, Zeiss operating microscope and belt driven dental drill in position on special table having foot operated relay switches for dental drill, suction and cautery.

Fig. 4. Ovary at day 11 of the cycle, with marked corpora lutea. Corpora at this time regularly protrude evenly from the surface, and are highly vascularized.

Fig. 5. Ovary from an estrogen treated hypophysectomized rabbit, 18 days after ovulation. Estrogen treatment results in papilliform, highly vascularized corpora lutea, even in the absence of pituitary hormones.

## PLATE II



### EXPLANATION OF PLATE III

Fig. 6. A ventral view of a rabbit skull with mandibles removed demonstrates the position of the drilled 5 mm hole with respect to the landmarks; basisphenoid synchondrosis and foramen cavernosum (small hole) 2X.

Fig. 7. Surgical exposure show the base of the tongue, salivary gland (middle bottom) and white hyoid bone posteriorly attached via stylohyoid muscle. 2X.

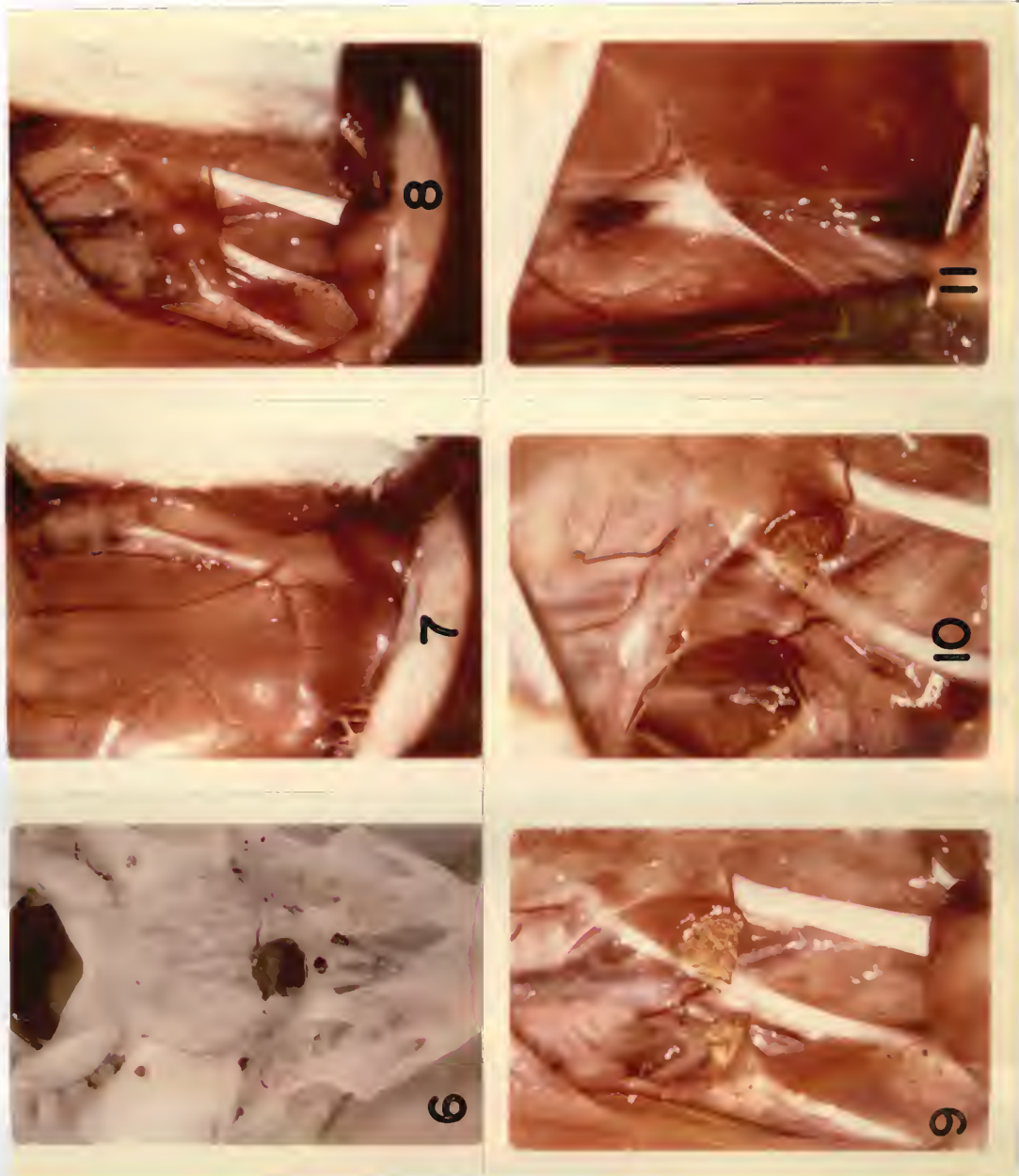
Fig. 8. The hyoid bone fascia is loosened spreading the stylohyoid muscle over the lingual nerve, carotid bundle and (white) tendon. 2X.

