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Title PHYSIOLOGY OF THE CORPUS LUTEUM OF THE RAT

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Rat luteal cells from functional and non-functional corpora lutea (corpora lutea rendered non-functional by hypophysectomy) were preserved for light and electron microscopy in Bouin's and glutaraldehyde-osmium fixation respectively. Material for electron microscopy was dehydrated with ethanol, embedded in Araldite and stained with lead hydroxide or uranyl acetate-lead citrate. Examination of functional and three day non-functional luteal cells by the electron microscope revealed no detectable differences in ultrastructure. The appearance of dense bodies demarcated the one week non-functional luteal cells which also contained a more dilated endoplasmic reticulum and more spherical mitochondria. Luteal cells, non-functional for two weeks, were similar to one week non-functional cells except for more exaggerated structural differences. Dilation of the endoplasmic reticulum may have been due to poor fixation. Luteal cells which were non-functional for one month showed marked changes from functional luteal cells: i.e. mitochondria of low

density and a sparse endoplasmic reticulum; dense bodies, and ribosomes were still evident. From these results, functional luteal cells cannot be distinguished from early non-functional cells by electron microscopy. Distinctions can be noted only after a period of three to seven days following cessation of function. Several hypotheses are presented in relation to the mechanism of action of luteotropic hormone.

PHYSIOLOGY OF THE CORPUS LUTEUM OF THE RAT

by

JAMES MICHAEL SHUMAKE

A THESIS

submitted to

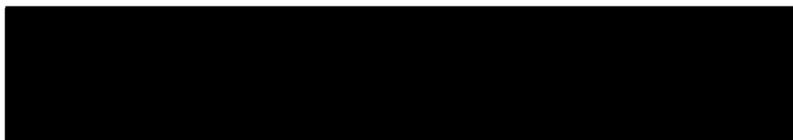
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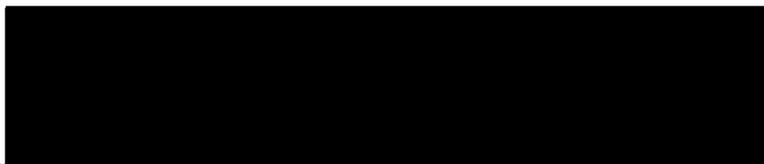
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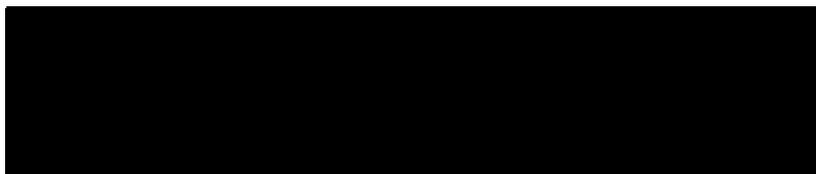
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## TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION.....	1
MATERIALS AND METHODS.....	9
RESULTS.....	11
Control and 72-Hour Luteal Cells.....	15
One-Week Experimental Luteal Cells.....	18
Two-Week Experimental Luteal Cells.....	20
DISCUSSION.....	24
SUMMARY.....	30
BIBLIOGRAPHY.....	31
APPENDIX.....	36

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	Light photo-micrograph of a section from a control ovary from Rat 17, fixed at time of hypophysectomy.....	11
2	Electron micrograph showing a survey view of control luteal tissue from Rat 13.....	12
3	Electron micrograph from a control corpus luteum (Rat 17).....	13
4	Electron micrograph from a 72-hour post hypophysectomy experimental luteal cell (Rat 17).....	14
5	Electron micrograph of a section from a control luteal cell (Rat 19).....	16
6	Electron micrograph of a section of a 72-hour experimental luteal cell (Rat 19).....	17
7	Electron micrograph of a seven-day experimental luteal cell (Rat 33).....	19
8	Electron micrograph of a two-week experimental luteal cell (Rat 35).....	21
9	Electron micrograph of a one-month experimental luteal cell (Rat 39).....	23

LIST OF TABLES

<u>Table</u>		<u>Page</u>
1	Decidual Reactions.....	36

# PHYSIOLOGY OF THE CORPUS LUTEUM OF THE RAT

## INTRODUCTION

The structure known as the corpus luteum is of primary importance in mammalian reproduction. The corpus luteum was first observed by Vesalius in 1543. However, credit for its discovery was given to Coiter in 1573 by Harrison in 1948.

The corpus luteum usually begins with the rupturing of the follicle and discharge of the ovum. There was a great deal of controversy as to the origin of the luteal cells. However, Sobotta in 1896 (Brambell, 1956) described the luteal tissue of the mouse as hypertrophied membrana granulosa cells. In later years this was confirmed by a number of workers. Pederson (1951) described, in detail, the histogenesis of the granulosa and theca interna into luteal cells.

Long and Evans (1922) were the first to describe, in detail, the ovary, the estrus cycle, and pregnancy of the rat. According to them the corpus luteum of ovulation is 1.0 mm. in diameter and increases to 1.2 mm. before regression begins. They pointed out that the corpus luteum of pregnancy cannot be distinguished from that of a non-pregnant rat until the 10th to 11th day of pregnancy. Observations by Weichert and Schurgast (1942) substantiated Long and Evans' finding and also noted that

injections of estrogen influenced the size of the corpus luteum. According to Bassett (1949), the increase in the corpus luteum size is due to an enlargement of the luteal cells and an increased mitotic activity.

Long and Evans (1922) noted that in the cyclic rat, osmiophilic substances (lipids) begin to accumulate within the luteal cells two days after ovulation. This was indicated by them as a sign of retrogression. Everett (1945) observed that lipids in the cyclic rat do not progress uniformly, but increase gradually during diestrus, diminish slightly during proestrus, and reappear in quantity by 24 hours. Everett also stated that the lipids are mostly cholesterol and its esters and that probably cholesterol serves as a precursor to progesterone. Dawson and Velardo (1955) observed that during pseudopregnancy lipids increase 0 to 72 hours, remain relatively the same during the remaining days of pseudopregnancy, and increase greatly during regression.

The changes in the vascular pattern of the rat were studied by Bassett (1943). Soon after ovulation the luteal tissue is invaded by a capillary wreath from the theca externa and development continues until 96 hours after ovulation. The pattern is retained during regression, while the whole structure continuously shrinks.

The gonadotropic control of the ovary is in essence considerably complex. However, it may be stated that in

the rat, follicle stimulating hormone (FSH), which stimulates the growth of follicles (Simpson, Li, and Evans, 1951), is secreted throughout the estrus cycle (Dempsey, 1937). Luteinizing hormone (LH) synergizes with FSH for the synthesis and release of estrogens. In high titers LH causes ovulation (Greep, Van Dyke, and Chow, 1942). Luteinizing hormone synergizes with the luteotropic hormone (LTH) to cause growth of the corpus luteum. High concentrations of LTH maintain the function of the corpus luteum with the secretion of progesterone (Everett, 1947). Small amounts of estrogen inhibit LH (Greep and Jones, 1950), while small titers of progesterone enhance LH secretion and high titers suppress it (Hisaw, 1947).

The function of the corpus luteum is to produce progesterone permitting implantation and maintaining pregnancy. If implantation does occur the functional life of the corpus luteum is maintained (Young, 1961). As previously stated, LTH is the hormone directly responsible for maintenance of the corpus luteum in the rat and mouse.

Long and Evans (1922) observed that the corpora lutea of the cyclic rat would not sustain placentomata unless the cervix was stimulated first. They also showed, at this time, that pituitary extracts from pregnant rats would prolong the cycle. Astwood (1941) reported a pituitary fraction which produced functional corpora lutea. He called this pituitary fraction luteotropin. In the

same year, Evans, Simpson, and Lyons (1941) also stated that the pituitary extract, identical to Astwood's, was the only substance that would maintain placentomata. They called the substance lactogenic hormone. Astwood noted that rats with functional corpora lutea must be given supplementary LTH within six hours after hypophysectomy for luteal function to be sustained.

