THE EFFECT OF PURIFIED GONADOTROPHINS ON THE OVARIAN STRUCTURE

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ABBREVIATIONS

FSH	Follicle Stimulating Hormone
LH	Luteinizing Hormone
Hypox.	Hypophysectomy
-	Same as hypophysectomized control
±	Doubtful change/reaction
+	Weak change/reaction
++	Moderate change/reaction
+++/ ++++	Strong change/reaction

PREFACE

This study was undertaken in the Department of Obstetrics and Gynaecology of the University of Glasgow, and in the Research Department, Wolfson Centre, Glasgow Royal Maternity Hospital, Rottenrow, Glasgow.

The work was performed over a period of three years while the candidate was a Research Student at the University of Glasgow.

This work was supervised by Professor M.C. Macnaughton, Department of Obstetrics and Gynaecology, University of Glasgow, and was under the direction of Dr A.D. Telford Govan, Director of Research, Wolfson Centre, Glasgow Royal Maternity Hospital, Rottenrow, Glasgow.

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SUMMARY

Mature female Sprague-Dawley rats were hypophysectomized towards the end of oestrus as indicated by daily vaginal smears. All animals exhibited regular four-day cycles.

At the seventh day after hypophysectomy, all ovarian structures had atrophied leaving small pre-antral follicles and apparently healthy looking corpora lutea.

Apart from intact and hypophysectomized animals which were used as controls, the hypophysectomized animals were divided into three main groups:

Group I were given increasing doses of a highly purified FSH preparation.

Group II were given increasing doses of a highly purified LH preparation.

Group III were given the minimal effective dose of FSH and increasing doses of a highly purified LH preparation.

All hormone injections were given as a single sub-cutaneous injection on the seventh day after hypophysectomy. By this time the only normal structures in the ovaries of the experimental animals were corpora lutea and very small pre-antral follicles. Graafian follicles had either disappeared or were in the advanced stages of

atresia. The interstitial tissue was inactive and showed marked fatty change.

The administration of FSH was followed by changes suggesting reactivation of the corpora lutea - accumulation of cytoplasmic RNA and an increase in 3β steroid dehydrogenase activity. Following this, follicular growth occurred and the interstitial cells lost their degenerate appearance but showed no sign of luteinization. Antral follicles were produced and the thecal cells made their appearance. The latter also did not luteinize. From these results it is argued that FSH stimulates the corpus luteum to produce the oestrogen necessary to initiate the first stages of follicular growth and stimulate the production of FSH receptors in granulosa cells. Although thecal cells were formed and the interstitial tissue became healthy in appearance there was no evidence of excessive androgen production. It was deduced from this that the unluteinized spindle-shaped thecal cells and the similarly unluteinized interstitial cells were a morphological form which may be mainly associated with oestrogen production.

LH caused hyperaemia and some increase in the size of the corpora lutea. There were also indications of increased functional activity as with FSH. Interstitial tissue was "repaired" and showed signs of partial luteinization. Although it is known that LH

stimulates the production of oestrogen in hypophysectomized rats provided corpora lutea are present in the ovaries there was no evidence of folliculogenesis. Indeed the process of atresia appeared to be hastened. It is suggested that the absence of follicular growth was due to the production of androgens by the stimulated interstitial cells.

From this it was concluded that the occurrence of luteinization in these cells was an indication of a high production rate of androgens.

FSH and LH administered together restored the ovarian structure to a reasonably normal condition. Folliculogenesis was active and the interstitial tissue became active in appearance. The corpora lutea, obviously pre-existing, were much enlarged and active. These changes were within the range of normality provided the dose of LH remained small. With increasing doses of LH the theca and the interstitial tissue became hyperplastic and fully luteinized. Granulosa cell growth ceased and ultimately the granulosa cells disappeared leaving theca lutein cysts. These results seemed to indicate that the main action of these larger doses of LH was on the thecal and the interstitial cells, resulting in the production of large quantities of androgen which counteracted the folliculogenic activity of the small dose of FSH. They help to support the hypothesis suggested as an

explanation for the results of the experiments with LH alone. An analogy is drawn to the polycystic ovarian syndrome in humans.

From the above results a further hypothesis is put forward to provide one reason for the increase in FSH at the time of the ovulation surge of LH. It is suggested that the increase in FSH acts to protect the granulosa cells from the atretic action of androgens produced under the influence of LH, by converting the androgens to progesterone.

INTRODUCTION

Much of the current knowledge of ovarian function has been derived from a study of animals under both normal and pathological conditions. Direct information about the human ovary is scanty since there are relatively few opportunities to examine the organ in completely normal women either during or before their productive phase of life.

There is, however, substantial evidence that the human ovary does not differ in many of its developmental and functional aspects. The ovary is remarkably uniform in vertebrates in general.

For a full review of the development and structure of the ovaries in vertebrates, reference should be made to Cole and Cupps (1969); Zuckerman, Mandl and Eckstein (1962) and Brambell (1956).

While the morphology and function of the ovaries were described in general terms as far back as the 17th century (de Graäf) it was not until 1926 that ideas on the control of these functions first began to be formulated. In that year Smith and Engle in New York demonstrated the effect of hypophysectomy on the gonad of rats and the efficiency of hypophyseal implants in restoring these gonads to normal. Independently, Zondek and Ascheim (1926) demonstrated the existence of specific gonadotrophic hormones in human urine during and after menopause and during pregnancy.

They named the pregnancy hormone prolan-B while the human menopausal gonadotrophin was called prolan-A.

The idea of blood-born gonadotrophic stimuli was not new at the time, but its realisation led to the foundation of our knowledge of pituitary-ovarian relationships.

Investigations showed that laboratory rats and mice are sensitive to gonadotrophic stimulation and prepared the way for the introduction of methods of estimating both qualitative and quantitative differences between gonadotrophins.

At first it was assumed that the gonad stimulating hormone from the pituitary gland was the same as that of pregnancy urine and a few years later it was shown beyond any doubt that the chorionic hormone (a term coined by Hamberger, 1931) and pituitary gonadotrophins were different hormones. The term gonadotrophic hormone was coined by Weisner and Crew (1930). An important discovery at this time was that of Phillipp (1930) which showed that the pituitary gland during human pregnancy was almost devoid of gonadotrophic effects.

Further work indicated the likelihood of two pituitary gonadotrophins and in 1930 Evans and Cole established the identity of these in extracts of pituitary glands.

It was not until the 1960's when the onset of immunological determinations and new methods of protein separation were devised that any real progress was made.

The great majority of publications dealt with attempts at the purification of gonadotrophins, bio-assays and determination in urine.

Studies on the purification of pituitary preparations were reported by Reichart and Parlow (1964), Hartree (1966) and Ryan (1968). By their method, Reichard and Parlow purified FSH by a factor of 6 and LH by a factor of 3, but each preparation was still contaminated by the other. Hartree (1966) succeeded in further purifying both hormones, especially LH. Butt (1967) using Hartree's FSH preparation produced a preparation almost free of LH contamination. With the production of a "pure" preparation, it became possible to employ radio-immunoassays with confidence, and these have replaced older biological methods. Chorionic gonadotrophins have proved easier to purify, and since it is antigenically very similar to LH, it has been used as a standard for LH estimation. Radio-immunoassay methods have been described by many investigators, among them Saxena et al (1968) and Donini et al (1969a) for FSH and Donini et al (1969b) for LH.

Smith and Engle (1926) and Zondek and Ascheim (1927) discovered the presence of gonadotrophic hormones in the pituitary gland. Fevold et al (1931) described two active substances, one caused follicular growth in the ovary and was called follicle stimulating

hormone (FSH), the other caused stimulation of the interstitial tissue and was called (I.C.S.H), it also caused luteinization in the ovary and was named luteinizing hormone (LH).

There is good evidence to believe that the release of these gonadotrophins is under strict neuro-endocrine control from the hypothalamus (Guillemin and Schally, 1963) via releasing substances.

Gonadotrophins are secreted by the basophilic cells of the anterior pituitary. Work using bio-immunological techniques with isotopically labelled antibodies localised FSH and LH to within the same individual cell (Phifer, Midgley and Spicer, 1972). The gonado-trophins, FSH and LH are glycoproteins with a molecular weight of between 25,000 and 40,000. Gonadotrophins have at least two subunits (A and B).

The plasma level of FSH and LH at any one time consists of the sum of different secretory components: first, the basal or tonic secretion which represents a fairly constant production not influenced by the releasing hormones but only modified by a feedback effect of circulating steroid directly on the pituitary; second, the small day to day or hour to hour episodic component which is more prominent for LH and is controlled by secretory pulses of releasing hormones or alteration in pituitary sensitivity for LH releasing hormone (direct feedback at pituitary

level); third, the mid-cycle or surge secretion is controlled by a positive feedback mechanism through LH releasing hormones but still modulated at both hypothalamic and pituitary levels by the ovarian steroids.

Studies of hormonal inter-relationships in the human have been carried out by many investigators, among them Bell et al. (1966), Adlercreutz and Luukkainen (1968), Neill et al. (1969), Franchimont (1967), Burger et al. (1968), Johannson and Wide (1969), Goebelsmann et al. (1969) and Dufau et al. (1970). All these authors have shown a recognisable pattern of FSH and LH levels in the plasma and urine during the menstrual cycle.

FSH increases as menstruation approaches, falls gradually during the proliferative phase of the cycle, rises to a peak around mid-cycle and falls once more. At all times in the cycle there are measurable quantities of FSH. Small quantities of LH are also present throughout the cycle but the concentration varies little except at mid-cycle when there is an abrupt and short lived release of a large quantity (Midgley and Jaffe, 1966 and Goebelsmann et al., 1969). There is some controversy regarding the timing of the mid-cycle peaks of FSH and LH. There is considerable evidence to show that an LH peak occurs 12-14 days before the next menstruation

(Brown et al, 1958; McArthur et al, 1958 and Fukushima et al, 1964). Studies of FSH patterns have yielded more variable results. Several workers using bio-assay methods, have found that the mid-cycle peak of FSH production occurs after the LH peak (Bell et al, 1966 and Franchimont, 1967). Others have claimed that the two peaks coincide. Radio-immunoassay has also revealed a variation in the time of the FSH peak (Faiman and Ryan, 1967; Cargille et al, 1969 and Taymor et al, 1968). However, this was considered to be of minor importance.

These findings have served to reinforce the conventional ideas regarding the sequence of events and mechanism thought to be operating in the ovary. At the end of menstruation, a high secretion of FSH stimulates the small pre-antral follicles to grow and develop antra. Around the twelfth day a sudden rise in LH production induces one of the enlarged follicles to ovulate and thereafter form a corpus luteum. When the corpus luteum degenerates menstruation occurs, the FSH has risen and a new cycle is started. While such a statement is true, closer inspection of the curves of secretion of gonadotrophins, and of FSH in particular, indicates that some elaboration of this simple concept is required if the mechanisms involved are to be understood.

Two phases of FSH secretion are of particular interest (Fig.1). The first phase is the fall in FSH production prior to ovulation (Faiman and Ryan, 1967; Taymor et al, 1968; Midgley and Jaffe, 1968 and Cargille et al, 1969). During this fall the oestradiol output rises progressively. According to Parlow (1964) and Bogdanove et al (1971) oestrogens suppress the output of FSH and LH in animals. Stevens and Vorys (1967) and Jaffe et al (1969) have demonstrated a similar action in human subjects. Findings such as these have given rise to the hypothesis of the negative feedback effect of oestrogens. Whether the oestrogens act directly on the pituitary or on the secretion of the gonadotrophin releasing substance by the hypothalamus is not completely clear. The second phase is the peak of FSH output which occurs and almost coincides with the ovulation surge of LH despite rising concentrations of oestrogen (Fig. 1). It has been known for a considerable time that oestrogen will induce ovulation in animals (Sawyer et al, 1949; Everett and Nichols, 1968 and Brown-Grant, 1969).

In the rat the surge of LH is preceded by a rise in plasma oestradiol (Brown-Grant et al, 1970; Shaikh, 1971; Dupon et al, 1973 and Lindner et al, 1974).

The LH surge is produced by hypothalamic releasing factors (Ramirez and Sawyer, 1965 and Everett, 1969).

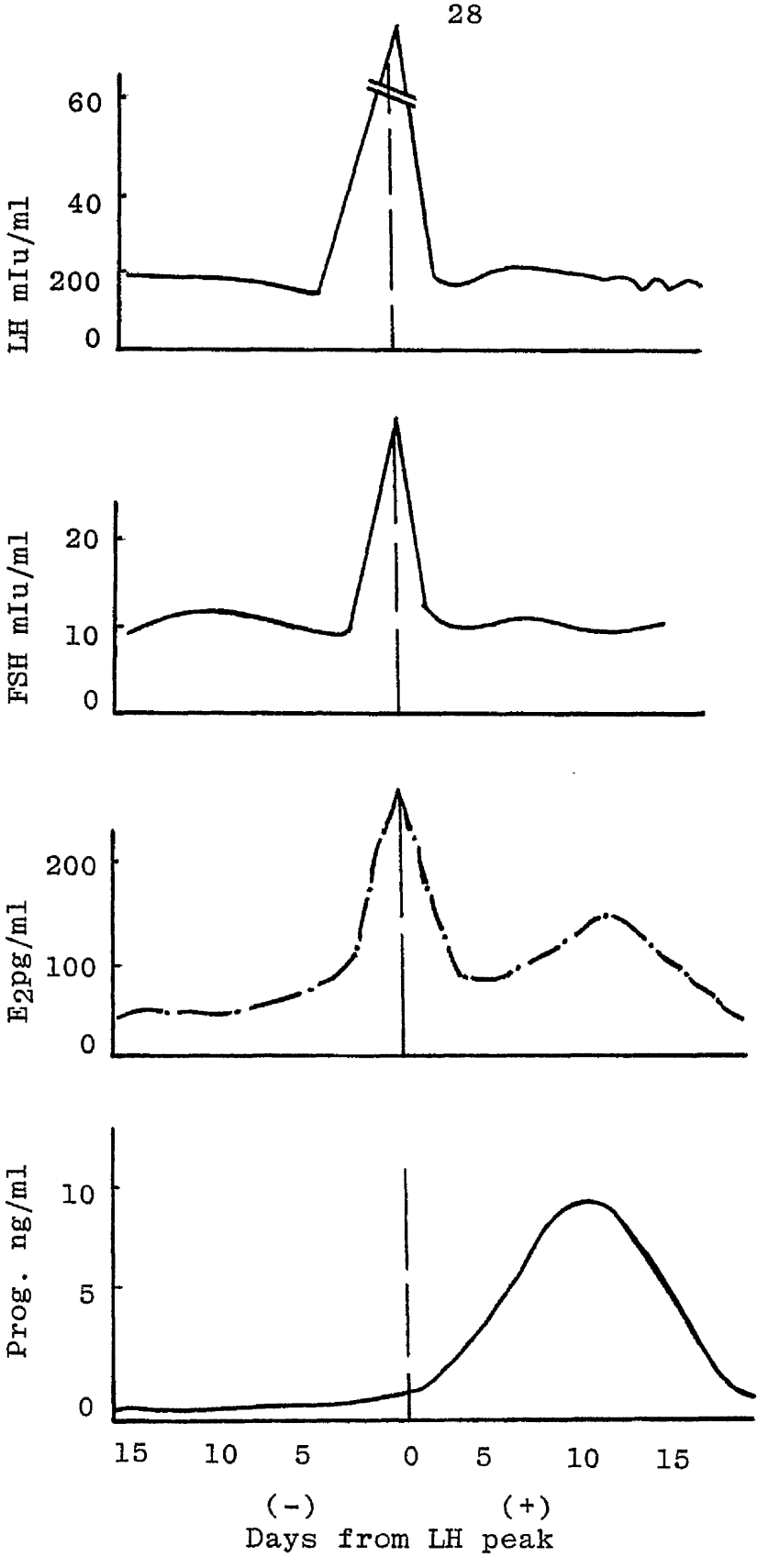


Fig. 1.

Mean values of LH, FSH, Estradiol (E_2), in daily serum samples of 9 women during ovulatory menstrual cycles (Thorneycroft et al., 1971).

All of these findings suggest that oestrogen has a positive feedback actively causing secretion of a releasing factor leading in turn to the LH surge and ovulation. So far there is no positive evidence regarding the peak of FSH secretion. It has been suggested that the hypothalamic releasing factor is not specifically related to LH secretion but acts on FSH as well.

While these biochemical findings and the arguments based on them have given rise to a neat persuasive hypothesis, it is as well to remember that much of the evidence is biochemical and therefore in one sense indirect. There is less evidence of what is actually happening in the ovaries and there is a considerable amount of experimental biological work which is confusing and some of it contradicts accepted ideas.

Hypophysectomy is always followed by a regression of reproductive functions and atrophy of the ovaries. It has been reported that the number of oocytes is greater in hypophysectomized than in normal animals. Swezy (1933) suggested that this was due to increased oogenesis. Young (1961) stated that in the hypophysectomized rat follicles can develop to the pre-antral stage without gonadotrophic stimulation. Nakano et al (1975) went even further and stated that follicles can grow to an early antral stage after hypophysectomy. It could be that FSH is only necessary beyond this

point. Certainly in normal animals many follicles start development at the beginning of the cycle but most undergo atresia in the early phases prior to ovulation (Boling et al, 1941; Young, 1961 and Ingram, 1962). However, doubt has been cast on the validity of many of these statements by the findings of Ingram (1953) who showed beyond any doubt that in the rat (i) the population of oocytes falls after hypophysectomy as it does in the intact animal but the rate of decline is slower and (ii) although medium and large follicles are present their number is very much lower in the hypophysectomized animals. In other words, after hypophysectomy oocytes are no longer lost by atresia or by ovulation of large follicles. Ingram's studies involved actual follicle counts in the ovaries of hypophysectomized animals whereas other investigators merely reported qualitative results. Paradoxically it has been claimed that pure FSH administered to hypophysectomized animals had no action on the ovary (Lostroh and Johnson, 1966). Less pure FSH produced follicular growth in hypophysectomized animals but only in large doses (Malven and Sawyer, 1966). Oestrogen was actually more effective in producing granulosa proliferation (Smith and Bradbury, 1961 and Wallach and Noriega, 1970). Aron et al (1965) and Krey and Everett (1971) showed that in intact

adult female rats oestrogen on day 1 speeds up the cycle. Smith and Bradbury (1961) also claimed that oestrogen sensitized the ovary to the subsequent action of FSH, and Goldenberg et al. (1972) demonstrated that oestrogen increased the incorporation of FSH in the follicles of hypophysectomized rats. Whether the lack of response to pure FSH noted above (Lostroh and Johnson, 1966) was due to the absence of preceding oestrogen stimulation per se or to a lack of follicles at a suitable phase of development was not apparent. Most observers are of the opinion that the source of oestrogen produced in the follicular phase is the theca interna (Corner, 1938; Dubreuil, 1953; Falck, 1959 and Harrison, 1962). In support of this are the findings of Deane et al. (1962) and Konig (1965) who demonstrated that the enzyme 3β hydroxy-steroid dehydrogenase was confined to the theca interna in the developing follicle. According to Balboni (1976) this tissue only begins to form after the follicle has a well-developed antrum. Eshkol et al. (1970) were of the opinion that FSH was responsible for granulosa cell proliferation and the development of a follicular basement membrane whereas LH promoted antrum formation, thecal differentiation and vascular development. It may be that the low levels of LH which are present in the follicular phase are sufficient for this purpose but the interplay of oestrogen, FSH and LH in promoting follicular development is far from clear. In particular

it remains to be shown decisively that LH has an influence on the theca interna. Lostroh and Johnson (1966) found that pure LH had no influence on the ovaries of hypophysectomized rats. Similar problems exist in regard to ovulation and corpus luteum formation. Both Aron et al (1969) and Schwartz and Ely (1970) stated that pure FSH will produce luteinization of suitably sized follicles although ovulation may not occur. Lostroh and Johnson (1966) stated that pure FSH or pure LH are both equally effective in causing ovulation of follicles in a suitable condition. Ryle (1972) cultured mouse ovaries and showed that FSH stimulated the growth of follicles only on days 2 and 5 of culture, and diminished thecal activity; LH stimulated the growth of follicles only on day 5 and only if they had 3-4 layers of granulosa cells, and promoted growth of the theca interna. In addition, it was noted that some follicles grew rapidly on day 5 without gonadotrophic stimulation.

Channing (1970) harvested and cultured granulosa cells from follicles of varying size from monkeys and found: granulosa cells from large pre-ovulatory follicles luteinized in culture without any specific stimulus and secreted progesterone for 6 days; those from medium-sized follicles luteinized partially without stimulation but if either FSH or LH were added to the culture medium luteinization was complete and

progesterone was secreted for periods of up to 27 days; cells from small follicles showed no evidence of luteinization in culture even when treated with FSH or LH.

Another perplexing feature regarding the mechanism of ovulation is to be found in the work of Schwartz and Gold (1967) who found that a single injection of anti-LH will block ovulation and also delay the subsequent ovulation. They suggested that the surge of LH was necessary for the development of the next set of follicles.

Many follicles undergo atresia during one oestrous cycle and Richards (1975), working with hypophysectomized animals, claimed that LH, far from promoting follicular growth, actually induces atresia if given before stimulation by FSH. It will be remembered that in the human, the LH surge probably antedates the mid-cycle rise in FSH so that it becomes difficult to prove that LH has a stimulatory effect on follicles after ovulation.

These observations deal with the problem of ovulation but there is a lack of knowledge regarding the mechanisms responsible for the maintenance and activity in the corpus luteum. The corpus luteum is a very persistent structure. In the rat, X-ray irradiation will destroy ovarian follicles but the corpus luteum survives, and if stimulated by

gonadotrophins, will produce progesterone (McDonald et al, 1969). Selye (1948) and Malven and Sawyer (1966) described the persistence of the corpus luteum after hypophysectomy while Levy et al (1959) also observed the persistence of 3β steroid dehydrogenase activity in the corpus luteum of the hypophysectomized rat and contrasted it with the rapid disappearance of the enzyme from the interstitial tissue. In an ultrastructural study Flerko et al. (1967) demonstrated the disappearance of the organelles of steroidogenesis from the interstitial cells after hypophysectomy.

Interstitial cells occur in small masses between the follicular structures. These cells were first described by Pfluger (1863). The appearance of the interstitial cells varies during the oestrus cycle. Evidence has been presented that they may secrete both oestrogens and androgens (Rennel, 1951 and Claesson et al., 1954). Histological studies by Deane (1962) and Guraya (1974) and electron microscope studies by Flerko (1967) showed that the interstitial tissue had all the characteristics of a steroid producing tissue. Motta and Bourneva (1970) noted that the interstitial tissue activity showed cyclic changes and reached a peak at pro-oestrus and oestrus. It is not yet known whether these cells pass through a definite cycle of changes ending in degeneration or persist indefinitely (Brambell, 1956).

The persistence of 3β steroid dehydrogenase activity

in the corpus luteum of the hypophysectomized rat is a very interesting phenomenon. It seems peculiar that the enzyme should persist when the substrate on which it acts is presumably diminishing. The methods used in demonstrating the presence of dehydrogenases are full of pitfalls. In all of them the presence of lipid is apt to give rise to false reactions and strenuous efforts have been made to eliminate this fault. Baker and Klapper (1961) used an acetone n-butanol and ether mixture at 65°C to extract the lipid. This was successful and did not appear to have interfered with the activity of succinic dehydrogenase and diphosphopyridine nucleotide diaphorase. While this method removed fine droplets of lipid, problems still remained when the deposits of lipid were large and attempts at removal interfered with the enzyme activity. This might be the case in a corpus luteum persisiting after hypophysectomy, making interpretation of the results difficult. The observations of Levy et al (1959) in regard to the persistence of 3β steroid dehydrogenase activity in the corpus luteum after hypophysectomy may have to be viewed with some caution.

In many of the studies of hormonal function and follicular activity quoted it is not always clear what type of follicle is involved. A number of classifications of follicles have been proposed but none have proved completely satisfactory. Lintern-Moore et al

(1974) classified follicles according to the total number of granulosa cells in any follicle at its largest cross-section. This is satisfactory for pre-antral follicles but cannot be applied to antral follicles. A simpler method (Mandl and Zuckerman, 1950, 1951a-c; Green, Mandl and Zuckerman, 1951) which depends on counting the number of granulosa layers in the follicle can be applied to both pre-antral and antral follicles and thus has much to commend it.

OBJECT OF INVESTIGATION

From a brief and selective review of gonadal-pituitary relationships it is apparent that many problems still remain. A number of questions seemed worth considering:

1. Do follicles develop to the pre-antral or early antral stage without the influence of gonadotrophins as suggested by Young (1961) and Nakano (1975) or are the follicles which they observed merely ones which persisted after hypophysectomy due to the slowing of the atretic process as suggested by Ingram (1953)?

2. Does pure FSH have indeed no activity at all? If it has any influence on follicle growth is this direct or indirect and does it depend on the stage of the development of the follicle?

3. Since there is a peak in FSH secretion around the time of ovulation does this hormone have an influence on the corpus luteum?

4. Similarly, does pure LH have no activity? Does it induce follicular maturation as stated by Lostroh and Johnson (1966) or, on the contrary, does it cause follicular regression (Richards, 1975)? Does it stimulate thecal activity in the absence of FSH?

5. Do either FSH or LH have any influence on the process of atresia of follicles or degeneration of the corpus luteum?

The following account deals with attempts to obtain answers to some of these questions.

MATERIAL AND METHODS

EXPERIMENTAL ANIMAL

Mature female non-pregnant Sprague Dawley rats (supplied by Batin and Kingman Limited, Hull, England) were kept in the animal house of Glasgow Royal Maternity Hospital until they were fully adapted to the new environment. Optimum room temperature of 20-22°C was maintained and a controlled 14/10 hour lighting cycle was operated.

At the start of the experiment these rats were 48-52 days old and those used in any particular experiment were in the weight range of 180-200 grams.

The laboratory rat is sexually mature at 6-7 weeks old and the female will have her first oestrous cycle at that time. The oestrous phase in the female rat lasts for about 10-12 hours and recurs every 4-5 days. Female rats usually have regular cycles provided they are housed in even numbers - with uneven numbers oestrus may be suppressed.

THE RAT OESTROUS CYCLE

The rat oestrous cycle is roughly divided into four stages (Long and Evans, 1922).

1. OESTRUS (late follicular phase)

   This is the period of heat; this phase lasts from 9-15 hours and is characterized by a high rate of running activity. Behavioural changes

include quivering of the ears and lordosis or arching of the back in response to handling. The uteri undergo progressive enlargement due to an accumulation of luminal fluid. Ovulation occurs during this phase.

2. METOESTRUS

This occurs shortly after ovulation and lasts for 10-14 hours. The ovaries contain corpora lutea and small follicles, and the uteri have diminished in vascularity.

3. DIOESTRUS

This lasts 60-70 hours, during which functional regression of corpora lutea occurs. The uteri are small and the vaginal mucosa is thin.

4. PROESTRUS (follicular phase)

This lasts 8-12 hours. Characterized by functional involution of corpora lutea and pre-ovulatory swelling of the follicle. Fluid collects in the uterine horns.

It must be remembered that the change from one phase to the other is gradual.

It was necessary to carry out the experiments in the same phase of the oestrous cycle and to this end vaginal smears were used to monitor the animals and determine the length of the cycle and the time of onset of oestrus.

VAGINAL SMEAR IN RATS

Cyclic changes in the rat vaginal smear were first described by Long and Evans (1922). They were confirmed by Turner and Bagnara (1971) and related to blood levels of hormones.

Technique of Vaginal Smears

A specimen of vaginal secretion was obtained from the posterior wall of the vagina by means of a wooden spatula, the fluid was smeared on to a clear dry glass slide and immediately fixed in 90% alcohol containing a small amount of glacial acetic acid and carbowax.

Staining Method

The slide dried in 5-10 minutes leaving a smear with a protective covering of wax which was easy to transfer to the laboratory (Papanicolaou, 1957). There it was stained by the Papanicolaou technique (1942).

Types of Cells seen in Normal Smear

1. Cornified cells: Large polygonal cells with abundant pink stained cytoplasm and small pyknotic nuclei (Fig.2). The presence of this type of cell in a smear indicates oestrogen activity.
2. Intermediate non-cornified cells: They are smaller than the cornified cells with basophilic cytoplasm and small rounded vesicular nuclei (Fig. 3 and 4).

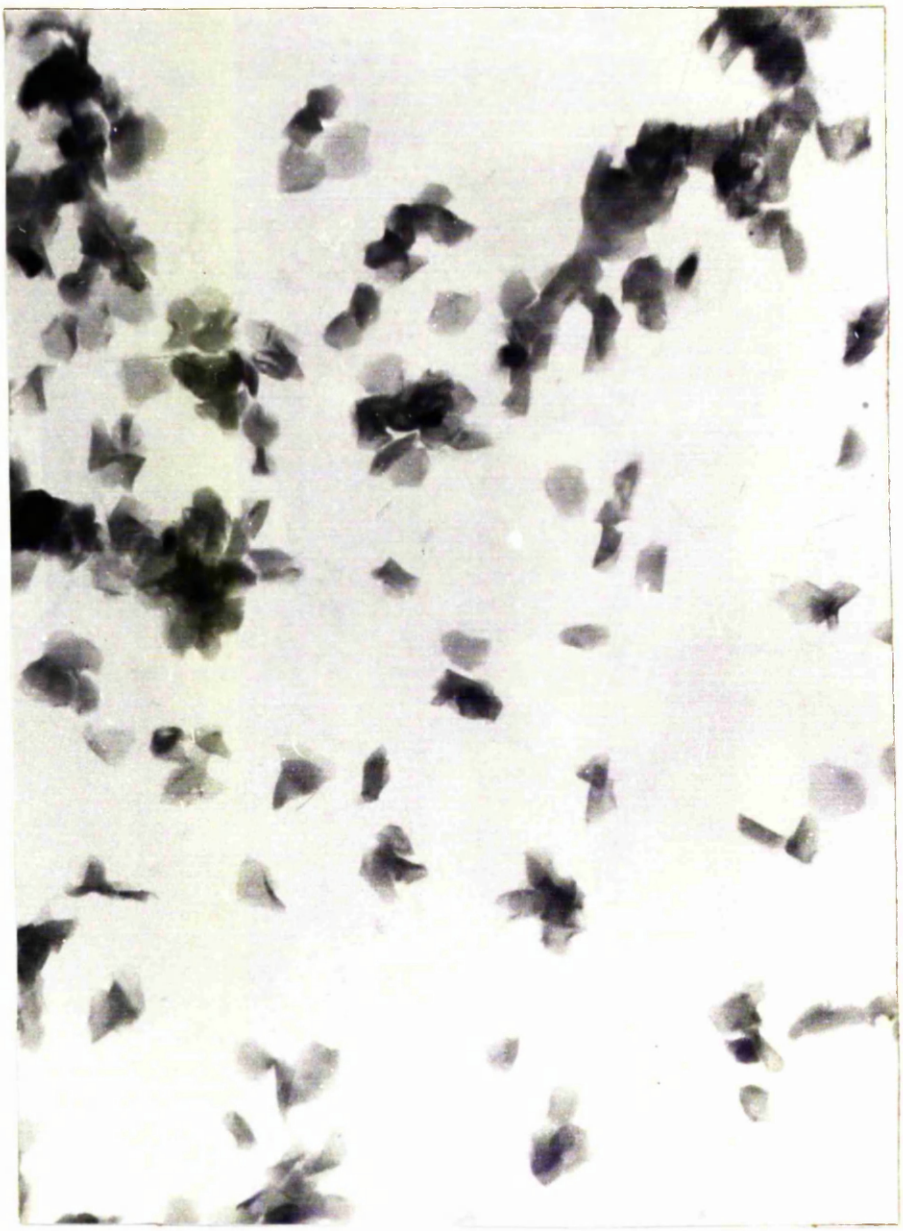


Fig. 2

Cornified cells in vaginal smear from normal rat at time of oestrus. Papanicolaou technique X 400.

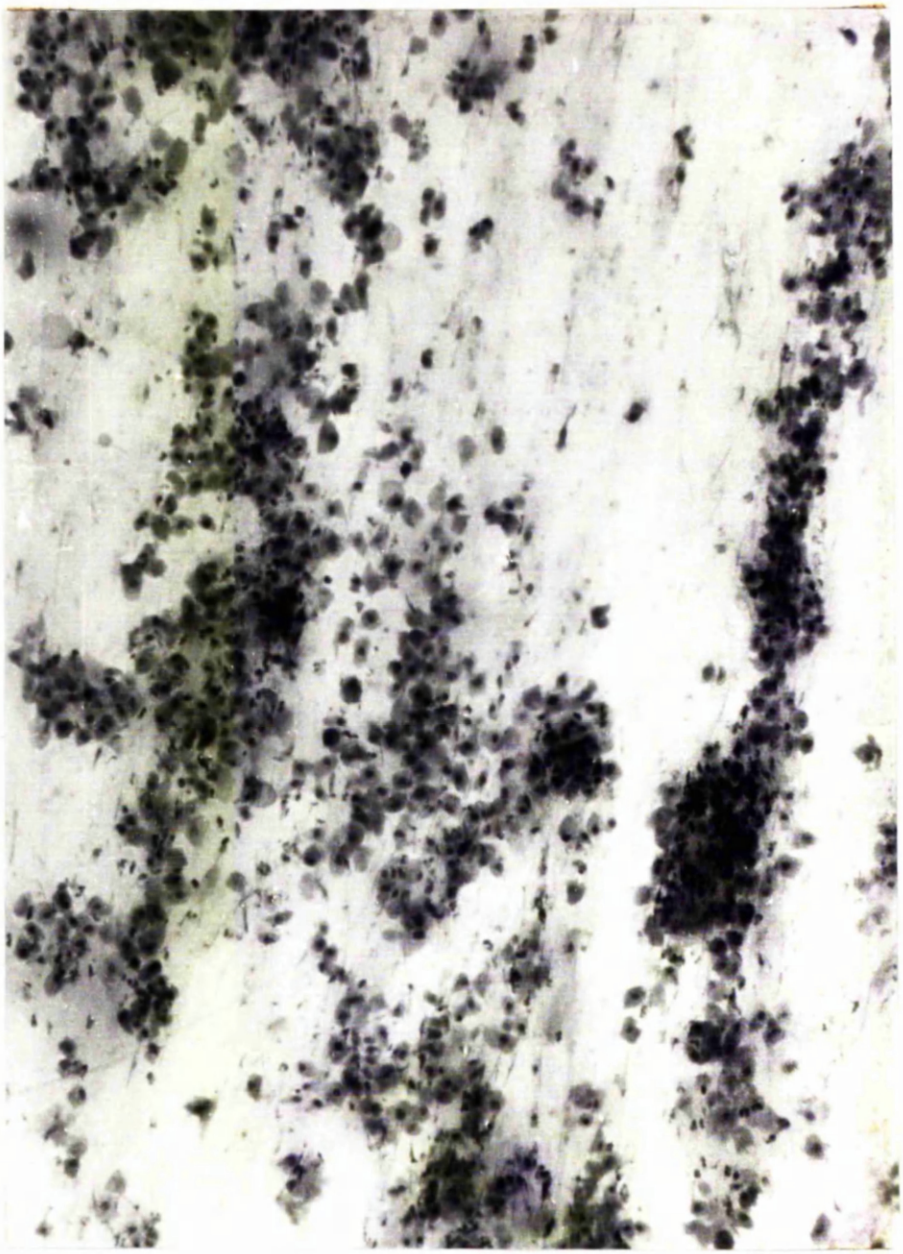


Fig. 3

Vaginal smear from normal rat at metoestrus, showing cornified and non-cornified cells. Papanicolaou technique X 300.

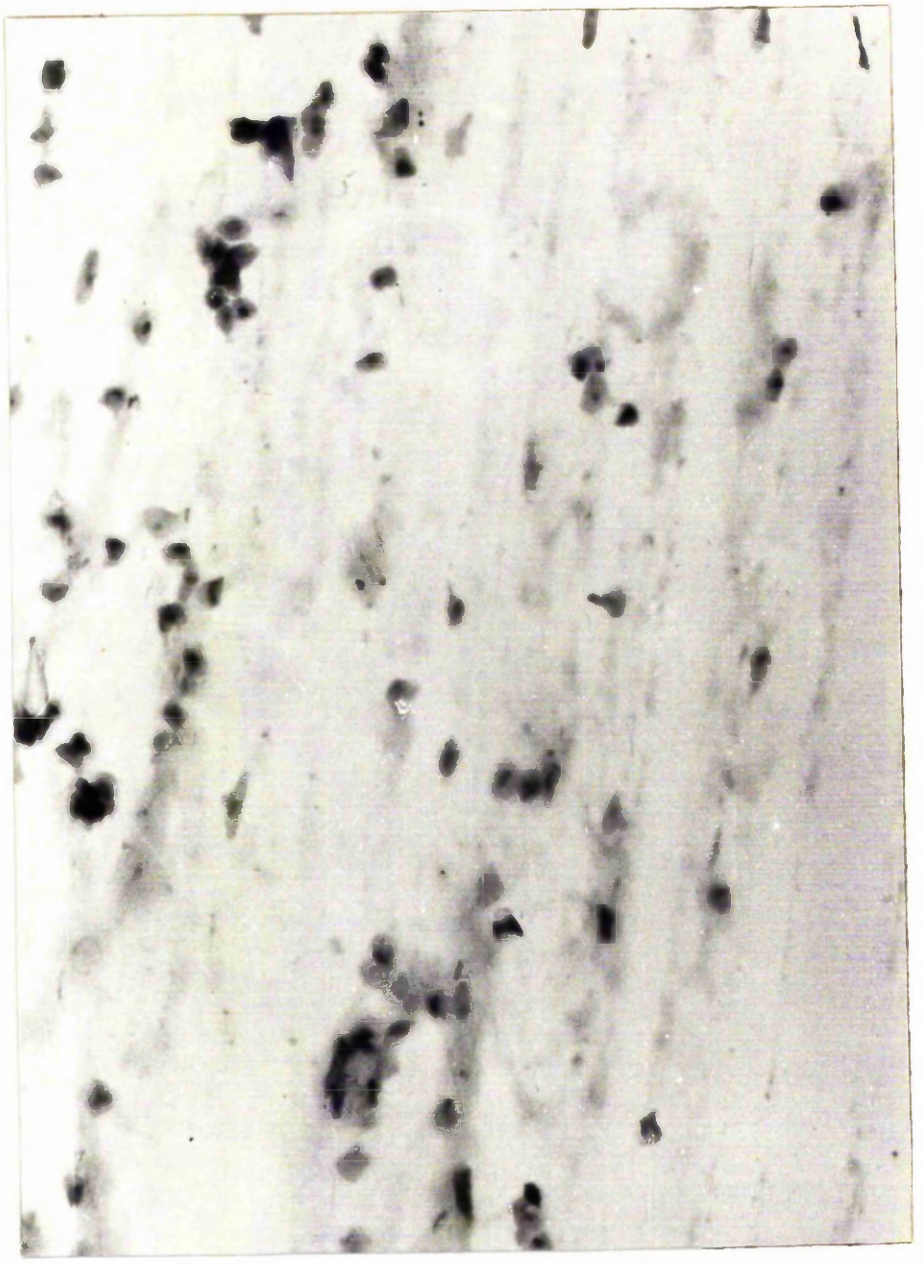


Fig. 4

Vaginal smear from rat at dioestrus. There is a large amount of mucus, some leucocytes and epithelial cells mainly of non-cornified type. Papanicolaou technique X 300.

3. Parabasal cells: They are small and round or oval with relatively large vesicular nuclei and constitute less than 5% of the smear population.

4. Basal cells: They have large nuclei and sometimes show mitosis and a little cytoplasm but they are practically never seen in a normal smear.

Table 1 gives, in brief form, the main types of cells seen in each of the four phases of the oestrous cycle together with histological changes in the ovary and uterus.

PHASE OF CYCLE	SMEAR	OVARIES	UTERUS
OESTRUS 9-15 hours	Cornified cells	Large follicles with cavity formation. Small granulosa cells surrounded by theca interna - ovulation at the end of this phase.	Uterus, large distended filled with clear fluid. Hyperaemia. No leucocytes.
METOESTRUS 10-14 hours	Leucocytes. Non-cornified and cornified cells.	Young corpora lutea. Small fully formed follicles.	Uterus, small with thin walls and narrow lumen. Glands are small interspersed with leucocytes. No mitosis.
DIOESTRUS the resting phase 60-70 hours	Mucus, leucocytes and non-cornified cells.	Small follicles and corpora lutea of previous cycles.	Small and anaemic narrow lumen. Leucocytes present in epithelium.
PROESTRUS phase of proliferation	Non-cornified cells	Large follicles with beginning of antrum formation. Corpora lutea of previous cycles show fatty degeneration.	Uterus, larger and becoming distended with fluid at the end of the stage. Increase in mitosis.

Table 1. The phase of cycle, the predominant vaginal cell and the corresponding ovarian and uterine changes.

EXPERIMENTAL DESIGN

Only animals with a four-day cycle were selected for study. The immediate post ovulation phase as determined by the vaginal smear pattern was chosen as the point of beginning all experimental studies. On this basis animals were divided into two main groups: (a) control animals and (b) animals subjected to hypophysectomies.

All animals were kept for a minimum period of two consecutive four-day cycles prior to being entered into any experiment.

Control and hypophysectomized animals were kept under exactly the same conditions. All animals were weighed daily. Vaginal smears were also done daily. The animals were housed as usual,one pair to a cage.

The animals were grouped in one of the following groups:

A. Control Normal Intact Animals

Two mature female rats were used per experiment. After determining the time of ovulation by the method of vaginal smear, pairs of rats were sacrificed towards the end of oestrous and every 6 hours for 96 hours (to cover a full oestrous cycle), a total of 34 rats. The object of such an exercise was to show the normal pattern of ovarian histology during the normal cycle of the mature female

rat, and then to compare it with changes found in the hypophysectomized group.

B. Control Hypophysectomized Animals

Hypophysectomy was carried out towards the end of oestrus as indicated by cornification of the vaginal smear.

One pair of animals was killed immediately after the operation and every six hours over a period of ninety-six hours and then further pairs were killed every day up to twenty-eight days, a total of 82 rats. The object of this experiment was to show the effect of removal of the pituitary gland on the ovarian histology of mature female rats.

Great care was taken to eliminate incompletely hypophysectomized animals. If the operation is carried out with care this condition is unusual.

Following hypophysectomy there are considerable changes in the animal behaviour such as irritability, an exaggerated response to stimuli, puckering of the skin, and a lack of interest in food. There is also a loss of body weight and muscle tone, and with the passage of time atrophy of the genital organs (Selye, 1948).

These changes were taken as indirect evidence of complete removal of the pituitary gland. In addition, at autopsy, careful inspection of the

region of the sella turcica was routinely performed. In a proportion of cases serial histological sections of the sella turcica were prepared to make sure that no pituitary tissue had been left behind.

C. Control Sham-Operated Animals

Eight animals were anaesthetised and the operation was carried on as far as the drilling of the base of the skull but with the pituitary gland left intact. The animals were sacrificed four days later.

D. Hypophysectomized Animals - Buffer Injected Controls

Animals in pairs each received a single subcutaneous injection of 0.5 ml of sodium phosphate buffer pH 8.0 on the seventh day at 9.00am and were sacrificed at time intervals of 1, 6, 12, 24 and 48 hours, a total of 10 animals, to form a further control group to evaluate the effect, if any, of the buffer solution.

E. Hypophysectomized Animals - Hormone Injected

Two animals were used at each time interval and each animal received a single subcutaneous injection of the preparation under experiment, dissolved in 0.5 ml of phosphate buffer, on the seventh day between 9.00 and 10.00am.
In experiments dealing with N.I.A.M.D. Rat FSH-B1, four groups of experiments were

performed using the minimal effective dose and its multiples. The dose schedule was:

Group I	25 µg
Group II	50 µg
Group III	75 µg
Group IV	100 µg

Each group consisted of 10 rats and the total number of animals in the test series was 40.

In experiments dealing with the LH preparation (I.R.C., 24-6-69) it was decided to use a dose schedule similar to that employed for FSH as experiments utilizing both FSH and LH injected into hypophysectomized rats with a dose of 25µg of the LH fraction seemed to have a stimulatory effect. Four groups of experiments were performed and the dose schedule was:

Group I	25 µg
Group II	50 µg
Group III	75 µg
Group IV	100 µg

Each group consisted of 10 rats and the total number is this series was 40.

In experiments dealing with both FSH and LH it was decided to use the minimal effective dose of a highly purified Rat FSH (N.I.A.M.D. Rat FSH, B-1) in combinations of multiples of a similar

dose of a highly purified LH preparation (I.R.C., 24-6-69).

Four groups of experiments were performed, the dose schedule was:

Group	I	25 µg FSH + 25 µg LH
Group	II	25 µg FSH + 50 µg LH
Group	III	25 µg FSH + 75 µg LH
Group	IV	25 µg FSH + 100 µg LH

Each group consisted of 10 rats and the total number in this series was 40.

All injections were given in the loose tissue in the back of the neck.

In all the experimental groups, two rats were sacrificed at specific times - 1, 6, 12, 24 and 48 hours post-operation.

All animals were killed by an overdose of ether, and the following routine was carried out:

1. Vaginal smear made, subsequently stained by the Papanicolaou technique.
2. The usual criteria for evaluating the stages of the oestrous cycle in terms of vaginal smear and uterine "ballooning" were observed.
3. The ovaries were removed, cleared of fat, weighed and immediately placed in a transport medium (Histocon* supplied by Bethlehem Trading Ltd., Gothenberg, Sweden) and sent to the laboratory

to be prepared for histological and histochemical staining. One ovary in each pair was serially sectioned for a follicle count.

4. The uterus was removed, stripped of fatty tissue, weighed and kept for histological study.

5. The sella turcica was examined by exploration of the region to confirm the completeness of the hypophysectomy operation.

*Histocon is a trade-name and the manufacturers, having applied for a patent refuse to reveal the composition. It is a biologic tissue transport solution which enables histological and histochemical analysis to be carried out by the use of the same biopsy specimens after freezing and cold microtome sectioning. In summary the properties of Histocon are as follows:

(1) Bacteriolytic effect
(2) Inhibition of autolysis
(3) Reduced leakage of protein and other cellular components
(4) Preservation of catalytically active enzymes at $+^{o}C$ within 48 hours of excision of tissue.

OVARIAN AND UTERINE WEIGHTS

A study of ovarian and uterine weights was made to provide a rough indication of the effects of gonadotrophins on ovarian mass and on the production of ovarian hormones.

At autopsy, both ovaries of each animal were dissected out, trimmed of fat and weighed together and expressed as a single figure of ovarian weight which was corrected to the nearest 0.0001gm.

The uterus of each animal was also dissected out and trimmed of fatty tissue and weighed and expressed as a uterine weight which was corrected to the nearest 0.0001gm.

As detailed previously, control intact animals and control hypophysectomized animals were killed in pairs at 6 hourly intervals each day for four days. For comparative statistical purposes the ovarian weights of all of the animals killed each day were summated. Means and standard deviations were derived from these figures.

In the case of hypophysectomized animals a further one pair of animals were killed each day from the fifth post-operative day until the twenty eighth day. To study the trend of change the ovarian weights of all animals killed in each four-day period, i.e. day 5 to day 8, day 9 to day 12 etc. were summated and means and standard deviations were derived.

The experimental period for hypophysectomized animals given injections of hormones was forty eight hours for each dose level. For statistical purposes the ovarian weights over the first twenty four hours from all animals given a particular dose of hormones were summated. It was realised that the mean value obtained from this procedure would be diluted by the weights at 1 hr and 6 hrs in most instances but this would in a sense emphasise the difference between the stimulated animals and the controls.