Fig. 9. The stylohyoid muscle is severed exposing the carotid bundle and sympathetic chain. 2X.

Fig. 10. A hole was made in fascia exposing the prevertebral muscles and basisphenoid vein. 2X.

Fig. 11. The nasopharynx fascia was retracted then cut to allow the nasopharynx to be retracted anteriorly. 2X.

## PLATE III





#### EXPLANATION OF PLATE IV

Fig. 12. Cellular configuration of a normal corpus luteum shows from left to right; interstitial tissue, theca externa, endothelial cells lining the capillary and luteal cells interspersed with companion cells. (200X).

Fig. 13. Section of normal corpus luteum. Nine countable large normal luteal cells are interspersed with companion cells with smaller nucleoli. (800X).

Fig. 14. Corpus luteum in early regression. Nuclear pycnosis results in cells which are uncountable. There is also an increase in amount of connective tissue intermixed. (650X).

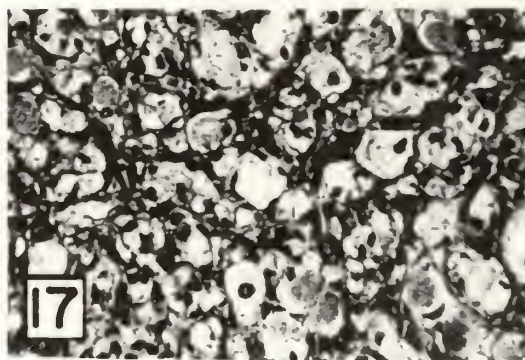
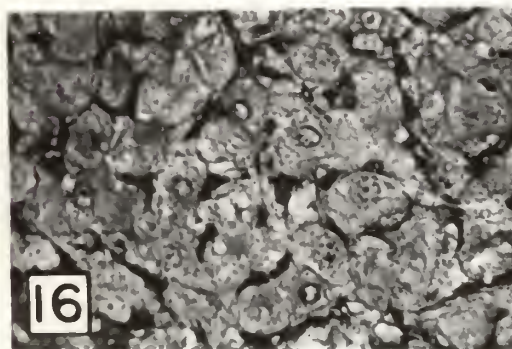
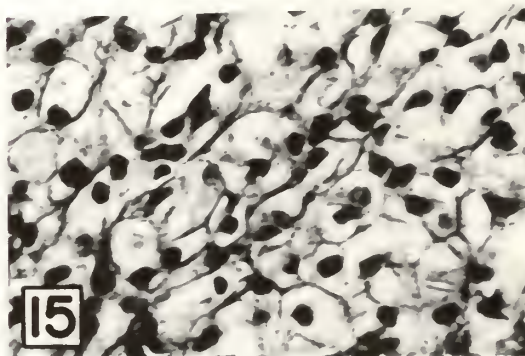
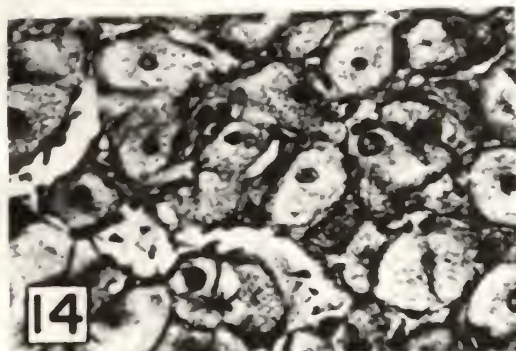
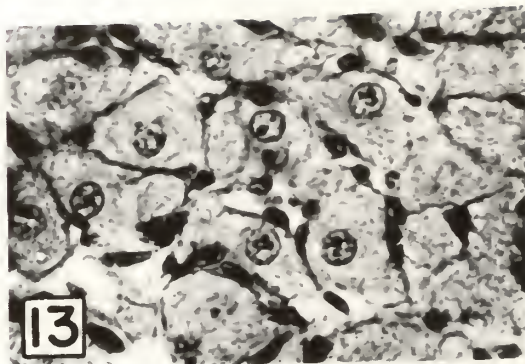
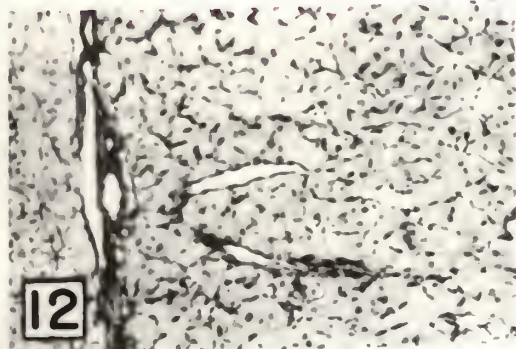
Fig. 15. Corpus luteum in definite regression stage. Post pycnotic cellular shrinkage increases the number of cells per field and makes luteal and companion cells less distinguishable. (800X).