In the rat the pituitary is essential for the maintenance of the first 10 to 11 days of pregnancy. Hypophysectomy does not interfere with pregnancy after this term. Astwood and Greep (1938) found that the placenta produces a substance which maintains luteal function after 10 to 11 days of pregnancy; therefore, the pituitary is no longer needed.

The regulation of LTH is under nervous control, since the secretion of LTH must first be initiated by cervical stimulation. Reserpine, a compound which suppresses higher nervous centers regulating gonadotropic secretion, when injected into female rats during estrus or metestrus induces pseudopregnancy (Zeilmaker, 1963). Transplants of the pars distalis under the renal capsule, maintain luteal function for months (Everett, 1956). Consequently, the hypothalamus controls luteal function by inhibition of the pituitary. Also, Everett's study shows that corpora lutea are not self-limiting and need only a luteotropic factor for

maintenance.

The factor, or factors, controlling the cessation of luteal function have been studied by several workers. First of all, progesterone does not seem to play a negative feedback role on LTH secretion (Wolthuis, 1963). However, Rothchild (1960 a, b) points out that progesterone may maintain LTH secretion by suppressing the central nervous system's inhibition of LTH. He further implies that LTH secretion will diminish if progesterone secretion is decreased. Therefore, a decrease in LTH or progesterone will lead to a decrease of each other and finally cessation of luteal function. Also, progesterone plays a role of negative feedback on FSH and LH secretion in which case FSH and LH diminish luteal function (van Rees, 1959).

Bradbury, Brown, and Gray (1950), noted that pseudopregnancy in the rat was prolonged by hysterectomy. They theorized that the uterus may produce a substance which inhibits LTH or luteal function. Silbiger and Rothchild (1963), working on this problem, state LTH secretion is not increased or prolonged by hysterectomy directly. They found, however, that hysterectomy may diminish both FSH and LH, in which case the luteolytic effect of the latter would be diminished and prolongation of luteal function would result. To support this theory de Jongh and Wolthuis (1964) demonstrated that the uterus utilizes

estradiol and progesterone. Extirpation of the uterus would, therefore, increase the titer of progesterone in the blood. Consequently, increased progesterone would diminish LH secretion. The function of the corpus luteum can be maintained for only two to three days by hysterectomy. Cessation of luteal function after this period must be due to primarily extra-ovarian and extra-uterine factors. The luteinizing hormone is probably one of the important factors resulting in cessation of luteal function according to de Jongh and Wolthuis (1964).

The fine structure of the corpus luteum of the rat was first studied by Lever (1956) in comparison to the adrenal cortex. He observed that there were light and dark luteal cells, large and numerous mitochondria with either villiform or lamelliform cristae. He noted that mitochondria were in close association with lipids and suggested the mitochondria are sites of lipid production or elaboration. Belt and Pease (1956) observed that the mitochondria of all steroid synthesizing cells are similar.

Enders (1962) studied the fine structure of the rat corpus luteum from the period of delayed implantation and early post implantation. He noted an extensive agranular endoplasmic reticulum, large and numerous mitochondria with mostly villiform cristae and highly irregular cell boundaries toward the perivascular space. There

were large lipid droplets and an absence of light or dark cells. Enders suggested at this time that the corpus luteum should be examined under different functional states with the electron microscope. The fine structure of the mouse's corpus luteum is similar to that of the rat (Yamada and Ishikawa, 1960).

Enders and Lyons (1964) followed Enders' previous suggestion of examining luteal cells in various functional states. They observed that luteal cells from pregnant or hypophysectomized LTH injected rats had an abundant agranular endoplasmic reticulum, and numerous disc-shaped mitochondria with dense matrices. The luteal cells of those rats which were hypophysectomized seven or fourteen days had a less developed endoplasmic reticulum, the mitochondria had irregular outlines, and "lipochrome granules" appeared. He emphasized that functional luteal cells could be distinguished from non-functional luteal cells.

Although the work of Enders and Lyons showed clearly that there are ultrastructural differences between functional and non-functional luteal cells, their work did not show whether these changes were direct results of LTH withdrawal or were secondary results. It has been confirmed by a number of laboratories that function and ultrastructure can be correlated directly. Hypophysectomy induces marked ultrastructural changes in the thyroid

(Dempsey and Pederson, 1955). Ten international units (I. U.) of thyroid stimulating hormone (TSH) injected into rats results in remarkable changes in thyroid cells one hour after injection (Braunsteiner, Fellingner, and Pakesch, 1953). Also, the ultrastructure of pancreatic cells from starved mice show marked changes of structure shortly after feeding (Weiss, 1953). The literature contains numerous other accounts correlating ultrastructure to function.

The objectives of this thesis are to first clarify Enders' and Lyons' (1964) observations by noting whether the function of luteal cells is directly correlated to their ultrastructure or to their chemistry, and second, to observe the luteal cells after long periods of non-function.

## MATERIALS AND METHODS

All rats used were of the Harvard strain; reared in the laboratory of Oregon State University. The rats were maintained on a diet of Purina Lab Chow, and placed on a twelve-hour day.

Twenty-two to twenty-four day old female rats were initially administered subcutaneously ten I. U. of pregnant mare's serum (PMS) to induce follicular development. Forty-eight hours later 20 I. U. of human chorionic gonadotropin (HCG) were injected in the same manner to bring about ovulation and development of corpora lutea. On the sixth day after the PMS injection, the right uterine horn was traumatized by scratching its full length four times with a No. 22 needle. Four days after traumatization, the uterus was checked for a decidual reaction which is indicative of luteal function. Rats without a substantial decidual reaction were discarded. The right ovary was removed and fixed for light and electron microscopy. This tissue was used as control material. The rats were then hypophysectomized and given an injection of 50 mg ascorbic acid. These rats were also given drinking water containing one percent sodium chloride, one percent sucrose and terramycin (two teaspoons per gallon).

Experimental luteal tissue (left ovary) was removed at 6, 9, 12, 24, 48, 72 hours and 7, 14, and 28 days

following hypophysectomy. Three animals were used for each time interval. At the time the experimental ovary was removed the traumatized uterine horn was weighed and compared to the control horn (see appendix). At this time, the animals were also checked for completeness of hypophysectomy.

Luteal tissue used for light microscopy was fixed in Bouin's, paraffin sectioned, and stained with hematoxylin and eosin. For electron microscopy, the corpora lutea were dissected from the ovary. Individual corpora lutea were then sliced with a razor blade into three parts. The middle piece, about 0.3 mm. thick, was fixed in glutaraldehyde at 0°C (Sabatini, Bensch, and Barnett, 1963) for three hours, washed in three changes of cold Sorensen's phosphate buffer (pH 7.4) and postfixed in osmium tetroxide at 0°C (Millonig, 1961a) for an hour and a half. The tissues were then washed with distilled water, dehydrated rapidly with ethanol, placed in two fifteen-minute changes of propylene oxide, infiltrated with Araldite, and embedded in Araldite.

Sections were supported with formvar film, stained with lead hydroxide (Millonig, 1961b) or stained with a saturated solution of uranyl acetate and restained with lead citrate (Reynolds, 1963). An RCA EMU-2 electron microscope was used for all ultrastructural observations.

## RESULTS

Control (functional) corpora lutea as well as all experimental corpora lutea had an average diameter of one mm. Luteal cells from control corpora lutea and non-functional luteal cells of one week had a maximum average diameter of about  $13\ \mu$ . Luteal cells from animals which had been hypophysectomized one month showed only a slightly smaller size of about 11 to  $12\ \mu$ .