A similar procedure was adopted for a comparative study of uterine weights. Details of the number of animals in each group are given in Table 2.

Group I: Intact Mature Female Rats

Total rats sacrificed on Day 1	= 8
" " " " " 2	= 8
" " " " " 3	= 8
" " " " " 4	= 8

Group II: Control Hypophysectomized

Total rats sacrificed on Day 1	= 8
" " " " " 2	= 8
" " " " " 3	= 8
" " " " " 4	= 8
" " " " " 5 - 8	= 8
" " " " " 9 - 12	= 8
" " " " " 13 - 16	= 8
" " " " " 17 - 20	= 8
" " " " " 21 - 24	= 8
" " " " " 25 - 28	= 8

Group III: Control Sham Operated Rats = 8

Group IV: Control Buffer Injected Rats = 10

Group V: Hormone Injected Hypophysectomized

(i) FSH: All rats sacrificed during Day 1

25 µg FSH	= 8
50 µg FSH	= 8
75 µg FSH	= 8
100 µg FSH	= 8

(ii) LH: All rats sacrificed during Day 1

25 µg LH	= 8
50 µg LH	= 8
75 µg LH	= 8
100 µg LH	= 8

(iii) FSH+LH: All rats sacrificed during Day 1

25 µg FSH + 25 µg LH	= 8
25 µg FSH + 50 µg LH	= 8
25 µg FSH + 75 µg LH	= 8
25 µg FSH + 100 µg LH	= 8

THE OPERATION OF HYPOPHYSECTOMY

General Anaesthesia in Rats

An anaesthetic should be efficient and as non toxic as possible. Various types are described in the literature. Halothane is an efficient anaesthetic but it requires complicated apparatus. Sodium pentobarbitone and Hexobarbitone are easily administered by intraperitoneal injection but only useful for short experiments. Ether, despite certain disadvantages such as copious salivation, increased bronchial secretion and cardiac stimulation, was selected as the anaesthetic of choice since it was easily available and could be administered in a simple manner. It provides a moderately long period of anaesthesia and the depth of anaesthesia is reasonably easily controlled.

The following anaesthetic method was used:
20 ml of ether were placed in the bottom of a glass dessicator and covered with a perforated platform. The rat was placed in the vessel which was then closed. The animal became comatose after a few minutes. When it was obviously unconscious the rat was gently removed from the vessel, and the operation quickly carried out.

Additional ether can be administered by a simple gauze face mask, when required.

Occasionally an animal may be particularly susceptible

to the toxic effect of the anaesthetic used and it may be necessary to resuscitate it. Artificial respiration may be carried out by manual compression of the thorax between the thumb and finger. In addition, the circulation may be stimulated by holding the animal by its tail and swinging it (Croft, 1960).

Operation Techniques

The animal was fastened on its dorsum to a specially designed dissecting table having a special light microscope attached to it. The rat was immobilised by rubber bands attached to its limbs, the head was kept extended by a rubber band attached to its upper incisors. The table was slightly inclined so that the head was at the lowest level to prevent the animal from aspirating mucous during the operation.

The operator sat at the tail end of the table; usually there is no need for an assistant.

The extended neck was shaved and then painted with antiseptic such as Cetavlon.

A midline longitudinal skin incision about three centimetres long was made extending from the submental papilla to the lower border of the submandibular gland. The tissues between the two salivary glands were separated to expose the triangle formed by the larynx and trachea medially, digastric muscle laterally, and the omohyoid

muscle caudally.

The closed lips of fine smooth curved forceps were forced through the omohyoid muscle at the point of its insertion under the sternomastoid muscle. The forceps lips were then directed toward the midline and upward, and then spread apart to expose the base of the skull covered by a thin strip of muscle which was scraped by a dental scrape to expose the bones. The trachea was retracted out of the operation field by a fine curved retractor.

By the use of a round bore burr, size 9, attached to a foot operated dental drill a hole was drilled in the midline of the occipito-sphenoid synchondrosis in a manner which allows $\frac{2}{3}$ of the hole to be cranial to the suture. The drill was not allowed to break through the bone, but was carefully controlled so that the last shell of bone was picked away by a fine dental hook without injury to the underlying dura (Figs. 5 and 6) The pituitary tissue was exposed by tearing away the dura with a dental pick.

A pipette of the largest size accommodated by the drill hole was placed against the pituitary tissue, and the gland was removed by controlled negative pressure. The strength of suction was controlled by a finger placed on a hole made in the suction pipette (Fig.7).

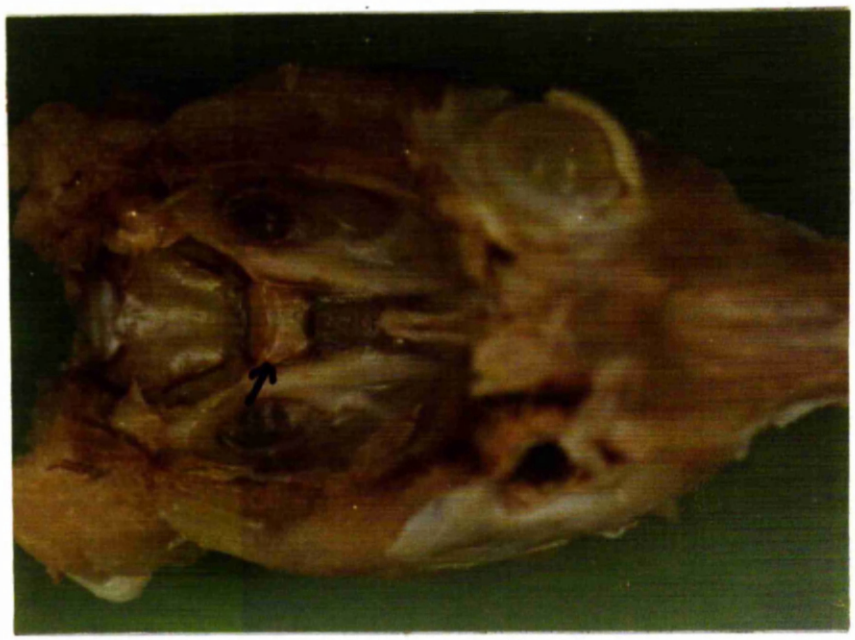


Fig. 5

Interior of base of skull exposed to show pituitary gland lying in its fossa (arrow).

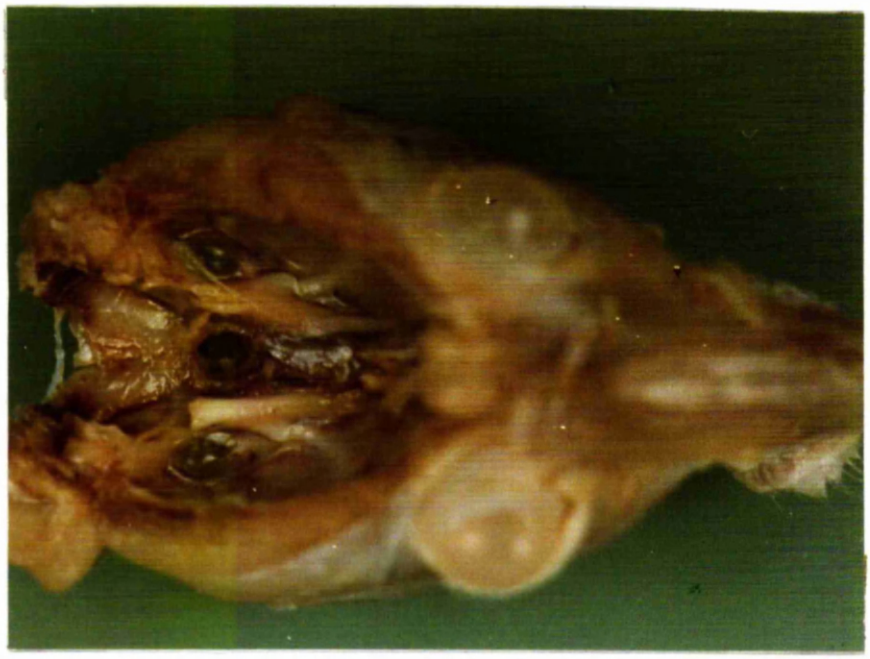


Fig. 6

Interior of base of skull exposed to show empty pituitary fossa following hypophysectomy.

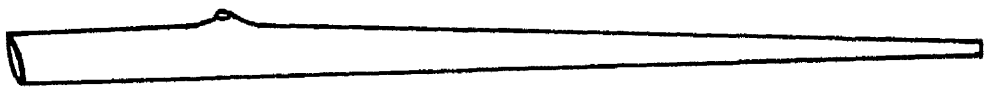


Fig.7 . Suction Pipette

The pipette was attached by means of a two holed rubber stopper to a glass tube having the dimensions of ¾" diameter and 4" high. This tube acted as a trap for the pituitary tissue and allowed examination of the pituitary tissue after removal.

Following removal of the pituitary tissue (Fig. 8), the exposed area was wiped free of blood with cotton wool; wax can be used to stop any active bleeding from the bone edge. The skin was then closed by interrupted silk sutures. With practice the whole operation took about 3-4 minutes.

During the operation there were two dangers: the first was damage to the nerves and vessels especially branches of the internal carotid artery and hence a fatal haemorrhage; the second was respiratory arrest produced by excessive or prolonged compression on the trachea. Tracheal retraction should be intermittent and of short duration. If respiratory movements cease, compression of the trachea should be relieved at once and a rubber tube promptly inserted

into the larynx of the rat and air or oxygen carefully blown directly into its lungs at low pressure.

Operations on dead animals were carried out to gain dexterity in manipulating the animal and to gain confidence in recognising anatomical landmarks. In the very first attempts at hypophysectomy on live animals the mortality rate was high (50%) but with practice this was reduced to less than 5%. Figure 8 shows a diagram of the various steps of the operation of hypophysectomy.

Table 2 illustrates the classified and total number of rats submitted for hypophysectomy. It also illustrates the number of operative, immediate and late post-operative (8 hours after operation) deaths.

Hypophysectomy and its After Effects

The stage of the cycle in rats was judged according to the appearance of the vaginal smear. Cornification of the majority of the cells was taken to signify that the oestrous phase had commenced and ovulation had taken place. In all cases hypophysectomy was carried out towards the end of oestrus.

The operations were carried out in the manner already described. Pairs of animals were kept in a dry clean cage and the temperature was maintained at 20-22^{o}C with special attention to the addition of sugar (0.03%) and sodium chloride (0.06%) to the drinking water.

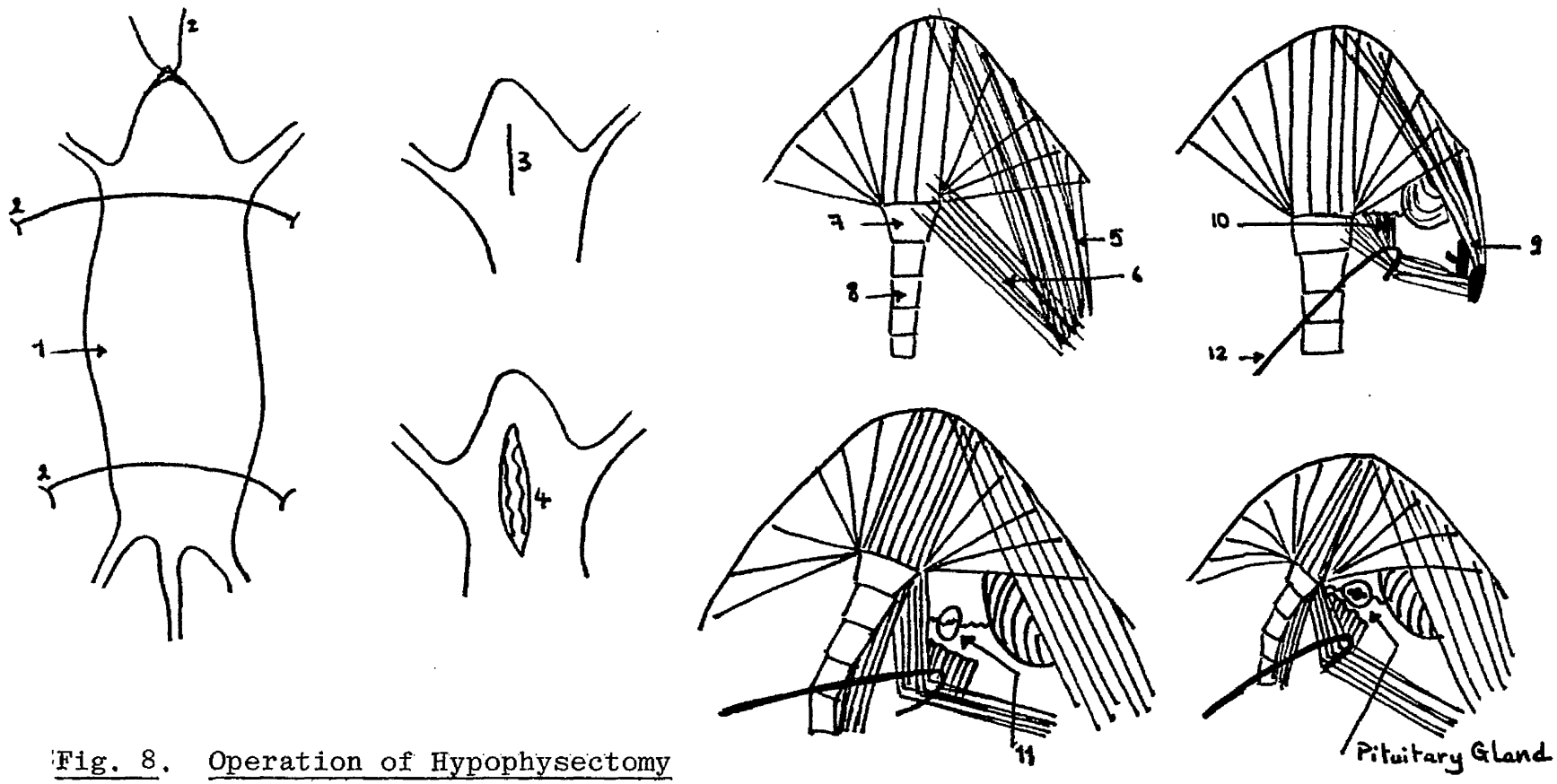


Fig. 8. Operation of Hypophysectomy

1. Mature female rat on the dissecting table
2. Rubber bands
3. Skin incision
4. Submandibular salivary gland
5. Digastric Muscle
6. Omohyoid Muscle
7. Larynx
8. Trachea
9. Internal carotid vessels
10. Muscle at the base of skull
11. Drill hole on occipito-sphenoid synchondrosis
12. Fine curved retractor

EXPERIMENTAL DESIGN	NO. OF RATS	
Sham-hypophysectomy	10	
Hypophysectomized Controls	82	
Buffer Controls	10	
FSH Dose Selection	90	
Hypophysectomized + FSH, B1	40	
Hypophysectomized + LH (IRC,24-6-69)	40	
Hypophysectomized + Combined FSH/LH	40	
Total number of successful hypophysectomies		312
Rejected as Incomplete Hypophysectomy	60	
Operative deaths	62	
Immediate post-operative deaths	22	
Late post-operative deaths	43	
Total number of unsuccessful hypophysectomies		187
Total number of hypophysectomies attempted		499
Intact Controls	34	

Table 2. Details of the classified and total number of rats submitted for study and the number of operative and immediate and late post-operative deaths.

The body temperature of hypophysectomized animals is usually slightly below normal and the basal metabolic rate considerably decreases (Selye, 1948).

After hypophysectomy the animals are relatively incapable of mobilising sugar from non-carbohydrate stores, and fasting or exposure to any stress will elicit an unusually pronounced and often fatal hypoglycaemia. If well fed the blood sugar can be maintained within the limits of normality.

Hypophysectomy also causes transient polyuria, with loss of sodium chloride in the urine and after the operation it is therefore important that sodium chloride is provided in the drinking water.

The animal is usually irritable and shows an exaggerated response to stimuli. The sexual cycle is immediately abolished and subsequently there is atrophy of the uterus and a reduction in the size of the ovaries. There is a loss of the total body weight, although this takes a considerable time.

Late Effect of Hypophysectomy

The skin atrophies and there is loss of hair. There is atrophy in the entire gastro-intestinal tract, including the liver. Ulcerative lesions appear in the intestine, with very loose stools. There is also a decrease in muscle tone.

The hypophysectomized rats were kept in the animal house for a period of not less than seven days prior to further experimentation.

GONADOTROPHINS

The gonadotrophins used in the experiments were as follows:

A. NIAMD-RAT-FSH, B-1

This is a highly purified preparation of FSH suitable for biological experiments, prepared from rat pituitaries at the Institute of Arthritis and Metabolic Diseases (N.I.A.M.D.), Bethesda, U.S.A. It was decided to use rat FSH to avoid the problem of antibody formation as there may be species difference. This preparation has a biological potency which is 3.7 times that of the National Institute of Health (N.I.H) FSH preparation on a weight for weight basis, as estimated by the Steelman-Pohley method (1953). It has minimal LH contamination, equivalent to 0.0045 of the N.I.H. LH, S-1 preparation, estimated by the Parlow method (1958).

The minimal effective dose of this FSH preparation was between 25 µg and 35 µg as indicated by a previous preliminary study. In such a study pairs of hypophysectomized rats were given the same preparation in doses of 5, 10, 15, 20, 25, 30, 35, 40 and 45 µg and the 25 µg pair were the ones that started to show a response after 12 hours.

B. I.R.C., 24-6-69

This is a highly purified preparation of LH of

human origin kindly supplied by Dr Anne Hartree of the Department of Biochemistry, Cambridge University. The biological activity of this preparation, measured by ovarian ascorbic acid depletion assay, with 95% confidence limit, was stated to be 3.21 (2.21-5.05) mg. of N.I.H. LH, S-1. The contamination by FSH was estimated at less than 0.05% based on the Steelman-Pohley assay method. According to Reichert and Parlow (1964), 1 mg N.I.H. LH, S-1 is approximately equal to 1540 i.u. of 2nd I.R.P.-HMG. Unfortunately it proved impossible to obtain a suitable preparation of rat LH. As the substance is not stable to repeated freezing and thawing, the material obtained was dissolved in distilled water, then divided among a large number of small tubes so that each tube contained 100 µg. of the LH preparation. These tubes were stored in a deep freeze at -20°C until required for use.

All preparations were dissolved in sodium phosphate buffer pH 8.0 at the time of injection. All injections were given between 9.00 and 10.00am, and were administered subcutaneously into the loose tissue in the back of the neck of the hypophysectomized rat. All injections were given as a single dose.

It will be noted that a human preparation of LH was used. Efforts were made to obtain purified

rat LH but this proved impossible. The use of luteinizing hormone from another species is to be regretted. It was felt however that since the experiments were short term, the period of observation being 48 hours after a single injection, this factor would have little bearing on the results obtained and would not invalidate any conclusions drawn therefrom.

HISTOLOGICAL TECHNIQUES

Four basic histological techniques were employed.

1. Haematoxylin and Eosin

This is a routine stain in all hospital Histology Departments and as such is the most important histological technique. With this method not only is it possible to gain a picture of the topography of a tissue and the relative changes occurring in it but also its cellular detail.

The tissue was fixed in 10% formol saline. In the case of rat ovaries this should not exceed 12 hours or be less than 4 hours. Excessive fixation toughens the tissue and makes it impossible to obtain a good histological preparation. The tissue was then processed through a series of dehydrating ethyl alcohol concentrations, cleared in xylol and embedded in wax. Sections were cut at a thickness of 7 μ on a rotary microtome. Sections were dewaxed and brought to water.

Staining with Mayer's haematoxylin was carried out employing times predetermined by test stainings with the particular batch of stain in current use. Differentiation was achieved with acid alcohol. The time and differentiation were such that the detailed structure of nuclei was easy to discern. Eosin was used as a counterstain for the cytoplasm.

2. Methyl Green Pyronin

Methyl Green is a good stain for deoxyribonucleic acid

(DNA). Pyronin stains ribonucleic acid (RNA) but it is not specific. Control sections must be treated with ribonuclease before staining to be sure that the red stained (i.e. pyronin stained) material is indeed RNA. The method of Trevan and Sharrock (1951) was used.

Staining Solution

5% aqueous Pyronin Y 17.5 ml.

2% aqueous Methyl Green 10.0 ml. was subject to partition in a separating funnel to remove Methyl Violet (a breakdown product of Methyl Green).

Distilled water 250 ml.

Concentration of Pyronin 0.16%.

Concentration of Methyl Green 0.036%.

The stains were diluted with an equal quantity of acetate buffer pH 4.8 before use.

Staining Method

1. Sections were brought to water.
2. The staining solution was poured on and left for 30 minutes.
3. The sections were rinsed quickly with distilled water and blotted dry.
4. The slides were flooded with acetone for a few seconds. At this point the process of differentiation was controlled under the microscope so that the stain was not

washed out excessively.

5. The acetone was drained and repeated when necessary.
6. The slide was flooded with equal parts of xylol and acetone and then drained and flooded with xylol and left until clear.
7. The slides were drained and mounted in D.P.X.

The object of using this method was to determine the turnover of ribonucleic acid in the various structures of the ovary which is an indication of protein turnover and a likely index of enzyme activity and general cellular activity. The small amount of tissue in the average rat ovary made it difficult to apply specific methods for the numerous enzymes associated with the various metabolic pathways.

Control sections were treated with ribonuclease before staining. Frozen sections were used in this method. Paraffin prepared sections of ovarian tissue did not provide such good results with the pyronin stain.

3. Oil Red O

This is a general stain for lipids. The method used was that of Lillie and Ashburn (1943).

Staining Solutions

A saturated solution of Oil Red O (0.25 - 0.5%)

was made up in isopropyl alcohol - this is a stock solution. It was used by diluting 3 parts of the stock solution with 2 parts distilled water. This was allowed to stand for 10 minutes and then filtered. This solution had to be used within 1 hour.

The staining solution will keep only for 1 to 1½ hours. After this time precipitation of the dye occurs.

Staining Method

1. The sections were cut.
2. Well washed frozen sections were placed in the staining solution in a small closed container for 10-15 minutes. Gentle agitation aided even staining of the lipids.
3. The sections were lifted from the stain and transferred quickly to another dish containing 60% alcohol. These were differentiated until the background was clear.
4. The sections were washed in water.
5. The nuclei were counterstained with haematoxylin. The time used was short to avoid the need for differentiation.
6. The haematoxylin was made blue with 1% disodium phosphate or tap water.
7. The sections were floated onto slides and mounted in glycerine jelly.

The lipids were stained various shades of bright red and the nuclei blue.

This is useful for a steroid producing tissue such as the ovary. During the period of active steroid production the turnover of lipids will be rapid, and stained deposits will be in the form of fine droplets forming an intra-cellular emulsion. In degenerative processes or when steroid production is low and lipid is not being utilised deposits are likely to consist of large droplets of varying sizes forming a coarse emulsion. This provided a rough guide to the rate of steroid production in the various structures in the ovary.

4. Steroid Dehydrogenases

The basis of demonstrating the sites of activity of these steroid dehydrogenase enzymes was as follows:

The tissue was incubated with a chosen steroid substrate mixture.

Co-enzymes in the form of nicotinamide adenine dinucleotide (NAD) were added.

The tissue dehydrogenase oxidised the steroid by splitting off hydrogen. This was accepted by the co-enzyme. In normal circumstances the co-enzyme would hand on the hydrogen to the general respiratory system of the cells. To prevent this a respiratory inhibitor was incorporated in the incubation mixture.

In addition, a colourless substance which on reduction became an insoluble pigment was also added to the incubation mixture. The reduced co-enzyme handed on its hydrogen to this colourless substance and pigment was deposited at the sites of enzyme activity. In the present investigations an attempt was made to demonstrate the activity of 3β steroid dehydrogenase.

Table 3 shows the incubation mixture for 3β steroid dehydrogenase acting on the conversion of Dehydro-epiandrosterone to Androstenedione.

Method

Materials had to be as fresh as possible to preserve enzyme activity. The tissue was placed on the cryostat block. A drop of O.C.T. compound (Lab-Tech Products) was applied and the whole was frozen by a spray of instant Freezing Aerosol (Dichlorofluoro).

Sections were cut on a Slee freezing microtome at -20°C and mounted on coverslips. Sections dried quickly in the microtome environment. Staining had to be carried out as quickly as possible.

1. The reagent mixture was filtered onto mounted sections and incubated for 1 hour at 37°C using a container as small as possible which closed, and contained a pad of cotton wool soaked in Tris-Tyrode solution. This prevented evaporation of the staining mixture.
2. Excess fluid was dried off.

	SUBSTANCE	CONCENTRATION	ACTION
1	Dehydroepiand-rosterone dissolved in dimethyl formamide	0.3 ml.	Steroid which dehydrogenase acts
2	Polyvinyl Priolidone	5.7 ml. 6.1% in Tris Tyrode solution	To prevent diffusion of reaction product
3	Potassium Cyanide	0.25 ml. of 0.91%	Respiratory inhibitor
4	Nicotinamide adenine	2.2 mg.	Co-enzyme hydrogen acceptor
5	Nitro Blue Tetrazolium	0.45 ml.	Dye
6	Dimethyl-sulphoxide	0.35 ml.	Increases substrate solubility and helps penetration of cells

Table 3. Steroid Dehydrogenase Incubation Mixture

Incubation mixture for 3β steroid dehydrogenase acting on the conversion of Dehydroepiandrosterone to Androstenedione.

3. The sections were fixed in 10% formol saline for 10 minutes and then washed for 5 minutes.
4. The sections were counterstained in carmalum for 5 minutes and then washed and mounted in glycerol jelly.

The sites of the enzyme activity appear as tiny black or dark blue spherical granules.

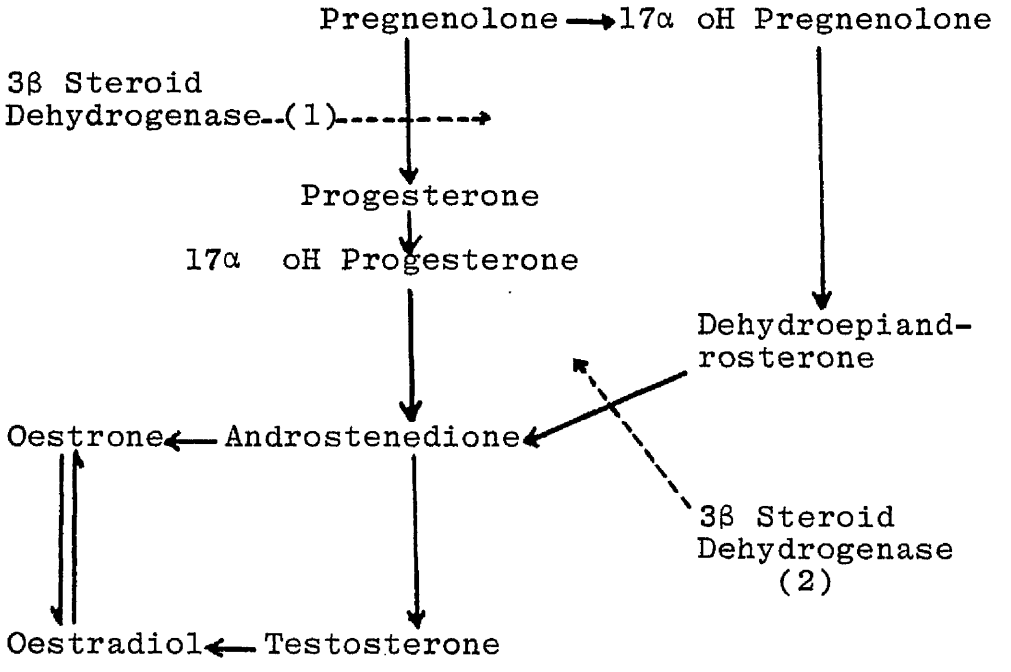
Control sections were incubated in the same mixture minus the specific substrate.

Interpretation of the results was difficult due to the presence of other reducing compounds in the tissue. False positive black deposits were common in the lipid bearing cells although they possessed no specific enzymes. These deposits were generally large and irregular. Another difficulty with this method was that the degree of staining and even the distribution varied with the time of incubation. Much of the confusion was eliminated by the use of control sections. An attempt at visual assessment was made but in view of the presence of large amounts of lipid in many of the structures in the ovary of the hypophysectomized animal this must be regarded as a rough and subjective estimate.

3β steroid dehydrogenase is a key enzyme in the sex steroid metabolic pathway, operating at two levels.

The method using DHA specifically tested for the action of 3β steroid dehydrogenase at point (2) in

the following diagram. This reaction also involved an isomerase enzyme in the tissues which converted the delta 5 compound DHA to the delta 4 compound Androstendione.



The site of action of 3β Steroid Dehydrogenase

FOLLICLE CLASSIFICATION AND ATRESIA

In order to make comparisons between the various groups of experimental animals it was necessary to provide some definitions of the structure and particularly the types of follicles and the changes they undergo. The following methods were adopted:

I. Classification of Follicles

For convenience of description a simple and efficient method of follicle classification (Mandl and Zuckerman, 1950, 1951a.b; Green, Mandl and Zuckerman, 1951) was used. It will be noted that the diameter of the oocyte increases very rapidly in the first four stages of follicle growth (Mandl and Zuckerman, 1951c), it then remains more or less stationary in size while the follicle continues to expand (Brambell, 1928).

Stage I - Oocytes covered by one layer of flattened or rounded granulosa cells. These follicles are seen mainly in the cortex just underneath the tunica albuginea. Each follicle consists of an oocyte varying in size from 10 to 20 μ in diameter and surrounded by a single layer of flattened or rounded pre-granulosa cells. The nucleus of the oocyte takes up most of the cell, being 8 to 12 μ in diameter. Chromatin is rather dense and the nucleolus, if present, cannot be defined. The pre-granulosa cells are spindle-shaped and have

little cytoplasm (Fig. 9)

Stage II - Oocytes covered by one layer of cubical granulosa cells. In these follicles the oocyte slightly increases in size, mainly by accumulation of cytoplasm. A nucleolus becomes apparent and the chromatin network is looser. The pre-granulosa cells change into a single layer of cubical granulosa cells. They have little cytoplasm at this stage but are epithelial in appearance (Fig. 10)

Stage III - Oocytes covered by two layers of cubical granulosa cells. The main changes in the succeeding stages of follicle growth are concerned with a progressive increase in the size of the oocyte and proliferation of the granulosa cells to form a two-layered mantle around the oocyte. By the time the granulosa cells have increased to two layers the oocyte has almost doubled in size. The increase is particularly in the cytoplasm which now shows an accumulation of ribonucleic acid and one or two tiny globules of lipid. The nucleus and the nucleolus are double in size. Granulosa cells are also increased in size.

Stage IV - Oocytes covered by three layers of cubical granulosa cells. At this stage three layers of granulosa cells have been produced. The oocyte is now three times its original size,

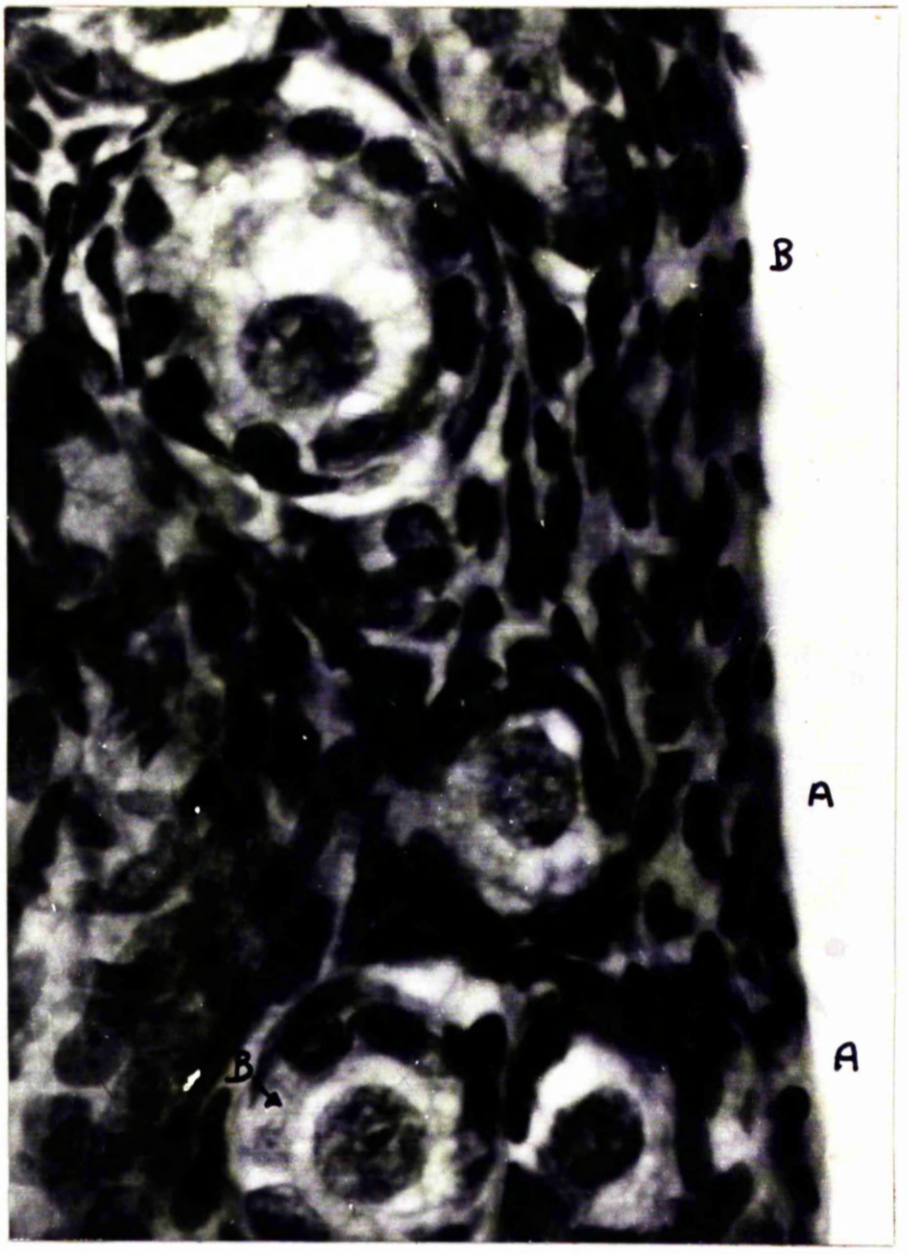


Fig. 9.

High power view of two normal Stage 1 (A) and two Stage 2 (B) follicles. In addition to the development of a granulosa layer, this shows the progressive enlargement of the oocyte in Stage 2 follicles. H. & E. X 400.

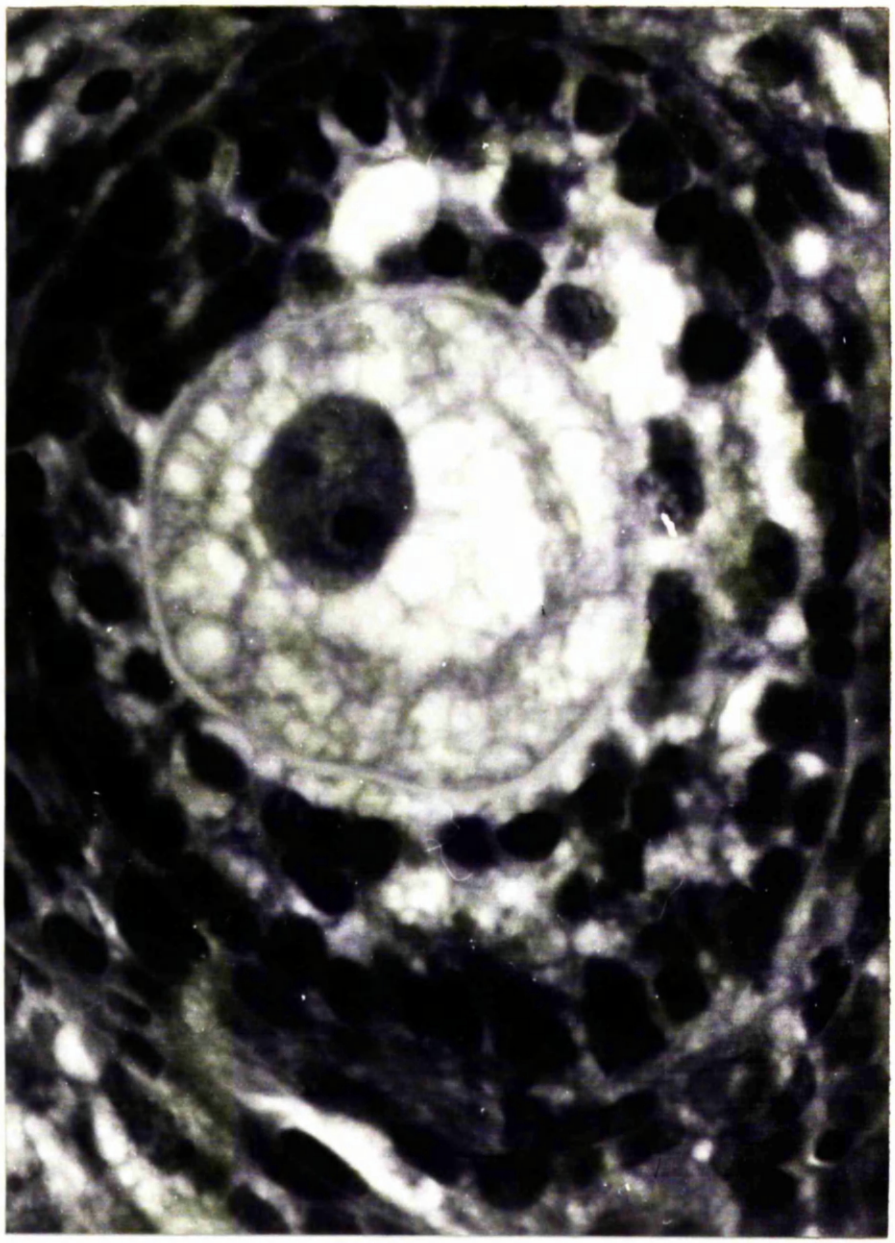


Fig. 10.

This is a normal relatively early pre-antral follicle with three layers of granulosa cells (Stage 4). H & E X 400.

again mainly due to cytoplasmic increase. The outermost layer of granulosa cells tends to have a pallisade arrangement with low columnar shape whereas the more internal cells are irregularly polygonal. Their nuclei are small and round. By this time the cytoplasm is more evident and gives a strong positive reaction for ribonucleic acid. There is no evidence of lipid in these cells and no reaction for 3β steroid dehydrogenase. The outermost layer of cells rests on the delicate basement membrane. At this stage the zona pellucida is beginning to form. Stromal cells around the follicle have no particular arrangement

Stage V - Oocytes covered by four or more layers of cubical granulosa cells but with antrum absent. At this stage the oocyte occupies a central position within the mass of granulosa cells. On average the oocyte reaches its maximum size when it is covered by four layers of granulosa cells and before an antrum appears (Mandl and Zuckerman, 1951). From the four layer granulosa stage onward the surrounding stromal cells become arranged in concentric layers, which increase in number to form a distinctive cell mantle around the follicle - the theca. The cells of this zone are initially plump spindles but with an increase in cytoplasm they become

polygonal and more epithelial in appearance. Fine lipid droplets develop in the cytoplasm of these cells and a positive reaction for 3β steroid dehydrogenase is obtained, especially in the polygonal cells. Small capillaries are present in this layer but they do not penetrate the basement membrane of the granulosa cells. The largest follicle of this type measured 0.2mm in diameter (Figs. 11 and 12)

Stage VI - Follicles with an antrum. This stage of development sees the appearance of two or three droplets of fluid among the granulosa cells to one side of the oocyte. Occasionally the granulosa cells bordering these droplets may show degenerative changes such as pyknosis of nuclei. In most instances however the cells are healthy and the increase in fluid does not appear to be accompanied by an undue increase in pressure. The droplets coalesce and the pool of fluid enlarges. The oocyte with an attendant mass of granulosa cells, the cumulus oophorus, is pushed to one side giving a signet-ring appearance to the follicle. The process of fluid accumulation and proliferation of cells continues. An arbitrary size was chosen to classify these follicles. The antral follicles were divided into three groups: small - 0.2mm, medium - 0.3mm and large - 0.4mm and over. The

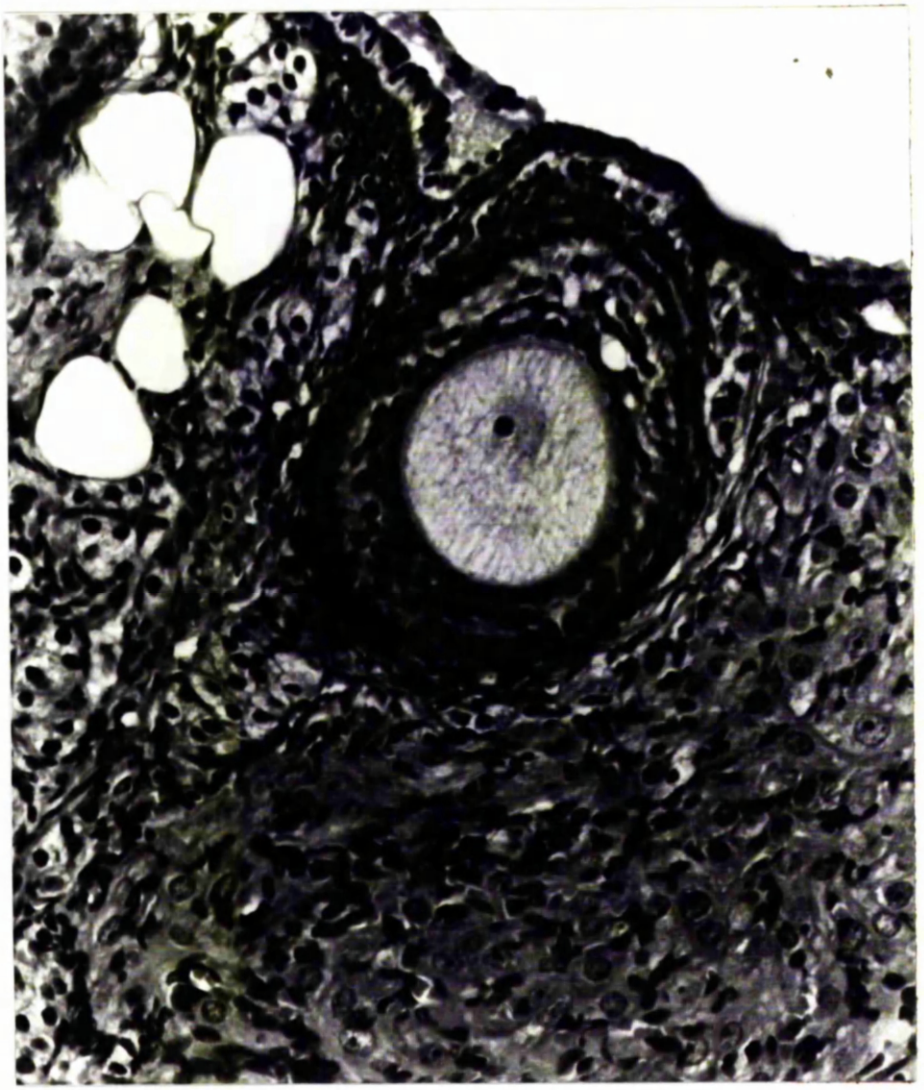


Fig. 11.

A Stage 5 pre-antral follicle with four layers of granulosa cells. H. & E. X 312.

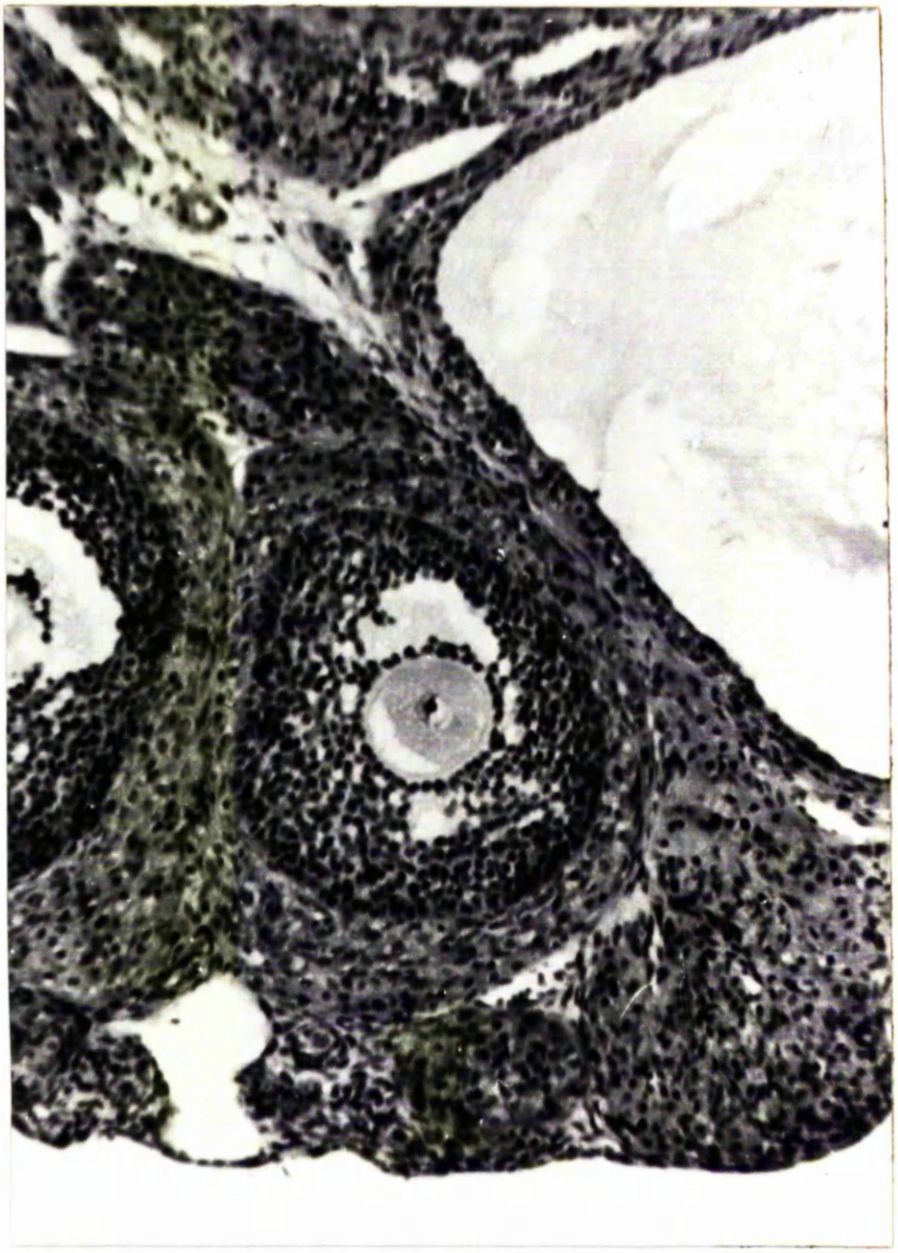


Fig. 12

Low power view of an early antral follicle. A zona pellucida has formed and the oocyte has reached its maximum size. H. & E. X 312.

granulosa cells of medium sized follicles form a layer of 8-10 cells thick in the wall of the follicle away from cumulus oophorus. The thecal cells of these follicles are arranged in three or four layers. Large antral follicles represent pre-ovulating follicles. The granulosa cells of these large antral follicles form six to eight layers deep but on one side of the follicle this may be reduced to three or four. The thecal cells are epithelial in appearance and are of the theca lutein type (Figs.13 and 14).