Fig. 16. Corpus luteum from hypophysectomized, estrogen treated rabbit. Some blossom cells exhibit cytoplasmic vacuolation presumably indicative of lipid storage. (650X).

Fig. 17. Corpus luteum in late regression. Some of the nuclei swell and others show pycnosis. Cytoplasm of all blossom cells is vacuolated. (400X).

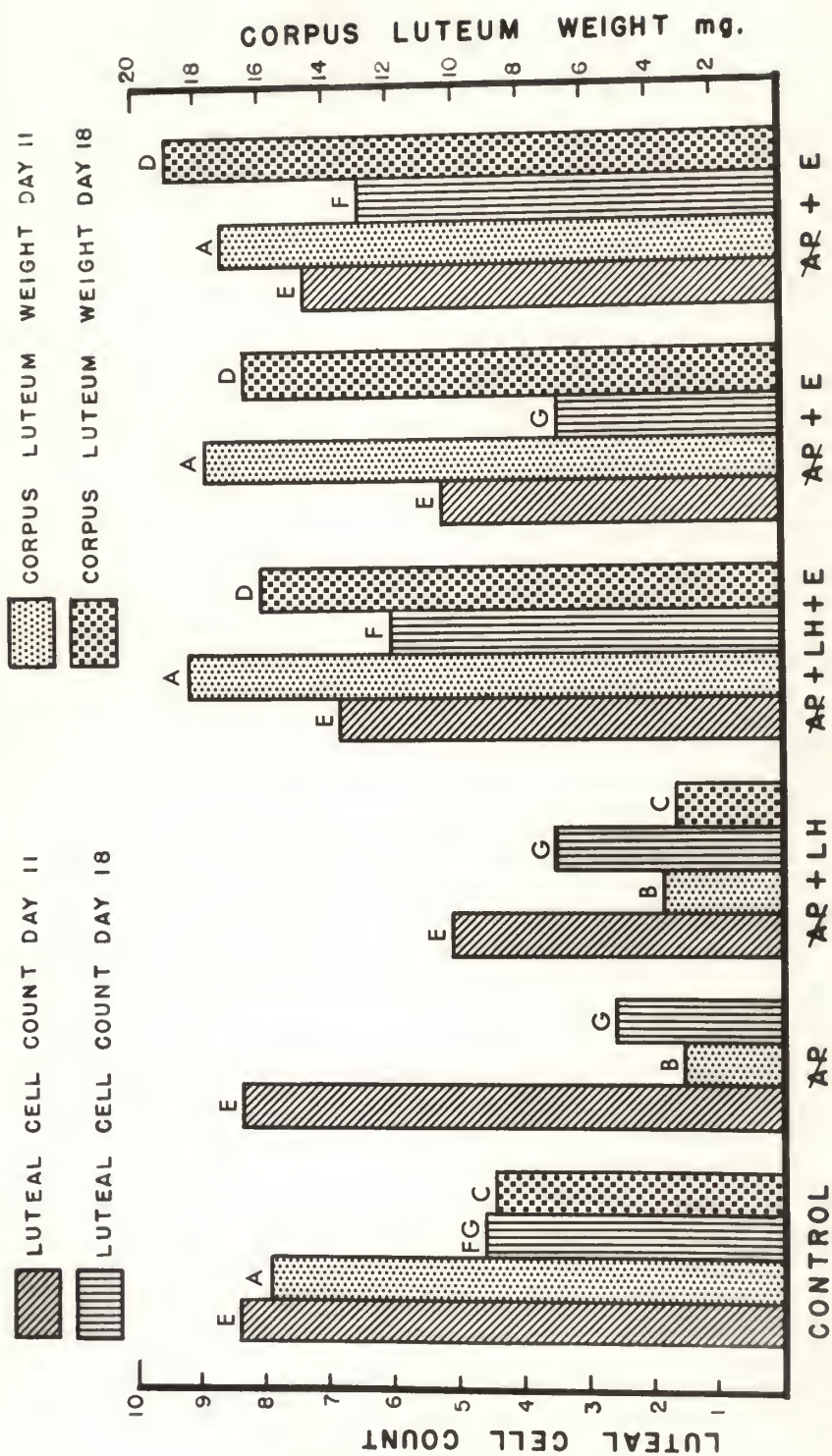


## PLATE IV



#### EXPLANATION OF PLATE V

Fig. 18 Luteal cell counts and corpus luteum weight at day 11 and day 18 under the various treatments described in the text. Bars having the same superscript are not significantly different.  $P < 0.05$ .



THE EFFECT OF EXOGENOUS LUTEINIZING HORMONE AND  
ESTROGEN ON THE CORPORA LUTEA OF THE  
HYPOPHYSECTOMIZED RABBIT

by

DON RICHARD WARREN

B.S., Kansas State University, 1964

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AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirement for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE UNIVERSITY  
Manhattan, Kansas

1966



## ABSTRACT

Six groups of rabbits, five to seven per group, were individually caged for an 18 day pretreatment period. Corpora lutea were induced in each animal by mating to a vasectomized buck on day 0 of the treatment period. Each corpus luteum was marked with India ink on day 3 or 4 for subsequent identification via paralumbar incision while the animal was lightly anesthetized with pentobarbitol.

On day 4 Groups II-VI were hypophysectomized by a modified infrahyoid parapharyngeal method of Rennie (1964), for which a retractor was designed and constructed. All animals were unilaterally ovariectomized on day 11 and sacrificed on day 18. Corpus luteum weights were recorded at both times and tissues were frozen and fixed for histological analysis.

The treatment groups were as follows:

- I. pseudopregnant control
- II. hypophysectomized pseudopregnant
- III. hypophysectomized and LH treated 6 hours post hypophysectomy with 50 ug daily thereafter.
- IV. treated same as Group III plus 1 mg estrone daily
- V. hypophysectomized plus 1 mg estrone daily.
- VI. hypophysectomized and received 50 ug estrone daily