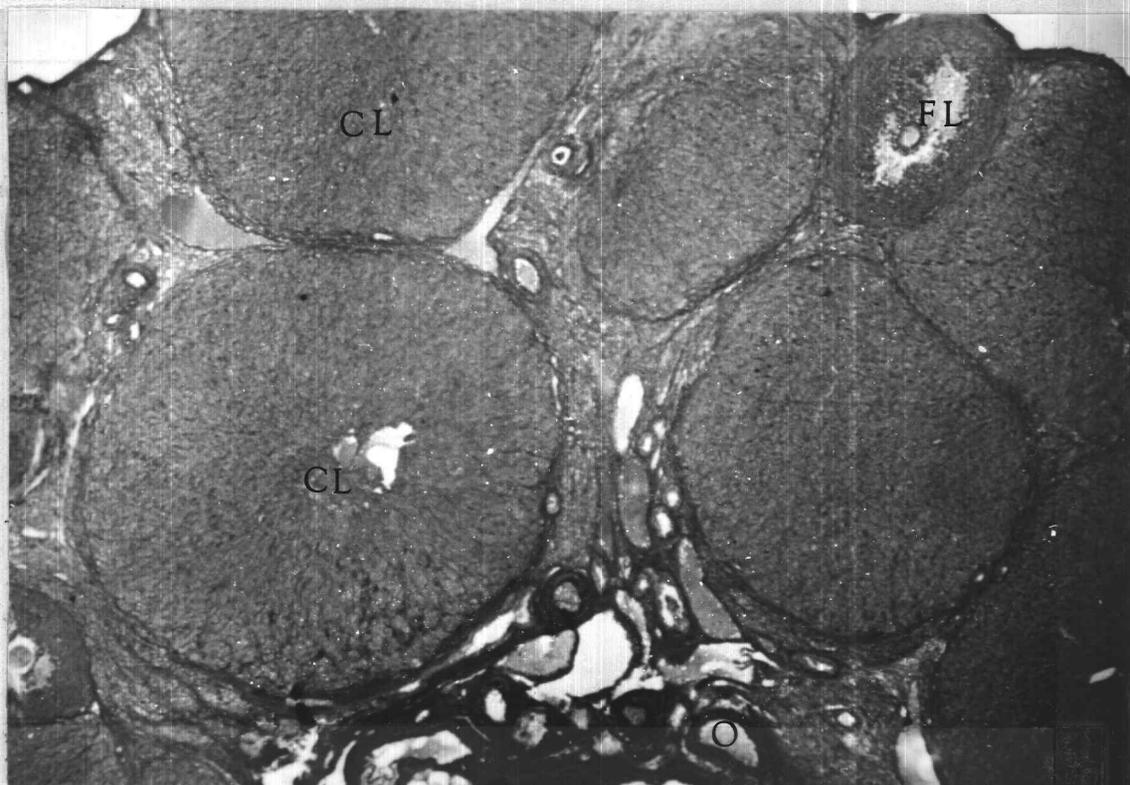


Figure 1. Light photo-micrograph of a section from a control ovary from Rat 17, fixed at the time of hypophysectomy. Structures shown are corpora lutea (CL), follicle (FL), and oviduct (O). X 17.5.

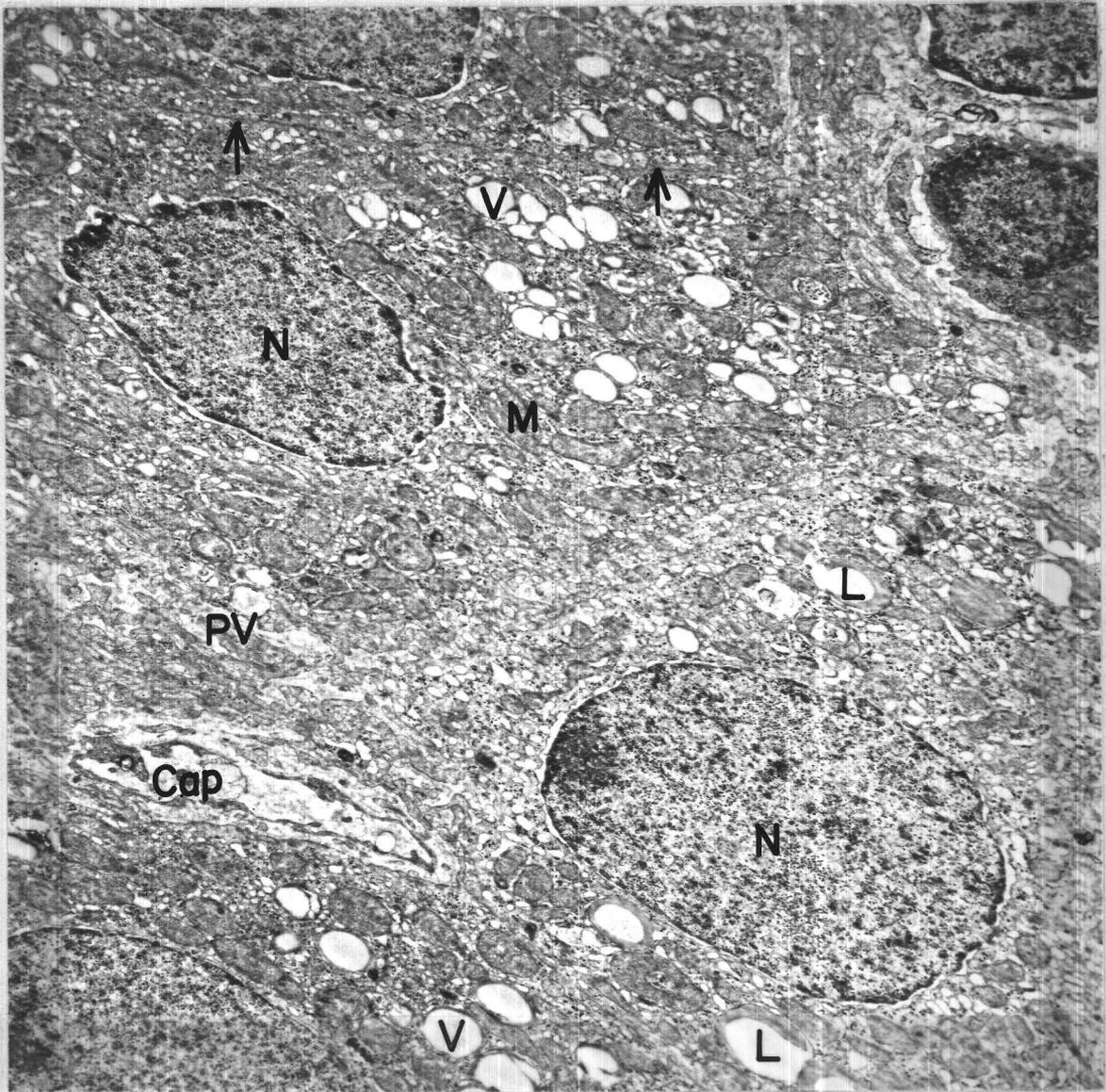


Figure 2. Electron micrograph showing a survey view of control luteal tissue from Rat 13. Notice the numerous mitochondria (M) and lipid vacuoles (L) from which the lipids have probably been partially removed due to poor fixation. V denotes a vacuole which probably contained lipids prior to dehydration. Other structures shown are capillary (Cap), perivascular space (PV), nucleus (N), and arrows showing plasma membrane. X 7500.