II. Follicular Atresia

Follicular atresia is the process by which oocytes are lost from the ovary other than by ovulation. Atresia is normally recognised on morphological grounds owing to changes in the oocyte and follicle around it.

Atresia can affect all stages of follicular growth from primordial to mature graafian, although atresia is most commonly observed in follicles with antra (Ingram, 1962).

Atresia is responsible for the elimination of most of the germ cells and regulates the number (and possibly the quality) of the oocytes which are ovulated (Baker and Wai Sum O, 1976).

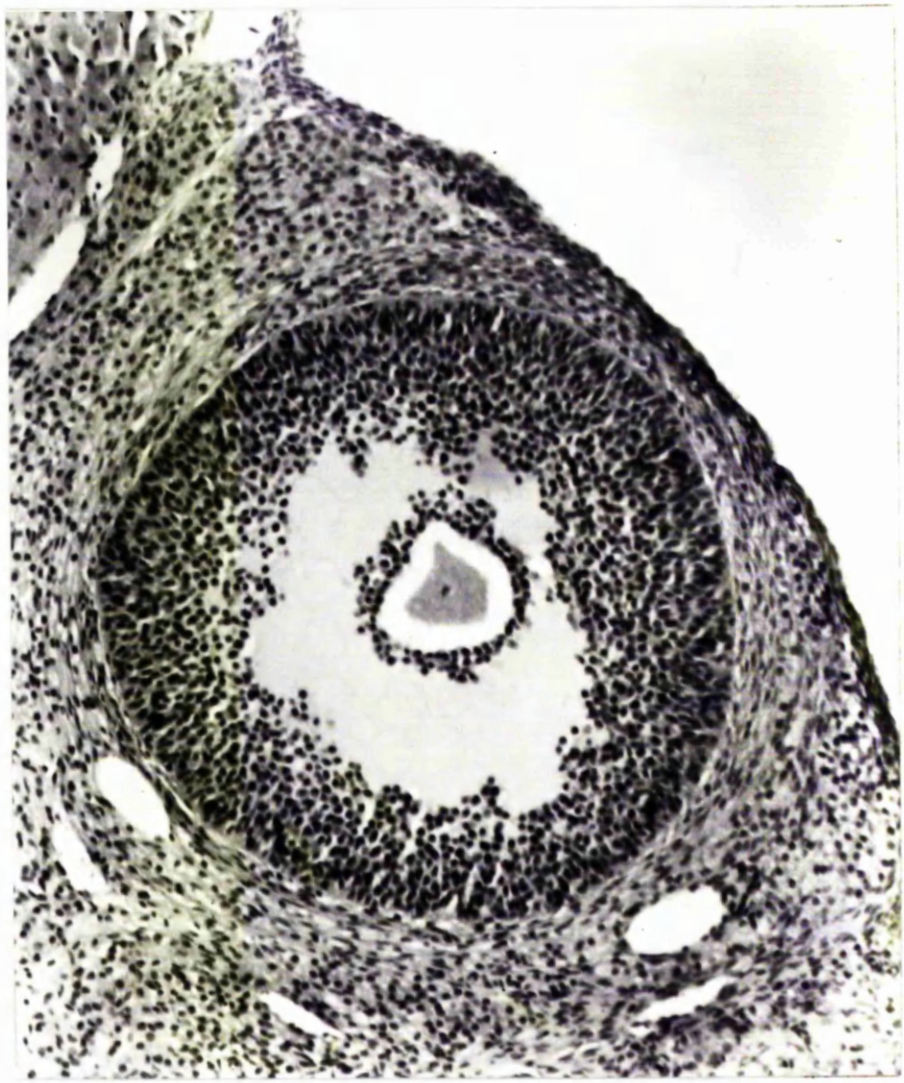


Fig. 13

A medium sized antral follicle. The thecal layer is well formed but the cells are still spindle-shaped and not as yet luteinized. H. & E. X 312.

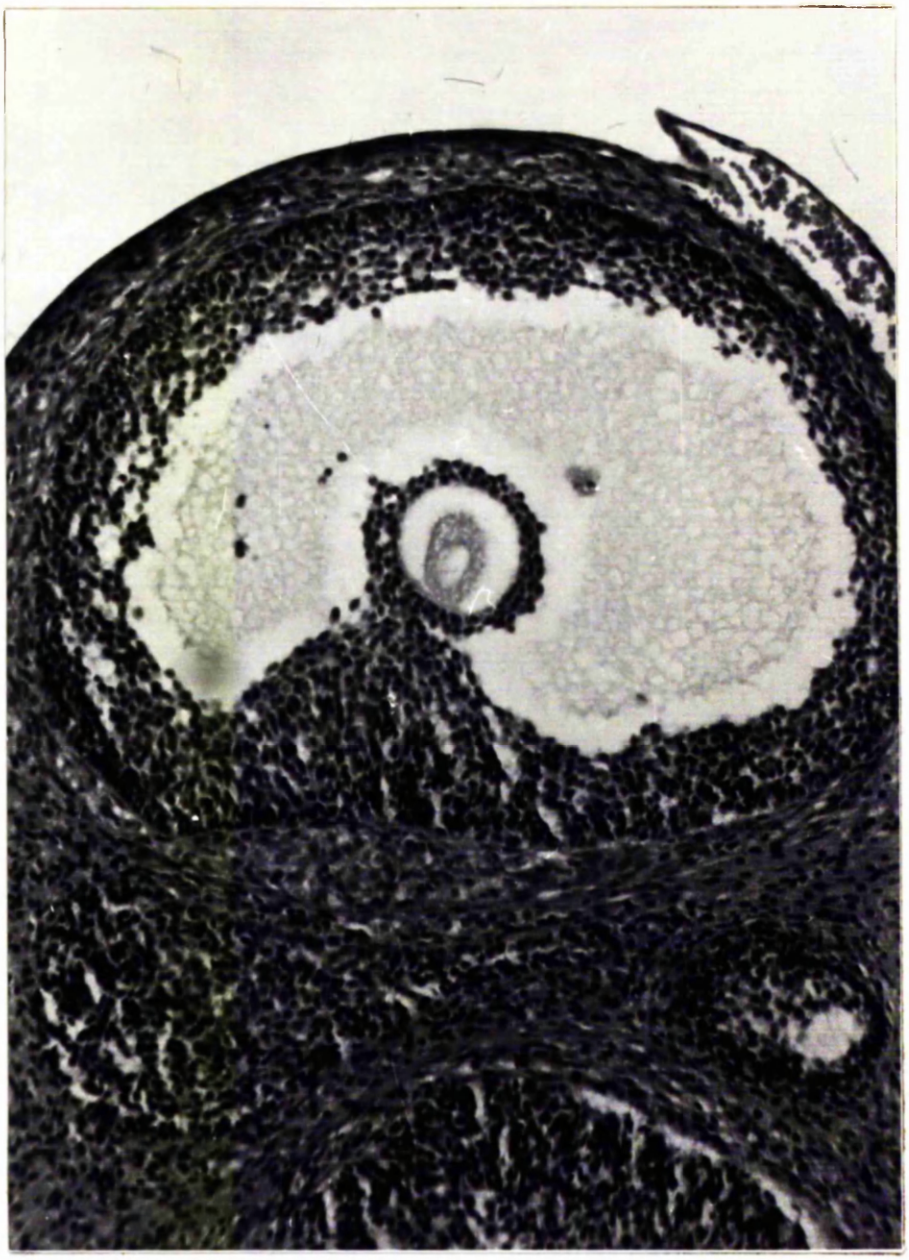


Fig. 14

Antral follicle with ten layers of granulosa cells on wall opposite the cumulus. The thecal cells are becoming cuboidal and luteinized. H. & E. X 300.

Atresia in Antral Follicles: The granulosa cells shrink, lose cytoplasm and their nuclei become smaller and darker. Tiny dark granules appear among the cells. These are fragments of chromatin (Matthews, 1941). The granulosa cells soon acquire lipid in their cytoplasm in large globules (Deane and Andrews, 1953). The granulosa cells loosen and some float into the antral fluid where they undergo further degeneration producing amorphous debris (Allen et al, 1930). A few leucocytes may appear among the cells and in the antral fluid (Stockard and Papanicolaou, 1917). The basement membrane of the granulosa cells thickens and is much more prominent. The thecal cells become swollen and their cytoplasm is filled with coarse emulsion of lipid (Brambell, 1956).

Ultimately the atretic follicle consists of a tiny space approximately the size of a primordial follicle surrounded by three or four layers of swollen fatty thecal cells with tiny pyknotic nuclei. Care has to be taken to distinguish these degenerate thecal cells from interstitial cells.

A large antral follicle may be converted into a cyst lined with a single layer of flattened or low cubical epithelial cells. The antral fluid

usually contains amorphous debris and a small number of leucocytes. Thecal cell layers are seldom present (Fig.15).

Atresia in Small Pre-Antral Follicles: Oocytes of follicles in Stages 1, 2 and 3 become distorted mainly by the crinkled appearance of the nuclear membrane (Ingram, 1962). The cytoplasm is deeply eosinophilic, while the nucleus becomes irregular in shape (Kramer et al., 1933) and dark and without internal structure (Stockard and Papanicolaou, 1917). The cytoplasm can sometimes become vacuolated and occasionally one or two mononuclear cells, possibly phagocytic (Clark, 1923), appear. The granulosa cells shrink and their nuclei become pyknotic and fragmentary. Ultimately the follicle disappears without trace.

Atresia in Large Pre-Antral Follicles: Follicles in Stages 4 and 5 show minor difference in the atretic process due to the presence of zona pellucida. The oocytes degenerate and then disappear leaving a tiny empty distorted cyst surrounded by the persisting zona (Harrison, 1948). The large pre-antral follicles with theca show a rather degenerate granulosa cell layer but a very prominent thick theca (Zuckerman and Parkes, 1932) (Fig. 16). Fig. 17 demonstrates many features of early atresia in large pre-antral follicles.

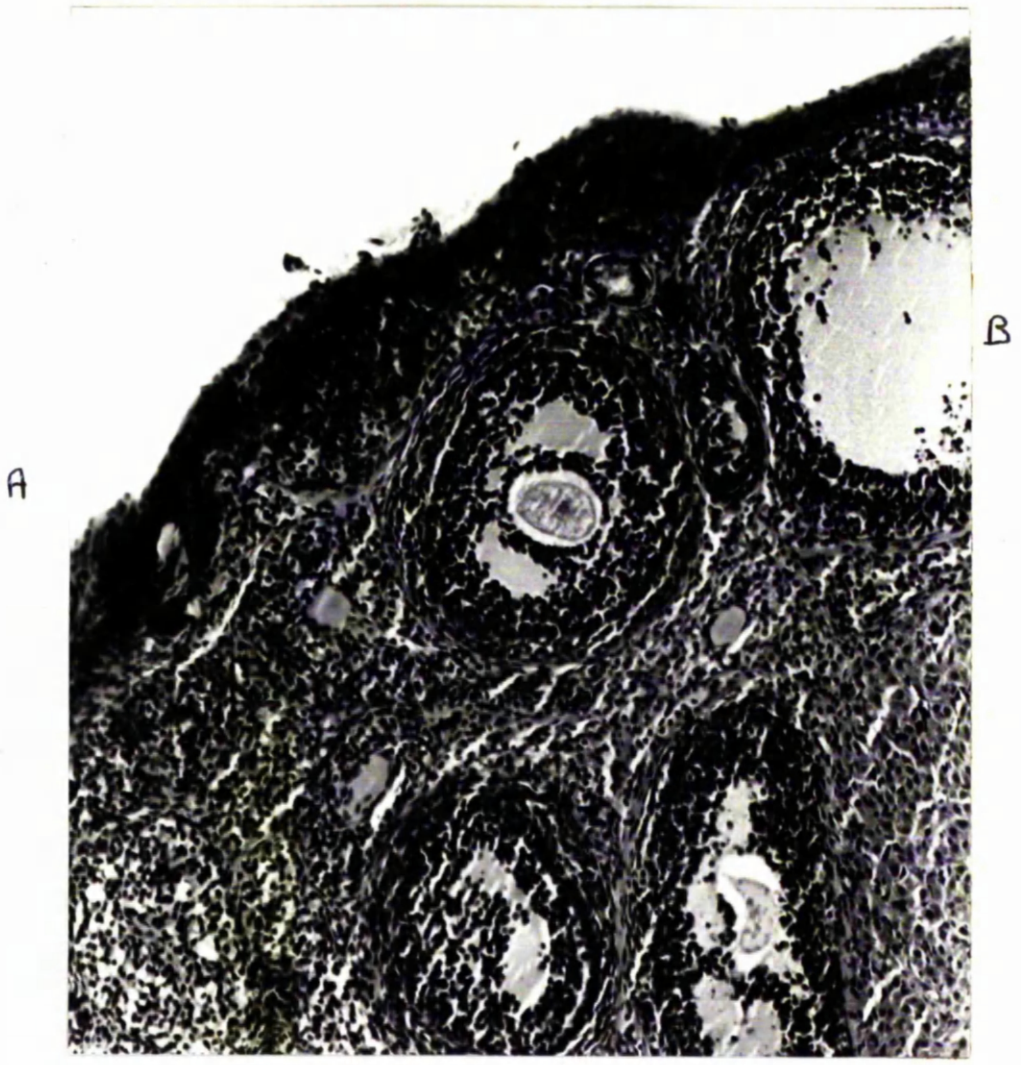


Fig. 15

This section shows a small antral follicle in the centre of the illustration (A) and three other antral follicles undergoing atresia. The follicle in the top right hand corner of the illustration (B) is fairly advanced in atresia; granulosa cells have desquamated and free granules of chromatin are present. H. & E. X 130.

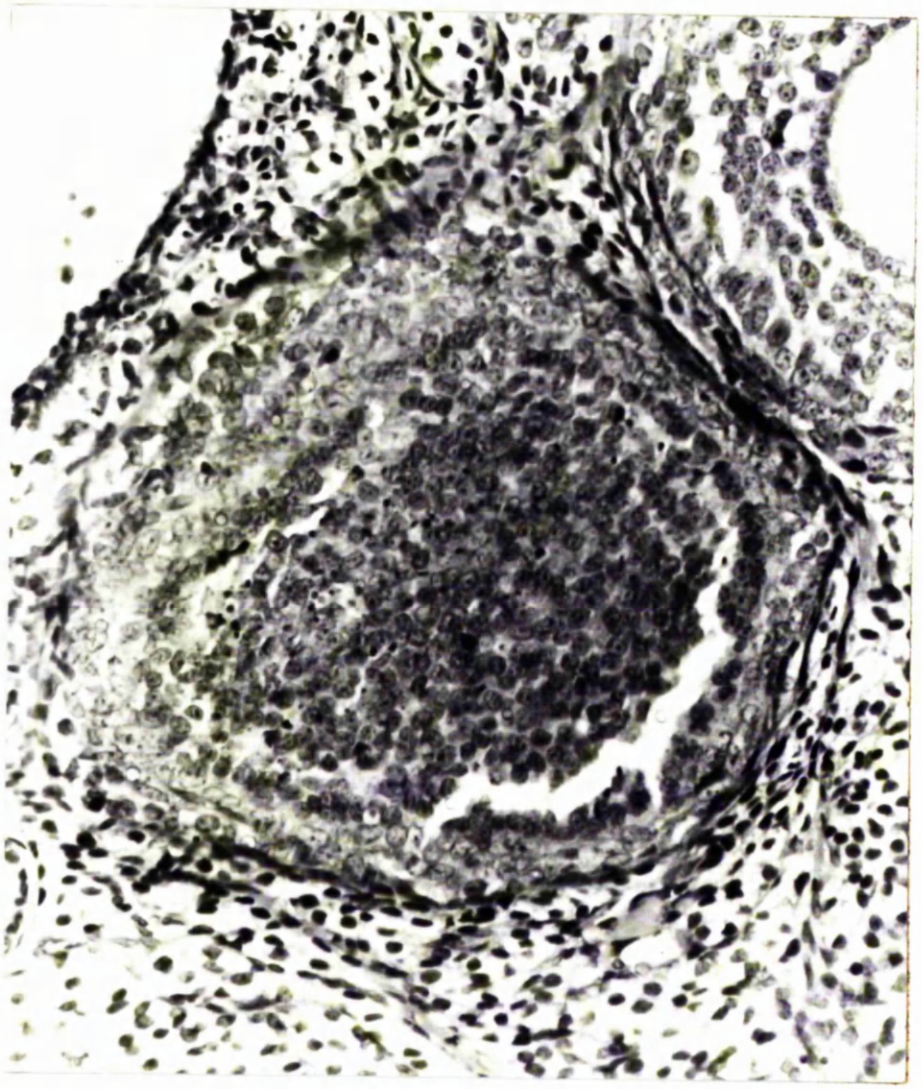


Fig. 16

This is an illustration of a large pre-antral follicle undergoing atresia, cut obliquely. It shows chromatin granules in the granulosa and a very prominent thecal layer. H. & E. X 312.

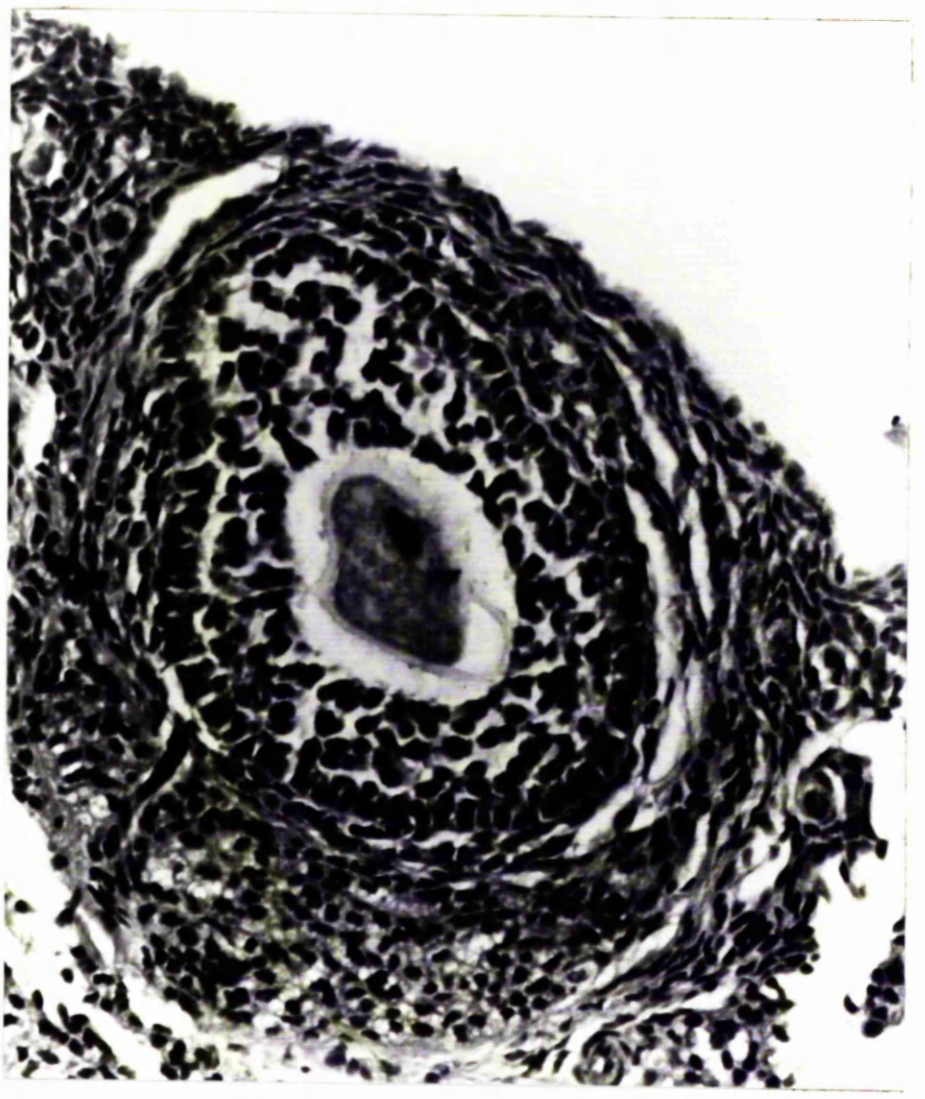


Fig. 17

Early atresia in a large pre-antral follicle. The oocyte nucleus is pyknotic, the zona pellucida is distorted. Granulosa cell nuclei vary in size and shape. H. & E. X 312.

FOLLICLE COUNTS

Counts of follicles in complete ovaries were made in the following manner:

1. Each ovary, after fixation and paraffin embedding, was serially sectioned, the sections being cut at a thickness of 7 μ.
2. All sections were mounted and stained by haematoxylin and eosin.
3. The oocytes were classified in developmental stages according to Mandl and Zuckerman, 1951.
4. Each follicle beyond Stage 2 was counted.
5. In order to avoid counting the same follicle twice the follicle had to contain not only a nucleated oocyte but the section had to pass through the nucleolus at its maximum diameter. This procedure made it unnecessary to apply a correction factor to the counts for these multi-layered follicles.

One ovary from each experimental animal was available for this purpose. Counts were carried out in the following groups:

1. Hypophysectomized animals killed seven days after operation.
2. Hypophysectomized animals given various doses of FSH and killed at intervals thereafter as specified in the design of experiment.

3. Hypophysectomized animals given various doses of LH and treated as in 2.

4. Hypophysectomized animals given a constant dose of FSH plus various doses of LH and treated as in 2.

STATISTICAL ANALYSIS OF FOLLICLE COUNTS

In the experimental groups two animals were used for each dose of hormone, e.g. 25 µg, 50 µg, 75 µg and 100 µg FSH, and each time interval, i.e. 1 hour, 6 hours, 12 hours, 24 hours and 48 hours. Follicle counts were carried out on one ovary from each animal at each dose level and time interval. Two ovaries were therefore used at each point. The follicle counts from these two ovaries were added together, and divided by two to provide an average figure.

Following the construction of graphs it was obvious that an increase in the follicle count with any particular hormone or mixture of hormones commenced at the 6 hour interval and reached a maximum at the 24 hour point. It was impossible, because of the number of specimens, to analyse statistically the values for each time interval. The following procedure was adopted. In order to compare the activity of different doses and different hormones the counts at 6, 12 and 24 hours were summated for each dose level. Means and standard deviations were derived from the individual values.

Follicle counts were carried out on animals killed on days 5, 6, 7 and 8 following hypophysectomy. Two animals were killed each day and one ovary from each animal was used for counting. A total of eight ovaries were analysed in this way. The counts were summated and a mean value and standard deviation calculated.

The mean values for each hormone injected group were statistically compared with the value obtained for the post-hypophysectomy group.

RESULTS

CYCLICAL CHANGES IN THE INTACT MATURE FEMALE RAT

The following is a simplified description of the cyclical changes observed in the ovary:

At oestrus large antral follicles were present, the granulosa cells of which appeared small in size and rounded in shape with a small pyknotic nucleus and very little cytoplasm. The nuclear membranes appeared wrinkled and nucleoli were not apparent. Cytoplasm varied in amount, many cells having little. Special stains showed that these cells lacked RNA and contained large amounts of lipid material. Staining for 3β steroid dehydrogenase gave a false reaction as positive staining was seen in the control sections. Sometimes small fragments of chromatin appeared in the place of the nucleus (apoptosis).

The theca cells of these large antral follicles were very much swollen with large vesicular nuclei and vacuolated cytoplasm. Special stains showed that these cells were deficient in RNA but rich in large globules of lipid, while the 3β steroid dehydrogenase reaction was falsely positive. The antra of these follicles contained a considerable amount of debris. At the same time, the ovary contained healthy small antral follicles, lined by cubical granulosa cells which showed mitotic activity. The associated theca

cells were plump and spindle shaped with finely vacuolated cytoplasm. Special stains showed that these follicles were rich in RNA both in the granulosa and theca layers. Fine lipid granules were present in the theca, the cells of which gave a positive reaction for 3β steroid dehydrogenase.

The interstitial tissue consisted of small polygonal cells with rounded nuclei and basophilic cytoplasm. Special stains showed that it was rich in RNA and contained fine lipid granules. A slight positive reaction for 3β steroid dehydrogenase was obtained.

Twelve hours later there was a mixture of medium and small antral follicles as well as pre-antral follicles. In the medium sized follicles the granulosa cells showed little sign of mitosis. The cells however were normal and showed no sign of degeneration. Thecal cells showed large swollen nuclei and vacuolated cytoplasm. Special stains showed that these follicles were rich in RNA but the granulosa cells may have contained fine lipid granules. Thecal cells may have contained large lipid granules. They still showed a positive reaction for 3β steroid dehydrogenase. The small antral follicles were rich in RNA and contained no lipid granules. Pre-antral follicles were compact structures with no thecal reaction and the granulosa cells were very rich in RNA and contained no lipid.

The interstitial tissue cells showed no change. Twenty four hours after oestrus the number of small antral follicles and pre-antral follicles were increased whereas medium sized follicles were less frequent. The large degenerate follicles noted at oestrus showed more advanced changes and some were in process of disappearing. There was a reduction in the size of the nuclei of the interstitial cells. Their cytoplasm, which was abundant, contained large lipid granules. Thirty hours after oestrus there was a mixture of atretic and growing medium sized follicles but the number of healthy follicles out numbered the atretic ones. There were only small numbers of small pre-antral follicles. Special stains showed that the degenerating follicles contained very little RNA and the theca cells were loaded with coarse lipid granules. Growing follicles were as usual rich in RNA and no lipid could be found in the granulosa layer. Even in the thecal layer it was scanty and in the form of very fine granules. The interstitial tissue cells now had a spindle-shaped nucleus with little cytoplasm. Such cytoplasm as was present looked pale and had lost its basophilic stain. Special stains showed that these cells contained little RNA. Fine droplets of lipid were present and a slight positive reaction for 3β steroid dehydrogenase was present. Thirty six hours after oestrus there was much more activity and the number

of medium and small antral and pre-antral follicles was much increased. One or two atretic medium sized antral follicles were present. The interstitial tissue cells were larger and contained slightly more RNA.

The picture in the ovary remained the same for the following twenty four hours but some pre-antral follicles with 2-3 layers of granulosa cells and an apparent zona pellucida had a thick thecal layer. The thecal cells possessed abundant vacuolated cytoplasm with little RNA but abundant coarse lipid granules. The reaction for 3β steroid dehydrogenase was falsely positive. Sixty hours after oestrus the follicles were mostly of small pre-antral and small antral type. There was no evidence of medium sized or large antral follicles. The interstitial cells contained very little RNA but numerous coarse lipid granules. Sixty six hours after oestrus medium sized antral follicles had again appeared. The granulosa cells of these follicles showed mitotic activity. The surrounding thecal cells had large nuclei and abundant cytoplasm. Both the theca and granulosa cells of these follicles were rich in RNA. The cytoplasm of the thecal cells contained fine lipid granules. More RNA and less lipid were present in the interstitial cells which had a less vacuolated cytoplasm. Small antral and pre-antral follicles

were also present at this stage. Seventy two hours after oestrus it was very similar to that at sixty six hours but more pre-antral follicles showing active mitosis in the granulosa layer were seen. The interstitial tissue cells were now basophilic and contained more RNA and less lipid. Eighty four hours and ninety hours after oestrus the follicular population consisted of large and medium sized antral types and a few of the pre-antral variety. In almost all of the large antral follicles there was an absence of mitosis in the granulosa layer. Many of the cells had little cytoplasm and indeed some showed apoptosis. The thecal cells of these follicles had large swollen nuclei and abundant vacuolated cytoplasm rich in RNA and contained fine lipid granules. These cells were now epithelialized; in the usual terminology, luteinized. The interstitial tissue cells had abundant basophilic cytoplasm, rich in RNA but with little lipid (Fig.18).

At the end of oestrus an average of five to six fresh corpora lutea could be seen on the outer surface of each ovary as pale rounded bodies. On cross section these bodies were surrounded by compressed stromal tissue. The corpus luteum contained large cells with abundant pale basophilic finely vacuolated cytoplasm, with a large rounded vesicular nucleus and prominent nucleoli (Figs. 19 and 20). Very

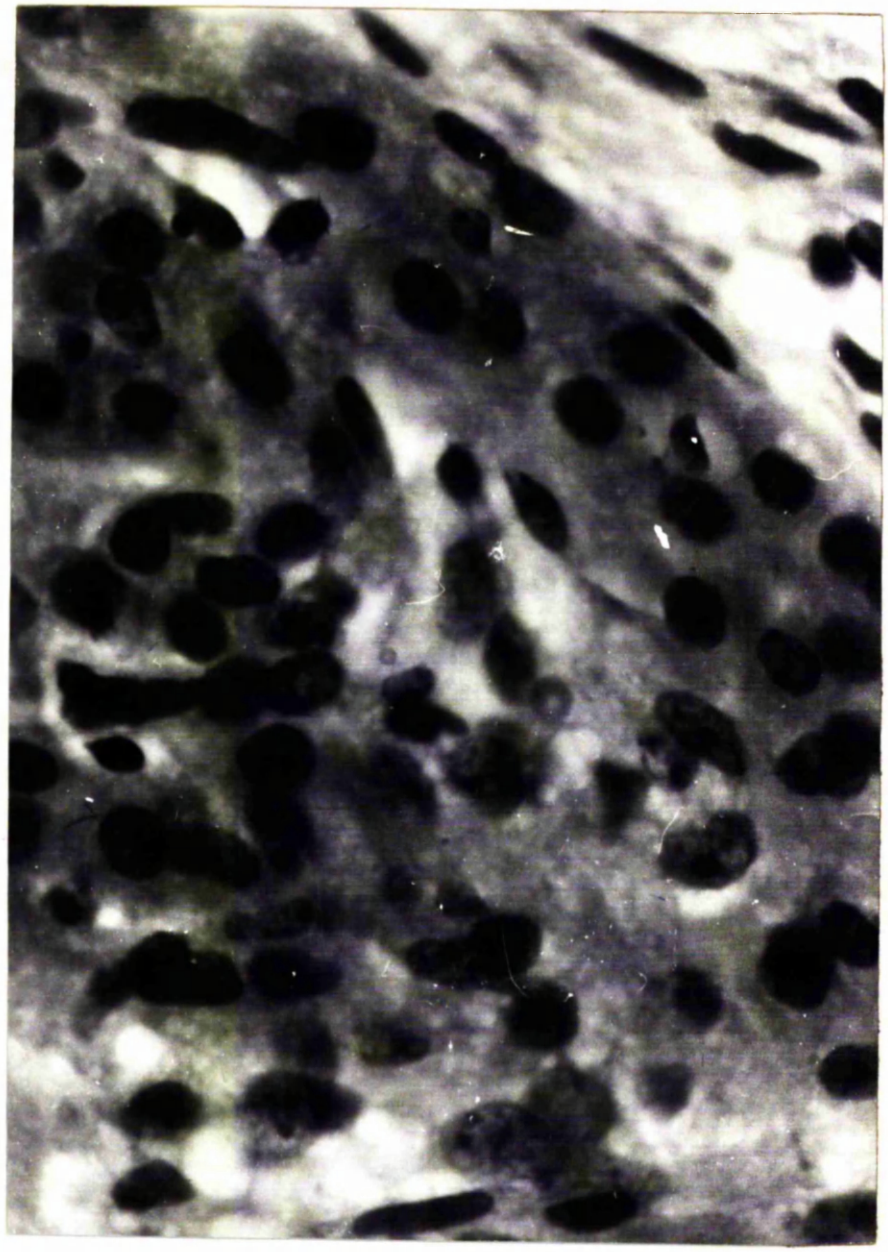


Fig. 18

High power view of normal interstitial cells. The cytoplasm is plentiful and basophilic. Nuclei are relatively large, round or oval with dispersed chromatin. H. & E. X 600.

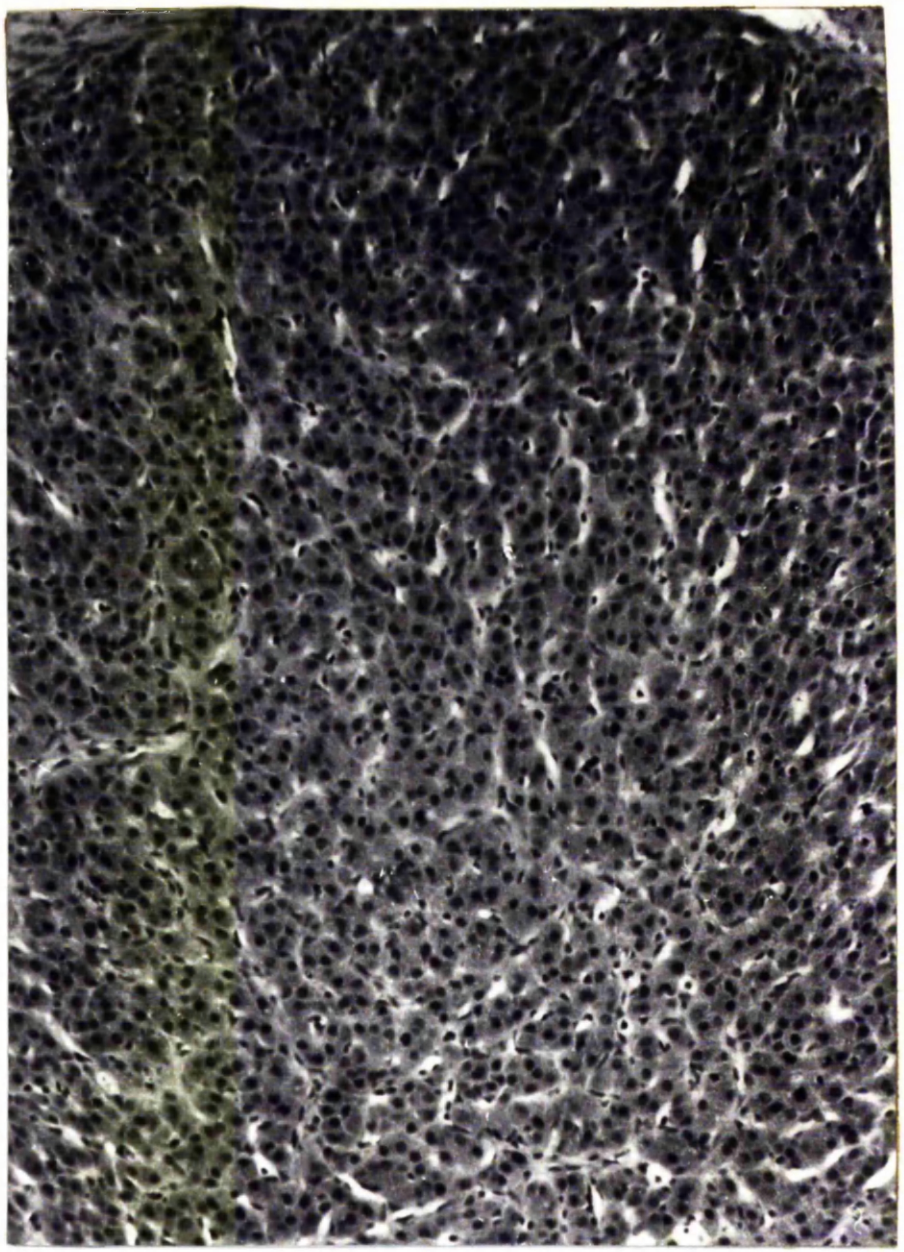


Fig. 19

Normal early corpus luteum. The cells are large with abundant cytoplasm which is basophilic. H. & E. X 150.

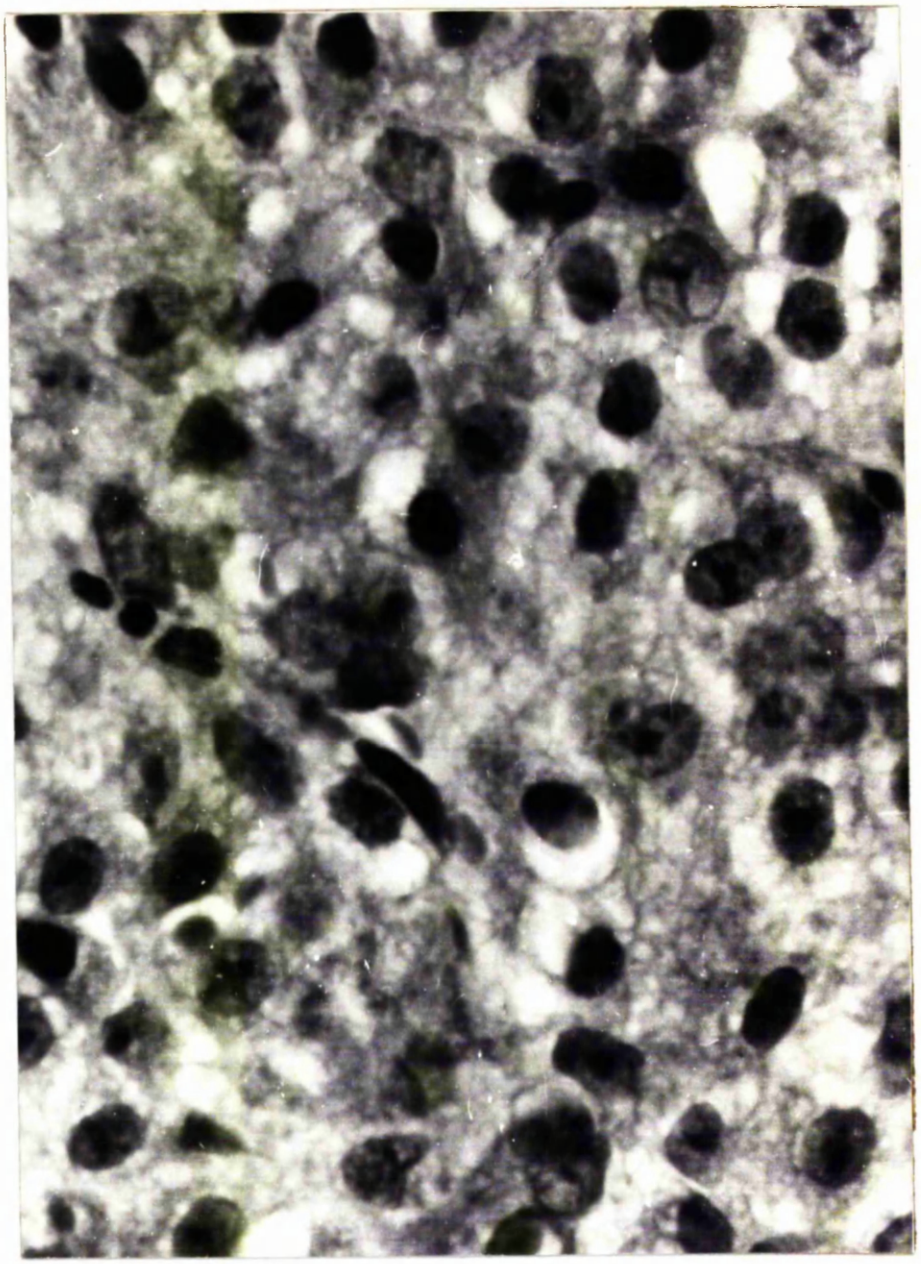


Fig. 20

High power view of fully functioning corpus luteum. The cells are large with abundant luteinized cytoplasm. Special stains reveal a high content of cytoplasmic RNA and a fine emulsion of lipid. H. & E. X 600.

few mitotic figures could be seen. The cells were supported by a fine reticular network with large sinusoidal capillaries. They were rich in RNA as evidenced by strongly positive PMG stain and contained fine lipid droplets. A very strong positive reaction was obtained in tests for 3β steroid dehydrogenase (Fig. 21).

No histological change was noticed in corpus luteum cells until thirty six hours after oestrus. The cytoplasm now contained slightly less RNA and more fine lipid granules. Their nuclei were possibly slightly smaller. Sixty hours after oestrus the luteal cells were smaller and the cytoplasm less in amount and more vacuolated. RNA was much reduced and coarse lipid globules appeared in the cytoplasm. 3β steroid dehydrogenase was difficult to assess. Between sixty six and seventy eight hours after oestrus, the luteal cells had become much smaller and the intracellular lipid granules were slightly reduced. The cells of the supporting reticulum were now prominent and had more the appearance of fibroblasts and the reticular fibres had become collagenous. Eighty four to ninety hours after oestrus there was a definite degeneration of luteal cells. They were very much smaller and irregular in shape, with nuclei of varying size, some of them pyknotic (Figs. 22 and 23). The phenomenon

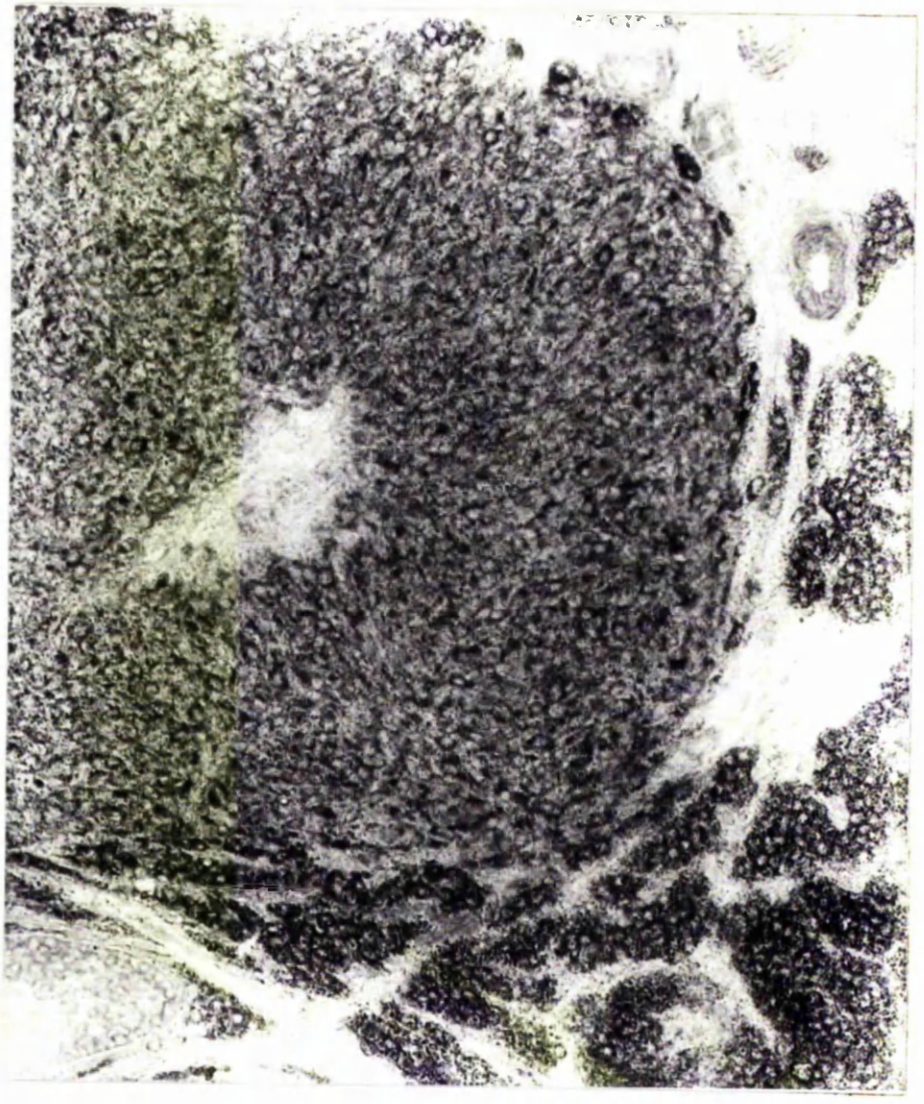


Fig. 21

This shows a positive reaction for 3β steroid dehydrogenase in an active corpus luteum and related interstitial tissue. X 130.

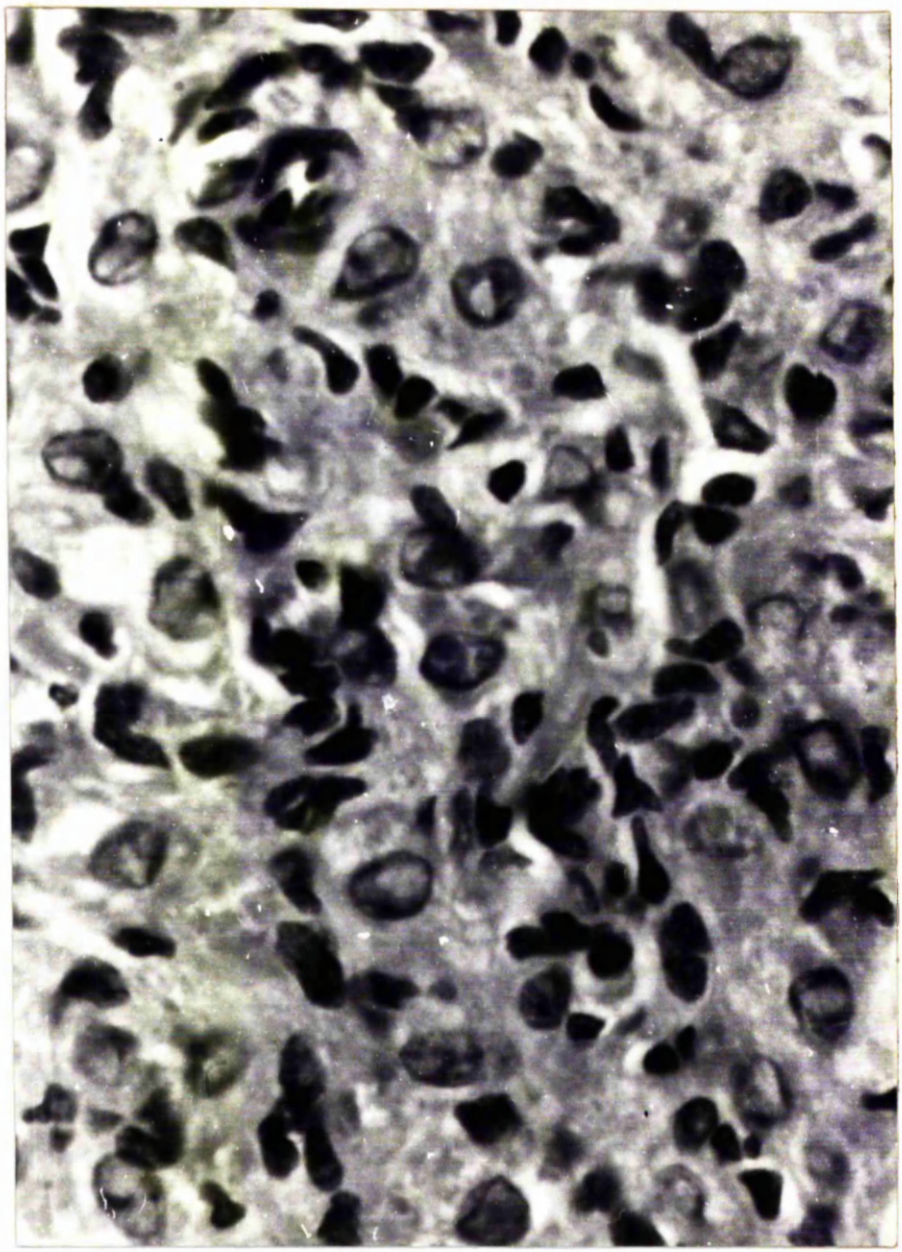


Fig. 22

High power view of degenerative corpus luteum (oestrus + 90 hours). The cells are small with reduced cytoplasm. Some show nuclear pyknosis. Numerous stromal nuclei are present. H. & E. X 600.