Corpus luteum blossom cell counts were made, averaging six 1000X fields of PAS and Mallorys Triple stained tissue. Both the blossom cell count and CL weights in groups lacking estrogen were not different at day 18, although estrogen treated groups were significantly increased in weight and count at day 18 approximating the normal functioning CL at day 11.

Hypophysectomized-LH treated animals exhibited a greater degree of aberrant nuclei, either swollen or pycnotic, as compared to the hypophysectomized group alone. Estrogen treatment maintained normal cellular configuration of the CL to sacrifice at

day 18. The 50 ug dosage of estrogen maintained the CL in a condition more comparable to normal in that there was less increase in connective tissue and cytoplasmic vacuoles than in the 1 mg dosage level. The high level of estrogen caused much edema in the endometrium.

These results suggest that the pituitary is not directly involved in the maintenance of the corpus luteum since the exogenous LH administration will not maintain, but rather permits regression of blossom cells and dominance of connective tissue.

Estrone at the 50 ug level maintained the CL at a more nearly normal configuration than did the 1 mg level, supporting previous observations that indicate direct effect of estrogen on luteal maintenance. Both luteolytic and luteotrophic activities have been ascribed to LH, indicating that the stage of formation of the CL may be important. Time and level of LH administration need further investigation.

The luteolytic effect observed for LH, injected at 12 hour intervals, may be explained by lack of estrogen production in the interstitial cells adequate to maintain the CL, resulting from repeated depletion of the steroid precursors before concentrations reach functional levels. A continuous trickle, or frequently repeated small doses of LH, might result in continuous mobilization of the steroid precursors and estrogenesis at effective levels.

Thus, luteotrophic action of the pituitary must be indirect, through estrogen production, rather than direct action of LH on the corpus luteum.