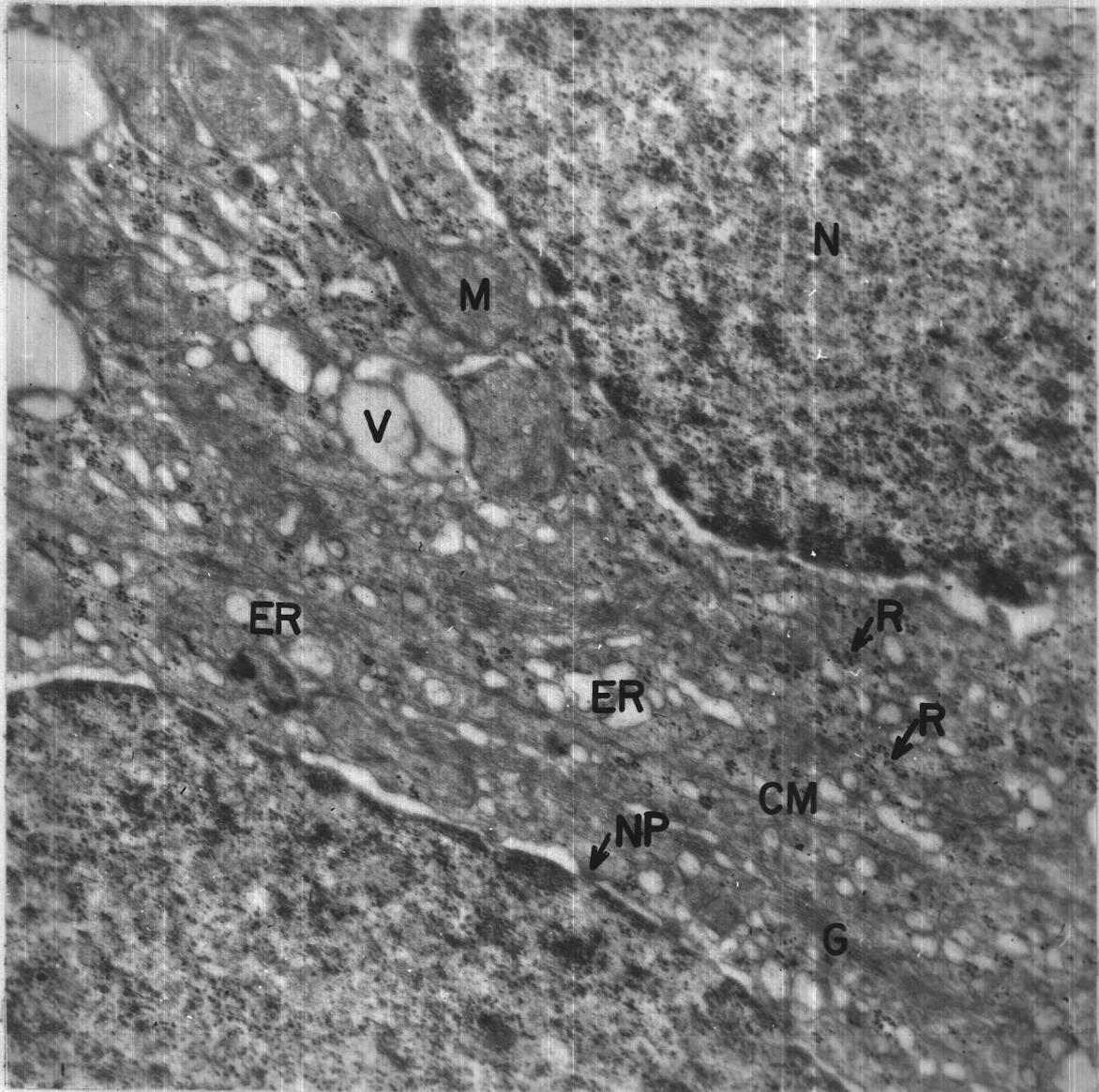


Figure 3. Electron micrograph from a control corpus luteum (Rat 17). Note the dumbbell shaped mitochondria (M), cristae are mostly villiform. Other structures are endoplasmic reticulum (ER), nucleus (N), nuclear pore (NP), cell membrane (CM), Golgi apparatus (G), ribosomes (R), and V indicating a vacuole which may have contained lipids. X 27,900.

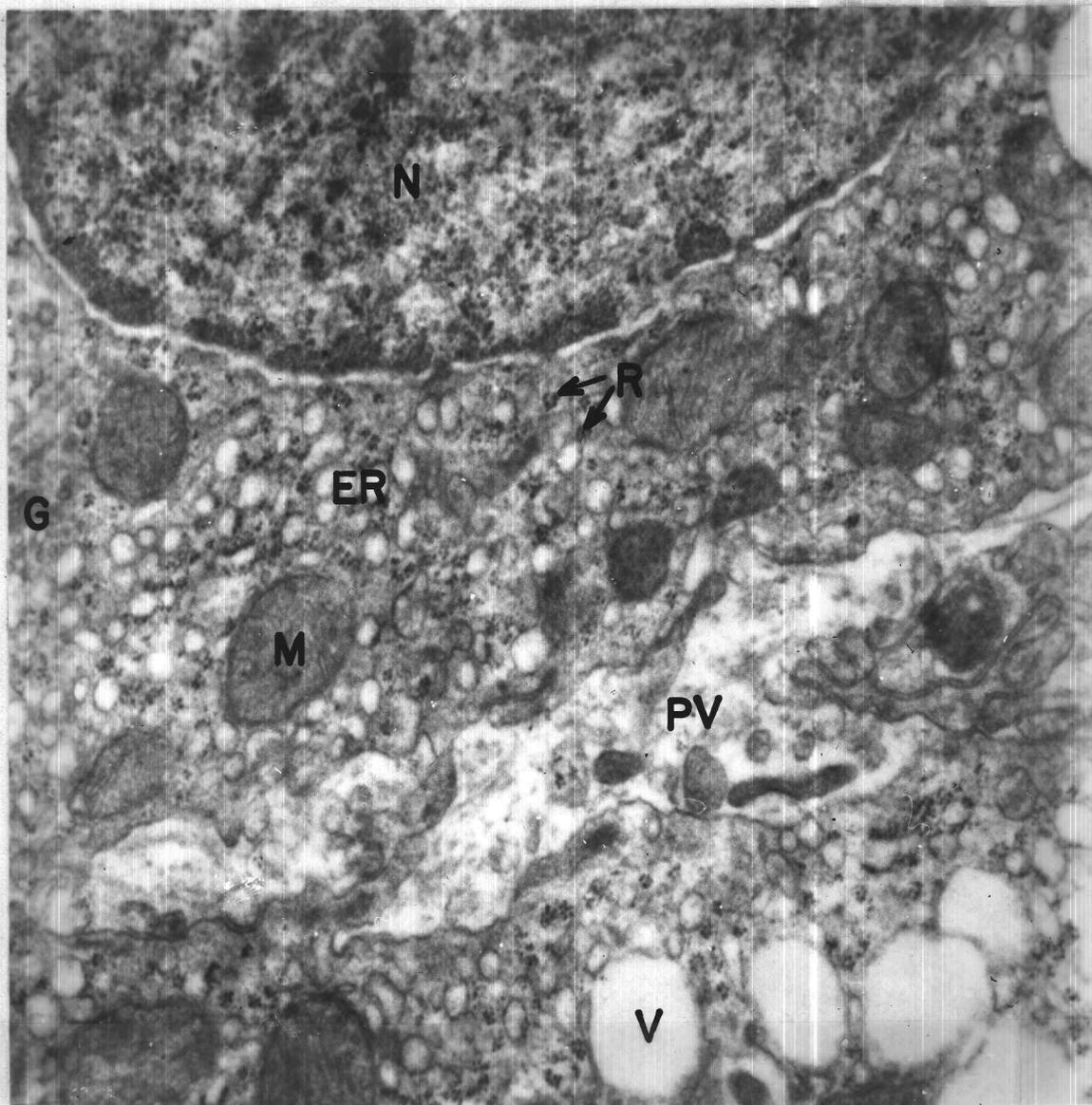


Figure 4. Electron micrograph from a 72-hour post hypophysectomy experimental luteal cell (Rat 17). Note the similarity in structure to that of the control shown in Figure 3. Other structures shown are nucleus (N), endoplasmic reticulum (ER), ribosomes (R), perivascular space (PV), and V showing a vacuole which probably contained lipids. X 27,900.