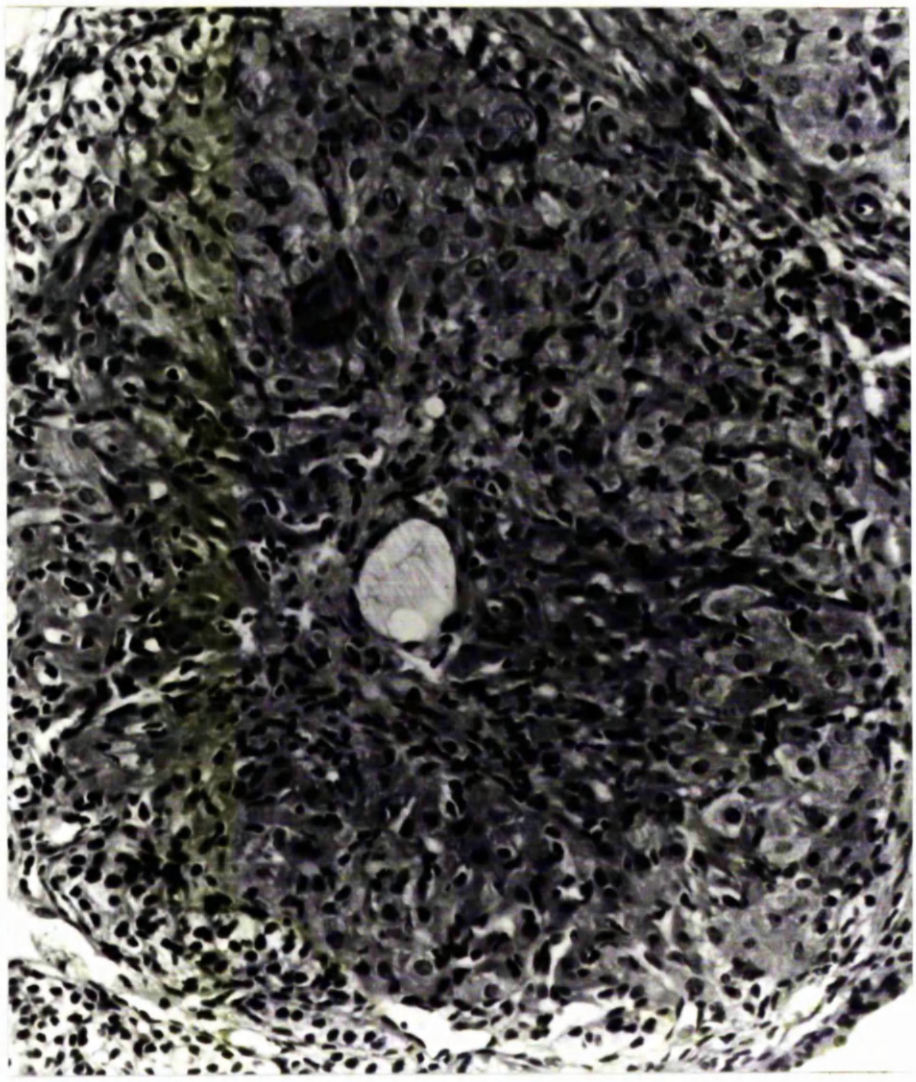


Fig. 23

Illustration showing corpus luteum 84 hours after oestrus. There is a small central space surrounded by fibrous tissue. The luteal cells are in general smaller and vary in size and shape. Spindle-shaped fibroblast nuclei are plentiful. H. & E. X 312.

of apoptosis did not seem to occur in luteal cells. The number of fibroblasts was much increased, and the corpora lutea as a whole shrunken by the effect of degeneration of the luteal cells and contracture of fibrous tissue.

The functional results of these cyclical ovarian changes were seen in the uterine horns and vagina of the animal. As the animal approached oestrus the uterine horns and vaginal tissue became more vascular. A progressive enlargement of the uterine horns took place. Following ovulation and corpus luteum formation the horns became greatly swollen, and transluscent due to distension with fluid (Fig.24).

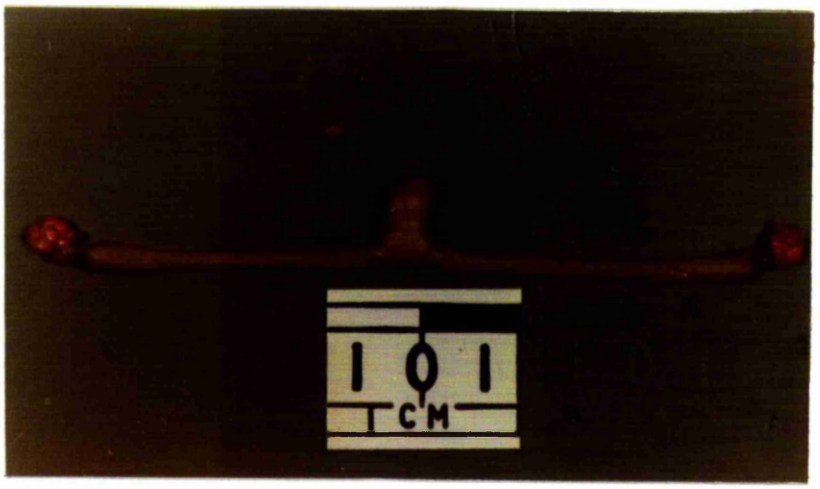


Fig. 24

Uterus and ovaries removed from normal rat killed 12 hours after oestrus. The ovaries are large and contain corpora lutea and the uterine horns are distended.

EFFECT OF HYPOPHYSECTOMY ON THE MATURE FEMALE RAT

EFFECT OF HYPOPHYSECTOMY ON TOTAL BODY WEIGHT

Hypophysectomy caused immediate cessation of any body growth and, apart from a temporary drop in the body weight immediately after the operation which can be explained by the effect of the anaesthetic and the immediate effect of hypophysectomy on carbohydrate metabolism, the animal will never reach the weight of the intact control and, if not well looked after, will never reach its weight at the time of operation.

It is known that a normal rat will gain an average of 4 grams per week (Donaldson, 1924) but the hypophysectomized animals failed to gain such weight and in fact they lost an average of 6 grams per week. As the animals in this experiment were well fed they gradually recovered some of this loss (Fig. 25 and Appendix II).

THE EFFECT OF HYPOPHYSECTOMY ON THE OVARIAN AND UTERINE WEIGHTS

Both ovaries and the uterus of the hypophysectomized rat showed a progressive loss of weight following the operation. The graph in Fig. 26 shows the very steep fall in the net weight of the ovaries during the first seven days following the operation. Thereafter the graph line is much less steep and by the twentieth day the line has levelled out and the ovarian weight remained constant over the next eight

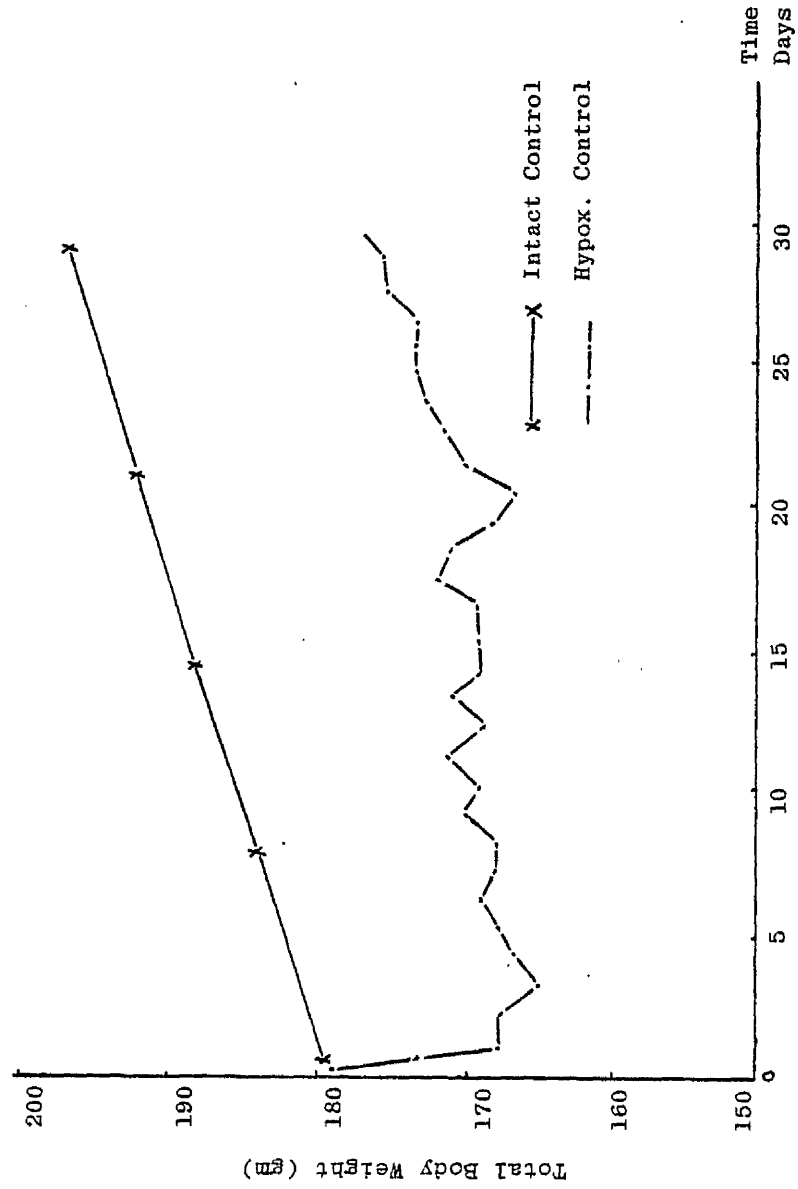


Fig. 25

Effect of hypophysectomy on the average total body weight of two hypophysectomized mature female rats at each time interval.

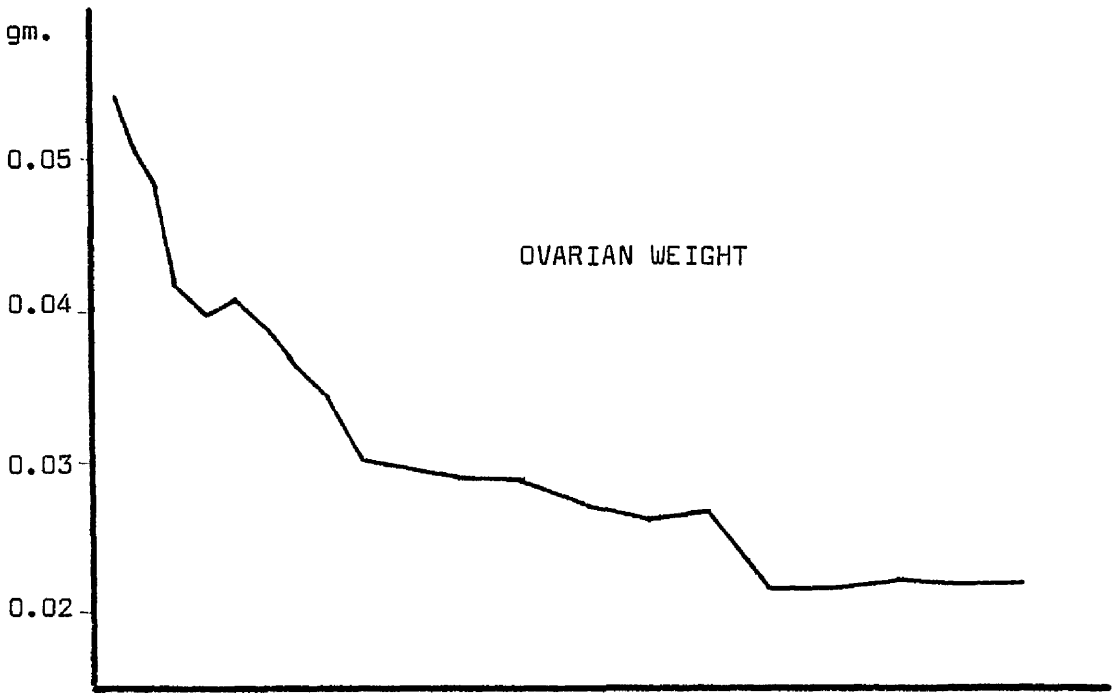


Fig. 26 This shows the changesin ovarian weight in hypophysectomized animals with the passage of time.

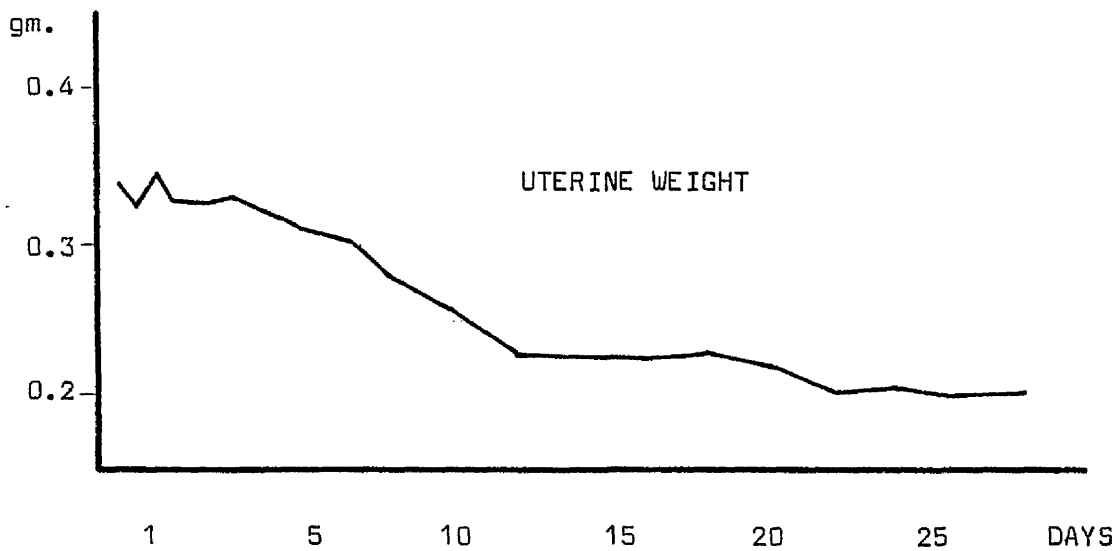


Fig. 27 This shows the changes in the uterine weight in hypophysectomized animals with the passage of time.

days. Comparison with the intact animals* showed that this fall in weight was significant in all cases (Tables 4 and 5). Forty eight hours after hypophysectomy the ovary had lost 25% of its weight and 10-12 days after hypophysectomy almost 50% of its weight. *See Appendix I.

In the case of the wet weight of the uterus of the hypophysectomized rat (Fig. 27) the changes were less dramatic but it can be seen that the steep fall in weight took place after the seventh day post-hypophysectomy when the ovarian changes were virtually maximal. Statistical analysis showed that the changes in the uterine weight were significant (Tables 6 and 7). Appendix II.

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.0581	0.0003
Hypox. Control			
Day 1	10	0.0539	0.0029
Day 2	8	0.0420	0.0021
Day 3	8	0.0391	0.0016
Day 4	8	0.0388	0.0006
Day 5 -8	8	0.0327	0.0029
Day 9-12	8	0.0296	0.0022
Day 13-16	8	0.0274	0.0011
Day 17-20	8	0.0251	0.0024
Day 21-24	8	0.0225	0.0015
Day 25-28	8	0.0223	0.0008

Table 4

The mean and standard deviation of the ovarian weight of Hypophysectomized Controls from day 1 to day 28 following operation compared with Intact Controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Intact Control Day 1 vs. Hypox. Control			
Day 1	18	4.3218	$P \leq 0.001$
Day 2	16	22.5715	$P \leq 0.001$
Day 3	16	43.4206	$P \leq 0.001$
Day 4	16	83.7105	$P \leq 0.001$
Day 5 -8	16	25.9402	$P \leq 0.001$
Day 9-12	16	60.9264	$P \leq 0.001$
Day 13-16	16	150.1074	$P \leq 0.001$
Day 17-20	16	40.6000	$P \leq 0.001$
Day 21-24	16	69.0538	$P \leq 0.001$
Day 25-28	16	123.0420	$P \leq 0.001$

Table 5

Comparison of the ovarian weights of the hypophysectomized control group (Day 1-28 after operation) with the intact control group (Day 1) using t. test.

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.3802	0.0081
Hypox. Control			
Day 1	10	0.3345	0.0042
Day 2	8	0.3240	0.0073
Day 3	8	0.3235	0.0066
Day 4	8	0.3138	0.0079
Day 5- 8	8	0.2951	0.0149
Day 9-12	8	0.2491	0.0172
Day 13-16	8	0.2258	0.0053
Day 17-20	8	0.2276	0.0077
Day 21-24	8	0.2027	0.0034
Day 25-28	8	0.2030	0.0049

Table 6

The mean and standard deviation of the uterine weight of Hypophysectomized Controls from day 1 to day 28 following operation compared with Intact Controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Intact Control Day 1 vs. Hypox. Control			
Day 1	18	15.0261	$P \leq 0.001$
Day 2	16	14.4048	$P \leq 0.001$
Day 3	16	11.7135	$P \leq 0.001$
Day 4	16	15.0855	$P \leq 0.001$
Day 5- 8	16	14.5513	$P \leq 0.001$
Day 9-12	16	20.1079	$P \leq 0.001$
Day 13-16	16	43.8705	$P \leq 0.001$
Day 17-20	16	38.2740	$P \leq 0.001$
Day 21-24	16	54.7076	$P \leq 0.001$
Day 25-28	16	51.3084	$P \leq 0.001$

Table 7

Comparison of the uterine weights of the hypophysectomized control group (Day 1-28 after operation) with the intact control group (Day 1) using t. test.

HISTOLOGICAL CHANGES IN RAT OVARIES FOLLOWING HYPOPHYSECTOMY

The results from sham-operated animals and intact animals were indistinguishable. (Appendix I)

It was found that the injection of sodium phosphate buffer, pH 8.0 on the seventh day after hypophysectomy had no effect on the histological appearance of the ovary or on the ovarian or uterine weight of hypophysectomized rats.

Following hypophysectomy there was a short initial period when the ovaries appeared to be unaffected but subsequently a progressive structural breakdown in all of the specialised tissues took place. This degenerative change followed a regular pattern with an orderly sequence of events.

During the first twelve hours after hypophysectomy in the oestrous rat no apparent change occurred. Primordial follicles were present but were relatively inconspicuous. Several Stage 2 follicles were present. Many pre-antral follicles could be seen. Early, medium and large antral follicles were present in small numbers but not more than two or three of the latter. All were structurally intact. The granulosa cells gave a strong reaction for ribonucleic acid. A positive staining for 3β steroid dehydrogenase was seen in the theca of small and medium sized antral follicles. In H. and E. sections these cells were

polygonal with a foamy cytoplasm. The thecal cells of large antral follicles gave a positive reaction for 3β steroid dehydrogenase and in an occasional follicle a few of the peripheral granulosa cells also reacted. Staining with Oil Red O showed the presence of fine lipid granules in the theca of small, medium and large antral follicles.

Corpora lutea were the same size as in normal animals (0.7-1.0mm). Ribonucleic acid and 3β steroid dehydrogenase were present in large quantities.

The interstitial cells were also normal in appearance, polygonal with small round nuclei and slightly basophilic cytoplasm. Ribonucleic acid was present in these cells.

Eighteen hours after hypophysectomy the first signs of degeneration appeared in the large antral follicles showing a loosening of the cells of the granulosa layer so that the orderly compact appearance was lost. The peripheral layer of cells ceased to have an orderly pallisaded arrangement. Apart from loosening there was an alteration in the shape of the cells which were now irregularly polygonal. Some nuclei were pyknotic and fragments of chromatin were seen. The cytoplasm was no longer basophilic and the ribonucleic acid content was much reduced. Although the thecal cells appeared to be relatively normal in preparations stained by

haematoxylin and eosin, there was a reduction in the RNA content of these cells also. Oil Red O revealed the presence of lipid in both the granulosa and theca with some large droplets. Control sections showed that the apparently positive reaction for 3β steroid dehydrogenase was false in these lipid laden cells.

No particular change could be found in smaller antral follicles or pre-antral follicles of all sizes and their histochemical reactions for RNA and steroid dehydrogenase remained normal. In some of the corpora lutea a few of the cells contained large globules of lipid but in general the histological appearance of these bodies was normal and the cells were still rich in RNA and gave a strong response for 3β steroid dehydrogenase. Similarly, the interstitial cells remained plump and polygonal with healthy nuclei and slightly basophilic cytoplasm. They too possessed RNA and gave a slight reaction for 3β steroid dehydrogenase.

Twenty four hours post-hypophysectomy more pronounced changes had taken place. Large antral follicles showed advanced atretic changes. In a few the granulosa layer had disappeared completely but in others numerous chromatin fragments floated free in the antral space. No intact cells could be seen. The thecal cells formed large bloated masses with clear cytoplasm and small pyknotic nuclei surrounding

the antrum. Oil Red O showed that these bloated cells were filled by a coarse emulsion of large lipid globules. They were completely devoid of RNA. The reaction for 3β steroid dehydrogenase was manifestly false and this was confirmed on examination of control sections.

Thirty hours post-hypophysectomy early degenerative changes, such as were seen in the large antral follicles at twenty four hours post-hypophysectomy, were now evident in small and medium sized antral follicles. The granulosa layers showed the same tendency to loosening of cells, pyknosis and accumulation of lipid. Lipid had also increased in the thecal cells and in both layers the ribonucleic acid content was reduced. Pre-antral and Stage 2 and 3 follicles were intact and the corpora lutea did not appear to have changed their morphology. There was no increase in the large lipid globules; RNA was still abundant and the reaction for 3β steroid dehydrogenase was positive. There was a loss of basophilia in the interstitial cells; the cytoplasm was now pale and slightly eosinophilic. Ribonucleic acid was much diminished and Oil Red O revealed an increase in the amount of cytoplasmic lipid.

In a few of the pre-antral follicles the thecal layer was very thick, consisting of plump spindle shaped cells with granular cytoplasm. At some points

these plump thecal cells appeared to have grown through the basement membrane and were invading and replacing the granulosa cells. This would appear to be an exaggerated form of the changes occasionally seen in pre-antral follicles undergoing atresia during the normal oestrus cycle.

A further six hours resulted in advanced changes in all antral follicles. Large antral follicles had virtually disappeared. Comparison of the appearances at this time with those in animals killed earlier indicated that the antra of these large follicles had shrunken and their presence was now indicated by collections of bloated lipid laden thecal cells surrounding small spaces. Small and medium sized antral follicles now exhibited all the changes which took place during atresia of the large follicles as previously described (Fig. 28). An odd intact small antral follicle was seen in some animals at this time but staining with Oil Red O revealed an increased accumulation of large lipid globules in the theca and a few small globules in the granulosa. Pre-antral and Stage 2 and 3 follicles were generally normal in appearance but one or two of the larger pre-antral follicles (Stages 4 and 5) showed loosening of the granulosa cells and there was a reduced content of RNA in their cytoplasm. Surprisingly little change was noted in the corpora lutea apart from a possible slight reduction in the cytoplasmic RNA. It was

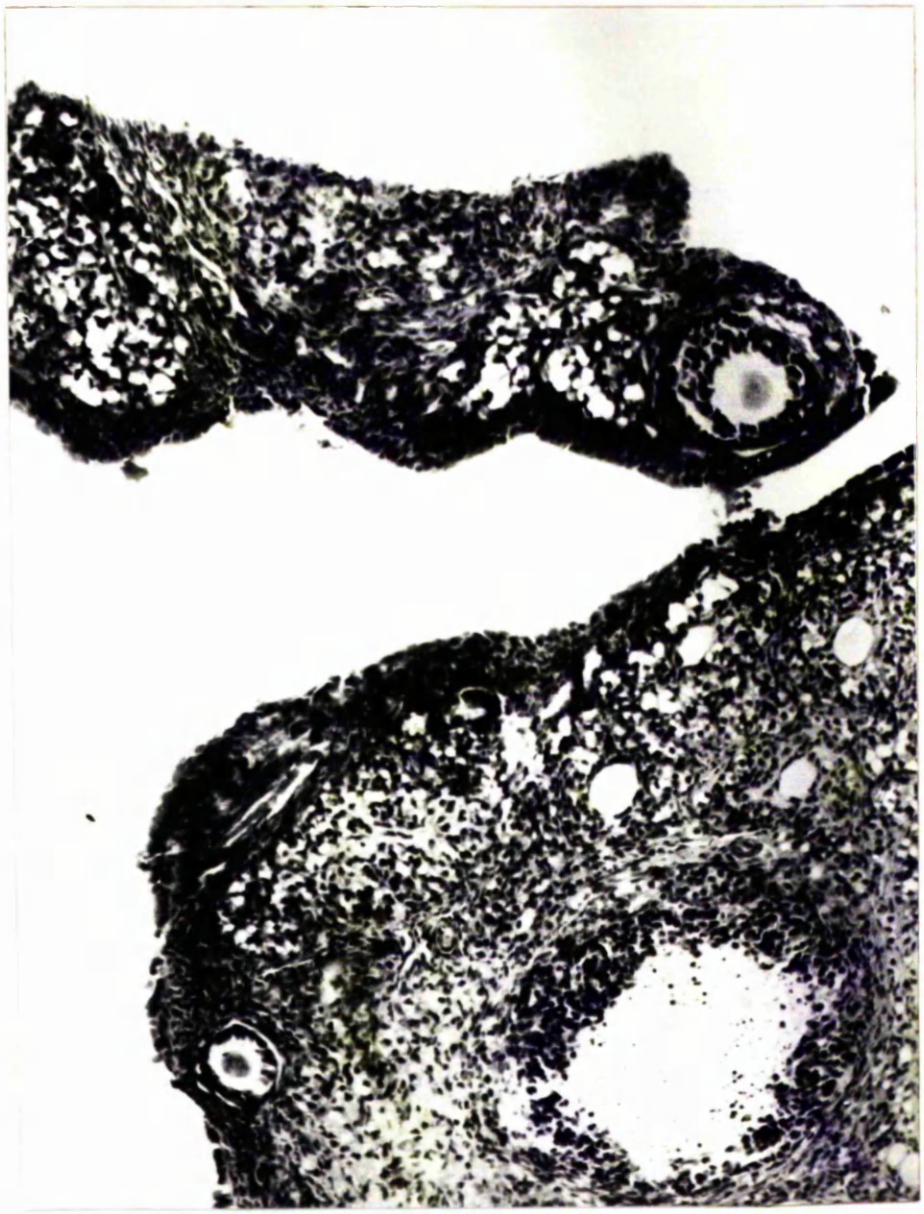


Fig. 28

Thirty-six hours after hypophysectomy large and medium sized follicles have degenerated. In this figure a medium sized follicle in advanced atresia is shown. A small two-layer pre-antral follicle is still present. H. & E. X 105.

difficult to judge this with the light microscope. The nuclei of the interstitial cells were now smaller and there were large globules of lipid in their cytoplasm (Fig. 29).

Forty eight hours post-hypophysectomy all antral follicles had undergone advanced degenerative changes and it became impossible to distinguish between follicles of different sizes (Fig. 30). The larger pre-antral follicles (Stages 4 and 5) were now showing signs of change. Again these consisted of loosening of cells, loss of pallisading and reduction of cytoplasmic RNA. Some cells showed pyknosis of nuclei and chromatin granules. The thecal cells around these large pre-antral follicles, normally spindle shaped, were now somewhat swollen. Smaller pre-antral follicles occasionally showed similar changes. Again there was little change in the corpora lutea apart from a diminution in cytoplasmic RNA which was now definite when compared with the fresh corpora lutea of intact animals. The interstitial tissue had lost all of its ribonucleic acid and had accumulated more lipid. It was now difficult to distinguish the interstitial cells from the degenerate thecal cells of atretic follicles.

During the succeeding twenty four hours degenerative changes increased in the pre-antral follicles. Seventy two hours post-hypophysectomy large

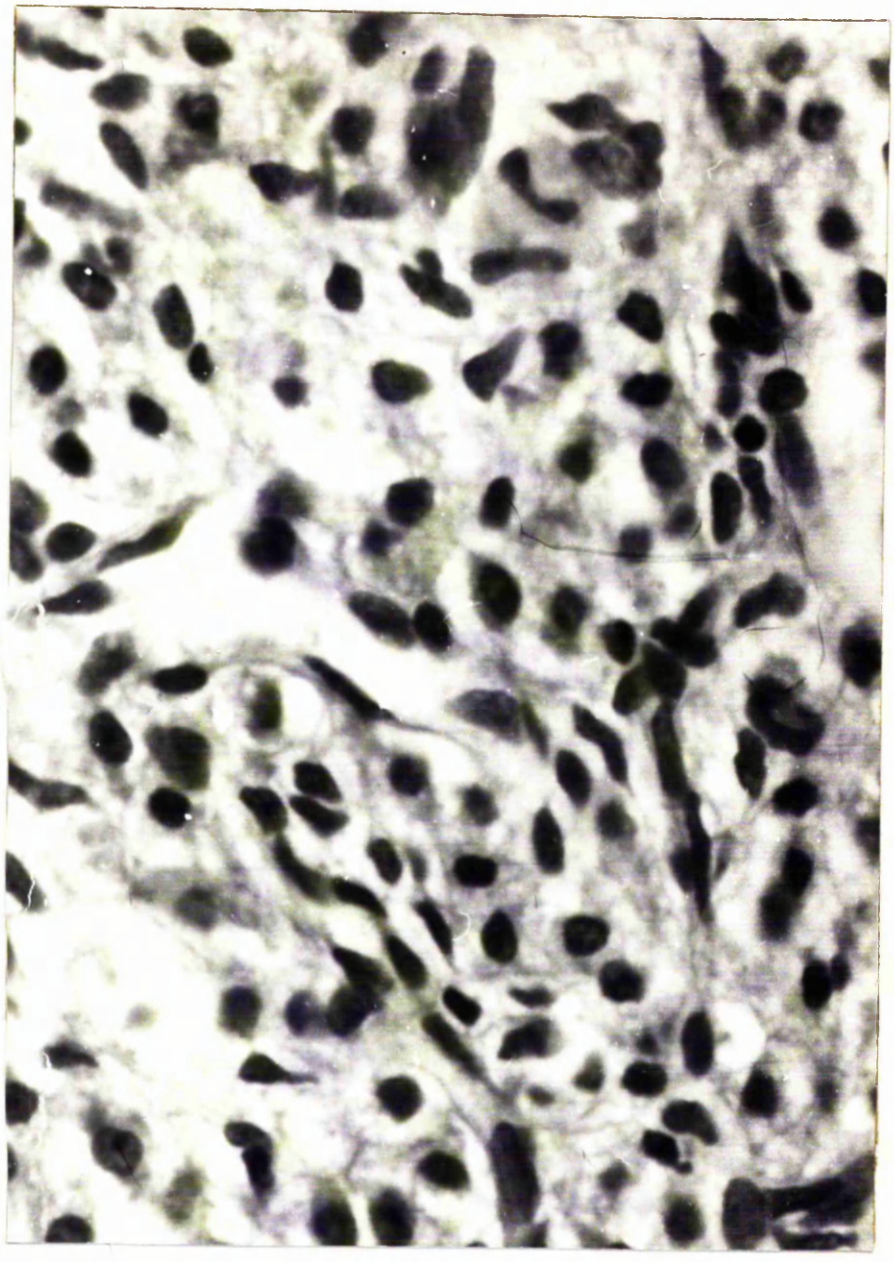


Fig. 29

High power view of interstitial tissue from an ovary of an animal 7 days after hypophysectomy. Note the foamy cytoplasm due to an accumulation of lipid. H. & E. X 400.

Fig. 30

Graafian follicles from an ovary 48 hours after hypophysectomy showing advanced atretic changes. H. & E. X 105.

pre-antral follicles had almost completely disappeared. Small pre-antral follicles (Stages 2 and 3) were still present but some of these showed loosening of the granulosa cells. There was loss of ribonucleic acid in these cells but as yet there was no definite evidence of cell death. Surprisingly, in one of the animals killed at sixty hours post-hypophysectomy a few small antral follicles were still present. In most degenerative changes had taken place but in others there was a peculiar appearance in the granulosa layers. Multiple round spaces, resembling Call-Exner formations, appeared around the whole circumference of the follicle. The appearance suggested that antral formation had become disorganised.

The interstitial tissue appeared to be reduced in amount but this may have been due to a reduction in the size of the individual cells (Fig. 31). These had clear cytoplasm and small nuclei. Oil Red O showed that the amount of lipid was now reduced in these cells. It was difficult to interpret the results of tests for 3β steroid dehydrogenase in these sections. The remains of atretic follicles gave a false reaction due to the presence of so much lipid and even in control sections reduction of the stain had taken place giving rise to deposits. In the corpora lutea however the results were still reliable and a fairly strong positive reaction was obtained.

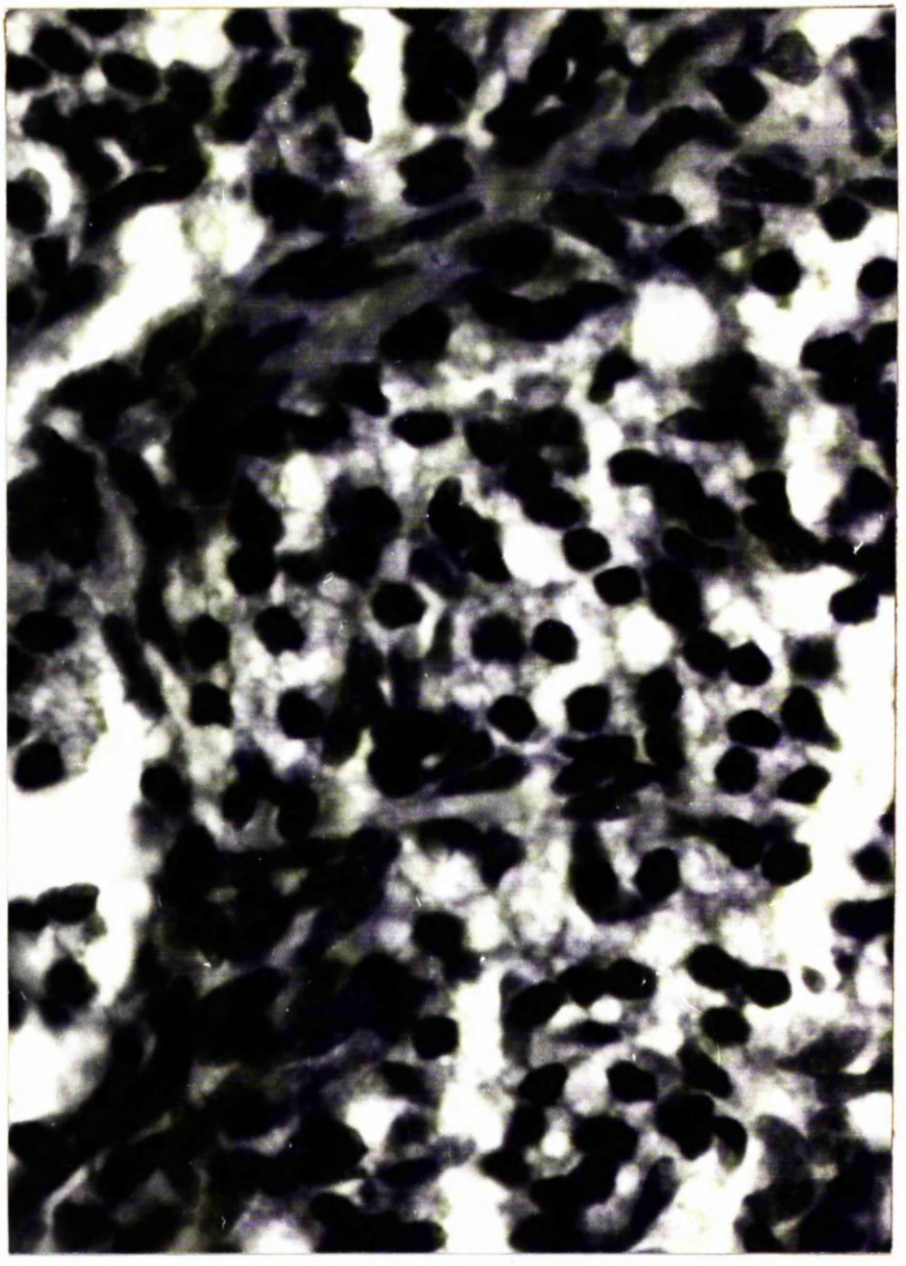


Fig. 31

Interstitial cells of an ovary 72 hours after hypophysectomy. High power view showing irregularity of nuclear shape and clumping of chromatin. H. & E. X 600.

The corpora lutea and some of the small pre-antral follicles were the only structures which contained a demonstrable amount of ribonucleic acid.

Ninety six hours post-hypophysectomy Stage 3 follicles with two layers of granulosa cells still remained. No follicle larger than this could be found. Stage 1 and 2 follicles were also present. The interstitial tissue had now more the appearance of fatty areolar tissue than a steroidogenic structure. Despite the advanced degenerative changes in other structures the corpora lutea were still structurally intact. However they did show an increased amount of lipid and the staining reaction with pyronin for RNA was much paler. There was still a considerable degree of staining for 3β steroid dehydrogenase.

In succeeding days the reduction in the number of follicles beyond Stage 1 became even more apparent. By day 6 primordial and Stage 2 and 3 follicles were still the only structures to be seen apart from corpora lutea which were structurally intact (Fig.32). The luteal cells were somewhat smaller but they still contained significant amounts of ribonucleic acid and tests for steroid dehydrogenase were positive if reduced. This situation remained until fifteen days post-hypophysectomy when the corpora lutea showed a definite shrinkage of cells and increased prominence of the supporting connective tissue. Most follicular structures had disappeared and only an occasional

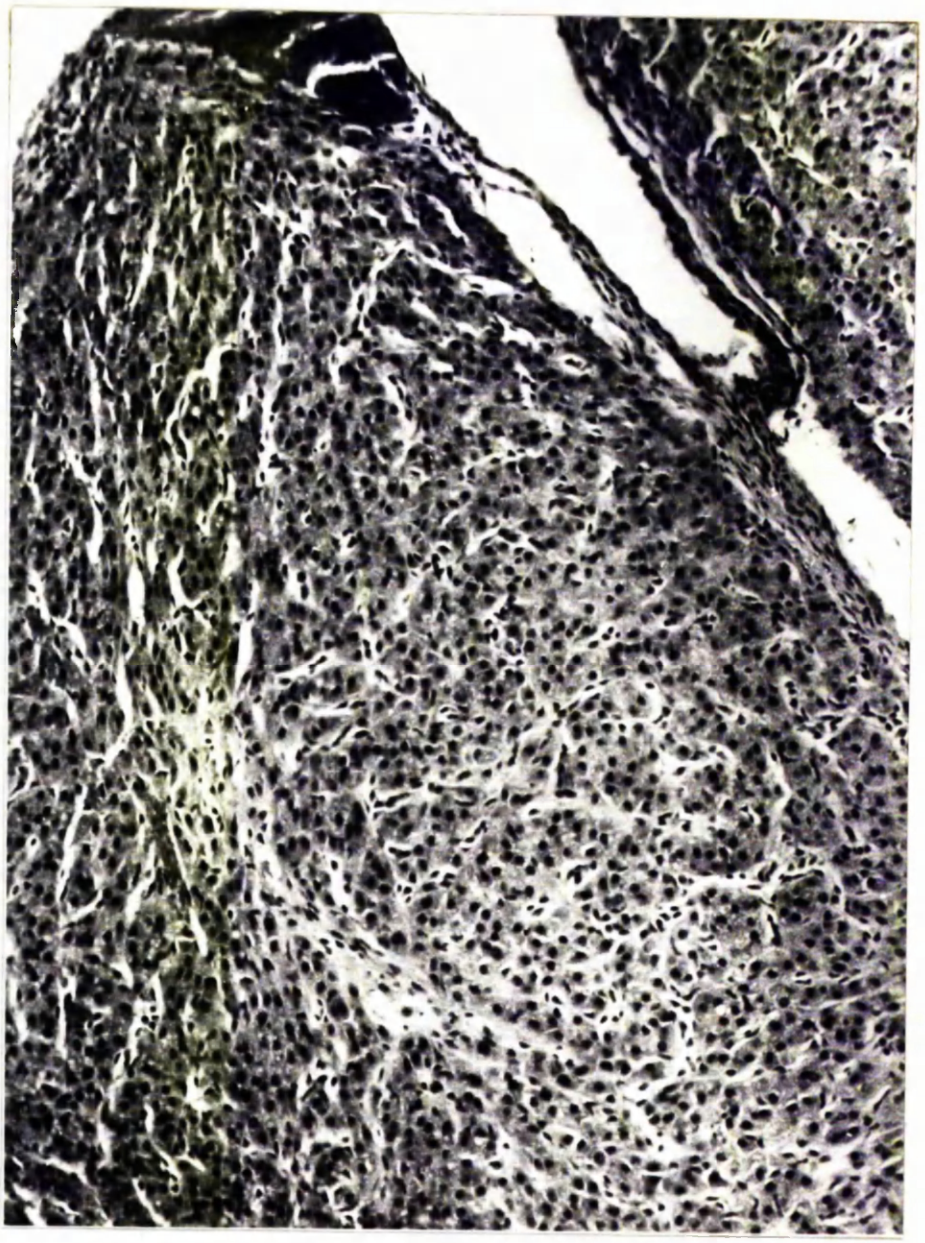


Fig. 32

Corpus luteum from an ovary 7 days after hypophysectomy. It is morphologically intact. H. & E. X 130.

degenerate primordial follicle could be seen. Apart from the persisting corpora lutea the ovaries were more or less converted into small masses of interstitial tissue of degenerate appearance. Subsequently the corpora lutea appeared to undergo acute necrosis by the end of four weeks.

While changes after hypophysectomy followed the general pattern described above it was noted that a considerable number of the ovaries presented a feature which was out of keeping with the main events. Very large antral follicles appeared in specimens within forty eight hours of the operation. These had a thin granulosa layer consisting of a single layer of flattened cells which showed degenerative changes and a relatively inconspicuous thecal layer. Similar cystic follicles but showing increasing degrees of degeneration of the granulosa were observed in the days following. Even seven days after hypophysectomy they presented as cystic structures with an incomplete lining of flattened cells and contained varying amounts of chromatin debris. The rate of degeneration of these flattened granulosa cells appeared to be slower than that of the granulosa cells of smaller follicles.

EFFECT OF HYPOPHYSECTOMY ON THE UTERINE HISTOLOGY

After hypophysectomy there was a detectable thinning of the lumina of the cornuae of the uterus (Fig. 33) with thinning of the endometrium and myometrium. The endometrial glands were narrow and short.

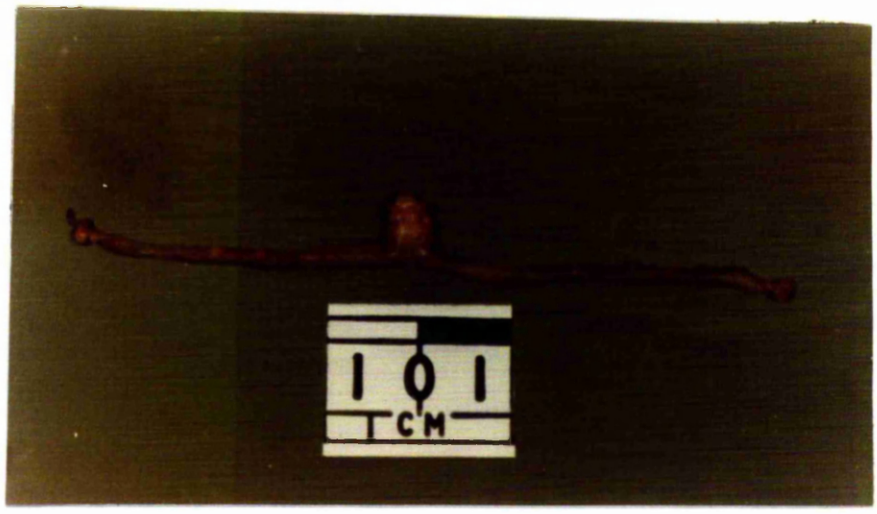


Fig. 33

Uterus and ovaries removed from rat killed 7 days after hypophysectomy. The ovaries are small and the tubes narrow.

EFFECT OF NIAMD RAT FSH, B-1 ON HYPOPHYSECTOMIZED MATURE FEMALE RATS

EFFECT OF FSH, B-1 ON THE OVARIAN WEIGHT

With all doses of FSH there was an increase in the ovarian weight. This increase was to some extent time related (Fig. 34). The rate of increase in every instance was greatest in the first twelve hours, there being little further change up to forty eight hours. Thereafter the graph levelled out. The increase in ovarian weight is most obvious with the higher doses but there is little apparent difference between the 50, 75 and 100 µg doses. (see Appendix).

Statistical analysis of the summated ovarian weights of all animals given a particular dose of FSH and sacrificed during the first twenty four hours showed that the increase in ovarian weight was significant for all doses except 25 µg (Tables 8 and 9).

EFFECT OF FSH, B-1 ON THE UTERINE WEIGHT

Following the administration of FSH there appeared to be a slight increase in the uterine weight (Fig. 35) but this proved to be of no significance (Tables 10 and 11). (See Appendix).

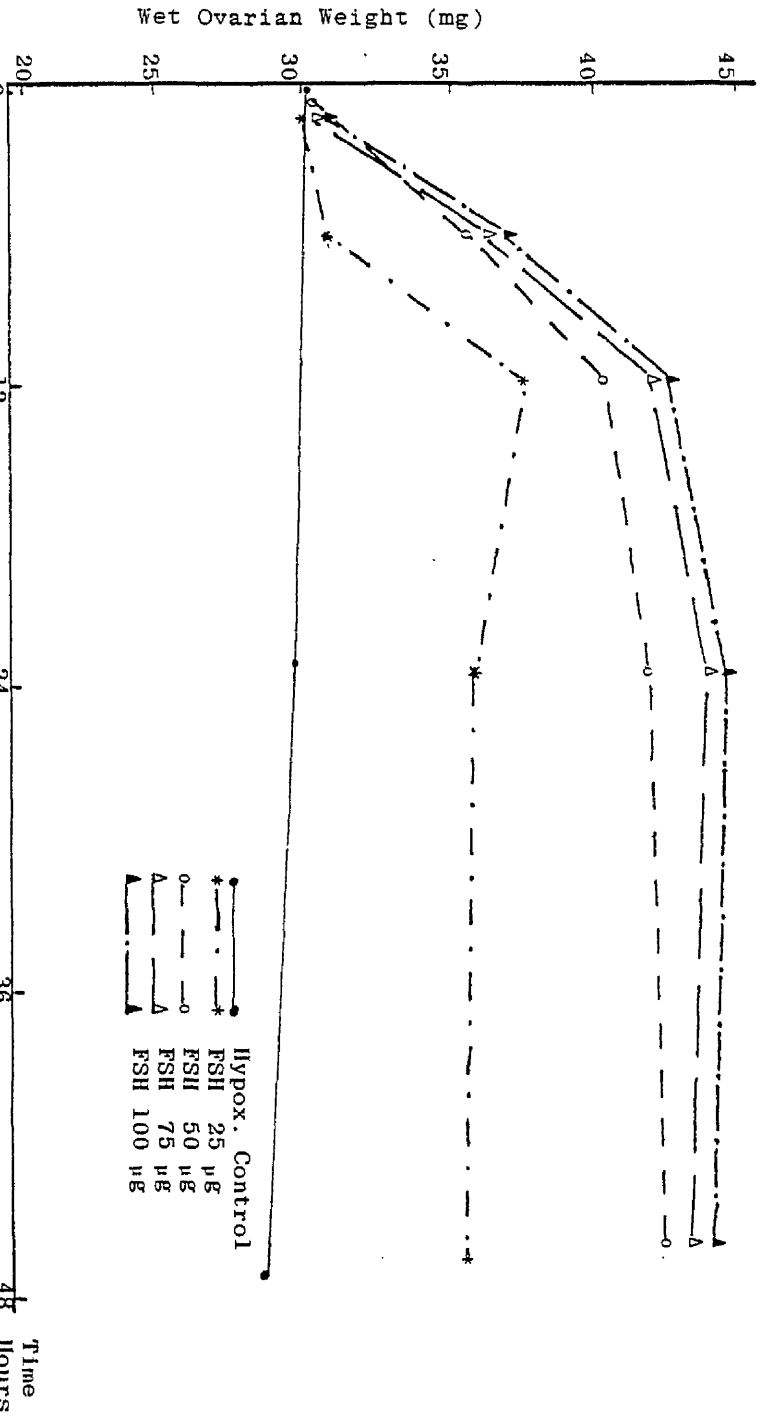


Fig. 34 The effect of varying doses of pure rat FSH, B-1 on the ovarian weight of two hypophysectomized mature female rats at each time interval.