### Control and 72-Hour Luteal Cells

Luteal cells from control corpora lutea as well as those from 72-hour hypophysectomized animals showed mitochondria of considerable variation and size randomly distributed; some being dumbbell shaped with elongated cristae. Elongated mitochondria usually have a parallel orientation about the nucleus. The mitochondrial matrix is in all cases denser than the cytoplasm. Cristae are mostly villiform, however, lamelliform cristae do occur especially in those mitochondria which are greatly elongated or dumbbell shaped. In the case of the 72-hour experimental luteal cells, no breakdown of mitochondria can be seen (Figures 3, 4, 5, 6).

For the most part the endoplasmic reticulum of both control and 72-hour experimental luteal cells is agranular (Figures 3, 4, 5, 6) with a vesicular or vacuolated form. There are some granular forms of the endoplasmic reticulum which are tubular in nature (Figure 6). There is an abundance of ribosomes not associated with the endoplasmic reticulum found in the cytoplasm. In both control and experimental 72-hour luteal cells, the abundance, distribution, and configuration of the endoplasmic reticulum is similar.

The plasma membrane of adjacent cells is always highly regular and contains no modifications such as

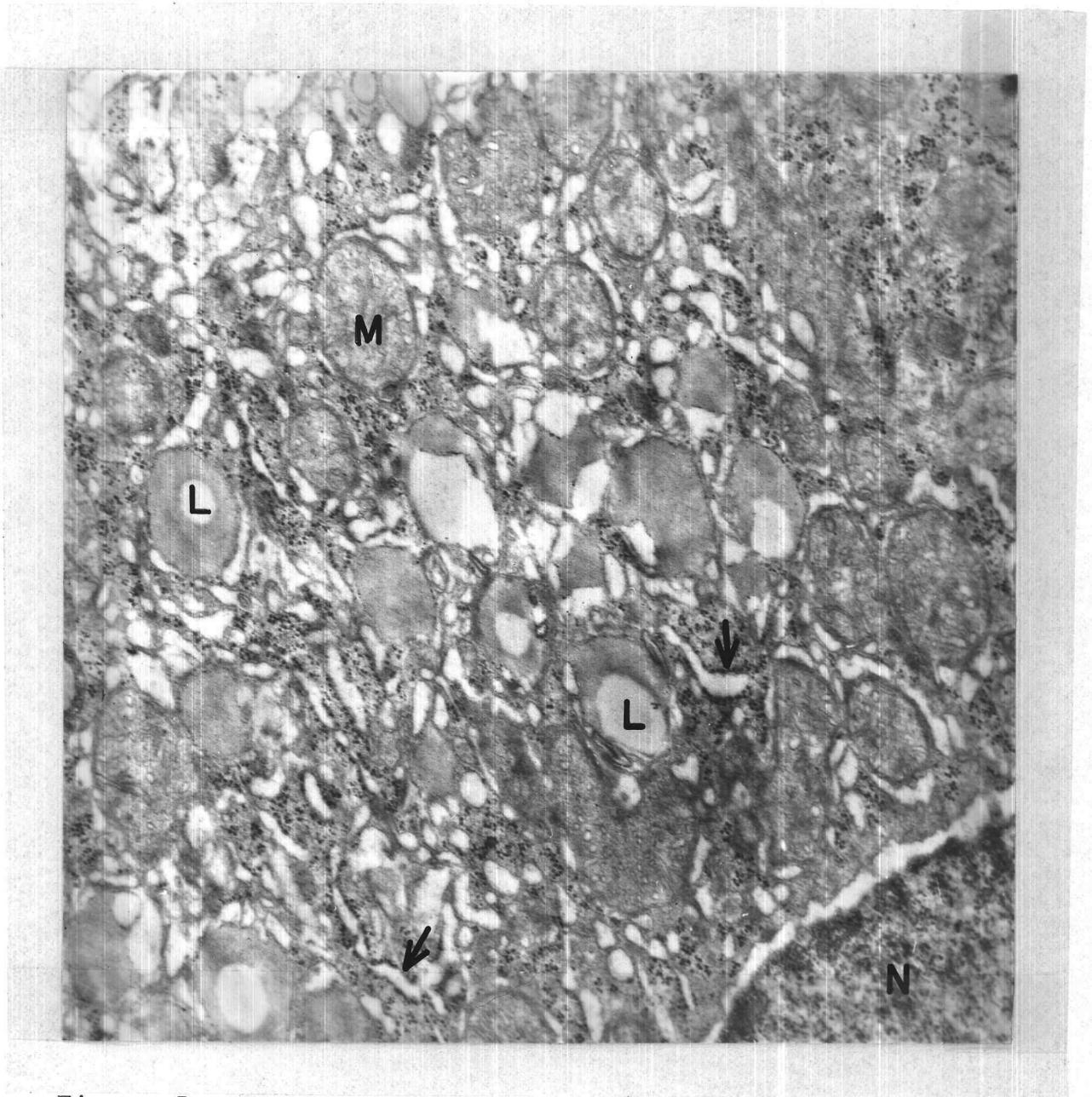


Figure 5. Electron micrograph of a section from a control luteal cell (Rat 19). Note the lipid vacuoles (L) containing a rim of lipid and the numerous mitochondria of various shapes (M). Arrows show tubular endoplasmic reticulum with attached ribosomes. The nucleus (N) is at the lower right-hand corner of the picture. X 27,900.