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.0581	0.0003
Hypox. Control	8	0.0327	0.0029
Hypox. Treated + 25 µg FSH	8	0.0330	0.0025
+ 50 µg FSH	8	0.0369	0.0049
+ 75 µg FSH	8	0.0383	0.0056
+100 µg FSH	8	0.0387	0.0051

Table 8

The mean and standard deviation of the ovarian weight of hypophysectomized rats treated with FSH, B-1 compared with intact and hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg FSH	14	1.9348	N.S
Hypox. Control vs. 50 µg FSH	14	3.1133	$P \leqslant 0.05$
Hypox. Control vs. 75 µg FSH	14	5.9854	$P \leqslant 0.05$
Hypox. Control vs. 100 µg FSH	14	3.8332	$P \leqslant 0.05$

N.S. = Not Significant

Table 9

Comparison of the ovarian weights of the hypophysectomized control group and the hypophysectomized rats treated with various doses of FSH using t. test.

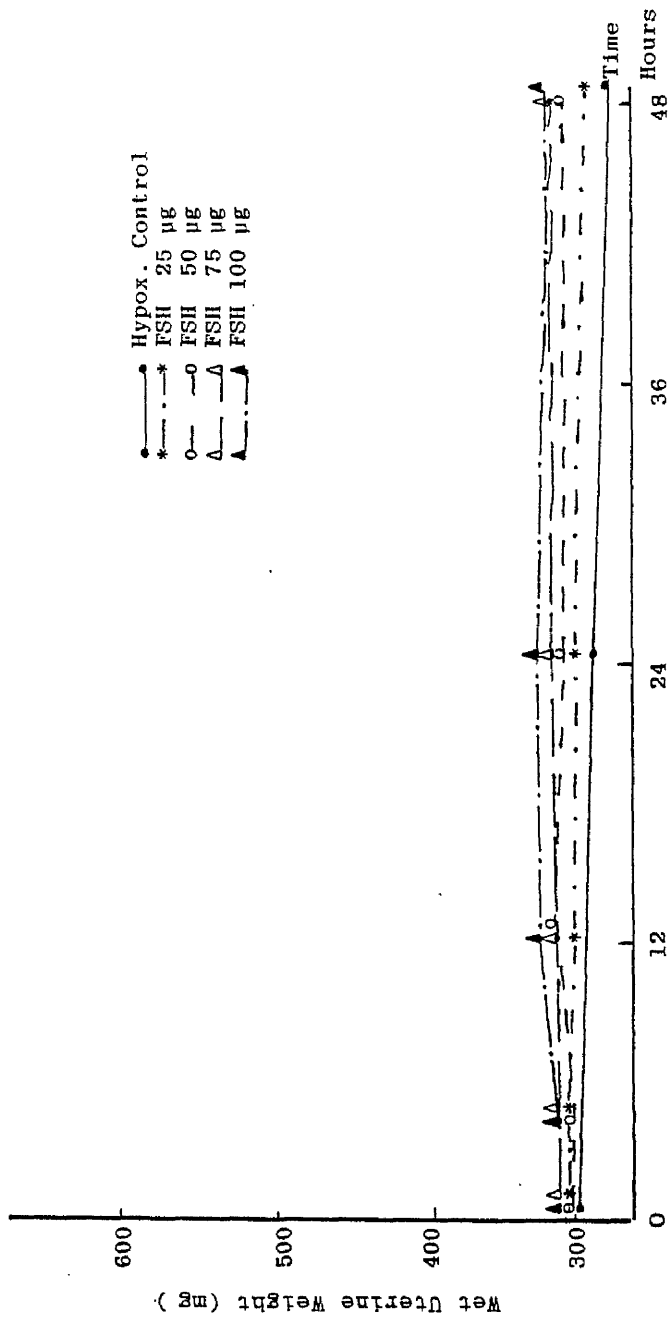


Fig. 35 The effect of varying doses of FSH, B-1 on the uterine weight of two hypophysectomized mature female rats at each time interval

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.3802	0.0081
Hypox. Control	8	0.2951	0.0049
Hypox. Treated + 25 µg FSH	8	0.3063	0.0061
+ 50 µg FSH	8	0.3096	0.0051
+ 75 µg FSH	8	0.3085	0.0053
+100 µg FSH	8	0.3106	0.0087

Table 10.

The mean and standard deviation of the uterine weight of hypophysectomized rats treated with FSH, B-1 compared with intact and hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg FSH	14	0.7066	N.S.
Hypox. Control vs. 50 µg FSH	14	1.2768	N.S.
Hypox. Control vs. 75 µg FSH	14	1.0874	N.S.
Hypox. Control vs. 100 µg FSH	14	1.3187	N.S.

N.S. = Not Significant

Table 11.

Comparison of the uterine weights of the hypophysectomized control group and the hypophysectomized rats treated with various doses of FSH using t. test.

THE EFFECT OF FSH, B-1 ON THE OVARIAN HISTOLOGY

Multiple changes occurred in the ovaries of the hypophysectomized rats given FSH, B-1. The effects on the follicles, corpora lutea and interstitial tissue are as follows:

Effect on the Follicles

Changes in the follicle varied with the dose of FSH preparation and the time interval involved. These changes will be described under Growth, Antral Formation and Thecal Formation.

Growth

There appeared to be a stimulation of growth not only in individual follicles (Stages 1 and 2) but also in an increasing number of follicles. A constant pattern of changes was obvious in all groups of animals, exemplified by 25 µg of FSH. One hour after the injection of FSH, B-1 there was no growth stimulatory effect on the follicles. Six hours after injection, Stage 3 follicles (with two layers of granulosa cells) which were present in the non-injected hypophysectomized animal diminished in number and Stage 4 follicles (with three layers of granulosa cells) appeared. At twelve hours, the Stage 3 follicles had increased in number once more while larger pre-antral follicles with five-six layers of granulosa cells (Stage 5) were present. The peak of stimulation

was twenty four hours after the injection of FSH. There was an increase in the number of pre-antral follicles at all stages of growth. The inference seems to be that the progressive growth initiated in Stage 3 follicles converted these follicles to Stages 4 and 5. An increased dose tended to increase the degree of growth and to some extent to speed the process. While the changes were slight with 25 µg FSH, B-1, they became very apparent when the dose was increased to 50 µg and even more when the dose was increased to 75 and 100 µg. With these larger doses follicles at Stages 2 and 3 showed mitotic figures among the granulosa cells and at Stages 4 and 5 this mitotic activity had increased (Fig. 36). At all these follicular stages there was a large amount of RNA in the granulosa cells but no lipid or any activity for 3β steroid dehydrogenase.

The oocyte size kept a constant relation to the follicular size which did not alter with the increase in the dose of FSH. At first, in Stages 2 and 3 of the follicles the ovum grew very rapidly in relation to the follicle, reaching three times its original size and almost half the size of the follicle of which it was part; the oocyte then remained more or less stationary in size while the follicle continued to expand.

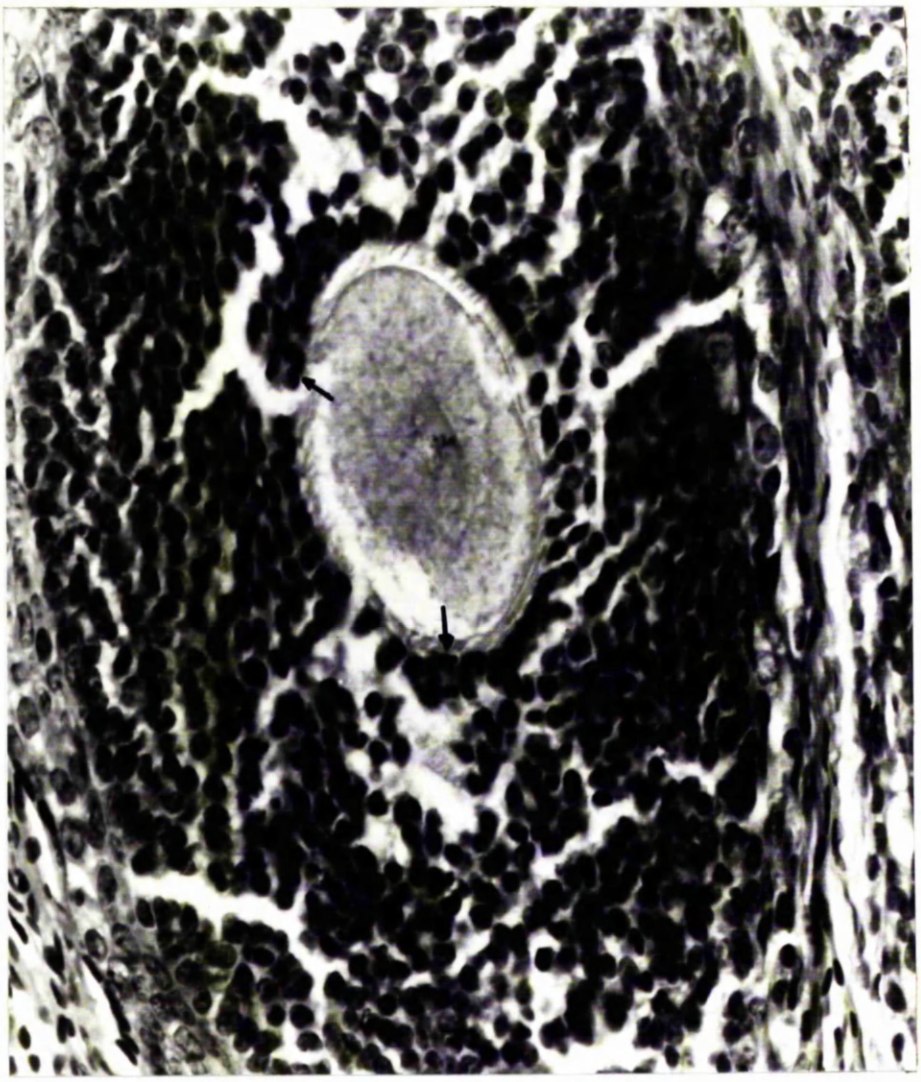


Fig. 36

Stage 5 follicle in an ovary from a hypophysectomized animal 24 hours after receiving 50 µg FSH. Mitotic figures can be seen in the granulosa layer (arrowed) H. & E. X 312.

The maximum stimulation of growth was obtained with 75 µg; increasing the dose to 100 µg did not alter the effect (Table 12).

With 25 and 50 µg dose schedules, proliferative activity as indicated by mitotic activity tailed off at forty eight hours but with 75 and 100 µg proliferation was still present at forty eight hours with no sign of tailing off (Table 12).

Follicle Count

The results of follicle counts obtained with various doses of FSH, B-1 and at intervals following administration are shown in Fig. 37. This indicates that an increase in the number of follicles beyond Stage 2 occurred with each dose administered starting at six hours post injection and peaking at twenty four hours. The effect was greatest with the 75 and 100 µg doses. After twenty four hours there was a fall off in the follicle count with the 25 and 50 µg doses while with the higher doses the follicle counts maintained a plateau. Comparison of the peaks of follicle counts (Fig. 38) emphasises the effect of increasing the dose of FSH.

Statistical analysis of the summated counts between six and twenty four hours showed that the increase in post-Stage 2 follicles was

Dose of FSH	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg	-	±	+	+	±
50 µg	-	±	+	++	+
75 µg	-	+	++	+++	+++
100 µg	-	+	++	+++	+++

Table 12 Follicular Changes - Growth

The effect of varying doses of FSH, B-1 on the follicular growth of the ovaries of two hypophysectomized rats at each time interval.

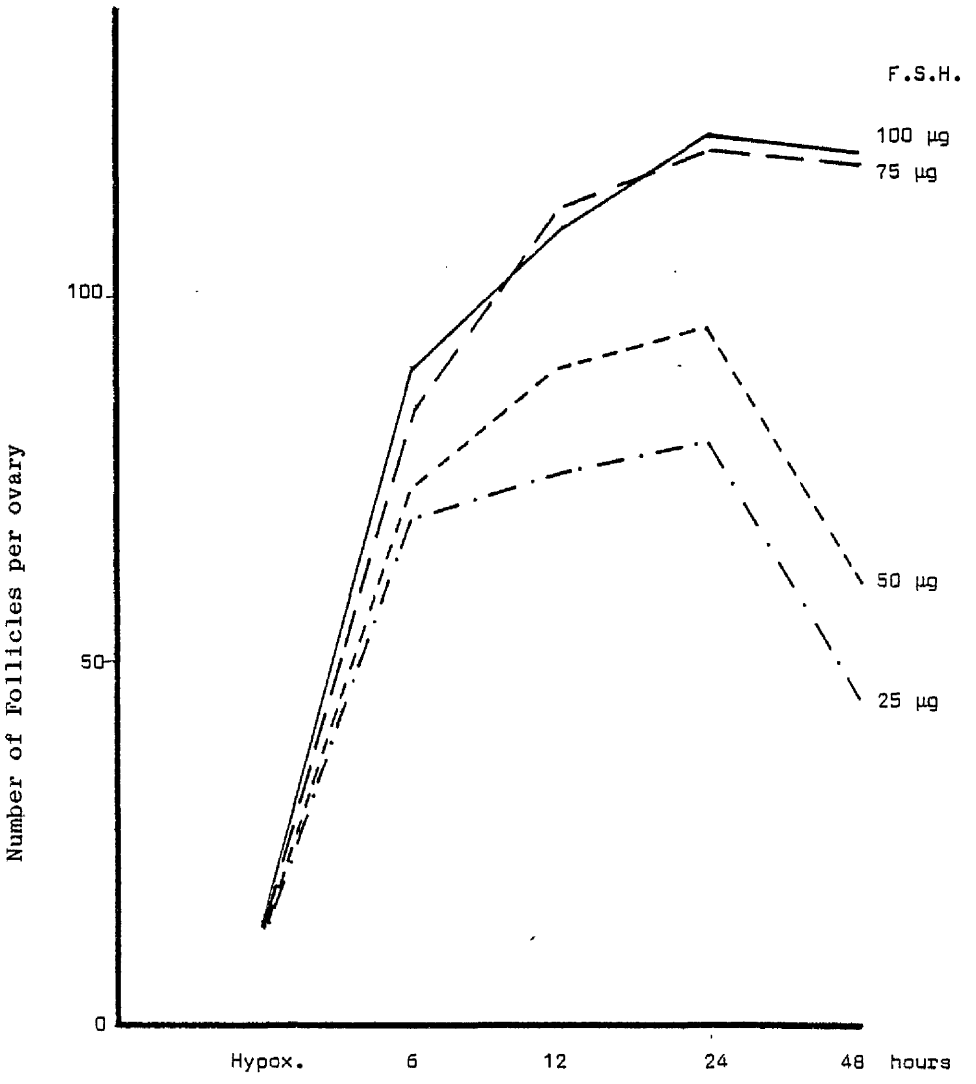


Fig. 37

The effect of the administration of varying doses of FSH, B-1 on the follicle count of one ovary from each of the two hypophysectomized rats at each time interval.

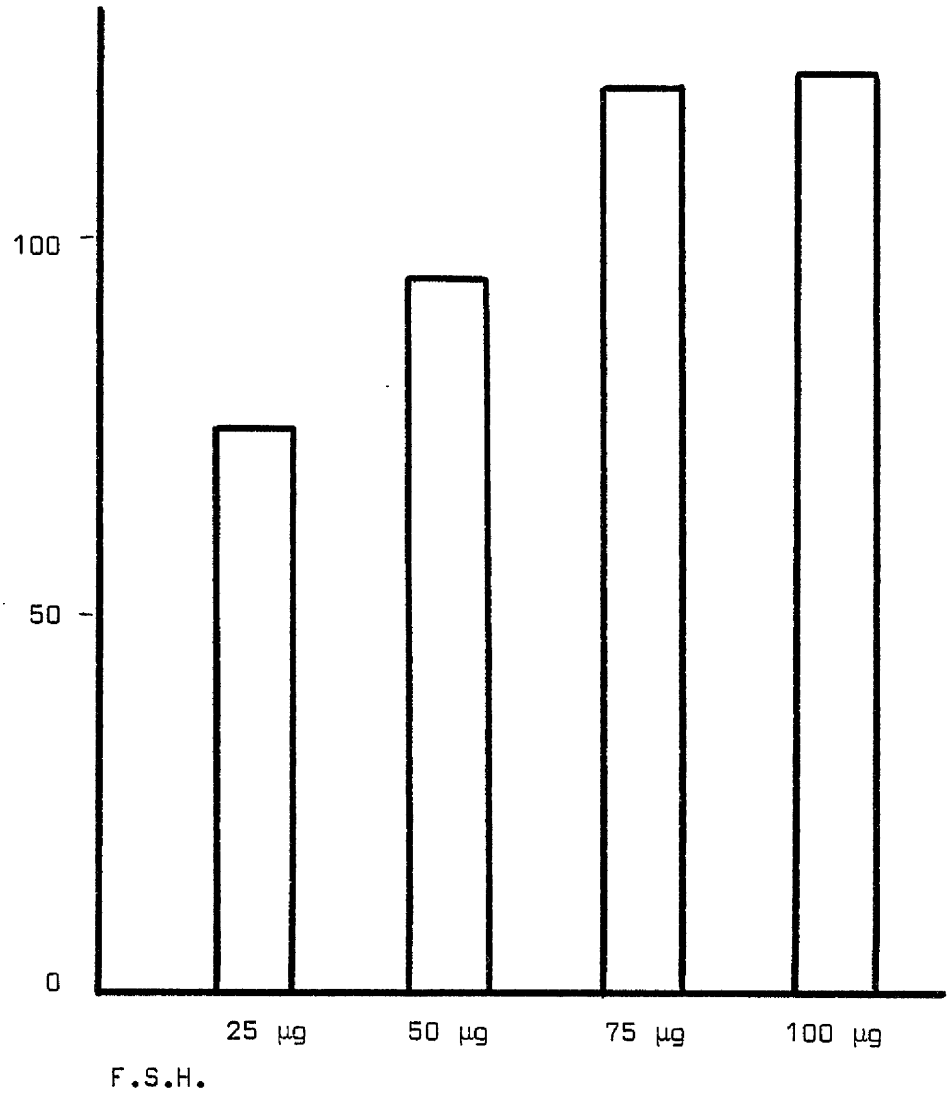


Fig. 38.

Comparison of the follicle counts at the peak of stimulation after the administration of varying doses of FSH, B-1.

significant for each dose of hormone ($P \leq 0.001$). This supports the impressions gained during histological examination of these ovaries (Tables 13 and 14). Table 15 shows the differentiated follicle count.

Antrum Formation

"Normal" antrum formation takes place in follicles with four or more layers of granulosa cells (Stage 5). Antral follicles were classified as Stage 6.

In the hypophysectomized mature female rat there was no antrum formation at one or six hours, but small antral follicles appeared twelve hours after the injection of 25 µg of FSH, B-1. The number and size of these antral follicles increased at twenty four hours (Figs. 39 and 41). In some of the antral follicles noticed at twenty four hours, the granulosa cells were loosened from each other and tended to vary in size. There was some vacuolation in the cytoplasm of these granulosa cells and Oil Red O showed that they contained lipid droplets. There was a definite reduction in the amount of RNA in these follicles when compared with the healthy looking antral follicles. At forty eight hours, more advanced degenerative changes were present in the granulosa cells of most of the antral follicles, small and large. The nuclei of

Experiment	No.	Mean	Standard Deviation
Hypox. Control	8	14.00	2.6186
Hypox. Treated + 25 µg FSH	6	72.33	10.0532
+ 50 µg FSH	6	84.33	11.5254
+ 75 µg FSH	6	106.00	16.5650
+100 µg FSH	6	107.66	17.0724

Table 13

The mean and standard deviation of the follicle count of hypophysectomized rats treated with FSH, B-1 compared with hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg FSH	12	14.550	$P \leqslant 0.001$
Hypox. Control vs. 50 µg FSH	12	15.444	$P \leqslant 0.001$
Hypox. Control vs. 75 µg FSH	12	14.307	$P \leqslant 0.001$
Hypox. Control vs. 100 µg FSH	12	14.146	$P \leqslant 0.001$

Table 14

Comparison of the follicle count of the hypophysectomized control group and the hypophysectomized rats treated with various doses of FSH using t. test.

Preparation and time of Administration		Follicular Stage			Normal Antral Follicles			Total	
		3	4	5	S	M	L	Normal	Abnormal
Control Hypophysectomized 7th Day		14	-	-	-	-	-	14	50
FSH, B-1 25 µg	6h	10	14	10	26	-	-	60	52
	12h	20	10	15	30	-	-	75	60
	24h	26	16	10	30	-	-	82	74
	48h	16	4	6	20	-	-	46	66
FSH, B-1 50 µg	6h	12	16	14	28	-	-	70	55
	12h	22	14	16	34	-	-	88	85
	24h	30	20	12	33	-	-	95	80
	48h	15	5	10	30	-	-	60	85
FSH, B-1 75 µg	6h	25	20	20	20	-	-	85	56
	12h	40	15	20	38	-	-	113	66
	24h	40	15	25	40	-	-	120	70
	48h	34	20	22	42	-	-	118	60
FSH, B-1 100 µg	6h	16	28	20	22	-	-	86	78
	12h	40	18	15	42	-	-	115	86
	24h	42	15	23	44	-	-	122	98
	48h	40	15	26	39	-	-	120	86

S - small M - medium L - large

Table 15.

Follicle count beyond Stage 2 in one ovary of each of two hypophysectomized rats treated with various doses of FSH compared with the hypophysectomized controls.

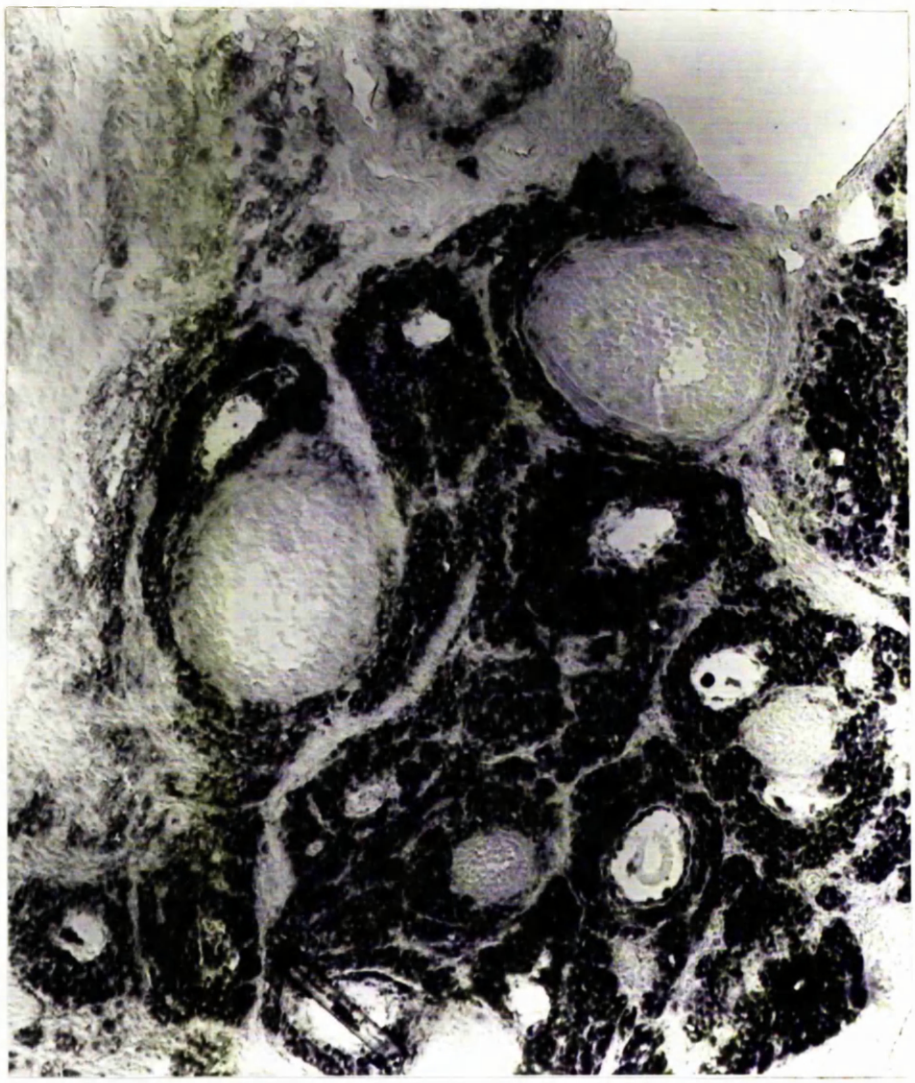


Fig. 39

Ovary of a hypophysectomized animal 24 hours after receiving 25 µg FSH. Antral follicles are present. Fine lipid can be seen in the theca of these follicles. Coarse lipid globules are present in the interstitial tissue. Oil Red O X 150.

of granulsoa cells were pyknotic and there was debris in the antral spaces. When the dose of FSH, B-1 was increased to 50 µg there was no change in the pattern described with 25 µg but there was a definite increase in the number of antral follicles recruited, and medium sized antral follicles made their appearance twenty four hours after injection. The tailing off activity noticed with 25 µg was more pronounced with 50 µg of FSH, B-1, and there were more degenerative changes in all follicles, especially the antral follicles (Fig. 40). When the dose of FSH, B-1 was increased to 75 µg the number and size of the antral follicles again showed an increase, a change which was apparent six hours after injection and which intensified at twelve, twenty four and forty eight hours. Medium and large sized antral follicles were seen at twelve, twenty four and forty eight hours. At forty eight hours, although most of the antral follicles maintained their healthy appearance, some of the large antral follicles (0.4mm or more) showed signs of atresia with pyknosis and apoptosis in the granulosa cells and an accumulation of large lipid droplets in their cytoplasm. A few degenerated granulosa cells could be seen in the follicular space. When the dose of FSH, B-1 was

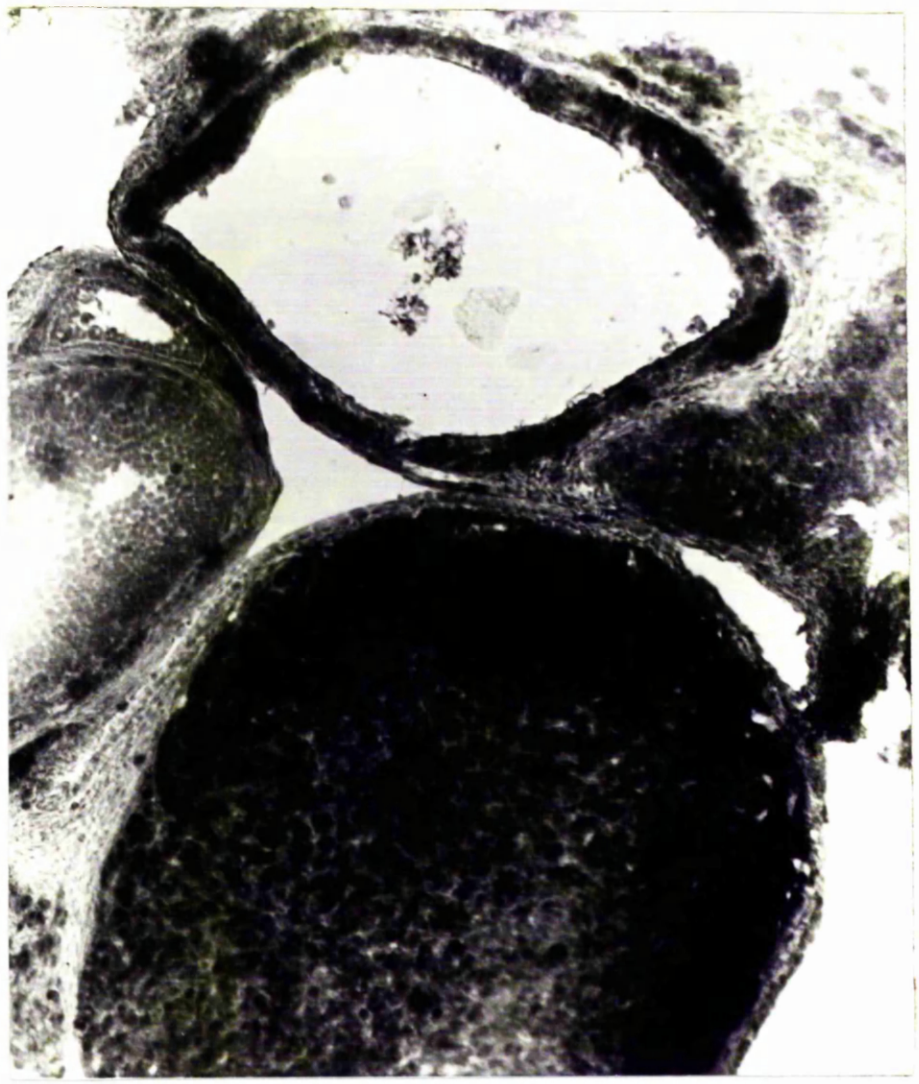


Fig. 40

The ovary from a hypophysectomized rat 48 hours after receiving 50 μg FSH. Numerous follicles have formed. A large antral follicle showing advanced atretic changes. The smaller follicles show no atretic changes. Corpus luteum appears morphologically intact.

increased to 100 µg there was no improvement in the response obtained by 75 µg (Fig. 41). Although large antral follicles showed some degeneration mitotic activity was still present in small antral follicles forty eight hours after receiving 75 and 100 µg of FSH, B-1. The relationships between antral formation and dosage are shown in Table 16.

"Abnormal" antrum formation is a term introduced to describe antra that are abnormal either in the time of appearance, number, shape or their size in relation to the size of the follicle. Normally the antrum forms in a follicle with four or more layers of cuboidal granulosa cells (Stage 5 follicles). Antra formed in follicles at Stages 4, 3 and 2 are described in this text as prematurely formed antra.

Abnormal antrum formation was noted in the hypophysectomized female rate twenty four hours after injection of 25 µg of FSH, B-1. Multiple small rounded spaces appeared round the whole circumference among the granulosa cells of Stage 4 and 3 follicles. The premature abnormal antrum formation was noticed on a larger scale at forty eight hours. In addition to premature and multiple formations the antra were often disproportionately large in relation to the total size of the follicle of which it was part. When

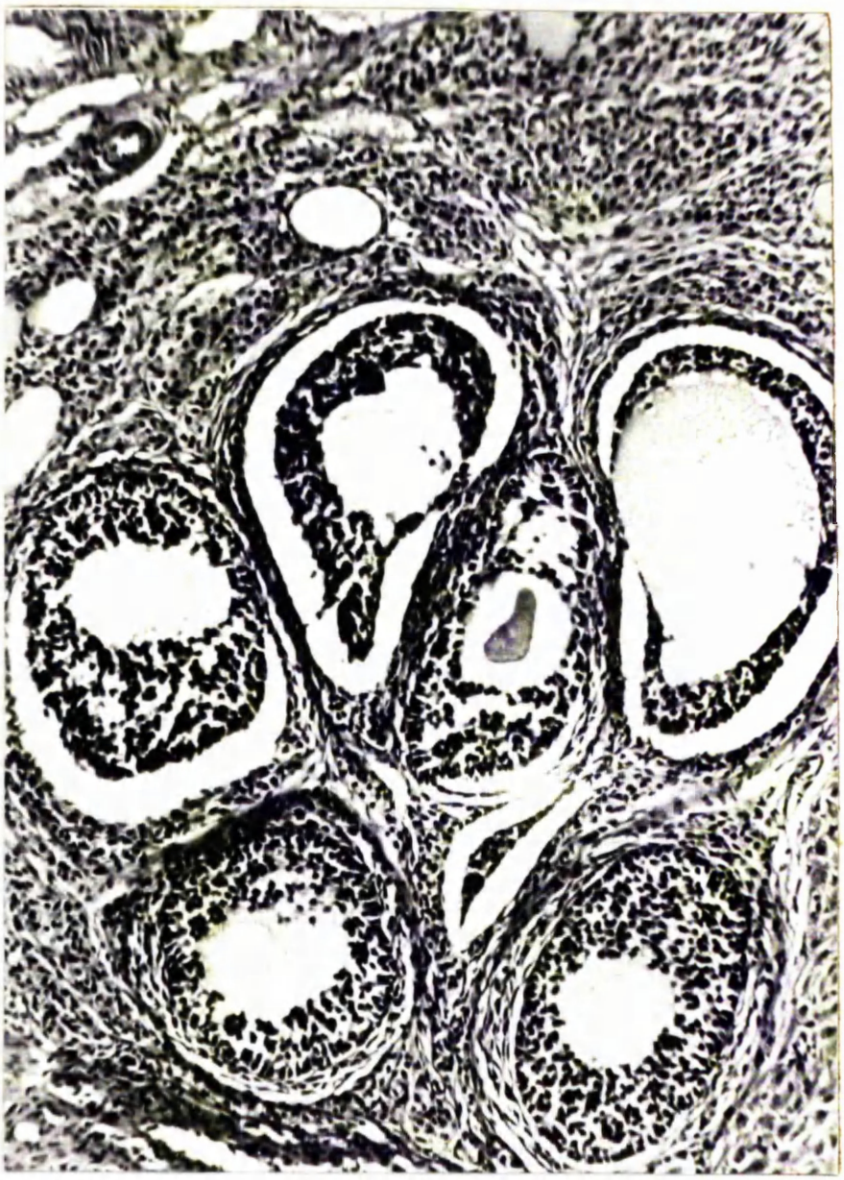


Fig. 41

Ovary from a hypophysectomized animal 24 hours after receiving 100 µg FSH. Numerous antral follicles have formed. H. & E. X 120.

Dose of FSH, B-1	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg	-	-	-	+	-
50 µg	-	-	+	++	-
75 µg	-	+	++	+++	++
100 µg	-	++	+++	++++	++

Table 16. Follicular Changes - Antral Formation

The effect of varying doses of FSH, B-1 on the antral formation in both ovaries of two hypophysectomized rats at each time interval.

the dose of FSH, B-1 was increased to 50, 75 and 100 µg the appearance of abnormal prematurely formed antral follicles was noted, first at twelve hours and then at twenty four hours and they became very noticeable forty eight hours after injection. When the dose of FSH, B-1 was increased to 75 and 100 µg there was an increase in the number of follicles with abnormal antra, to the extent that twenty four hours after injection all follicles at Stage 3 or more showed an irregular antrum formation (Fig. 42). Mitotic figures were common in these follicles which also contained a large amount of RNA with no evidence of lipid in their granulosa cells.

At twenty four hours, with 75 and 100 µg of FSH, B-1, a few large follicles with a large cystic antrum were present and they showed a well defined thecal formation (Figs. 43 and 44). In some of these follicles the granulosa cells showed early signs of degeneration. At forty eight hours, with 75 and 100 µg of FSH, B-1, some of the medium sized antral follicles had a rather thin granulosa layer and a disproportionately large antrum. Also, at forty eight hours, many empty spaces lined by a single layer of cubical cells were present here and there in the cortex of the ovaries. These spaces were about the size

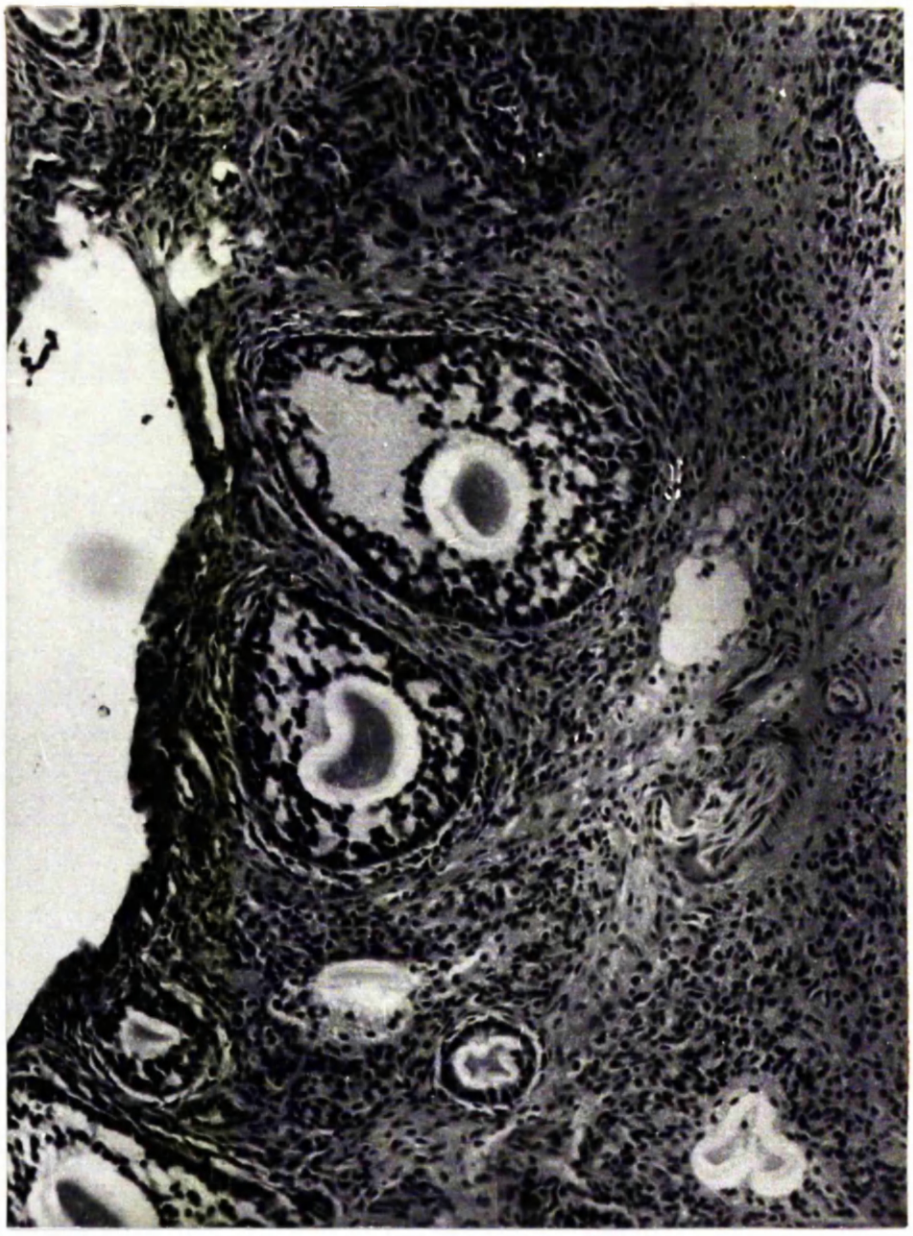


Fig. 42

This shows the numerous spaces in the granulosa of follicles of various sizes indicating rapid and irregular antral formation. These spaces occurred in animals receiving FSH. In this instance 24 hours after 75 µg FSH. H. & E. X 120.

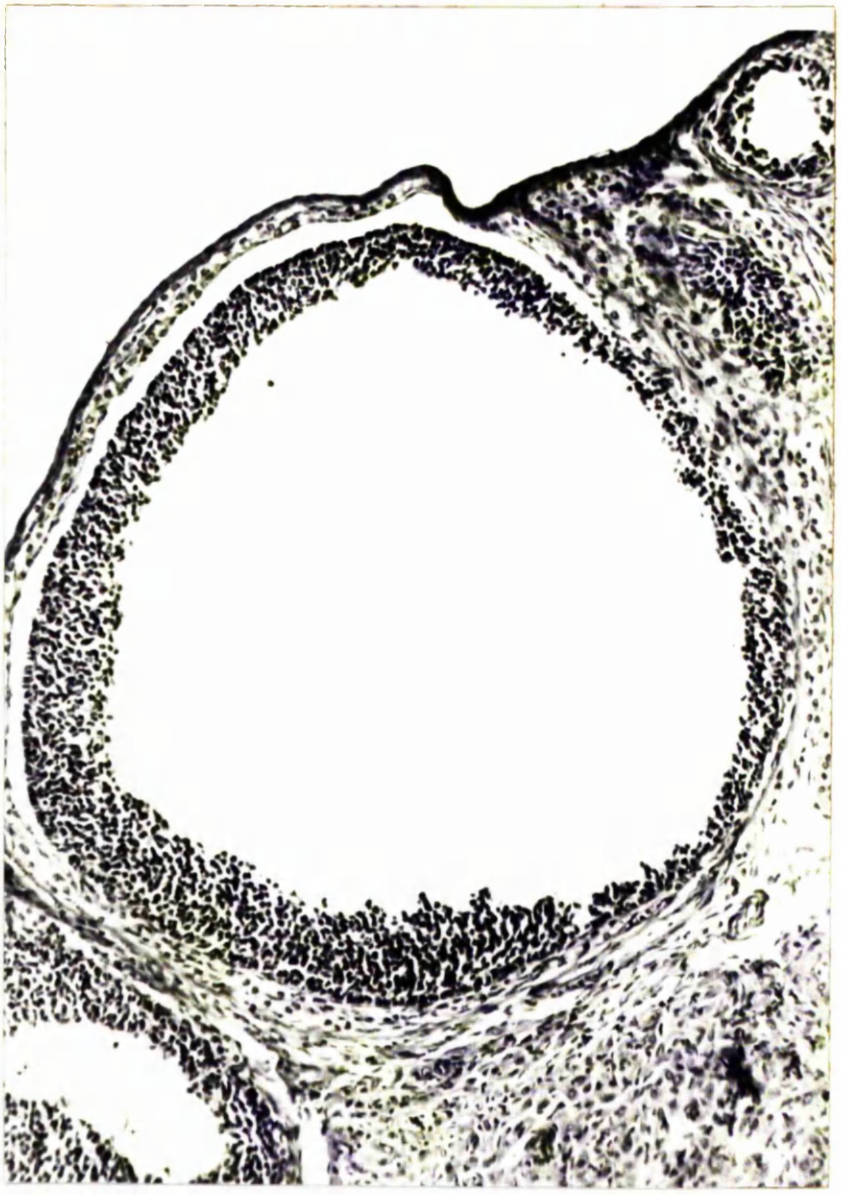


Fig. 43

Low power view of a large sized follicle from the ovary of a hypophysectomized animal 48 hours after receiving 100 µg FSH. A definite theca has formed and the interstitial tissue is healthier in appearance. H. & E. X 120.

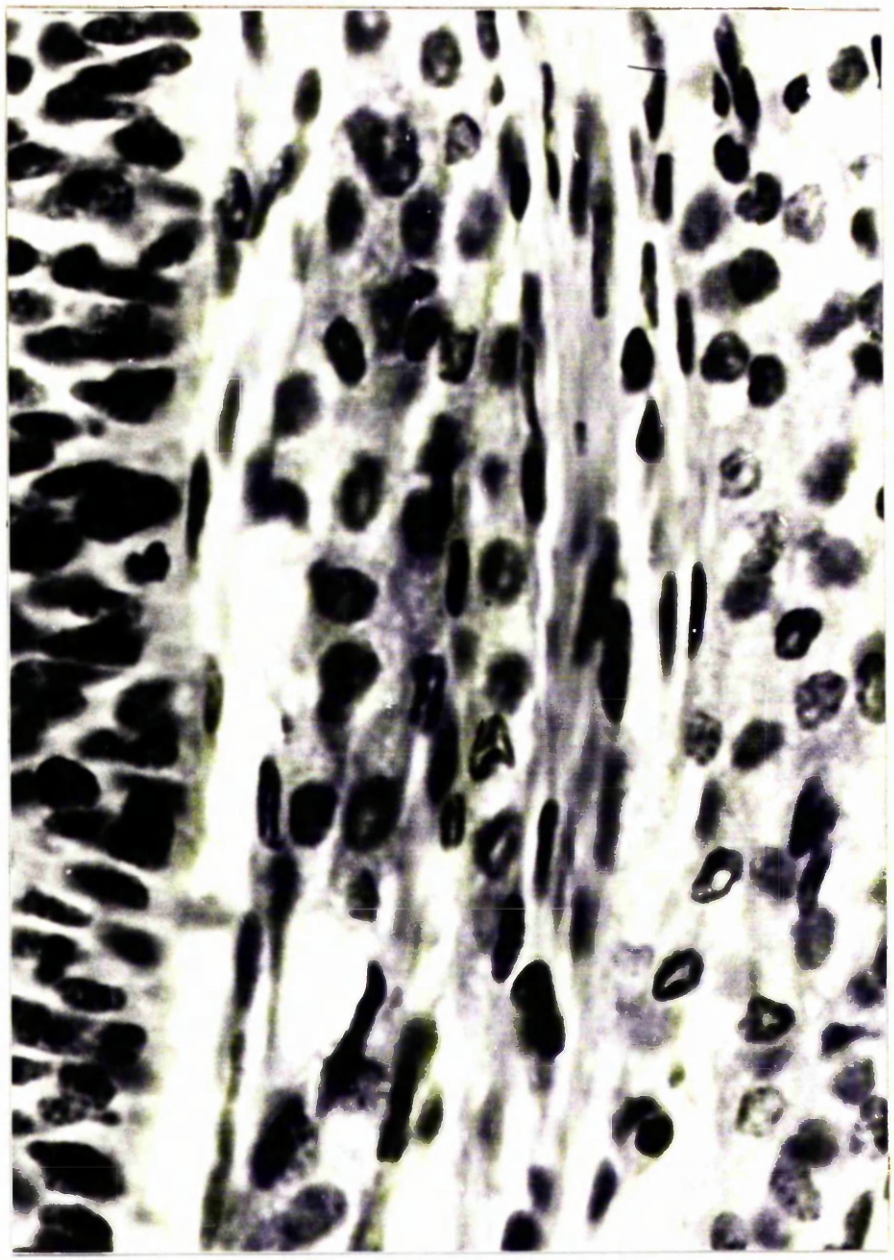


Fig. 44

High power view of the granulosa, theca and interstitial tissue seen in the previous low power figure. H. & E. X 600.

of primordial follicles and it was difficult to be sure if they actually represented the remains of primordial follicles from which oocytes have disappeared.

Thecal Formation

There was no sign of thecal formation with 25 µg of FSH, B-1 at one or six hours after injection. At twelve and twenty four hours, large pre-antral follicles acquired a thin concentric arrangement of thin spindle-type cells which did not show any real sign of differentiation.

The first sign of any real thecal activity was seen with 50 µg of FSH, B-1 at twelve, twenty four and forty eight hours, but only around the medium sized antral follicles where the surrounding stromal cells were arranged concentrically in three or four layers. The nuclei of the thecal cells were spindle-shaped. The thecal cells were rich in cytoplasm and in cytoplasmic RNA and contained fine lipid droplets. The reaction for 3β steroid dehydrogenase was weakly positive (Figs. 45 and 46). It is noteworthy that there was very little thecal stimulation around the follicles with abnormal prematurely formed antra. When the dose of FSH, B-1 was increased to 75 µg it caused thecal formation around the degenerate large antral follicles at six hours. At twelve

Fig. 45

Ovary from a hypophysectomized animal 24 hours after receiving 75 μg FSH stained for 3β steroid dehydrogenase. Strong reaction is present in the corpus luteum and interstitial tissue. Between these structures is a follicle showing a slight reaction in the theca layer. X 120.

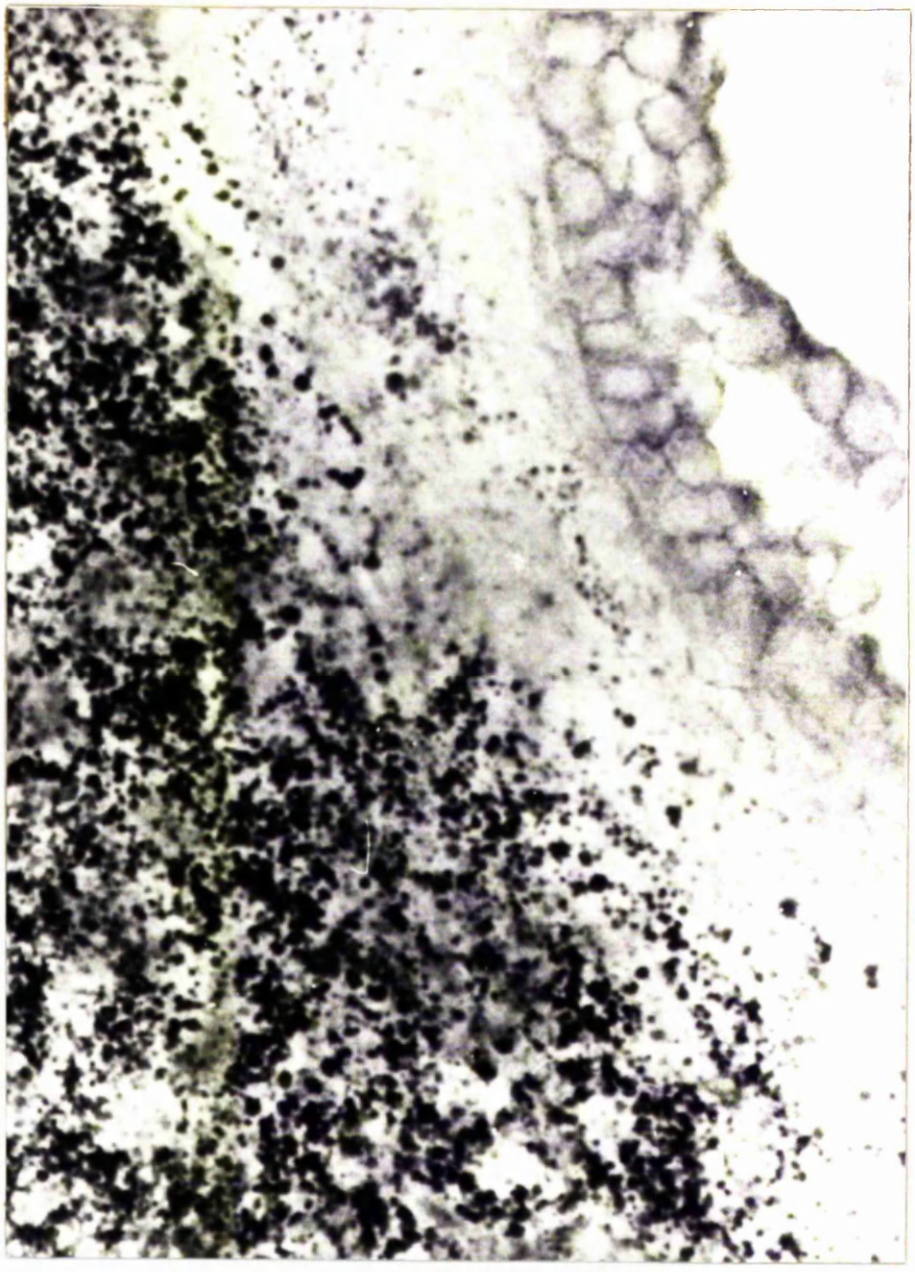


Fig. 46.

High power view of the corpus luteum and follicle seen in the previous figure. The reaction for 3β steroid dehydrogenase is positive in the corpus luteum. There is also a slight positive reaction in the theca of the follicle. 3β steroid dehydrogenase X 600.

hours medium sized antral follicles had acquired several layers of theca. The thecal cells were rich in cytoplasmic RNA and fine lipid droplets and gave a positive reaction for 3β steroid dehydrogenase. At twenty four hours there was marked thecal formation around the medium sized antral follicles. Again, there was no theca around the follicles showing abnormal prematurely formed antra. At forty eight hours the thecal cells had large amounts of large lipid droplets and relatively small amounts of cytoplasmic RNA. When the dose of FSH, B-1 was increased to 100 μg there was no change in the pattern or degree of response of the thecal tissue. The quantitative relationships of thecal stimulation is shown in Table 17.

Dose of FSH, B-1	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 μg	-	-	-	-	-
50 μg	-	-	±	+	+
75 μg	-	±	+	++	++
100 μg	-	±	+	++	++

Table 17. Follicular Changes - Thecal Stimulation

The effect of varying doses of FSH, B-1 on the thecal formation in both ovaries of two hypophysectomized rats at each time interval.

Effect on the Corpora Lutea

The corpora lutea of rats seven days after hypophysectomy (Animal Model) were reasonably normal and morphologically intact (Fig. 32). These were old corpora lutea as they had a fibrous central core. The cytoplasm of the luteal cells still contained significant amounts of cytoplasmic RNA and a large number of fine and coarse lipid droplets (Fig. 47). The test for 3β steroid dehydrogenase was positive.

The administration of FSH, B-1 was found to maintain and to stimulate the corpora lutea of the hypophysectomized mature female rat.

The administration of 25 µg of FSH, B-1 caused a slight but doubtful increase in the cytoplasmic RNA (Fig. 48) of the luteal cells with a proportionate reduction in the size of lipid droplets in the cytoplasm six hours after injection (Fig. 49). Twelve hours after injection the corpora lutea showed a definite increase in the cytoplasmic RNA of their luteal cells. Intracellular lipid was present in the form of a peppering of fine droplets (Fig. 50). The corpora lutea showed a definite strong reaction for 3β steroid dehydrogenase (Fig. 51). At twenty four and forty eight hours, there were no noticeable changes from those observed at twelve hours.

Although an increase in the dose of FSH, B-1 to

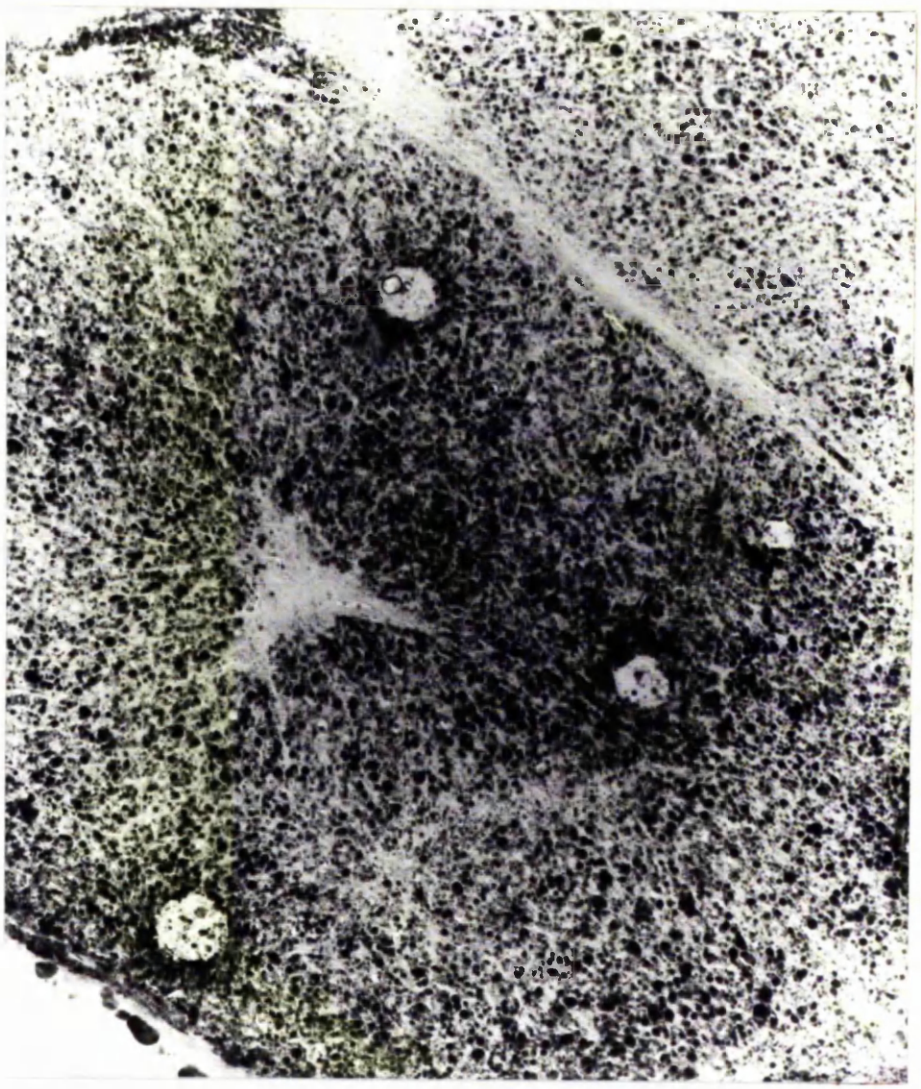


Fig. 47

Corpus luteum seven days post-hypophysectomy stained for 3β steroid dehydrogenase. Note the central core. X 105.

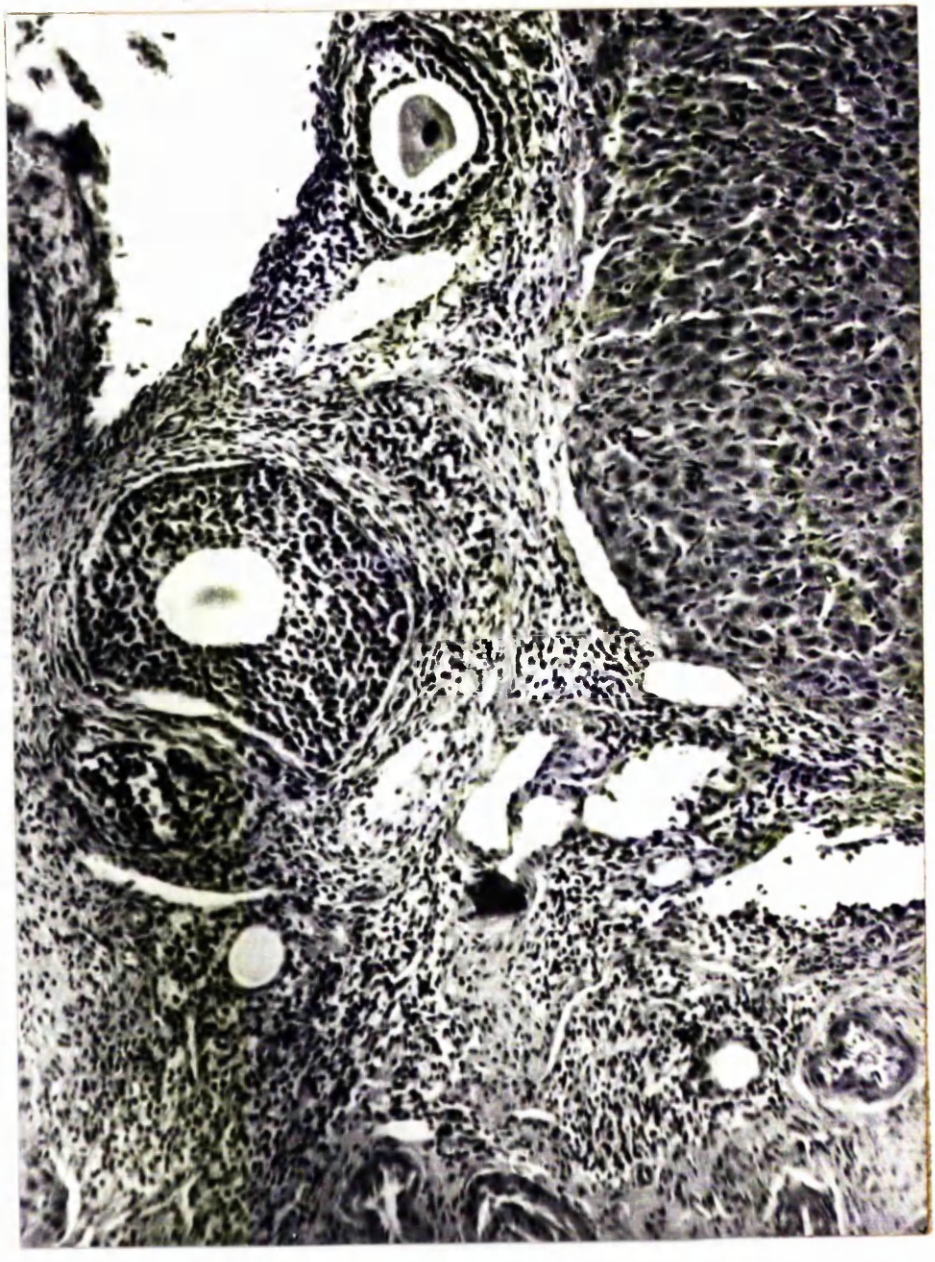


Fig. 48.

Corpus luteum from a hypophysectomized rat 24 hours after the administration of 50 µg FSH. The corpus luteum is histologically normal. Large pre-antral follicles have formed. X 150.

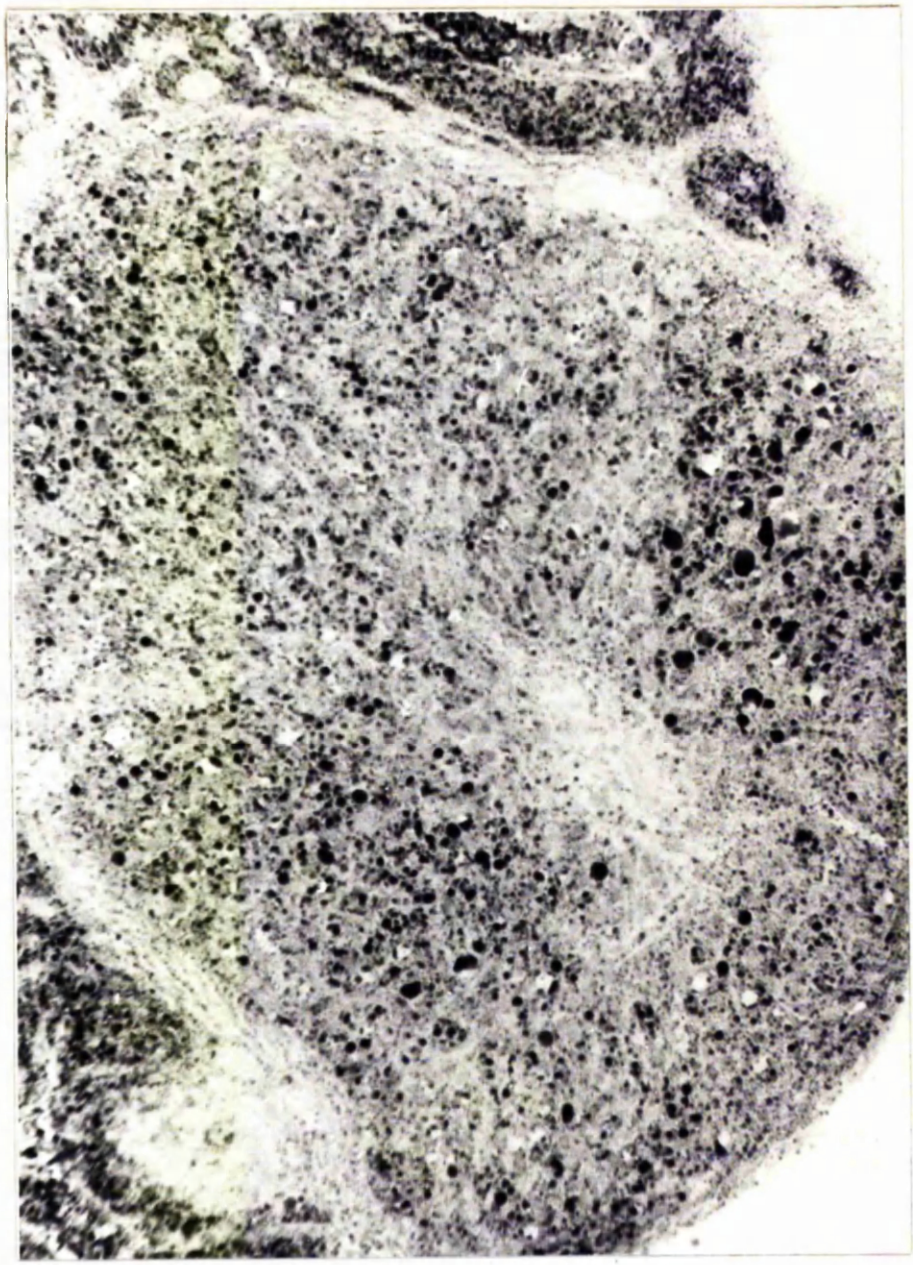


Fig. 49

Corpus luteum of a hypophysectomized rat 6 hours after receiving 25 µg FSH. The amount of lipid is somewhat reduced. Oil Red O X 120.

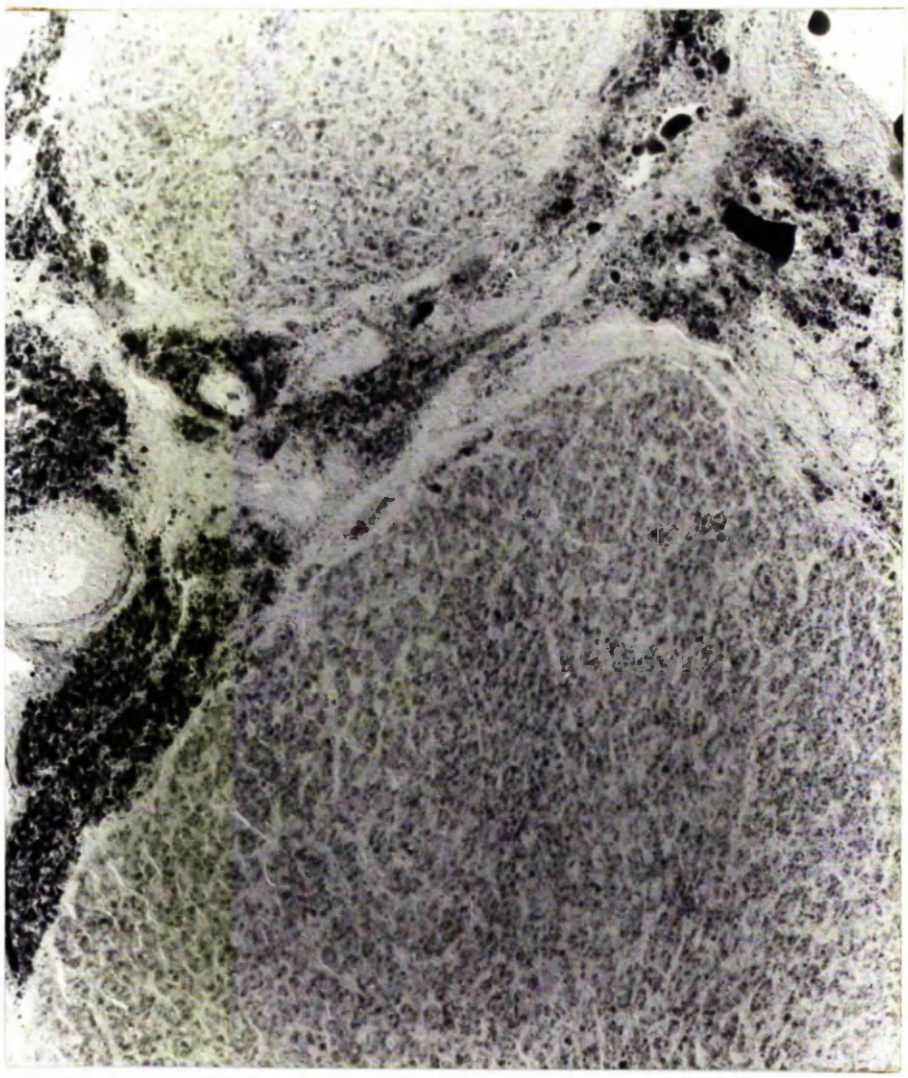


Fig. 50

Corpus luteum and interstitial tissue stained for lipid 12 hours after the administration of 25 µg FSH. The amount of lipid is greatly diminished in the corpus luteum cells but the interstitial cells still have a large amount of fat in the form of coarse globules. Oil Red O X 105.

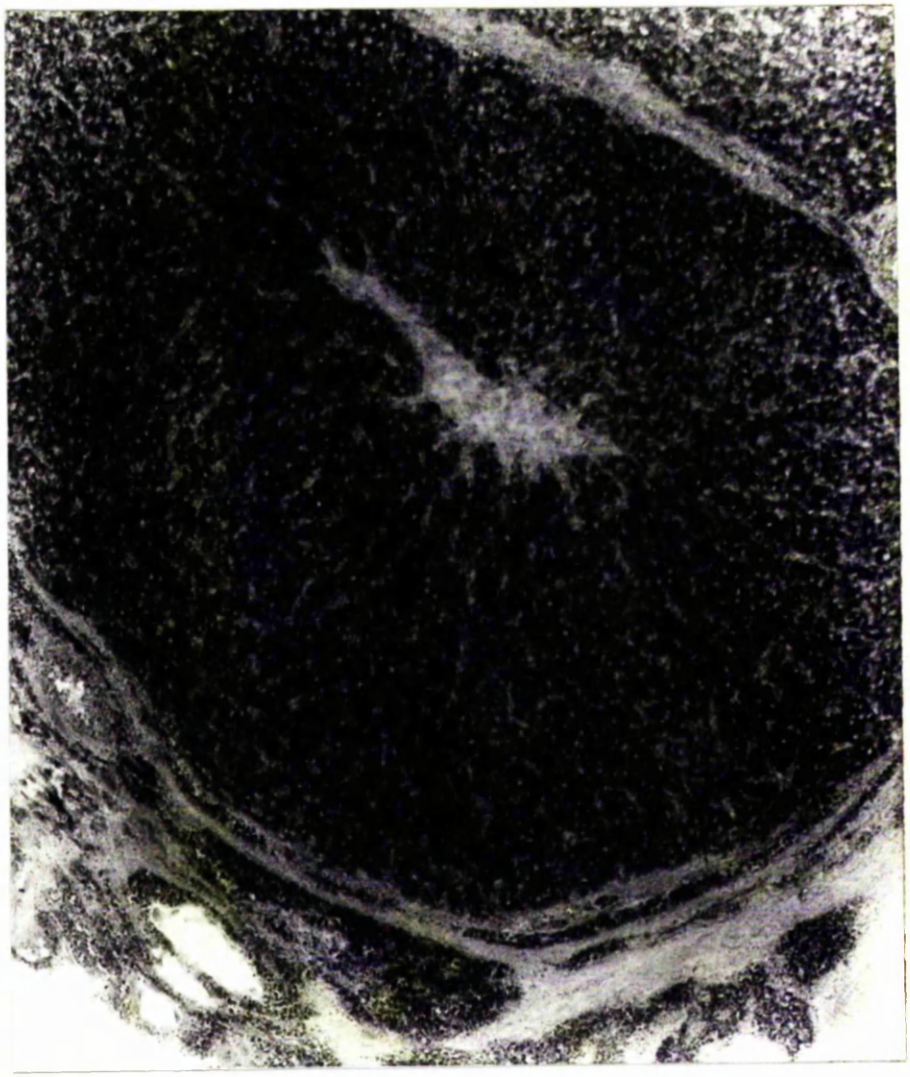


Fig. 51

Corpus luteum from hypophysectomized rat 12 hours after receiving 25 µg FSH. A strong reaction is shown. 3β steroid dehydrogenase X 105.

50, 75 and 100 µg did not change the above pattern it did however intensify the degree of response to the administration of FSH, B-1 by increasing the amount of cytoplasmic RNA and reducing further the amount of intracellular lipid (Fig. 52). The peak of activity was twenty four hours after injection; there was no change at forty eight hours. The relative changes in histochemical reactions are shown in Table 18.

Effect on the Interstitial Tissue

Seven days after hypophysectomy, the ovarian interstitial tissue of the hypophysectomized mature female rat had the appearance of a fatty areolar tissue rather than a steroidogenic structure (Figs. 28, 29, 31).

FSH, B-1 was found to cause stimulation of the interstitial tissue of the hypophysectomized female rat, varying with the dose and time.

After the administration of 25 µg FSH, B-1, no constant change was observed at any time interval. The cells still retained their degenerate appearance (Figs. 53 and 54). This contrasts with the definite changes noted in the corpora lutea twelve hours after an injection of 25 µg FSH, B-1 (see Fig. 50).

A slight change was noted twelve hours after the injection of 50 µg FSH, B-1, when the interstitial

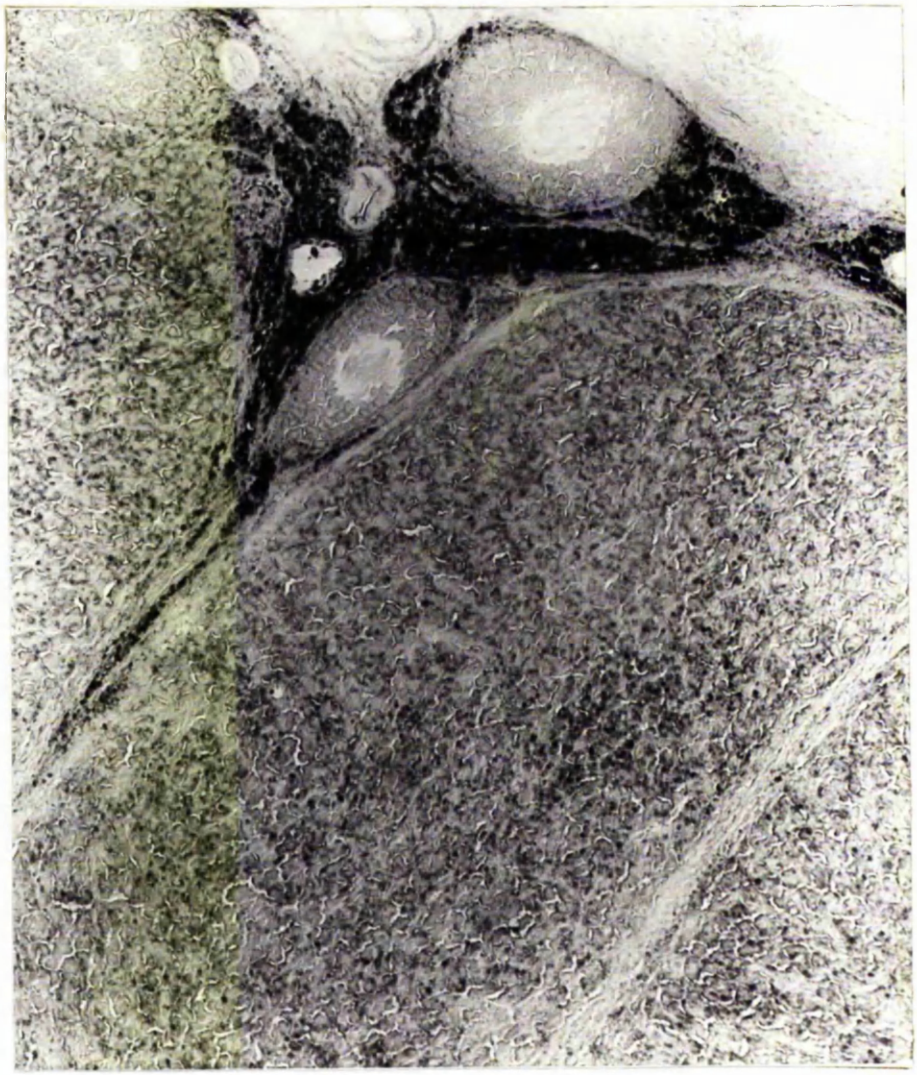


Fig. 52

The ovary of a hypophysectomized animal 12 hours after receiving 75 µg FSH. Very little lipid is present in the corpus luteum but some interstitial cells still contain coarse globules. Oil Red O X 105.

Hypophysectomized Rat + FSH, B-1	Histochemical Methods		
	Pyronin M.Green	Oil Red O	3β steroid Dehydrogenase
25 µg FSH, B-1			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	++	++	++
24 hours	++	+	++
48 hours	++	++	++
50 µg FSH, B-1			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	++	++	++
24 hours	++	+	++
48 hours	++	++	++
75 µg FSH, B-1			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	+++	++	++
24 hours	+++	+	+++
48 hours	+++	+	+++
100 µg FSH, B-1			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	+++	+	+++
24 hours	+++	+	+++
48 hours	+++	+	+++

Table 18 Corpora Lutea

The effect of varying doses of FSH, B-1 on the corpora lutea of both ovaries of two hypophysectomized rats at each time interval.

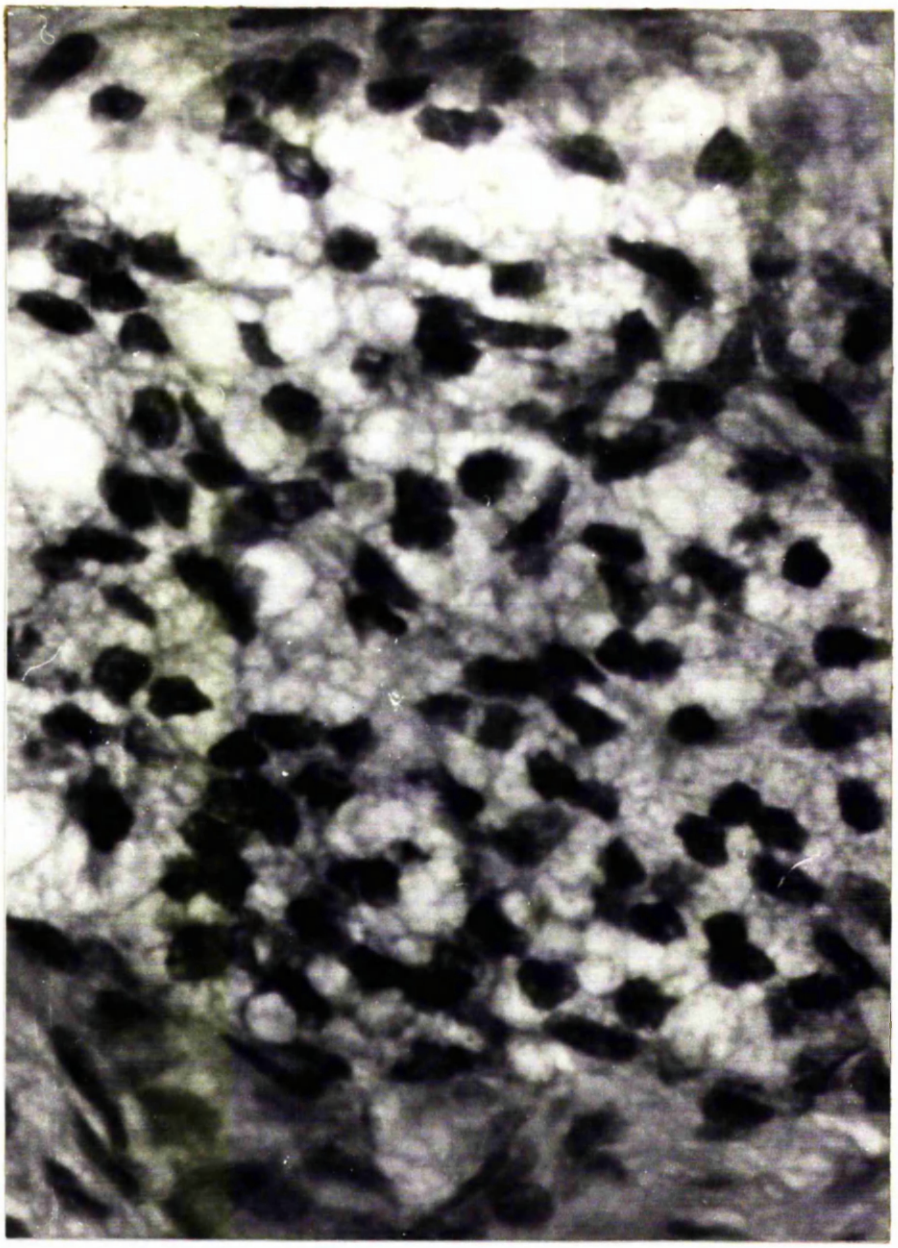


Fig. 53

Interstitial cells of an ovary of a hypophysectomized animal 24 hours after receiving 25 µg FSH. The nuclei are still irregular with clumped chromatin and the cytoplasm grossly vacuolated. H. & E. X 600.

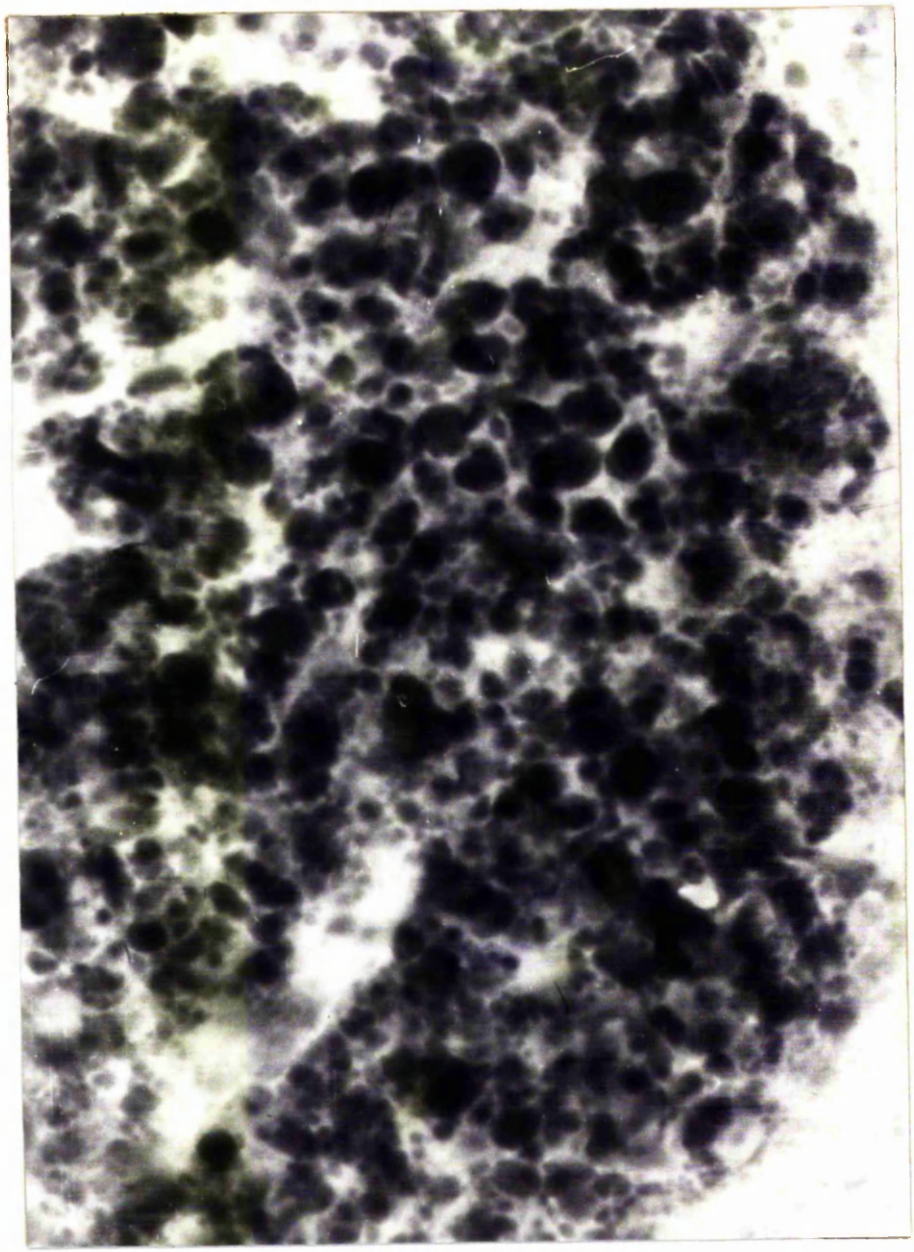


Fig. 54

Interstitial tissue of same ovary stained by Oil Red O. A heavy coarse lipid deposit is present in the cells. X 600.

cells were more prominent and less crowded. They had increased in size; nuclei were larger and more transluscent. Slight cytoplasmic basophilia was noted in some of the cells. Cytoplasmic RNA was present and the cytoplasm gave a weakly positive reaction for 3β steroid dehydrogenase. The interstitial tissue cells were more prominent around the large degenerate cystic follicles forming a mantle around them.

At twenty four hours the interstitial tissue appeared to have increased in amount but this was probably due to the swelling of individual cells which now had more cytoplasm. There was a considerable amount of cytoplasmic RNA with a noticeable reduction in the size and number of lipid droplets in the cytoplasm. The interstitial tissue cells gave a positive reaction for 3β steroid dehydrogenase. At forty eight hours there was a reduction in the amount of the cytoplasmic RNA in the interstitial tissue cells.

While increasing the dose of FSH, B-1 to 75 and 100 µg did not fundamentally change the above pattern, the intensity of stimulation was increased when the dose was increased (Fig. 55). There was stimulation of very few interstitial tissue cells six hours after the injection of 75 µg FSH, B-1. In contrast to all dose schedules at forty eight hours, the interstitial tissue cells continued to show the presence of RNA (no tailing off of activity) after the injection of 100 µg FSH, B-1. The degree of interstitial cell

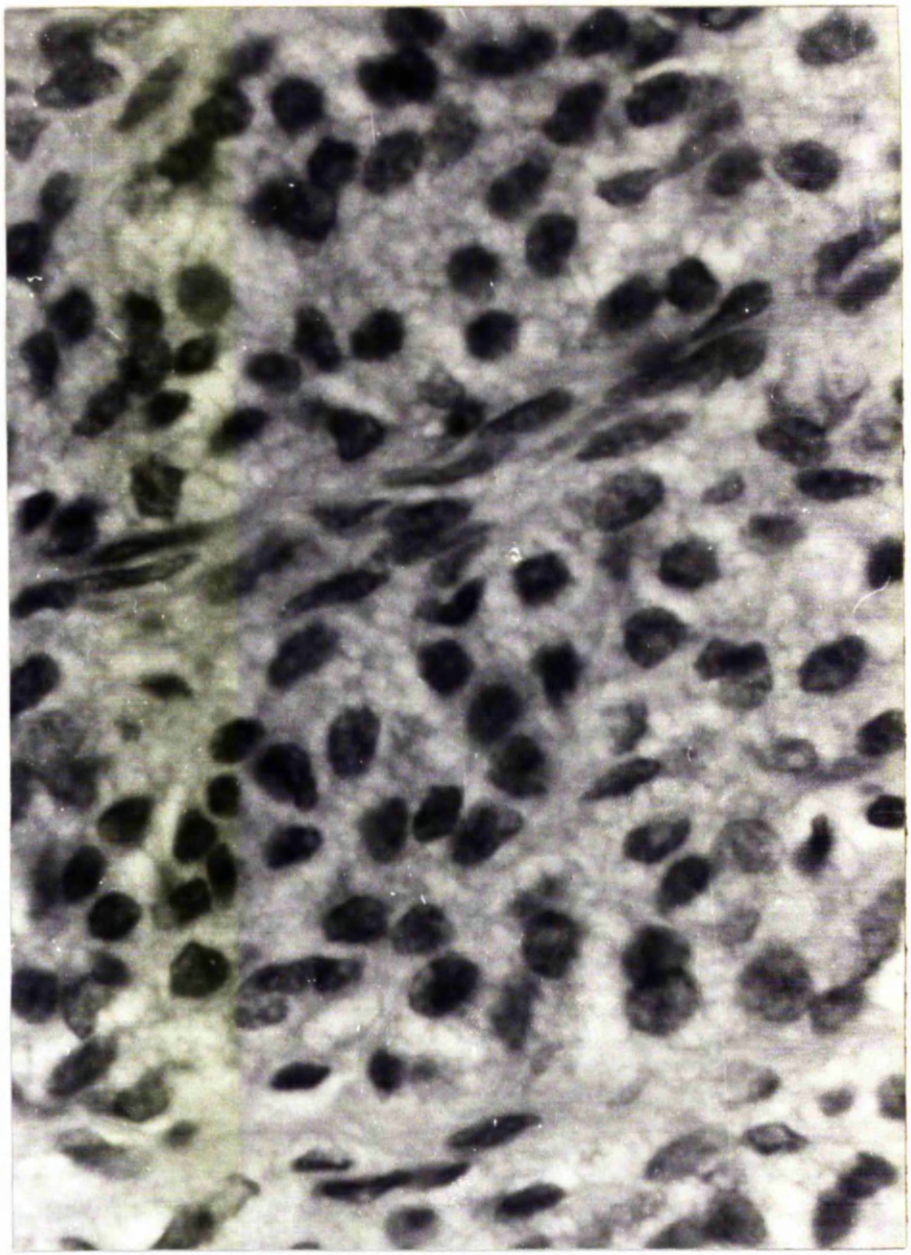


Fig. 55

High power view of interstitial cells 48 hours after the administration of 75 µg FSH. The cells now look healthier with larger nuclei showing dispersed chromatin and the cytoplasm is fully vacuolated. H. & E. X 600.

repair in relation to the dose of FSH is shown in Table 19 and the histochemical changes are tabulated in Table 20.

EFFECT OF FSH, B-1 ON THE UTERINE HISTOLOGY

With the administration of FSH, B-1 there was a detectable dilation of the lumina of the cornuae of the uterus (Fig. 56).

Histologically the endometrium was slightly thickened mainly due to oedema of the stroma. To some extent the glands were enlarged but there was only a little mitotic activity.

Dose of FSH, B-1	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg	-	-	-	-	-
50 µg	-	-	+	++	+
75 µg	-	±	+	++	+
100 µg	-	±	+	++	++

Table 19. Interstitial Tissue Cell Repair

The effect of varying doses of FSH, B-1 on the interstitial tissue of both ovaries of two hypophysectomized rats at each time interval.

Hypophysectomized Rat + FSH, B-1	Histochemical Methods		
	Pyronin M.Green	Oil Red O	3β steroid Dehydrogenase
25 µg FSH, B-1			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	-	+++	-
24 hours	-	+++	-
48 hours	-	+++	-
50 µg FSH, B-1			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	+	++	+
24 hours	++	+	++
48 hours	+	++	+
75 µg FSH, B-1			
1 hour	-	+++	-
6 hours	±	+++	-
12 hours	+	++	+
24 hours	++	+	++
48 hours	+	++	+
100 µg FSH, B-1			
1 hour	-	+++	-
6 hours	±	+++	-
12 hours	+	++	+
24 hours	++	+	++
48 hours	++	+	++

Table 20. Interstitial Tissue

The effect of varying doses of FSH, B-1 on the interstitial tissue of both ovaries of two hypophysectomized rats at each time interval.

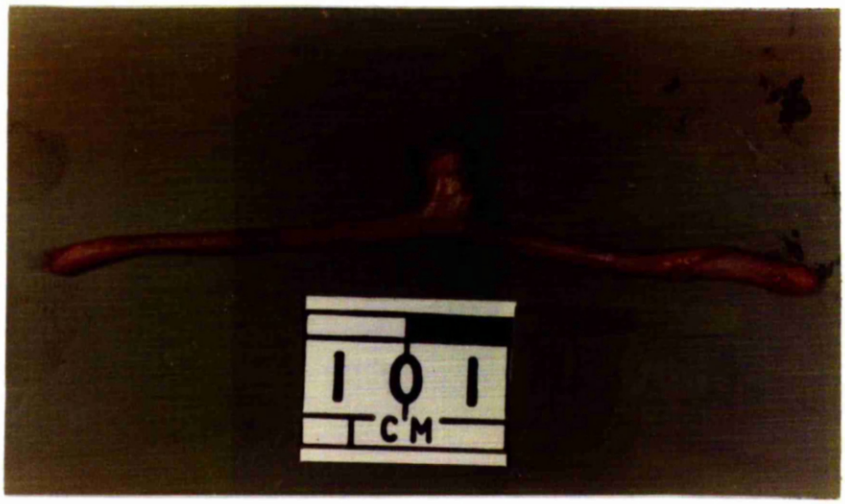


Fig. 56

Uterus of a hypophysectomized rat removed 24 hours after receiving 75 µg of FSH. The ovaries had been dissected off and fixed for histology. There is enlargement of the uterine horns not quite equal to that seen at oestrus.

THE EFFECT OF LH (IRC, 24.6.69) ON HYPOPHYSECTOMIZED MATURE FEMALE RATS

EFFECT OF LH ON THE OVARIAN WEIGHT

There was some increase in the ovarian weight following the administration of LH and as with FSH the greatest increase occurred in the early part of the experimental period (Fig. 57). There was some fall in weight during the second twenty four hour period, unlike FSH which produced a continuing response at this time. In addition the peak increase was less than half that produced by FSH alone (Figs. 57 and 34). Comparing the results at the twenty four hour interval (Fig. 34) indicates the modest degree of increase. (see Appendix).

Statistical analysis of the summated ovarian weights of all animals given a particular dose of LH and sacrificed during the first twenty four hours showed that none of the increases in weight were significant. (Tables 21 and 22).

EFFECT OF LH ON THE UTERINE WEIGHT

There was a very slight increase in the uterine weight but the change was of no significance (Fig. 58 and Tables 23 and 24). (see Appendix).