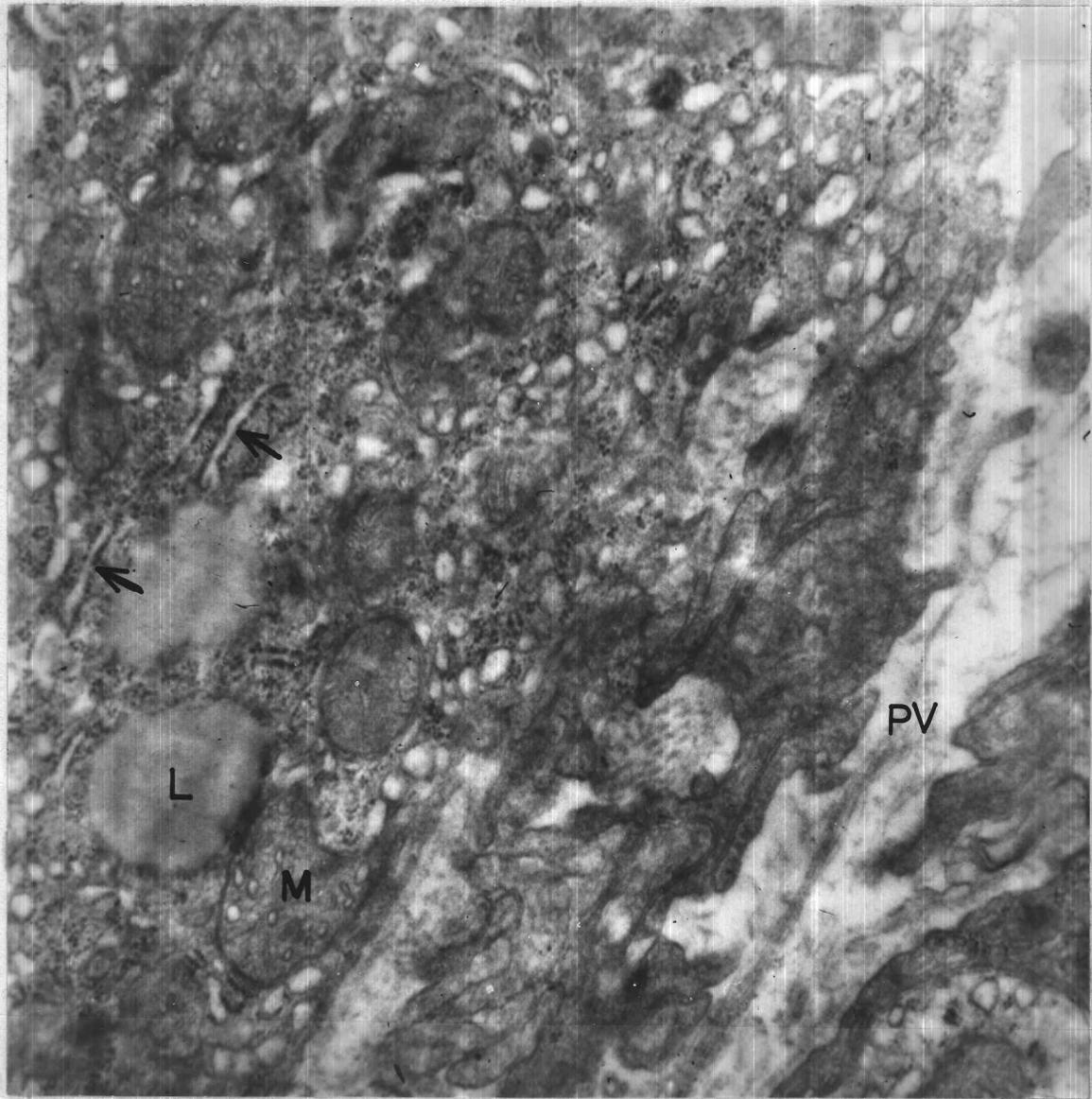


Figure 6. Electron micrograph of a section of a 72-hour experimental luteal cell (Rat 19). The cytoplasmic projections of the perivascular space (PV) are highly irregular. Arrows note ribosomes associated with a tubular endoplasmic reticulum. Mitochondria (M), and lipids (L) are also shown. X 27,900.

interdigitations or desmosomes (Figure 3). The cell surface towards the perivascular space on the other hand is typically irregular (Figures 4 and 6), however, the amount of irregularity is not consistent, varying from cell to cell.

The Golgi complex (Figure 3) is characterized by containing several small parallel membranes lacking any polarity. The parallel membranes usually occur in units of four to five.

In electron micrographs of luteal cells, numerous large vacuoles can be seen. Some of these vacuoles contain various amounts of lipids (Figures 2, 5, 6). Others appear as large, round or oval spaces devoid of any electron dense material (Figures 2, 3, 4). These vacuoles are not randomly distributed throughout the cytoplasm, but are positioned in groups or clusters towards one or two sides of the cell (Figure 2). The amount and distribution of the vacuoles in control and 72-hour experimental luteal cells is similar. Rarely were dense bodies observed.

#### One-Week Experimental Luteal Cells

Luteal cells from one-week hypophysectomized rats showed changes when compared to control luteal cells (Figure 7). The mitochondria of these cells are for the most part round or slightly oval. Elongated mitochondria

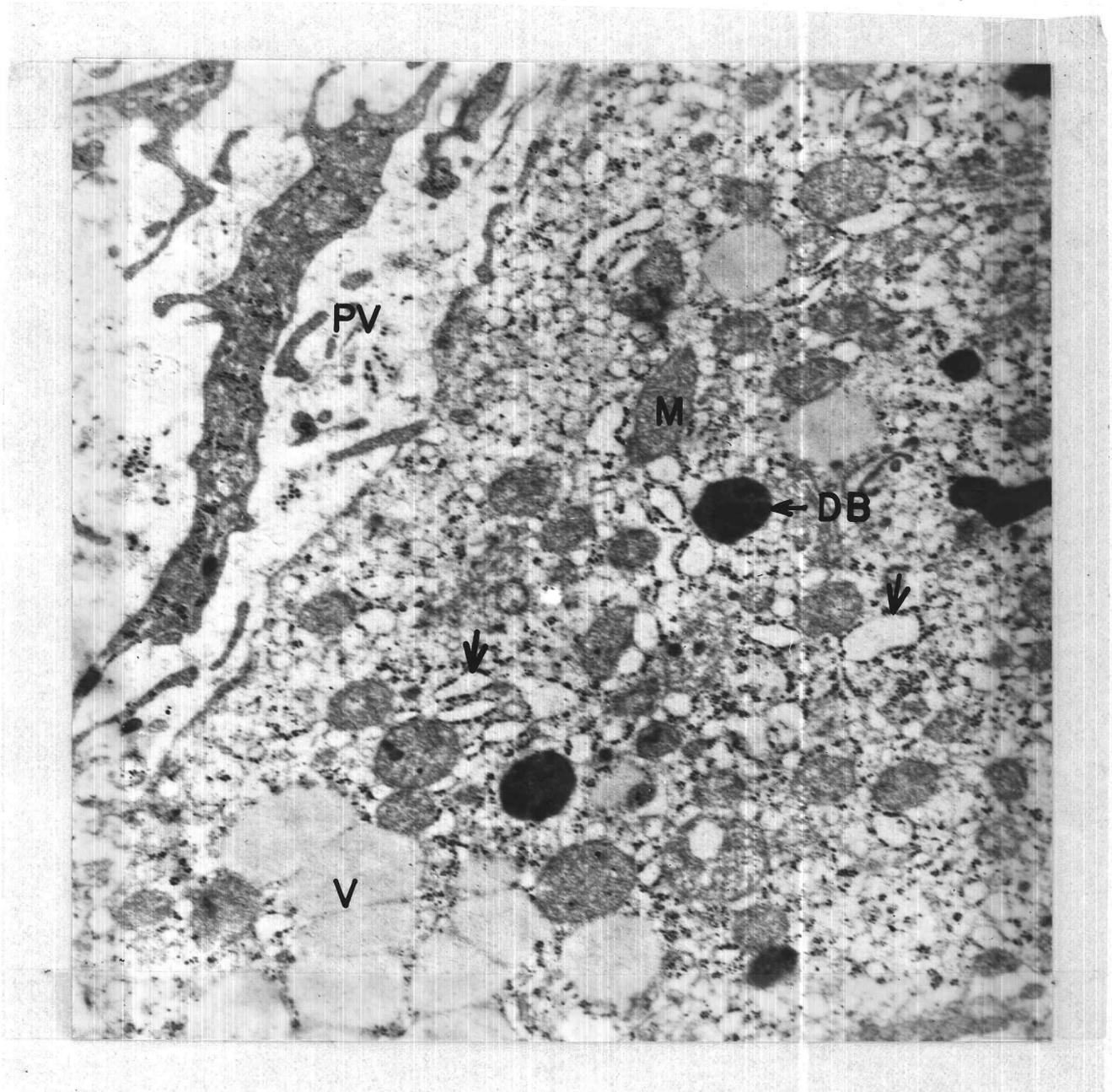


Figure 7. Electron micrograph of a seven-day experimental luteal cell (Rat 33). Notice the dilated appearance of the endoplasmic reticulum (arrow) and the dense bodies (DB). Other structures shown are the perivascular space (PV) and vacuoles (V). X 23,700.

were not observed. The mitochondrial cristae appear more irregular.

The endoplasmic reticulum appears dilated and frequently shows attached ribosomes suggestive of a granular endoplasmic reticulum. The cell surface towards the perivascular space is still irregular but not to the degree of control luteal cells. The cellular projection in this area gives the appearance of being shrunken.

The vacuoles contain no or very little lipids. The appearance of electron dense granules (dense bodies) is evident in these luteal cells. In nearly all luteal cells observed, dense bodies were seen randomly distributed throughout the cell consisting of a dense amorphous mass usually spherical in form and containing one or more darker areas within.

#### Two-Week Experimental Luteal Cells

In the case of two-week experimental luteal cells (Figure 8) the mitochondria are all round in form and show a degree of atrophy. Ribosomes are evident in the cytoplasm but are not distributed throughout the cell, instead they have a tendency to be located in groups or clusters. Dense bodies (not shown in electron micrograph) have increased somewhat in number, but have the same appearance as seen in one-week experimental cells.