EFFECT OF LH ON THE OVARIAN HISTOLOGY

Following the administration of LH certain changes appeared in the ovaries. There was no evidence of proliferation of any new follicles (Fig. 59) but the general impression was that LH does help to some

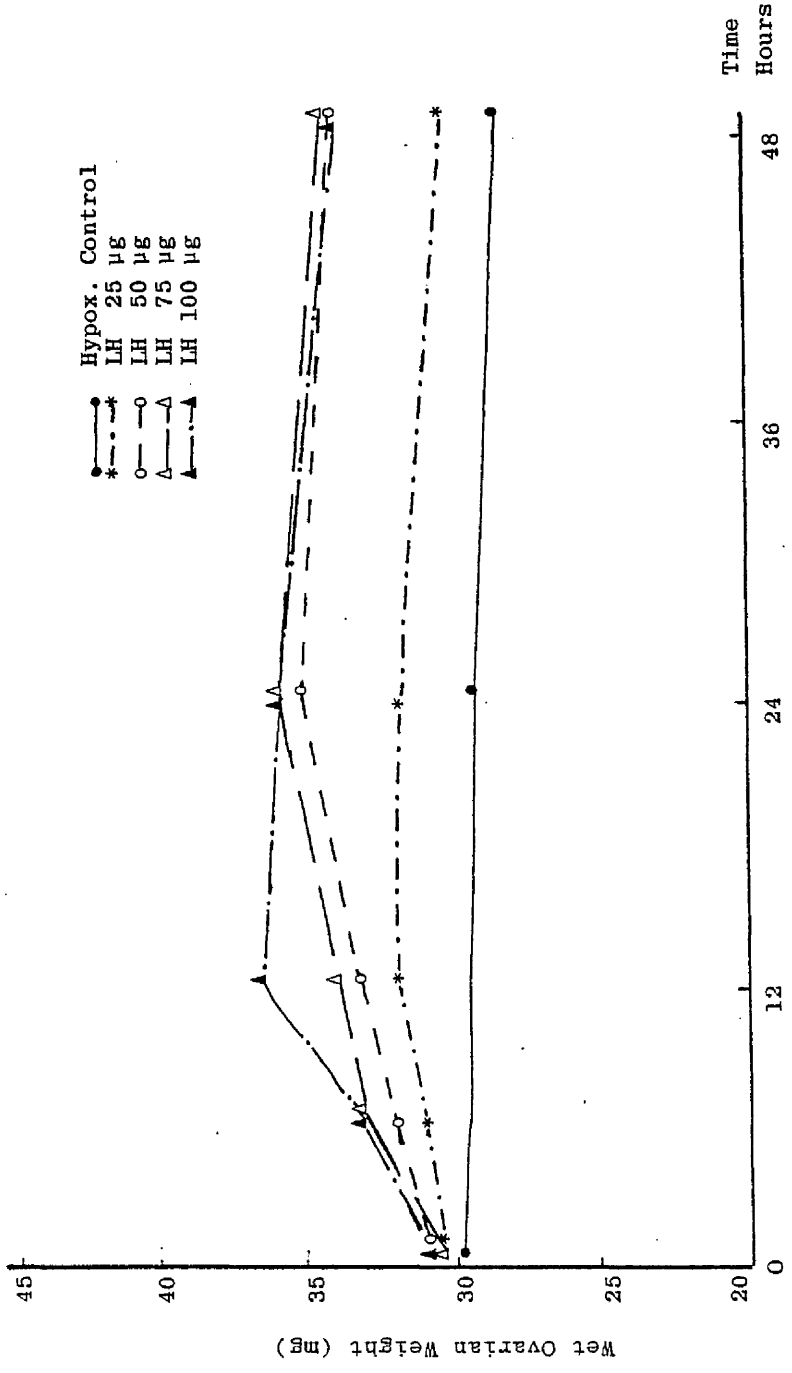


Fig. 57 The effect of varying doses of LH (IRC, 24.6.69) on the ovarian weight of two hypophysectomized mature female rats at each time interval.

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.0581	0.0003
Hypox. Control	8	0.0327	0.0029
Hypox. Treated + 25 µg LH	8	0.0318	0.0013
+ 50 µg LH	8	0.0330	0.0014
+ 75 µg LH	8	0.0338	0.0017
+100 µg LH	8	0.0343	0.0019

Table 21

The mean and standard deviation of the ovarian weight of hypophysectomized rats treated with LH (IRC,24.6.69) compared with intact and hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg LH	14	0.7493	N.S.
Hypox. Control vs. 50 µg LH	14	0.2465	N.S.
Hypox. Control vs. 75 µg LH	14	0.8658	N.S.
Hypox. Control vs. 100 µg LH	14	1.2210	N.S.

N.S. = Not Significant

Table 22

Comparison of the ovarian weights of the hypophysectomized control group and the hypophysectomized rats treated with various doses of LH using t. test.

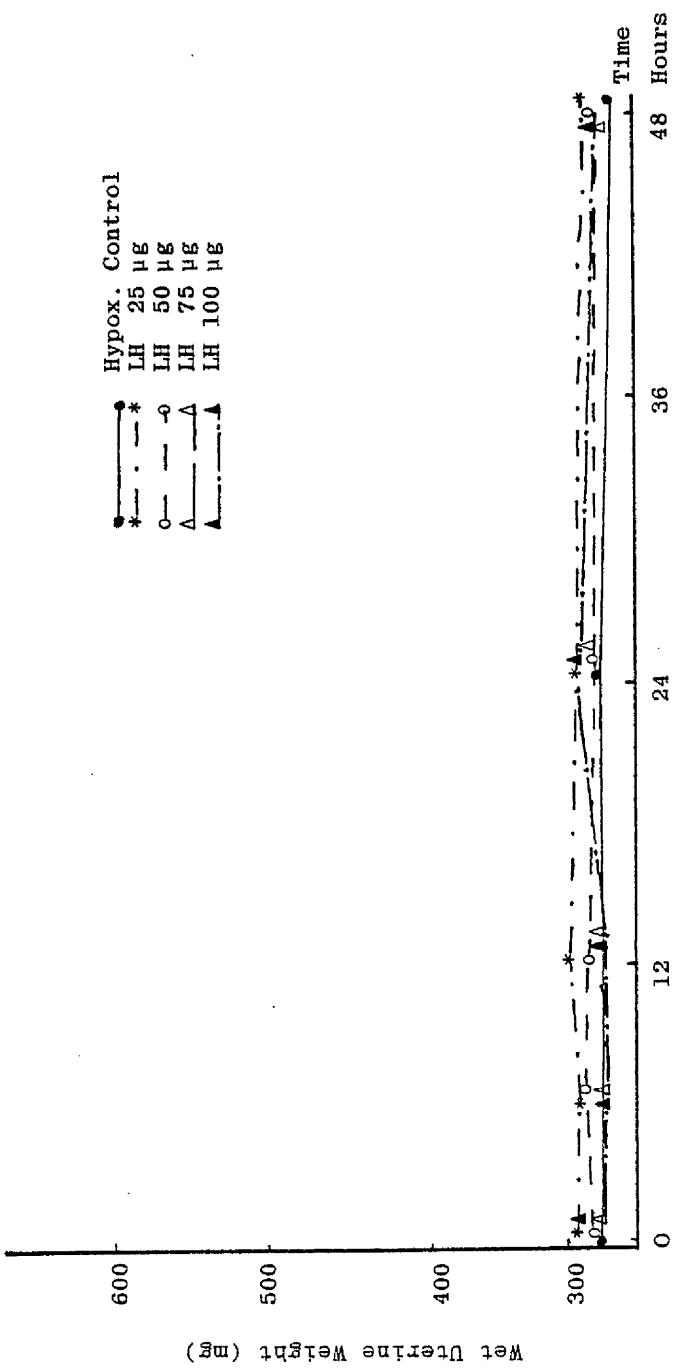


Fig.58 The effect of varying doses of LH (IRC, 24.6.69) on the uterine weight of two hypophysectomized mature female rats at each time interval.

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.3802	0.0081
Hypox. Control	8	0.2951	0.0149
Hypox. Treated + 25 µg LH	8	0.2925	0.0069
+ 50 µg LH	8	0.2871	0.0024
+ 75 µg LH	8	0.2821	0.0175
+100 µg LH	8	0.2889	0.0008

Table 23

The mean and standard deviation of the uterine weight of hypophysectomized rats treated with LH (IRC,24.6.69) compared with intact and hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg LH	14	0.4189	N.S
Hypox. Control vs. 50 µg LH	14	1.4025	N.S
Hypox. Control vs. 75 µg LH	14	1.4965	N.S
Hypox. Control vs. 100 µg LH	14	1.0993	N.S.

N.S. = Not Significant

Table 24

Comparison of the uterine weights of the hypophysectomized control group and the hypophysectomized rats treated with various doses of LH using t. test.

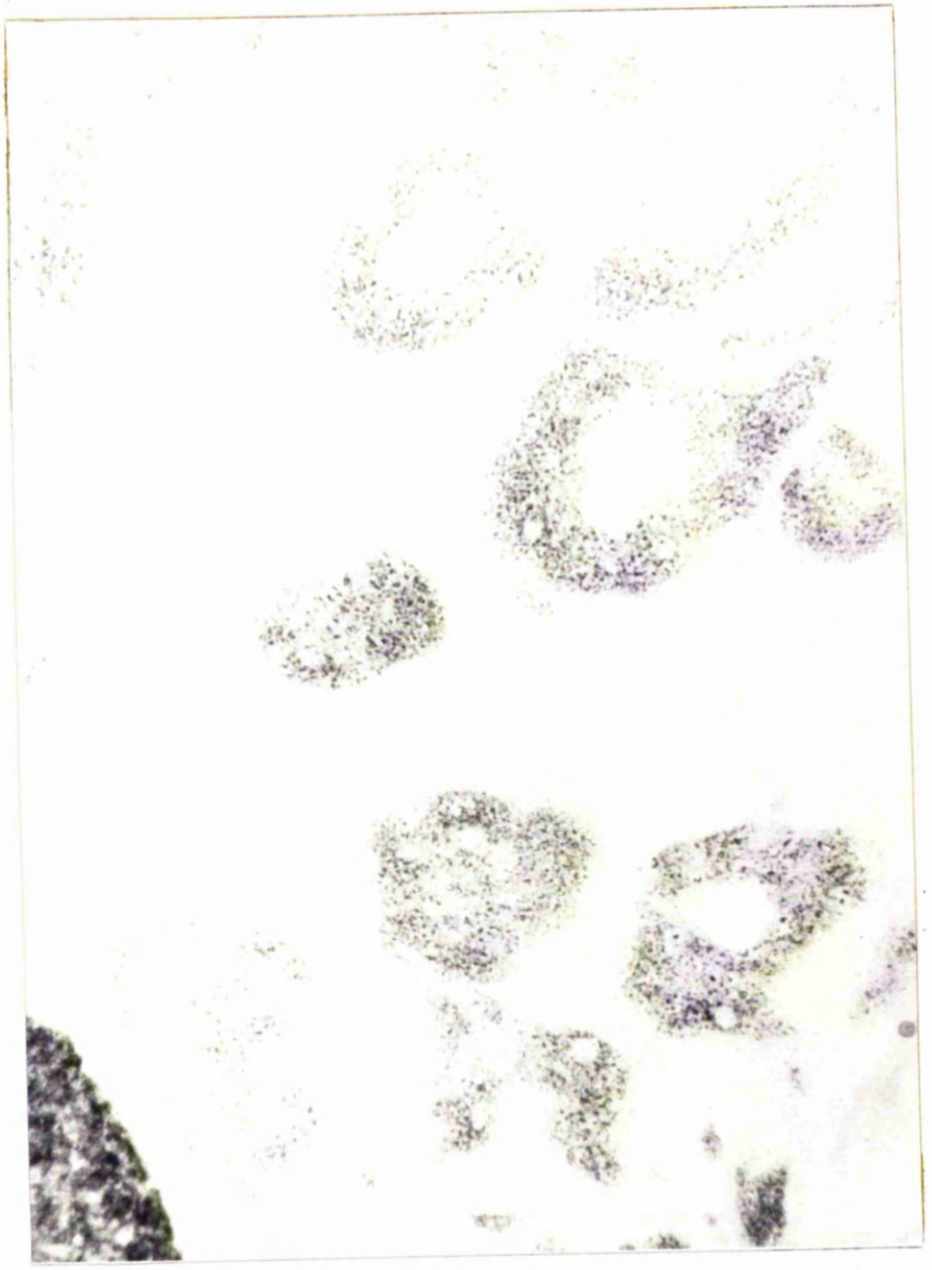


Fig. 59

A low power view of the ovary of an animal 24 hours after receiving 50 μg LH stained for 3β steroid dehydrogenase. Positive reactions are shown in the thecal tissue, the interstitial tissue and a corpus luteum the edge of which is at the lower left hand corner of the illustration. The absence of intact follicular structures should be noted. X 120.

extent in the repair of the damage inflicted by hypophysectomy on the corpora lutea and the interstitial tissue. An important yet not completely understood function is its possible help in the clearance of atretic follicles (an effect which is dose and time related).

The following is a detailed description of the effect of a highly purified preparation of human LH on the ovaries of the hypophysectomized mature female rat.

Effect on the Follicles

LH had no stimulatory effect on the ovarian follicular system of the hypophysectomized mature female rat with any of the dose schedules. It did not affect the number of small pre-antral follicles. Indeed, six and twelve hours after the administration of 75 and 100 μg the granulosa cell layer of small pre-antral follicles showed early degenerative changes, indicating that LH in these doses may have an inhibitory effect on these pre-antral follicles, either directly or indirectly. By the end of forty eight hours with the larger doses of LH all antral follicles were grossly atretic and their sites were indicated by round spaces surrounded by a thick layer of theca-like tissue. The cells of this tissue had large vesicular nuclei and abundant cytoplasm containing a fair amount of cytoplasmic RNA. Lipid was present in the form of fine droplets. Some 3β

steroid dehydrogenase was present in the thecal cells (Fig. 59). These changes in the thecal cells were apparent with all LH dose schedules except those of 25 µg and at all time intervals except those of one and six hours. They were most marked with the larger doses and reached a peak at forty eight hours. The thecal cells however were not fully luteinised.

In most sections of the ovaries of the hypophysectomized animals there were a few atretic antral follicles. The administration of LH appeared to increase the atretic changes (Fig. 60) but more important was the increased prominence of the thecal layer around these follicles (Figs. 61 and 62), particularly the larger follicles.

A study of the influence of LH on the follicle count of the ovaries of the hypophysectomized mature female rats showed no evidence of any increase in the number of follicles beyond Stage 2 and it was therefore impossible to apply any form of analysis to the results.

Effect on the Corpora Lutea

As previously stated the corpora lutea of the hypophysectomized mature female rat appeared relatively normal seven days after the operation. The luteal cells had considerable cytoplasm with abundant RNA, contained fine and coarse lipid droplets and had a moderately strong reaction for 3β steroid dehydrogenase.

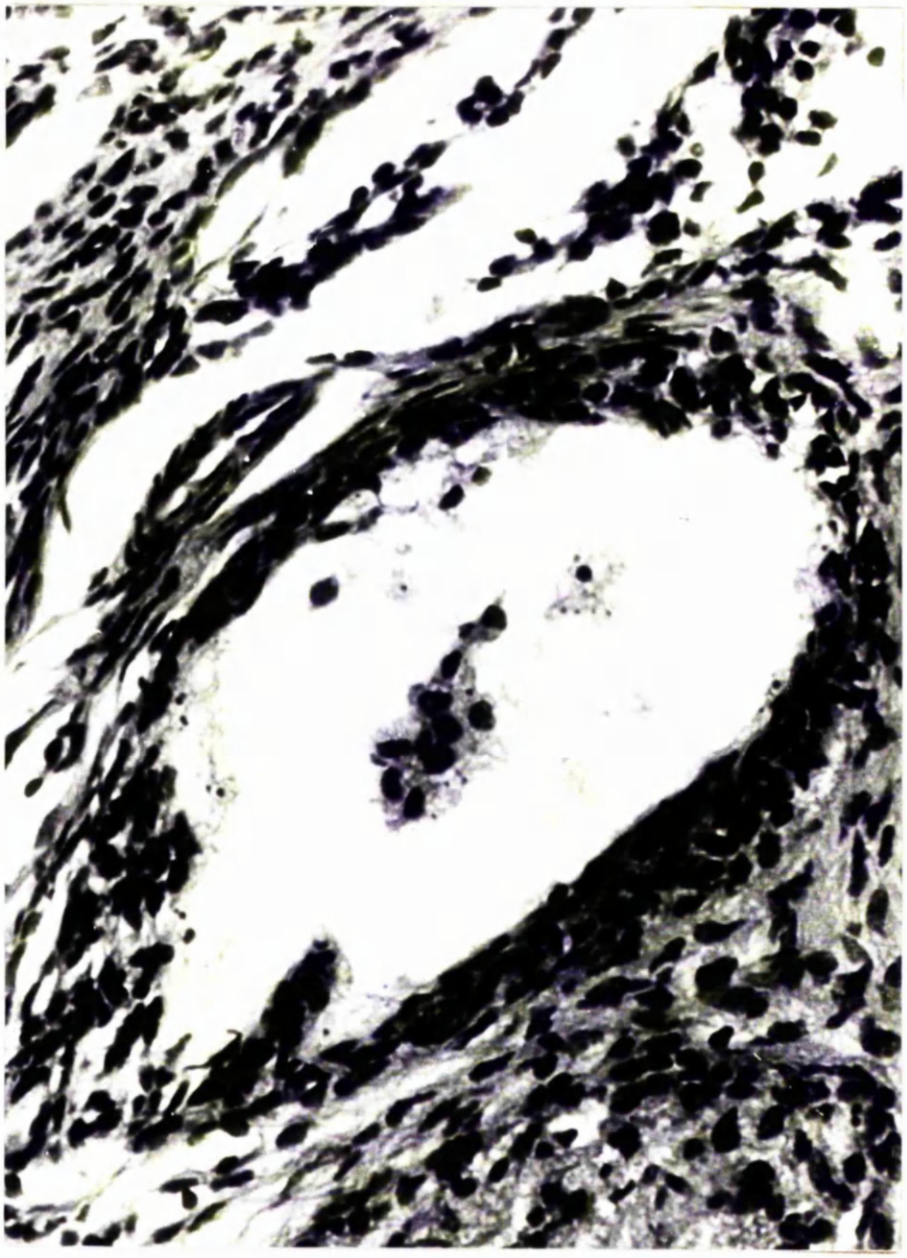


Fig. 60

A small antral follicle of a hypophysectomized rat 12 hours after receiving 50 µg LH. Macrophage type cells are present in the antral space; granules of chromatin can be seen and the granulosa cells are irregular in shape with pyknotic nuclei of varying size and shape. H. & E. X 300.

Fig. 61

Antral follicle of a hypophysectomized rat 48 hours after receiving 50 µg LH. There is a prominent thecal layer. H. & E. X 300.

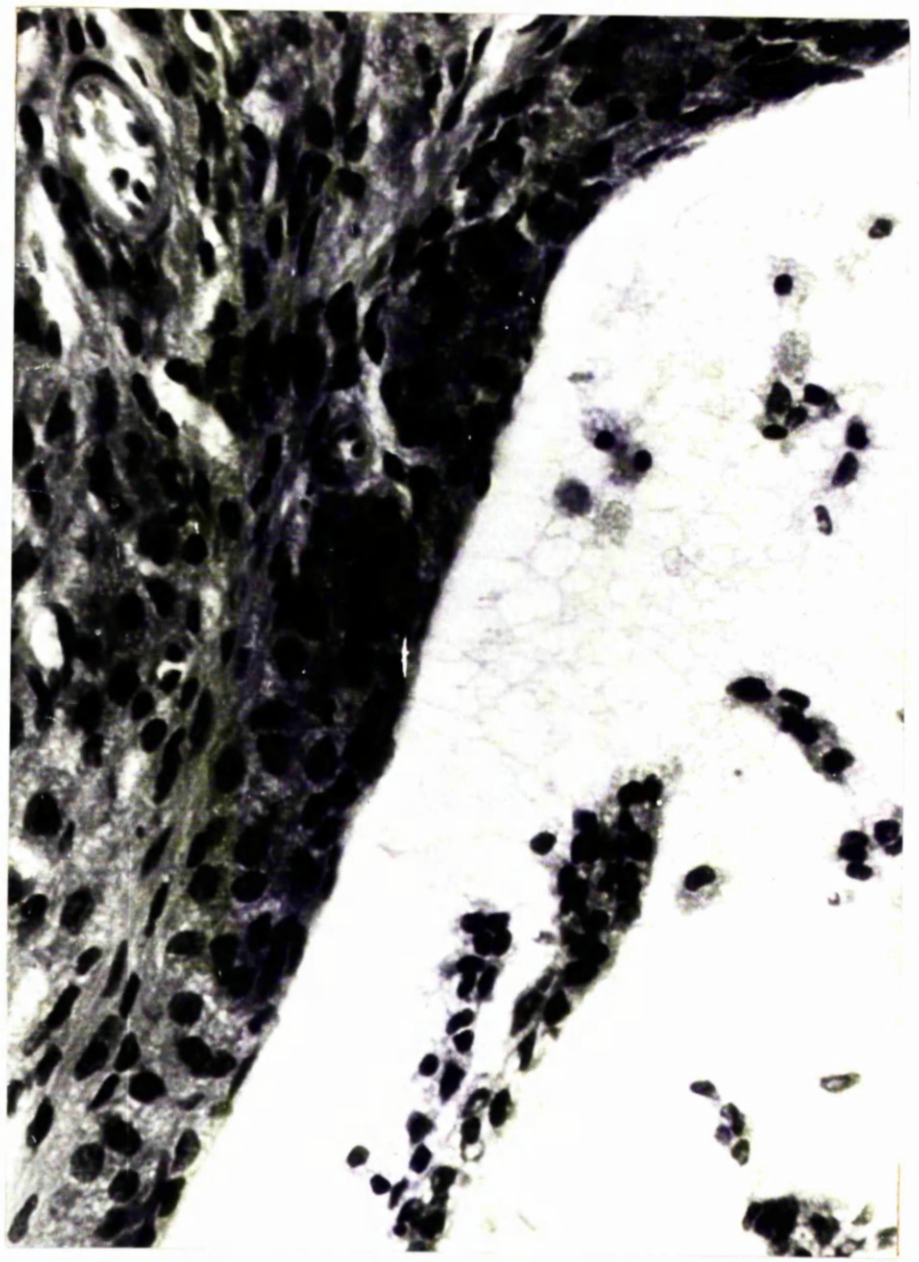


Fig. 62

High power view of follicle in previous illustration showing the thecal cells with abundant finely vacuolated cytoplasm. H. & E. X 600.

The ovaries of the injected animals showed hyperaemia. This was obvious with doses above 25 µg LH and at intervals of twelve and twenty four hours after injection. By forty eight hours, however, it had faded. Microscopically it was found that the hyperaemia was particularly noticeable in and around the corpora lutea, with the appearance of blood filled sinusoids among the luteal cells (Fig. 63). The effect appeared within twelve hours of the injection of 50 µg LH but this reaction time was reduced to six hours when the dose was increased to 75 and 100 µg LH. While increasing the dose of LH caused intensification of the hyperaemia effect of LH at twelve and twenty four hours the vascular dilation disappeared by forty eight hours even in the larger dose schedules. The congested corpora lutea were larger (0.9-1.1mm) than those of the untreated control animals (0.7-1.0mm). The comparative effects of the various doses are shown in Table 25.

The administration of 25 µg LH at all time schedules had little effect on the histological picture of the corpora lutea. At six hours, LH in all higher dose schedules caused an apparent increase in the amount of cytoplasmic RNA in the luteal cells and, at the same time, caused a reduction in the total amount of lipid. In these cells, the lipid droplets appeared finer with the increase in the dose and time and this effect reached its maximum at twenty four hours. By

Fig. 63

Corpus luteum of a hypophysectomized rat 24 hours after receiving 50 µg LH. Note the greatly dilated sinusoids. H. & E. X 300.

Dose of LH (IRC,24.6.69)	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg	-	-	-	-	-
50 µg	-	-	±	++	-
75 µg	-	±	+	++	-
100 µg	-	+	+	++	-

Table 25 - Ovarian Vascularity

The effect of varying doses of LH (IRC,24.6.69) on the vascularity of both ovaries of two hypophysectomized rats at each time interval.

forty eight hours, however, in all instances there was a significant reduction in cytoplasmic RNA, an increase in the size of lipid droplets and an increase in the total cytoplasmic lipid. There was a relative increase in 3β steroid dehydrogenase reaction in the luteal cells with increasing time and dose schedules (Table 26 and Fig. 64).

Effect on the Interstitial Tissue

LH in dose schedules of 25 and 50 µg had no effect on the microscopic appearance of the interstitial tissue but increasing the dose to 75 and 100 µg induced reparative changes (Table 27). There was no reaction one hour after the administration of these doses but after six hours some of the interstitial tissue cells showed an increase in size with slightly larger nuclei and an increase in cytoplasm containing small sized lipid droplets. Cytoplasmic RNA was, if anything, increased compared with the hypophysectomized controls and the 3β steroid dehydrogenase was doubtfully positive. At twelve hours, the interstitial tissue cells became more prominent. Cytoplasmic RNA was definitely increased and the lipid droplets previously large were now small. At twenty four hours, the interstitial tissue increased in amount (Fig.65) and the cells contained a fair amount of RNA and a small amount of fine lipid droplets. The reaction for 3β steroid dehydrogenase was now positive but not strong.

Hypophysectomized Rat + LH (IRC, 24.6.69)	Histochemical Methods		
	Pyronin M.Green	Oil Red O	3β Steroid Dehydrogenase
25 µg LH			
1 hour	+	+++	+
6 hours	+	+++	+
12 hours	+	+++	+
24 hours	+	+++	+
48 hours	+	+++	+
50 µg LH			
1 hour	+	+++	+
6 hours	+	++	++
12 hours	++	++	++
24 hours	+++	+	++
48 hours	++	++	+
75 µg LH			
1 hour	+	+++	+
6 hours	+	++	++
12 hours	++	++	+++
24 hours	+++	+	+++
48 hours	++	+	++
100 µg LH			
1 hour	+	+++	+
6 hours	+	++	++
12 hours	++	+	+++
24 hours	+++	+	+++
48 hours	++	+	++

Table 26 Corpora Lutea

The effect of LH (IRC, 24.6.69) on the corpora lutea of both ovaries of two hypophysectomized rats at each time interval.

Fig. 64

Low power view of a corpus luteum stained for 3β steroid dehydrogenase from the ovary of a hypophysectomized rat 24 hours after receiving 50 µg LH. A strong reaction is shown. X 120.

Dose of LH (IRC,24.6.69)	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg	-	-	-	-	-
50 µg	-	-	-	±	-
75 µg	-	±	+	++	+
100 µg	-	±	+	++	+

Table 27 Interstitial Tissue - Repair

The effect of varying doses of LH (IRC,24.6.69) on the interstitial tissue of both ovaries of two hypophysectomized rats at each time interval.

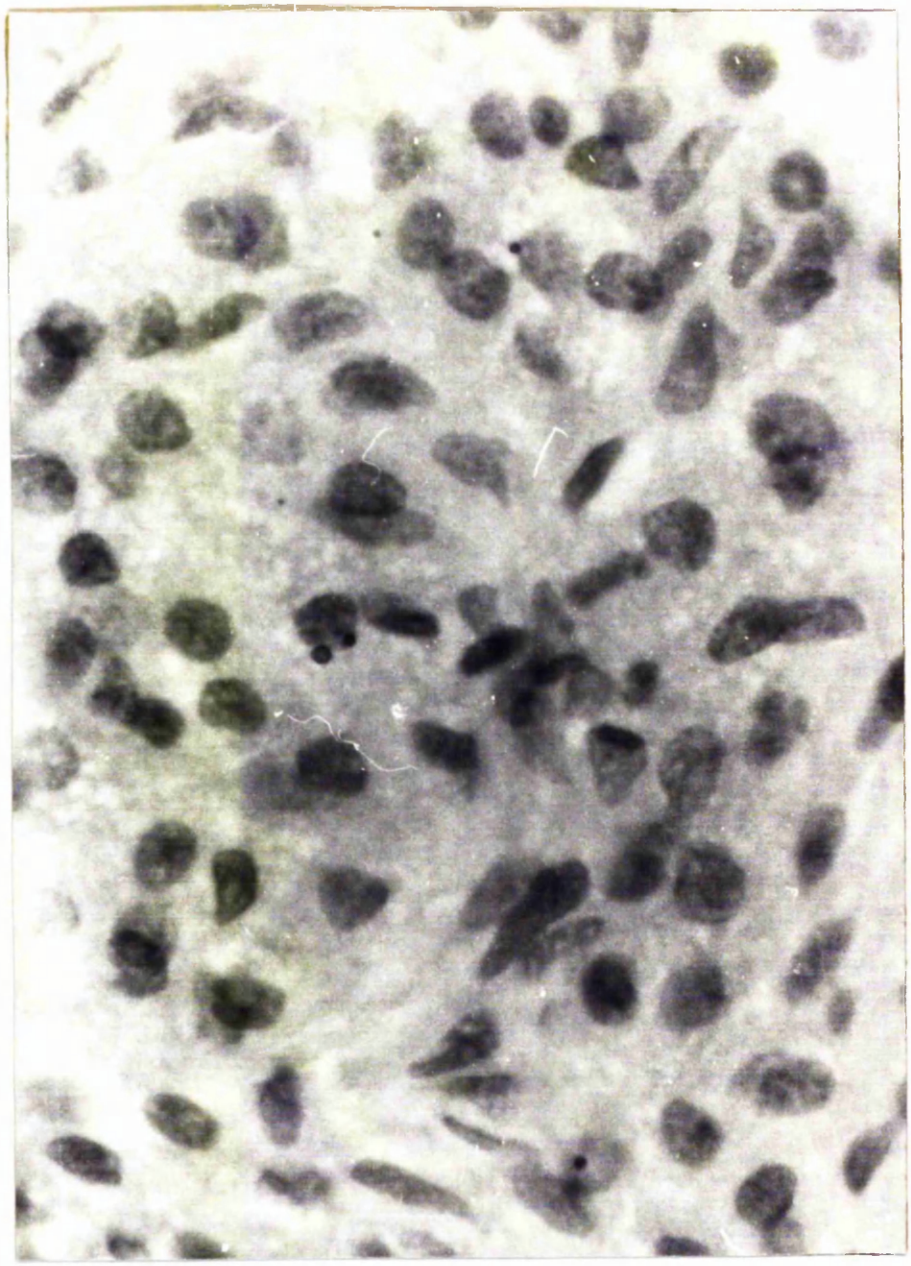


Fig. 65

High power view of an ovary of a hypophysectomized animal 24 hours after receiving 50 µg LH. The interstitial cells are now plump with regular nuclei containing dispersed chromatin and abundant finely vacuolated cytoplasm. H. & E. X 600.

At forty eight hours the interstitial tissue became less prominent, the cells being reduced in size; the nuclei were smaller and the cytoplasm was also reduced in amount. RNA was scanty and large lipid droplets had once more appeared. An estimate of the histochemical reactions in the interstitial tissue is shown in Table 28.

EFFECT OF LH ON THE UTERINE HISTOLOGY

No significant change could be seen in the myometrium or endometrium of the hypophysectomized animals treated with LH (IRC, 24.6.69) when compared with the untreated hypophysectomized controls. Fig.66 shows the uterus and ovaries of the hypophysectomized animal twenty four hours after receiving 75 µg of the LH preparation.

Hypophysectomized Rat + LH (IRC, 24.6.69)	Histochemical Methods		
	Pyronin M. Green	Oil Red O	3β Steroid Dehydrogenase
25 µg LH			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	-	+++	-
24 hours	-	+++	-
48 hours	-	+++	-
50 µg LH			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	-	+++	-
24 hours	-	+++	-
48 hours	-	+++	-
75 µg LH			
1 hour	-	+++	-
6 hours	+	++	±
12 hours	++	+	+
24 hours	++	+	++
48 hours	+	++	+
100 µg LH			
1 hour	-	+++	-
6 hours	+	++	±
12 hours	++	+	+
24 hours	++	+	++
48 hours	+	++	+

Table 28 Interstitial Tissue

The effect of varying doses of LH (IRC, 24.6.69) on the interstitial tissue of both ovaries of two hypophysectomized rats at each time interval.

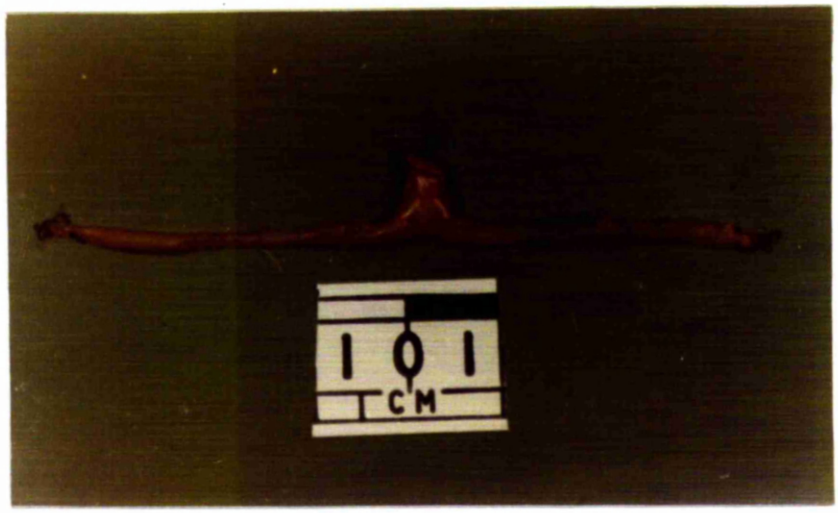


Fig. 66

Uterus and ovaries of hypophysectomized animal 24 hours after receiving 75 µg of LH. The ovaries are not quite as large as those of the untreated hypophysectomized rat. There is only slight enlargement of the uterine horns.

EFFECT OF A COMBINED PREPARATION OF THE MINIMAL EFFECTIVE DOSE OF FSH, B-1 AND VARYING DOSES OF LH, (IRC, 24.6.69) ON HYPOPHYSECTOMIZED MATURE FEMALE RATS

EFFECT OF FSH + INCREASING DOSES OF LH ON THE OVARIAN WEIGHT

There was a marked increase in the ovarian weight as a result of combining the minimal effective dose of FSH and varying doses of LH in injections and the greater the dose of LH the more marked the increase in the ovarian weight (Fig. 67). The rate of increase was greatest in the first twenty four hours, peaking at twelve hours with 50, 75 and 100 µg LH. After twenty four hours there was a decline with those doses of LH. In the case of the lowest dose of LH, 25 µg, there was by contrast a continuous increase in the ovarian weight throughout the forty eight hour experimental period. (see Appendix).

Comparison of the weights at twenty four hours (Fig. 67) showed that with the highest dose of LH ovarian weights not far short of those of the intact animal were achieved.

Statistical analysis of the summated ovarian weights of all animals given a particular dose of FSH + LH sacrificed during the first twenty four hours showed that the gains in weight were significant. The number of animals, mean ovarian weight, standard deviation and p_value for each dose level are shown in Tables 29 and 30.

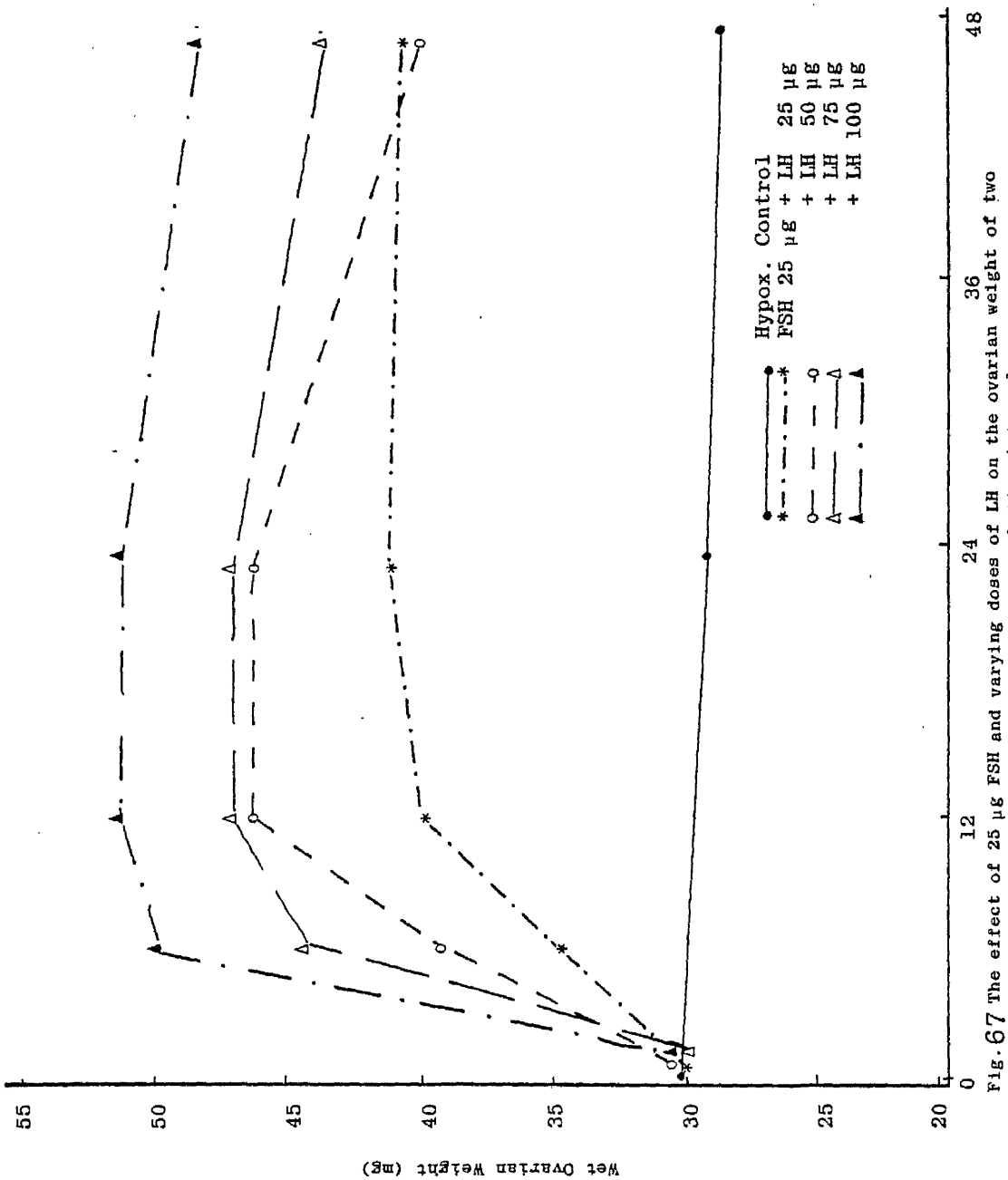


Fig. 67 The effect of 25 µg FSH and varying doses of LH on the ovarian weight of two hypophysectomized mature female rats at each time interval

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.0581	0.0003
Hypox. Control	8	0.0327	0.0029
Hypox. Treated 25 µg FSH + 25 µg LH	8	0.0366	0.0034
+ 50 µg LH	8	0.0390	0.0067
+ 75 µg LH	8	0.0419	0.0070
+100 µg LH	8	0.0456	0.0094

Table 29

The mean and standard deviation of the ovarian weight of hypophysectomized rats treated with 25 µg FSH, B-1 and various doses of LH (IRC, 24.6.69) compared with intact and hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg FSH + 25 µg LH	14	2.3090	$P < 0.05$
+ 50 µg LH	14	2.2831	$P \leqslant 0.05$
+ 75 µg LH	14	3.2125	$P \leqslant 0.01$
+100 µg LH	14	3.4695	$P \leqslant 0.01$

Table 30

Comparison of the ovarian weights of the hypophysectomized control group and the hypophysectomized rats treated with 25 µg FSH and various doses of LH using t. test.

THE EFFECT OF FSH + INCREASING DOSES OF LH ON THE UTERINE WEIGHT

The uterine weight showed a progressive increase with all dose levels and also with the elapse of time. The rate of increase was greatest in the first twenty four hours following the administration of the hormones (Fig.68) Statistical analysis showed that the increase in the uterine weight was significant (Tables 31 and 32). (see Appendix)

EFFECT OF FSH + INCREASING DOSES OF LH ON THE OVARIAN HISTOLOGY

The administration of a mixture of FSH and LH resulted in various changes in the histological and histochemical appearances of the ovaries of the hypophysectomized mature female rats. The main changes are summarised as follows:

1. A return to "normal" ovarian structure.
2. Enlargement of existing corpora lutea beyond the size of those found in the intact animal at oestrus.
3. Repair of the interstitial tissue and histological evidence of a return of function.
4. Resumption of folliculogenesis.
5. Increased thecal formation with larger doses of the LH fraction.
6. Degeneration of granulosa cells with large doses of the LH fraction but persistence of theca.

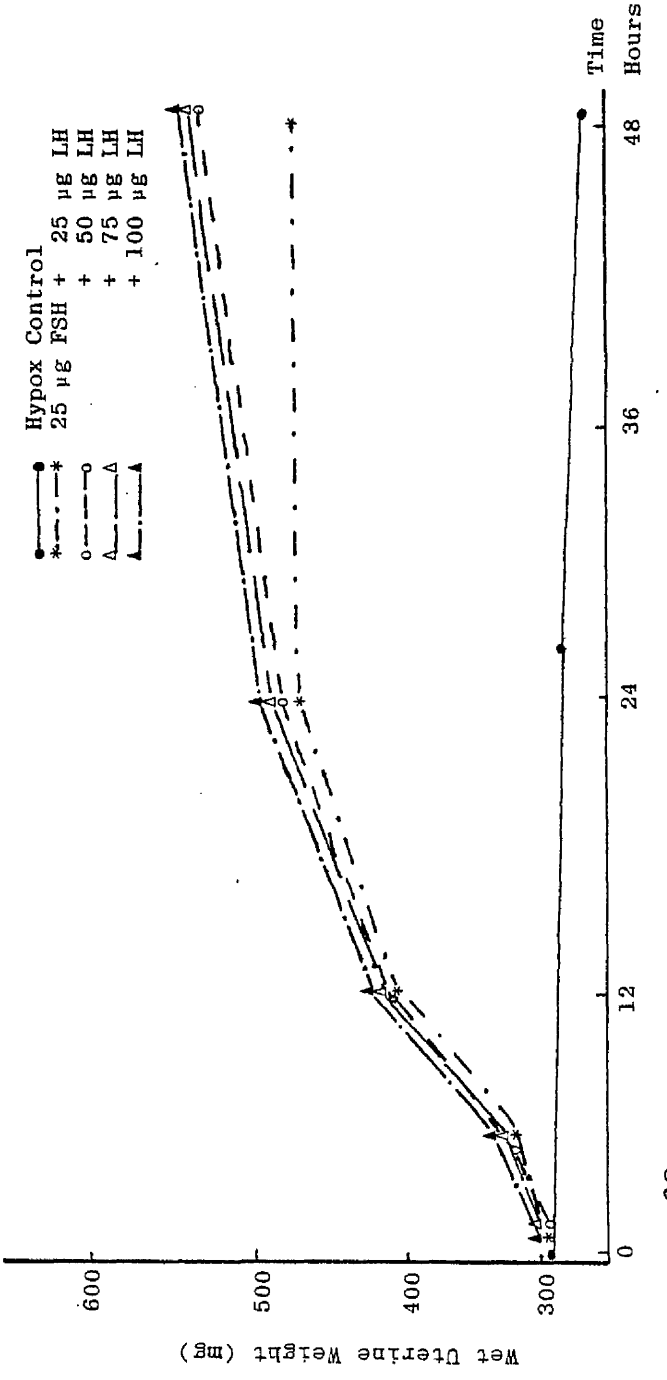


Fig.68 The effect of 25 µg FSH and varying doses of LH on the uterine weight of two hypophysectomized mature female rats at each time interval.

Experiment	No.	Mean	Standard Deviation
Intact Control	10	0.3802	0.0081
Hypox. Control	8	0.2951	0.0149
Hypox. Treated 25 µg FSH + 25 µg LH	8	0.3530	0.0678
+ 50 µg LH	8	0.3668	0.0749
+ 75 µg LH	8	0.3686	0.0841
+100 µg LH	8	0.3750	0.0869

Table 31

The mean and standard deviation of the uterine weight of hypophysectomized rats treated with 25 µg FSH, B-1 and various doses of LH (IRC, 24.6.69) compared with intact and hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg FSH + 25 µg LH	14	2.2068	$P \leq 0.05$
+ 50 µg LH	14	2.4840	$P \leq 0.05$
+ 75 µg LH	14	2.2768	$P \leq 0.05$
+100 µg LH	14	2.3976	$P \leq 0.05$

Table 32

Comparison of the uterine weight of the hypophysectomized control group and the hypophysectomized rats treated with 25 µg FSH and various doses of LH using t. test.

At autopsy, there was a surface hyperaemia of the ovaries. The hyperaemia was noticeable twelve and twenty four hours after injection but by forty eight hours the hyperaemia had faded.

The following is a detailed description of the effect of a combination of the minimal effective dose of a highly purified preparation of FSH, B-1 and varying doses of a highly purified LH, (IRC, 26.6.69) preparation on the ovaries of hypophysectomized mature female rats.

Effect on the Follicles

The administration of a combined preparation of 25 µg FSH, B-1 and varying doses of LH (IRC, 24.6.69) caused changes in the follicles. These changes varied with increasing doses of the LH fraction of the combined preparation and the time interval involved. These changes will be described under Growth, Antral Formation and Thecal Formation.

Growth

The administration of 25 µg FSH + 25 µg LH of the combined preparation had no effect on the follicular growth one hour after injection. Six hours after injection there was a marked increase in the number of Stage 4 and 5 follicles but not to the same extent as those at Stages 2 and 3. Twelve hours and twenty four hours after injection there was a further increase in the number of

Stage 3 and 4 follicles but there was no increase in the number of follicles at Stages 1 and 2 (Fig. 69). Forty eight hours after injection there was a reduction in the number of pre-antral follicles since most of the follicles had developed antrum and a thick theca. In all Stage 3, 4 and 5 follicles the oocytes showed an increase in size in proportion to the increase in the size of the follicles of which they were part. All Stage 2, 3 4 and 5 follicles contained a large amount of cytoplasmic RNA, with no lipid or 3β steroid dehydrogenase activity in the granulosa cell layer of these follicles.

Mitotic activity was present and most prominent at the six and twelve hour intervals after the administration of 25 and 50 µg of the LH fraction. Approximately three-four mitotic figures could be seen in each high power field of granulosa cells of Stage 3, 4 and 5 follicles. The number of mitotic figures was not so great with the higher doses of the LH fraction - 75 and 100 µg at these time intervals. At the twenty four hour period active mitosis was still present in the granulosa of the follicles of the animals receiving 50 µg of the LH fraction. Mitosis was absent at this time in the animals receiving 25, 75 and 100 µg of the LH fraction. No mitotic activity could be seen

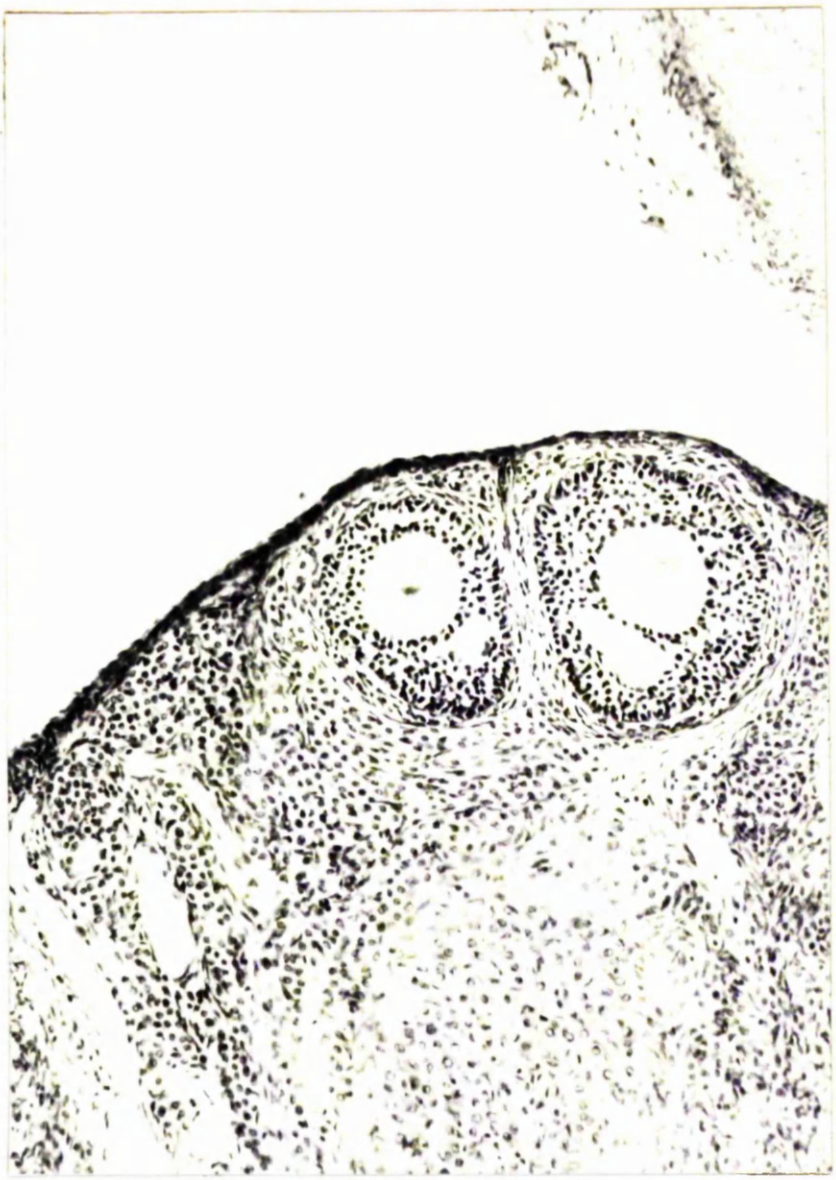


Fig. 69

Another view of an ovary from a hypophysectomized animal 24 hours after receiving 25 µg FSH plus 50 µg LH. This shows growing follicles. The interstitial tissue has recovered a more normal appearance.

in the granulosa layer forty eight hours after any dose of the LH fraction.