One-month experimental non-functional luteal cells

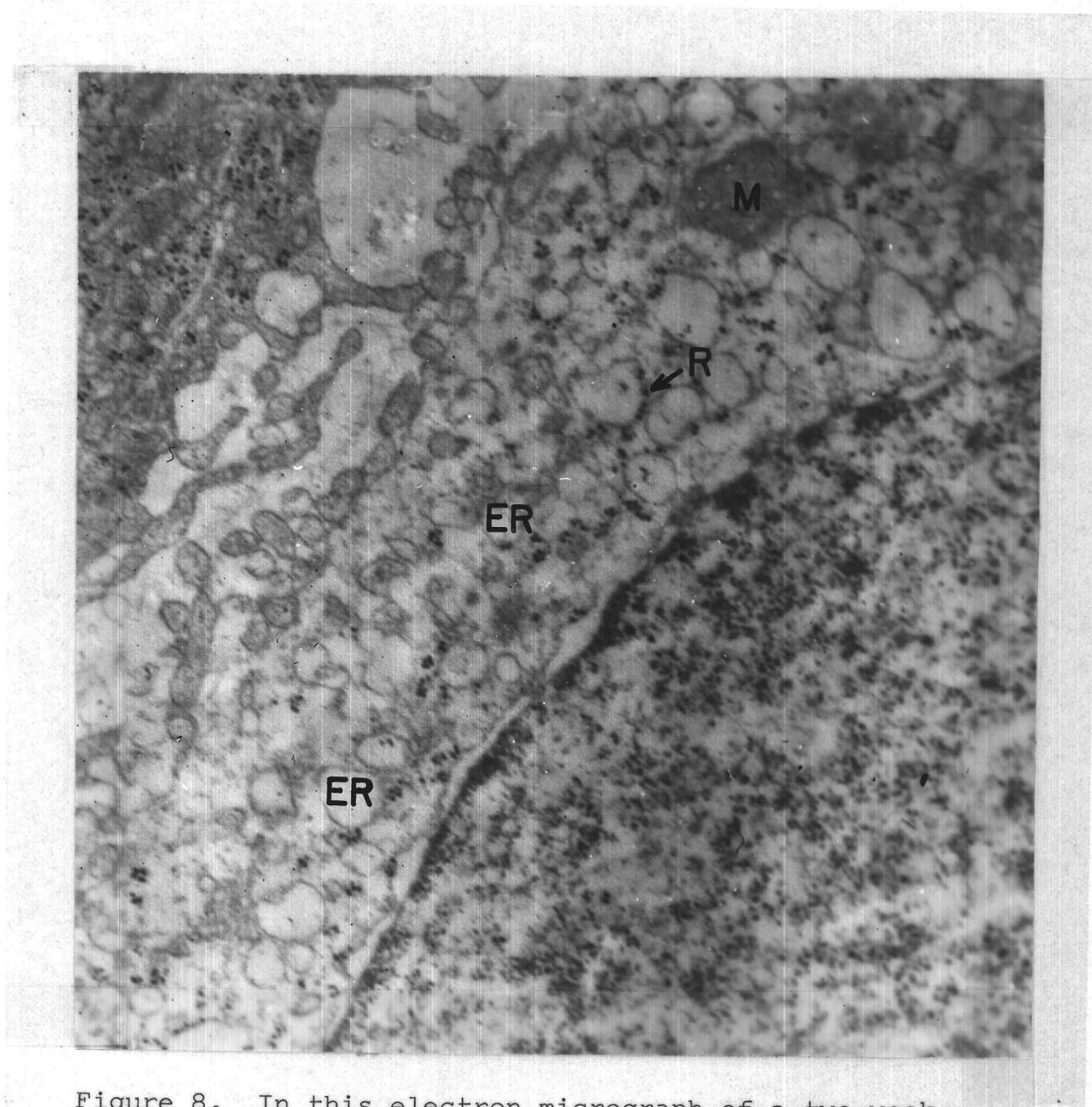


Figure 8. In this electron micrograph of a two-week experimental luteal cell (Rat 35) the endoplasmic reticulum is dilated (ER), but there are not too many changes from one-week experimental luteal cells. Although this micrograph does not show dense bodies, they do occur in this tissue. Other structures shown are mitochondria (M), and ribosomes (R). X 27,900.

are characterized by a lack of contrast of the cytoplasm. The mitochondria can be seen, but appear to be of low density. What endoplasmic reticulum remains consists of the granular type. Ribosomes are in abundance, but mostly in clusters (Figure 9). Dense bodies have increased in number and are evident in all cells.

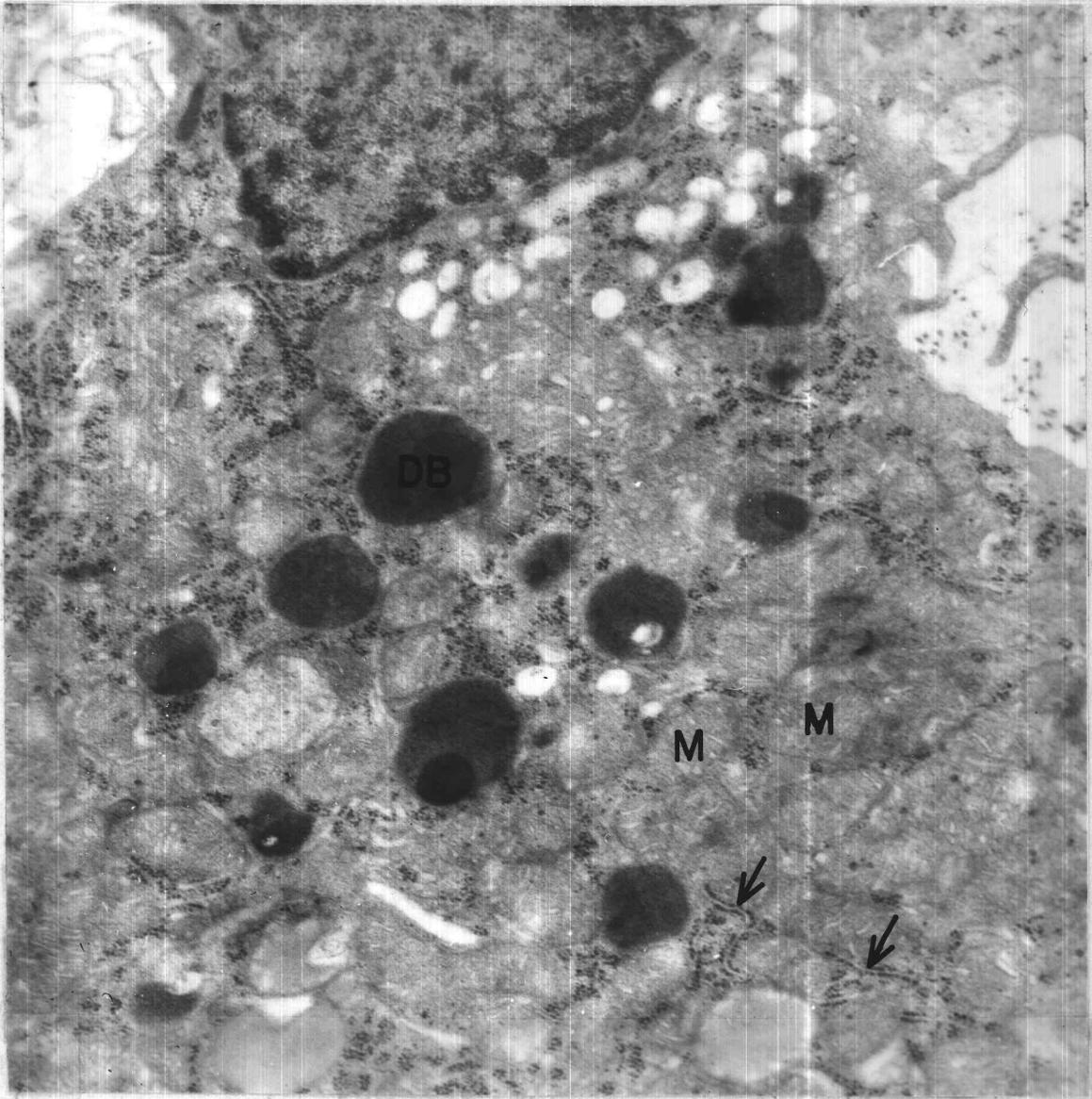


Figure 9. Notice in this electron micrograph of a one-month experimental luteal cell (Rat 39) the appearance of the cytoplasm which appears to be lower in contrast when compared to the previous electron micrographs. Mitochondria (M) and dense bodies (DB) can be distinguished as well as ribosomes which are in clumps or associated with what endoplasmic reticulum remain (arrows). X 28,900.

## DISCUSSION

Since there are no indications in the literature suggesting that there are functional differences between different areas of the corpus luteum, the center of the corpus luteum was used in order to insure that thecal, or interstitial tissue, was not being observed. Therefore, it was assumed that tissue used in this study was functional at the time of hypophysectomy.

Glutaraldehyde, followed by osmium fixation, has been shown to be at least as satisfactory as/or superior to osmium fixation alone (Sabatini, Bensch, and Barnett, 1963). The advantages of glutaraldehyde fixation are a higher degree of preservation of cytoplasmic membranes and cellular substances (i.e. glycogen).

Although the fine structure of luteal cells in this study was comparable to the work of Enders and Lyons (1964), it did differ in two respects. First, Enders and Lyons noted that lipids within the lipid vacuoles increased in amount during non-function. This study, on the contrary, showed a lower lipid content during the period of non-function. Factors which could account for this difference are the strain of rats and/or the longer period of function of corpora lutea prior to hypophysectomy (eight to nine days) compared to the four days of Enders and Lyons. A reason for the observed lower lipid

content of luteal cells in this study may be due to an actual decrease in lipids during luteal cell activity. Probably a more reasonable assumption for the lower lipid content of luteal cells was the poor fixation of the lipids by osmium post-fixation. Since the tissue remained in osmium for a short time (one and one-half hours) at 0°C, it is doubtful whether the osmium had sufficient time to fix all the lipids. Therefore, extraction of the lipids would occur during dehydration and embedding. To test this assumption one should fix for a longer period in osmium at 0°C and/or fix at room temperature. Histochemical staining for lipids would also indicate the quantity of lipids present. Secondly, Enders and Lyons did not note a dilation of the endoplasmic reticulum in non-functional luteal cells. This study, however, did show a dilation of the endoplasmic reticulum in these cells. There are cases in which the endoplasmic reticulum does dilate during non-function. For example, parietal cells from the intestinal epithelium of a mouse show a marked swelling of the endoplasmic reticulum when the animal is starved for 24 hours (Kurosumi, 1961). However, dilation of the endoplasmic reticulum may also be due to poor fixation. To test for poor fixation, one should test tissue with several concentrations of fixative and also supplement the fixative with substances (i.e. sucrose, NaCl, CaCl<sub>2</sub>) which will vary the osmolarity.

Enders and Lyons labeled the dense granules or bodies, found in non-functional luteal cells, as lipid pigment granules. Since the composition of the dense bodies found in luteal cells after one week of non-function is not known, the term lipid pigment granules should not be used. Histochemical or chromatographic studies of luteal tissue may elucidate the nature of the pigment.

Enders and Lyons did not observe luteal cells from animals that had been hypophysectomized one month. Corpora lutea as well as luteal cells which had been inactive for one month showed a static appearance when observed with the light microscope. There was very little volume change of the corpus luteum or luteal cells which is contrary to what one observes in the intact animal. Corpora lutea in the normal rat decrease in size with absorption of the luteal cells soon after function ceases. The pituitary, therefore, directly and/or indirectly must play a part in the absorption of non-functional luteal tissues.

It may be hypothesized that if any ultrastructural changes were directly associated with function of the corpus luteum, these changes would be observed shortly after hypophysectomy. Since no observable difference of luteal fine structure between control and 72-hour hypophysectomized rats could be detected, it may be assumed that fine structural changes due to hypophysectomy are

secondary. The changes which are brought about by non-function take nearly a week to express themselves.

However, it must be remembered that the limiting factor of the electron microscope is the resolving of macromolecules (15-20 Å). Beyond this range the electron microscope cannot detect further detail of structure. Since structure and function are so closely correlated, in fact many times inseparable, it is reasonable to assume that structural changes, which cannot be detected with the electron microscope, do occur with function.

The mechanism by which a hormone acts is for the most part little understood. However, recent work with steroid hormones indicates that these substances affect the genes directly which ultimately controls message ribonucleic acid (mRNA) synthesis (Davidson, 1965). On the other hand, insulin is believed to control cell membrane permeability to glucose (Levine and Goldstein, 1955). Davidson's work showed that steroids can penetrate the nucleus. Vasopressin, a peptide, has been shown to attach to the target cell membrane surfaces at certain receptor sites (Fong et al. 1960). Since LTH is a large protein molecule, M.W. 24,000 (Li, 1961), it is probable that it does not penetrate the cell membrane. The cellular site of action could be demonstrated by autoradiography if and when it becomes possible to label LTH.

If LTH does have its effect on the genes directly or

indirectly, the use of actinomycin D would render LTH non-functional. On the other hand, if actinomycin D has no effect on LTH, it is probable that the action of LTH is in the cytoplasm and/or cellular permeability.

The more perplexing problem concerning the physiology of the rat's corpus luteum is the irreversible effect of LTH removal for six or more hours (Astwood, 1941). There are few explanations in the literature which could indicate the mechanism of irreversibility in reference to a hormone. In considering this phenomenon, however, one may suggest several hypotheses to the underlying mechanism. First, removal of LTH may result in the synthesis, or activation of a repressor substance which either blocks the synthesis of mRNA or protein synthesis. Talwar et al. (1964) proposed that in the absence of estradiol in the rat's uterus, a repressor substance blocks the transcription of genetic information. Assuming that LTH does act on the genes, or by the activation of specific enzymes for progesterone synthesis, a repressor substance is conceivable. The repressor substance would not be inactivated by administration of LTH.

A second hypothesis for the irreversibility phenomenon may be based on the concept of LTH binding to specific receptor sites on the luteal cell surface. If this is the case, six hours after removal of LTH, these sites may close, no longer permitting LTH to attach. If this is

true, autoradiography with labeled LTH would provide evidence.

## SUMMARY

1. No ultrastructural changes could be noted in luteal cells which were non-functional for three days, when compared to fully functional luteal cells.

2. Non-functional luteal cells of one week duration were noted as possessing a dilated endoplasmic reticulum, a tendency for more spherical mitochondria, and the appearance of dense bodies.

3. Those luteal cells which were non-functional for two weeks were similar to one week non-functional cells, differing by having more pronounced characteristics described under two above.

4. Luteal cells quiescent for one month contained mitochondria of low contrast. Little agranular endoplasmic reticulum remained. Ribosomes and dense bodies were still evident.

5. The low quantity of lipids in most luteal cells and the dilation of the endoplasmic reticulum found in one and two week non-functional luteal cells was probably due to poor fixation.

6. Theoretical considerations were undertaken to explain the mechanism of LTH action and the phenomenon of irreversibility of luteal cell function by LTH once this gonadotropin is withdrawn for six hours.

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## APPENDIX

Table 1. Decidual Reactions.

Rat Number	Duration of Time From Hypophysectomy to Autopsy	Control Uterine Horn (mg.)	Traumatized Uterine Horn (mg.)
5	6 hours	52.9	264.4
6	6 hours	65.2	315.0
7	6 hours	49.1	298.3
15	48 hours	45.5	593.1
16	48 hours	55.4	199.9
17	48 hours	50.2	225.5
13	72 hours	50.1	227.6
17	72 hours	50.5	225.5
19	72 hours	45.9	189.7
32	7 days	37.2	39.8
33	7 days	45.1	50.2
34	7 days	39.6	49.5
35	14 days	25.6	37.9
36	14 days	31.2	48.6
38	14 days	27.8	30.3
32	28 days	18.5	17.6
39	28 days	21.4	25.7
40	28 days	22.3	24.2