The number of pre-antral follicles was reduced and most of the follicles had developed an antrum and a very thick theca twenty four hours after the injection of 25 μg FSH + 75 μg LH and 25 μg FSH + 100 μg LH and forty eight hours after the injection of all dose schedules. At both these time schedules most of the follicles showed an advanced degree of degeneration. The degenerated follicles showed no mitotic figures and a proportionately large antrum. The granulosa cells were degenerate with smaller nuclei and scanty cytoplasm. Many of the granulosa cells had been desquamated in the follicular space. They had very little cytoplasmic RNA and increasingly large amounts of lipid. With the larger doses of the LH fraction advanced cases of atresia could be seen where all that was left of a follicle was a space lined by a thick layer of hyperstimulated theca with no trace of the granulosa cell layer.

When the dose of the LH fraction of the combined preparation was increased from 25 μg to 50, 75 and 100 μg the atretic changes in the antral follicle were both increased and advanced twenty four and forty eight hours after injection. Table 33 shows the effect of dose and time on follicular growth.

Dose of FSH + LH	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg FSH + 25 µg LH	-	+	++	+++	-
25 µg FSH + 50 µg LH	-	+	++	+++	-
25 µg FSH + 75 µg LH	-	++	++	±	-
25 µg FSH +100 µg LH	-	++	++	±	-

Table 33 Follicular Changes - Growth

The effect of 25 µg FSH, B-1 + varying doses of LH (IRC,24.6.69) on the follicular growth of the ovaries of two hypophysectomized rats at each time interval.

Follicle Count

The influence of a combined preparation of 25 µg FSH, B-1 and varying doses of LH (IRC, 24.6.69) on the follicle count of the ovaries of hypophysectomized mature female rats was studied (Table 34).

Plotting the number of follicles beyond Stage 2 against time revealed the marked stimulatory effect of combining FSH with LH (Fig. 70). It should be emphasised that the dose of FSH was constant at 25 µg but the addition of the lowest dose of LH (25 µg) trebled the increase in the number of follicles obtained with 25 µg of FSH alone. With doses of 25 and 50 µg of the LH fraction the peak of growth occurred at twenty four hours whereas with 75 and 100 µg the peak was at six hours. A decline in the number of follicles occurred in each instance after the peak was achieved.

A comparison of the peak values (Fig. 71) helps to emphasise the peculiar inhibitory effect obtained with the higher doses of the LH fraction. This contrasts with the effect of FSH alone (see Fig. 38).

The counts for the intervals six hours, twelve hours and twenty four hours were summated for each dose of the LH fraction. Means and standard deviations were calculated and comparison with

Preparation and time of Administration		Follicular Stage			Normal Antral Follicles			Total	
		3	4	5	S	M	L	Normal	Abnormal
Control Hypophysectomized 7th Day		14	-	-	-	-	-	14	50
25 µg FSH + 25 µg LH	6h	20	20	25	75	4	-	144	80
	12h	45	15	15	110	6	-	191	120
	24h	40	30	20	120	2	-	212	134
	48h	24	20	12	40	-	-	96	194
25 µg FSH + 50 µg LH	6h	35	20	15	95	3	-	168	90
	12h	40	14	16	120	2	-	192	104
	24h	50	10	8	128	3	-	199	116
	48h	20	12	12	96	-	-	140	172
25 µg FSH + 75 µg LH	6h	30	20	10	80	2	-	142	90
	12h	20	16	15	80	-	-	131	96
	24h	15	10	-	95	1	-	120	100
	48h	10	2	-	66	-	-	72	112
25 µg FSH +100 µg LH	6h	30	10	5	80	-	-	125	80
	12h	20	8	10	70	-	-	108	90
	24h	15	2	2	40	-	-	59	98
	48h	14	-	-	30	-	-	44	84

S - small M - medium L - large

Table 34

Follicle count beyond Stage 2 in one ovary of each of two hypophysectomized rats treated with 25 µg FSH and various doses of LH compared with the hypophysectomized controls.

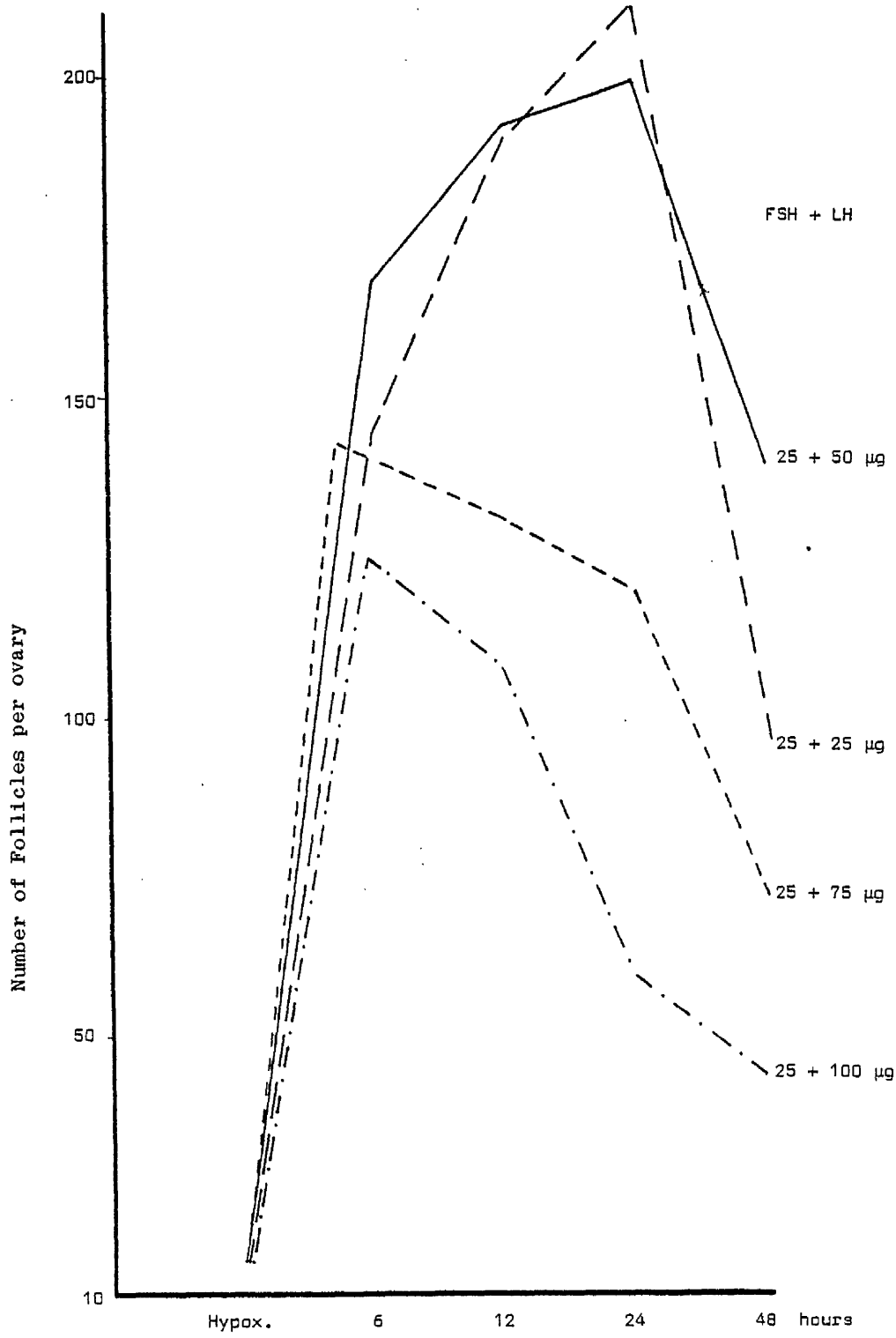


Fig. 70.

The effect of the administration of 25 µg FSH and various doses of LH on the follicle count of one ovary from each of the two hypophysectomized rats at each time interval.

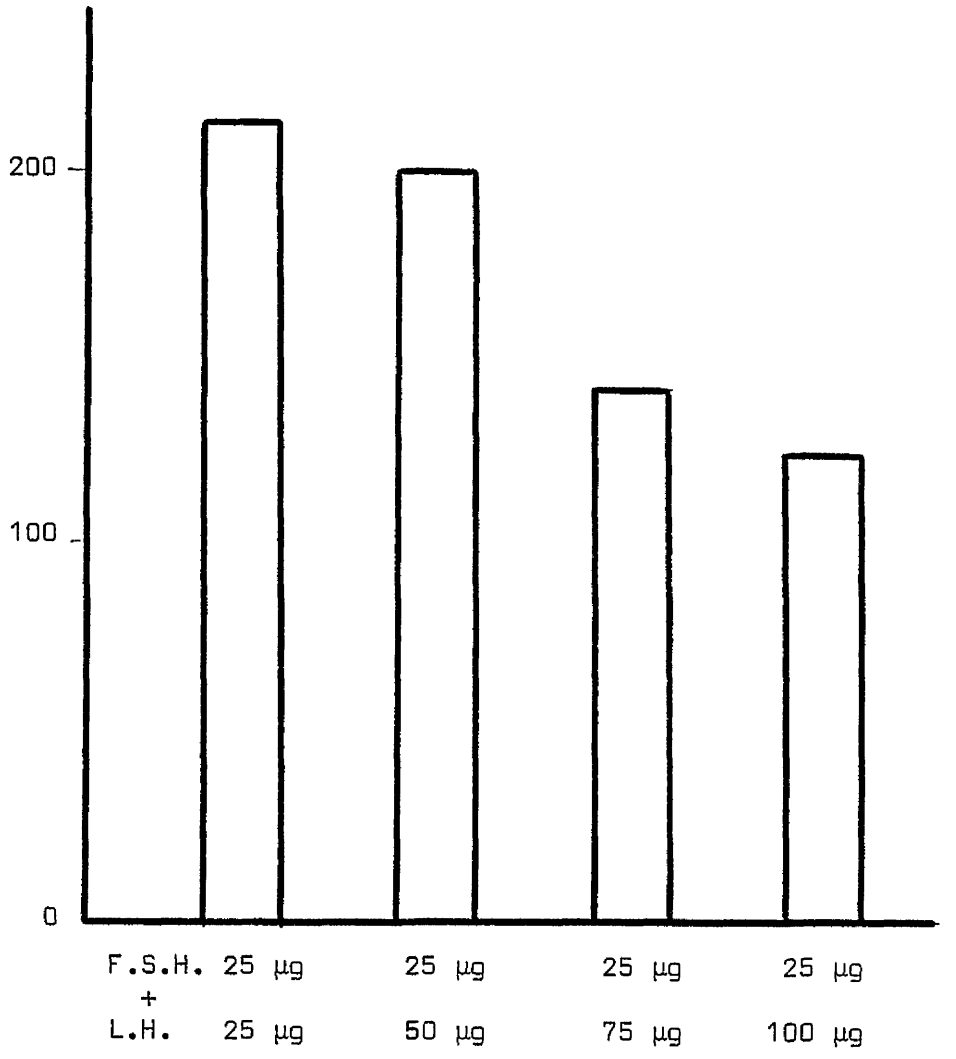


Fig. 71.

Comparison of the follicle count at the peaks of stimulation after the administration of 25 µg FSH and varying doses of LH.

hypophysectomized controls showed that the follicle increase was significant in each case. This underlines the impressions received on histological examination. ($P \leq 0.05$ - see Tables 35/36)

With the material available it was not possible to analyse the apparent inhibition of growth caused by the higher doses.

Antral Formation

Normal antrum formation took place in follicles with four or more layers of granulosa cells. In the hypophysectomized mature female rat "normal" antrum formation occurred six hours after injection of 25 µg FSH, B-1 + 25 µg LH (IRC, 24.6.69).

When the dose of the LH fraction was increased from 25 µg to 50, 75 and 100 µg many Stage 5 follicles formed antra of normal appearance. Normal antral formation was noted to involve a large number of Stage 5 follicles at twelve hours after injection at all dose schedules, and at twenty four hours after the injection of 25 and 50 µg of the LH fraction. Antral formation tended to be abnormal at the twenty four hour interval after injection of 75 and 100 µg of the LH fraction of the combined preparation and with all dose schedules at forty eight hours (Table 37).

In some of the follicles with a normal antrum at twenty four and forty eight hours after the

Experiment	No.	Mean	Standard Deviation
Hypox. Control	8	14.00	2.6186
Hypox. Treated 25 µg FSH + 25 µg LH	6	182.33	31.1427
+ 50 µg LH	6	186.33	14.5420
+ 75 µg LH	6	131.00	9.8387
+100 µg LH	6	97.33	30.6507

Table 35

The mean and standard deviation of the follicle count of hypophysectomized rats treated with 25 µg FSH, B-1 and various doses of LH(IRC, 24.6.69) compared with hypophysectomized controls.

Experiment	Degree of Freedom	t. Value	Degree of Significance
Hypox. Control vs. 25 µg FSH + 25 µg LH	12	14.088	$P \leqslant 0.001$
+ 50 µg LH	12	30.382	$P \leqslant 0.001$
+ 75 µg LH	12	29.766	$P \leqslant 0.001$
+100 µg LH	12	7.085	$P \leqslant 0.001$

Table 36

Comparison of the follicle count of the hypophysectomized control group and the hypophysectomized rats treated with 25 µg FSH and various doses of LH using t. test.

Dose of FSH + LH	Time After Injection				
	1 hr	6 hr	12 hrs	24 hrs	48 hrs
25 µg FSH + 25 µg LH	-	+	++	++	+
25 µg FSH + 50 µg LH	-	++	++	++	+
25 µg FSH + 75 µg LH	-	++	++	+	+
25 µg FSH +100 µg LH	-	++	++	+	+

Table 37. Follicular Changes - Antral Formation

The effect of 25 µg FSH and varying doses of LH on the antral formation in the ovaries of two hypophysectomized rats at each time interval.

injection of all dose schedules the granulosa cells were loosened from each other and tended to vary in size. Some of the granulosa cells were seen in the follicular space. There was a definite reduction in the amount of cytoplasmic RNA in the granulosa cells of these follicles with an increase in the amount of their intracellular lipid. All these follicles were surrounded by a thick layer of theca which was very apparent with larger doses, i.e. 75 and 100 µg of the LH fraction specially at twenty four and forty eight hours.

Abnormal antrum formation took place twenty four hours after the injection of 25 µg of the LH fraction. This abnormality took the form of disproportionally large antral spaces or multiple small rounded spaces among the granulosa cells round the whole circumference of the Stage 3 and 4 follicles (prematurely formed antra). This was noted on a larger scale at forty eight hours (Fig. 72). When the dose of the LH fraction was increased from 25 µg to 50, 75 and 100 µg these abnormal antra formed to a lesser extent in the Stage 3 and 4 follicles six hours after injection. The number of follicles with abnormally shaped and prematurely formed antra were found to extend progressively to involve a larger number of follicles twelve, twenty four and forty eight

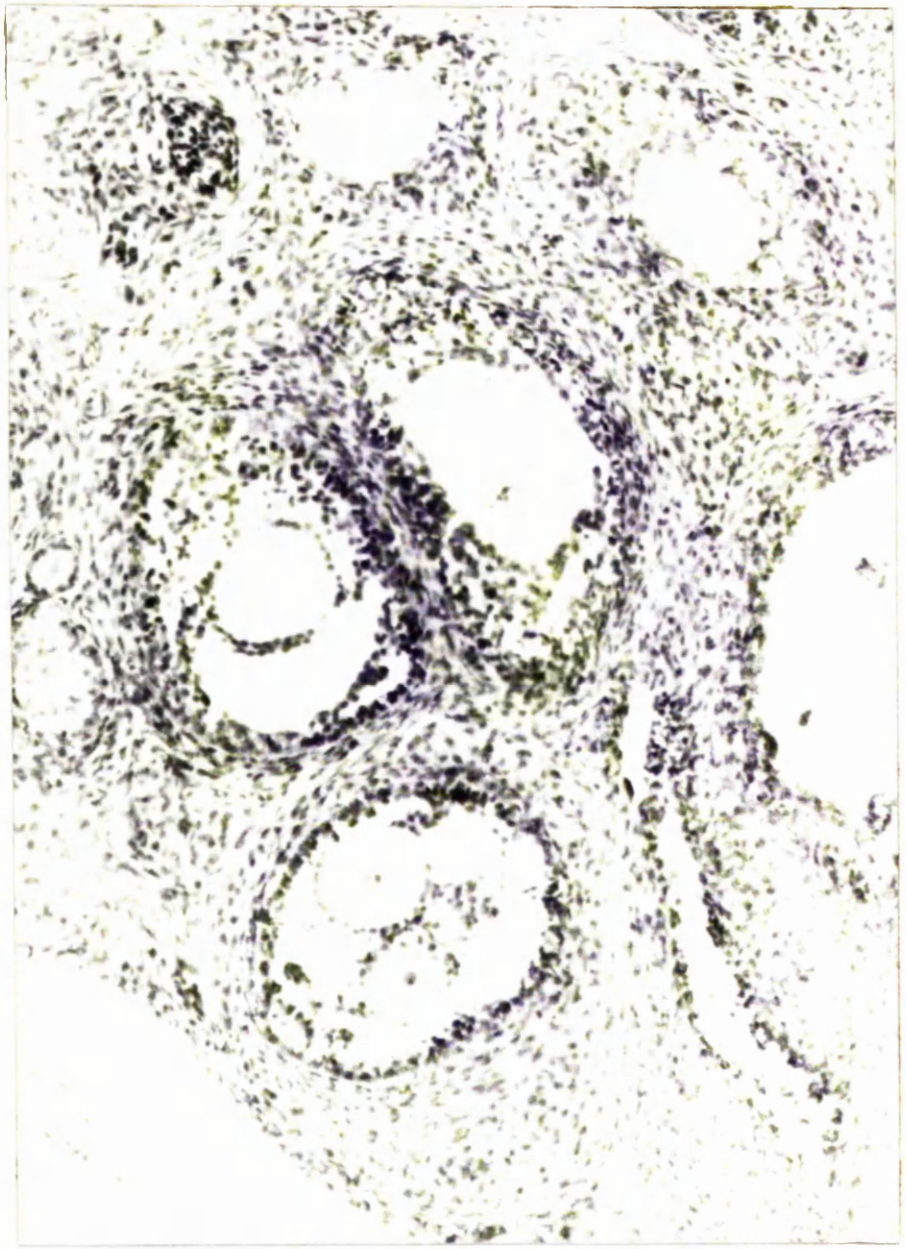


Fig. 72

Ovary of a hypophysectomized rat 48 hours after receiving 25 µg FSH plus 50 µg LH. Many small follicles have formed, one binovular. Degeneration of the granulosa can be seen in at least one follicle. There is also a degree of abnormal antral formation. H. & E. X 150.

hours after injection. At twenty four and forty eight hours after injection all Stage 3 or more follicles showed an irregularly formed antra.

Mitotic figures were common in the granulosa of follicles (5-7 per high power field) with the lower doses of the LH fraction (25 and 50 µg) at the six and twelve hour intervals. They were uncommon with the larger doses in follicles with abnormal antra irrespective of dose. At the twenty four hour interval no mitotic activity was observed with the larger doses and at forty eight hours no mitosis could be seen with any dose.

Thecal Formation

One hour after the administration of a combined preparation of FSH, B-1 and LH (IRC, 24.6.69) there was no sign of thecal formation.

Six and twelve hours after the injection of 25 µg of the LH fraction large pre-antral follicles had acquired a narrow concentric zone of thin spindle-type cells which did not show any sign of differentiation. At twenty four hours and especially round the antral follicles, the thecal cells were arranged concentrically in three or four layers. The nuclei of these thecal cells were spindle-shaped. The thecal cells were rich in cytoplasm and cytoplasmic RNA and contained

fine lipid droplets. The reaction for 3β steroid dehydrogenase was weakly positive. Thecal stimulation was extended to involve all follicles with an abnormal antrum.

When the dose of the LH fraction was increased from 25 µg to 50, 75 and 100 µg there was intensification of the thecal reaction around all the large and small antral follicles (dose dependent). The thecal stimulation was noted to intensify even further at twelve, twenty four and forty eight hours after injection (time dependent). These changes are shown in Table 38.

Forty eight hours after the injection of 50 µg of the LH fraction and forty eight hours after the injection of 75 and 100 µg all of the antral follicles and many of the large pre-antral follicles were surrounded by a thick hyper-stimulated thecal zone. The cells of this zone had a large vesicular nucleus and abundant foamy cytoplasm and had in fact the typical appearance of luteinization. When the dose of the LH fraction was increased from 50 µg to 75 and 100 µg this luteinized thecal reaction intensified.

It will be noted that the thecal reaction described above was completely different from that observed after the injection of FSH, B-1 or LH (IRC, 24.6.69) alone. The difference was

Dose of FSH + LH	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg FSH + 25 µg LH	-	+	+	++	++
25 µg FSH + 50 µg LH	-	+	+	++	+++
25 µg FSH + 75 µg LH	-	++	++	+++	+++
25 µg FSH +100 µg LH	-	++	+++	+++	+++

Table 38 Follicular Changes - Thecal Stimulation

The effect of 25 µg FSH and varying doses of LH on the thecal formation in the ovaries of two hypophysectomized rats at each time interval.

not only in morphology but also in the time of appearance. As the theca became more prominent the granulosa of these follicles degenerated and by forty eight hours with the high doses of the LH fraction the follicles had become theca lutein cysts (Fig. 73).

Effect on the Corpora Lutea

As already noted the corpora lutea of hypophysectomized mature female rats appeared relatively normal seven days after the operation. The luteal cells had abundant cytoplasm with a moderate amount of RNA, contained fine and coarse lipid droplets and gave a moderately strong reaction for 3β steroid dehydrogenase.

With the administration of the combined FSH and LH preparation the corpora lutea appeared to increase in size. These copora lutea were not new ones and this was obvious for two reasons, (i) there was no sign of new formation of corpora lutea with the lower doses of the LH fraction and (ii) there was a small mass of dense collagenous tissue in the centre of the corpora lutea. The diameter of the largest corpus luteum encountered in the present series was 1.42mm and it was seen twenty four hours after the injection of 25 µg FSH and 50 µg LH. This contrasted with the size of the corpora lutea in the hypophysectomized controls seven days after the operation

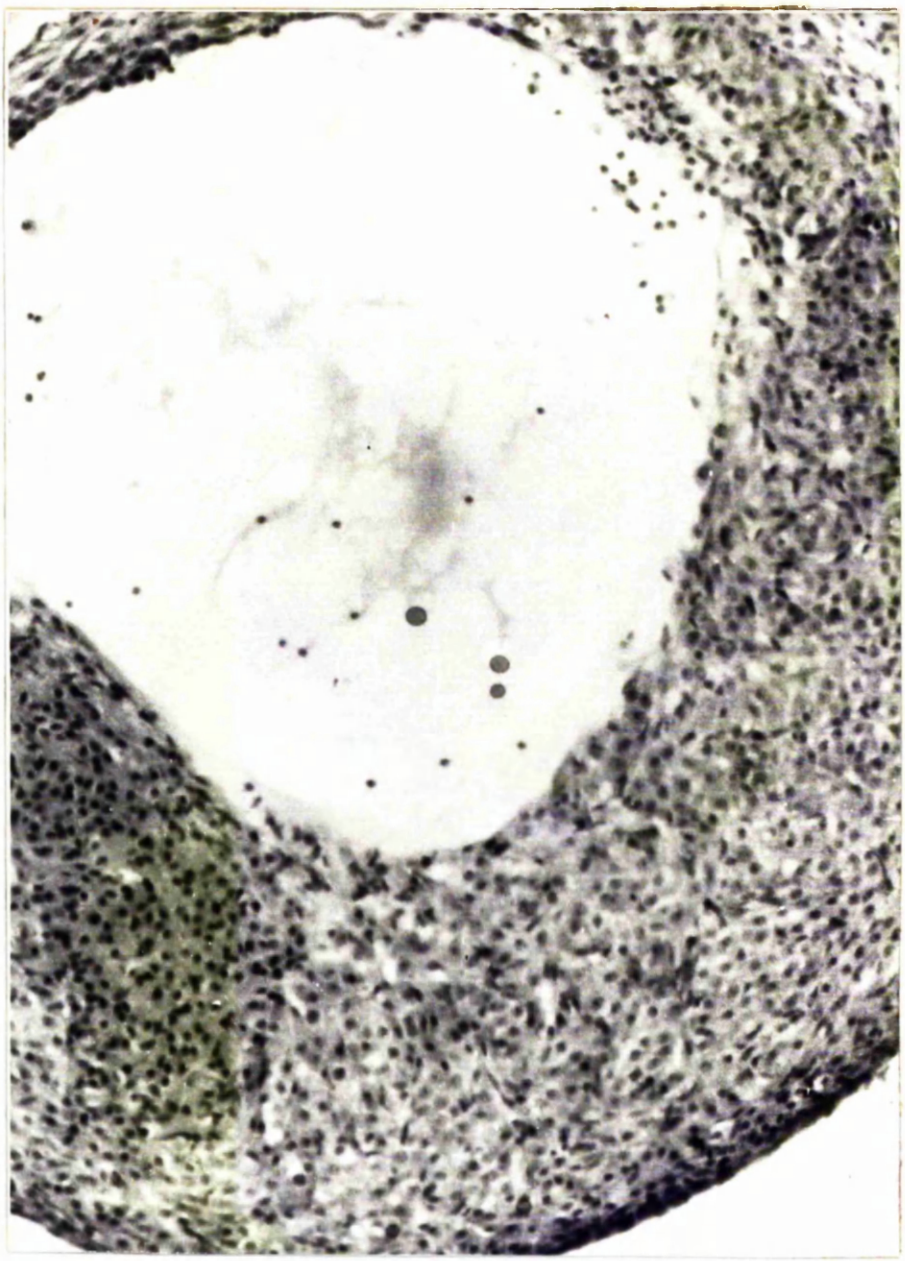


Fig. 73

Ovary from a hypophysectomized animal 24 hours after receiving 25 µg FSH plus 75 µg LH. This shows a small cyst containing granules of chromatin, the remains of necrotic granulosa cells. The wall of the cyst is a thick layer of luteinized thecal cells.

when the largest corpus luteum found was 1.00mm.

The surface hyperaemia of the ovaries noticed at autopsy and the increased vascularity of the corpus luteum was microscopically obvious with 25 µg FSH + 25 µg LH at twelve hours and progressively increased when the dose of the LH fraction was increased to reach a maximum of 100 µg at twenty four hours. In all dose schedules there was no apparent evidence of hyperaemia at forty eight hours (Table 39).

In addition to the increase in vascularity and the size of the corpora lutea, combined FSH and LH in all dose schedules except those at one hour caused a progressive increase in the amount of cytoplasmic RNA. This was apparent by six hours and had progressively increased by twelve and twenty four hours. Proportionately, with the increase in cytoplasmic RNA, there was an apparent reduction in both the number and size of the lipid droplets in the luteal cells.

At forty eight hours this effect had continued with all doses except 25 µg FSH + 100 µg LH when there was a reduction in the cytoplasmic RNA and an increase in the size and number of lipid droplets.

The reaction for 3β steroid dehydrogenase showed alterations with the administration of FSH and increasing doses of the LH fraction. The reaction was positive each time with a tendency to increase

Dose of FSH + LH	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg FSH + 25 µg LH	-	-	+	+	-
25 µg FSH + 50 µg LH	-	-	+	++	-
25 µg FSH + 75 µg LH	-	-	++	++	-
25 µg FSH +100 µg LH	-	-	++	+++	-

Table 39 Ovarian Vascularity

The effect of 25 µg FSH, B-1 and varying doses of LH (IRC, 24.6.69) on the ovarian vascularity of the ovaries of two hypophysectomized rats at each time interval.

in positivity with the increase in the LH fraction (Table 40).

Effect on the Interstitial Tissue

The administration of a combined preparation of FSH, B-1 and LH (IRC, 24.6.69) in all dose schedules had no influence on the interstitial tissue at one hour. The earliest sign of the repair of this tissue occurred twelve hours after the administration of 25 µg FSH + 25 µg LH and at all other dose schedules except 25 µg FSH + 100 µg LH when stimulation was noted as early as six hours. The degree of stimulation increased parallel with the dose of the LH fraction (Table 41). The cells increased in size, gained in cytoplasm which became basophilic and showed large vesicular nuclei (Figs. 74-76).

Cytoplasmic RNA progressively increased with the increased dose of the LH fraction. There was a corresponding decrease in the lipid droplets. The intensity of the reaction for 3β steroid dehydrogenase was parallel with that for RNA. It was noticeable that these changes were more marked than those observed after treatment with FSH or LH alone. The relative changes in the histochemical reactions are shown in Table 42. Fig. 77 illustrates the strong reaction for 3β steroid dehydrogenase.

Hypophysectomized Rat + FSH, B-1 + LH (IRC, 24.6.69)	Histochemical Methods		
	Pyronin M.Green	Oil Red O	3β Steroid Dehydrogenase
25 µg FSH + 25 µg LH			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	+++	+	+++
24 hours	+++	+	+++
48 hours	+++	++	++
25 µg FSH + 50 µg LH			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	+++	+	+++
24 hours	+++	+	+++
48 hours	+++	+	+++
25 µg FSH + 75 µg LH			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	+++	+	+++
24 hours	+++	+	+++
48 hours	+++	+	+++
25 µg FSH +100 µg LH			
1 hour	+	+++	+
6 hours	++	++	++
12 hours	+++	+	+++
24 hours	+++	+	+++
48 hours	+++	++	+++

Table 40 Corpora Lutea

The effect of FSH, B-1 25 µg and varying doses of LH (IRC,24.6.69) on the corpora lutea of the ovaries of two hypophysectomized rats at each time interval.

Dose of FSH + LH	Time After Injection				
	1 hr	6 hrs	12 hrs	24 hrs	48 hrs
25 µg FSH + 25 µg LH	-	-	+	++	-
25 µg FSH + 50 µg LH	-	-	+	++	+
25 µg FSH + 75 µg LH	-	-	+	++	+++
25 µg FSH +100 µg LH	-	+	++	+++	+++

Table 41. Interstitial Tissue Repair

The effect of 25 µg FSH and varying doses of LH on the interstitial tissue of the ovaries of two hypophysectomized rats at each time interval.

Hypophysectomized Rat + FSH, B-1 + LH (IRC, 24.6.69)	Histochemical Methods		
	Pyronin M.Green	Oil Red O	3 Steroid Dehydrogenase
25 µg FSH + 25 µg LH			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	±	+++	±
24 hours	+	++	+
48 hours	±	+++	+
25 µg FSH + 50 µg LH			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	±	+++	±
24 hours	+	++	+
48 hours	+	++	+
25 µg FSH + 75 µg LH			
1 hour	-	+++	-
6 hours	-	+++	-
12 hours	+	++	+
24 hours	++	+	++
48 hours	++	+	++
25 µg FSH +100 µg LH			
1 hour	-	+++	-
6 hours	±	+++	±
12 hours	+	++	+
24 hours	++	+	++
48 hours	++	++	++

Table 42. Interstitial Tissue

The effect of FSH, B-1 25 µg and varying doses of LH (IRC, 24.6.69) on the interstitial tissue of the ovaries of two hypophysectomized rats at each time interval.

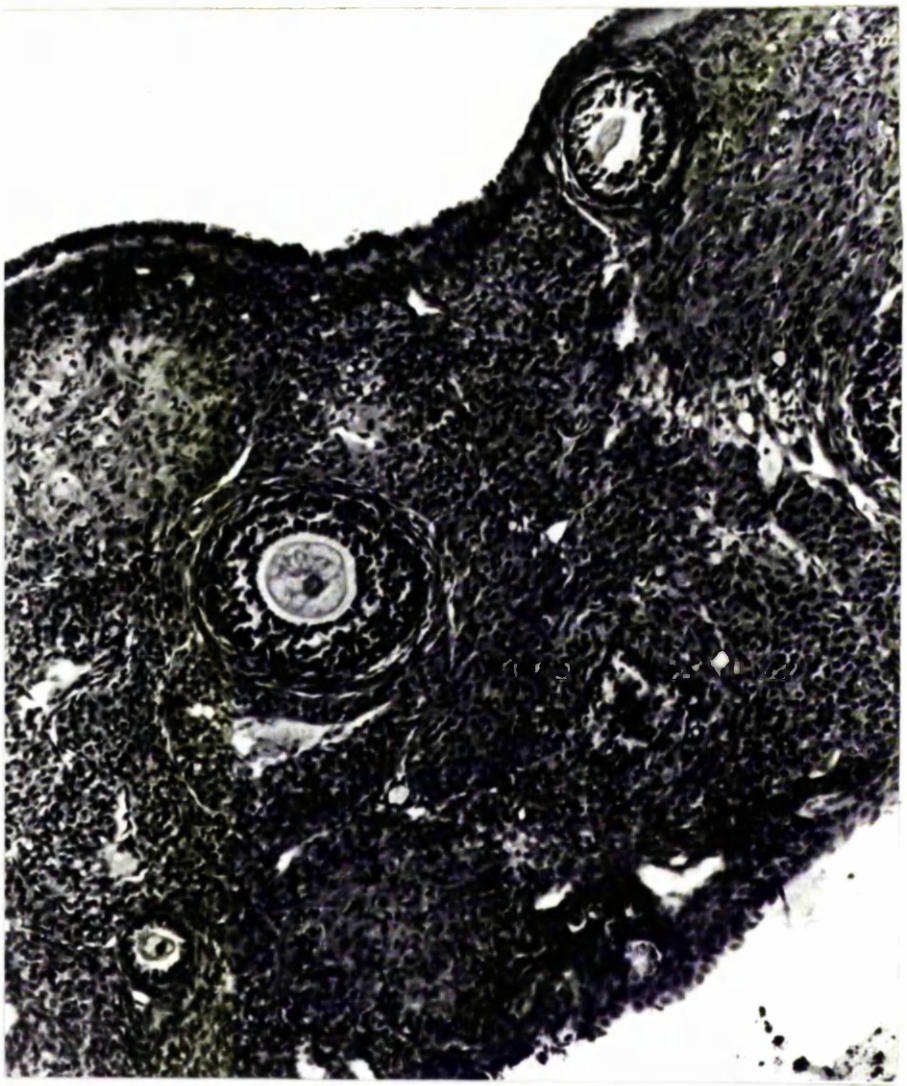


Fig.74

An ovary from an animal 12 hours after receiving 25 µg FSH and 100µg LH. Three small pre-antral follicles are present. Degeneration of the granulosa cells is taking place in the largest follicle. Note the prominent interstitial tissue. H. & E. X 130.

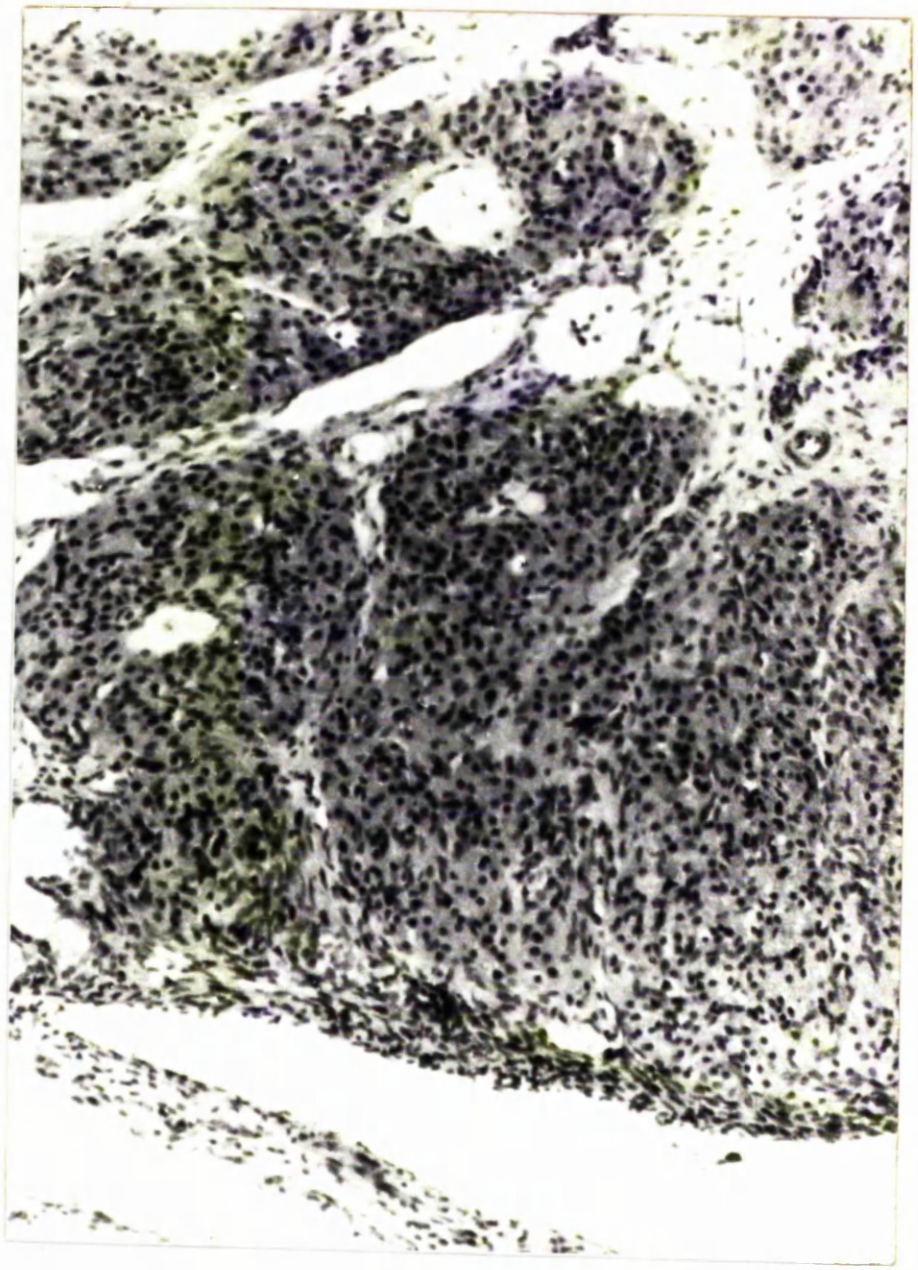


Fig. 75

Interstitial cells from an ovary 48 hours after the administration of 25 µg FSH and 75 µg LH. The cells are fully luteinized and very numerous.

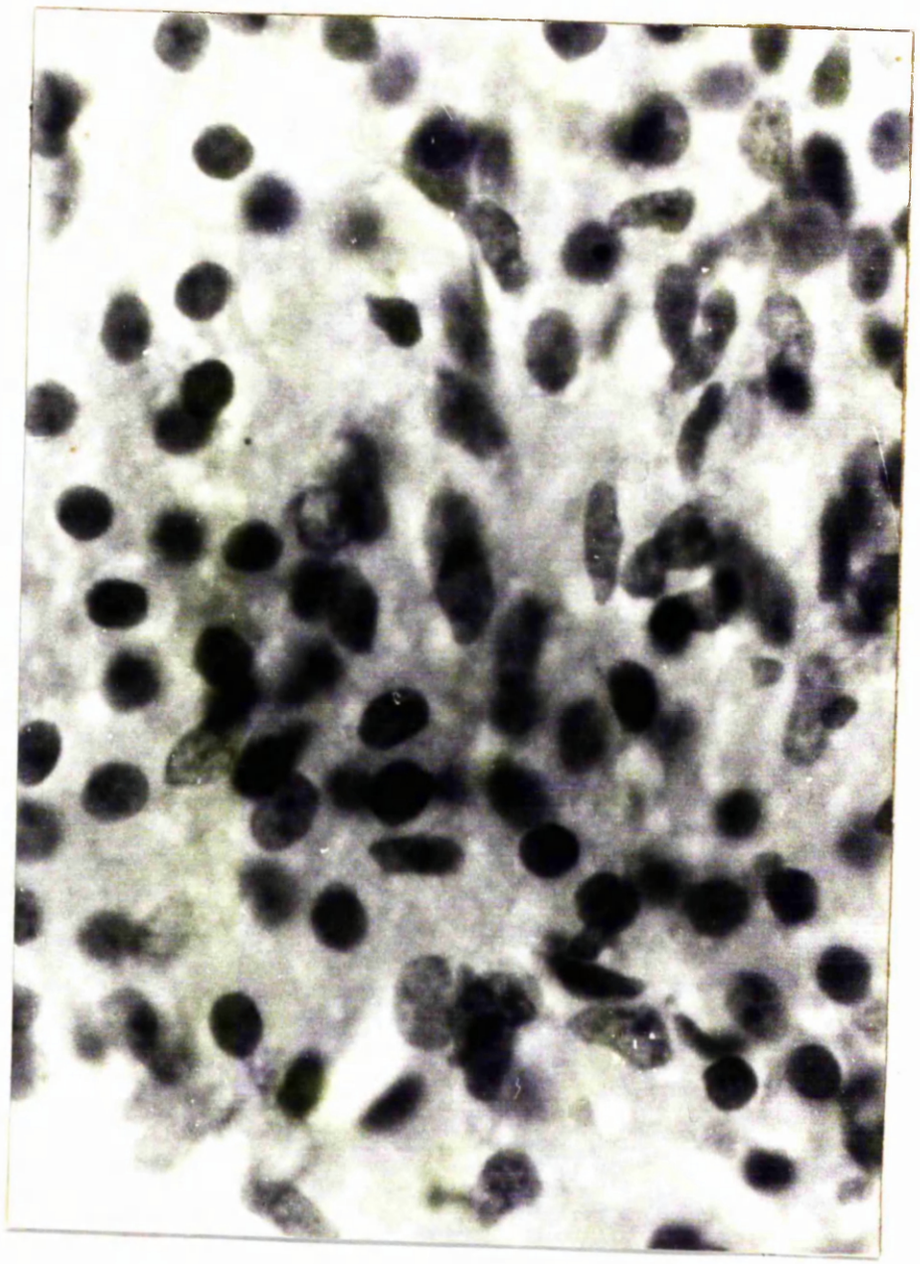


Fig. 76

High power view of the luteinized interstitial cells seen in the previous illustration.

Fig. 77

A high power view of the interstitial tissue stained for 3β steroid dehydrogenase from an animal receiving 25 µg FSH plus 50 µg LH. A good positive reaction is obtained. X 300.

EFFECT OF FSH + INCREASING DOSES OF LH ON THE UTERINE HISTOLOGY

The same changes noticed with FSH alone were apparent with FSH + LH but in much greater degree. Marked dilatation of the lumina of the cornuae had occurred. The uterine wall was greatly thickened; this was mainly due to oedema of the tissue which had affected both the myometrium and endometrium, part of this was probably intracellular since the myometrial cells appeared enlarged. The endometrial glands were elongated and the lining epithelium was somewhat taller than in the controls. The degree of change was directly proportional to the dose of gonadotrophins administered. Fig.78 shows the uterus and ovaries removed from the hypophysectomized rat twenty four hours after receiving 25 µg FSH plus 50 µg LH.

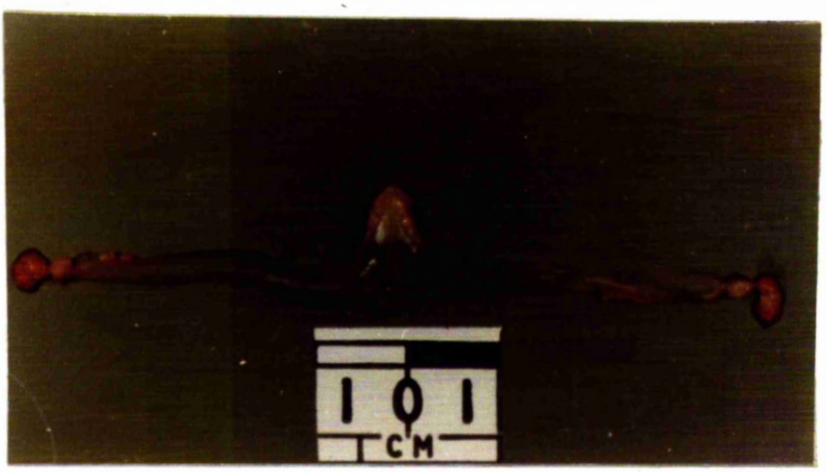


Fig. 78

Uterus and ovaries removed from a hypophysectomized rat 24 hours after receiving 25 µg FSH plus 50 µg LH. Note the enlargement of the ovaries and the uterine horns distended with fluid, and the congestion of the ovaries.

DISCUSSION

CHOICE OF ANIMAL

The use of proper control groups is essential. Careful standardization of experimental conditions helps to reduce variability and to increase the reliability of the experiment. A factor often neglected is the effect of the laboratory environment on the results of experiments with rats. For most work the experimental room should have the same conditions of lighting, temperature and humidity as the animal room in which the rats were reared.

The number of experimental animals housed together in the cage is important as this affects the temperature and humidity of the cage microclimate, and also has a marked effect upon the endocrine balance of these animals.

Usually the adult female rat has a four-day cycle but a percentage have five-day cycles. It was therefore necessary to eliminate these animals with five-day cycles. The length of the cycle was determined by examining vaginal smears. The basis for this was established by Courrier (1950) who demonstrated the sensitivity of rat vaginal epithelium to hormones. Schwartz and Bartosik (1962) extended these observations and showed that the vaginal epithelium established cyclical changes corresponding to those found in the ovary and endometrium.

The rat ovulates spontaneoulsy under normal conditions but the cycle and ovulation can be altered by changing the environment. If female rats are kept in constant light a persistent stage of oestrus is established but ovulation only occurs on copulation, i.e., the animal has become a "reflex ovulator" (Dempsey and Searles, 1943). Hoffmann (1970) has shown that provided the light period is less than twelve hours per twenty-four most rats have a four-day cycle, but if sixteen hours of light are provided the cycle is extended to five days. This of course will vary with the particular breed of rat and even with different colonies of the same breed. The majority of animals in our colony had a four-day cycle with fourteen hours of light.

The rat required was one with a four-day cycle and the ovaries had to contain corpora lutea which were fresh. In addition, so far as could be ascertained, the oestrogen production should not be high.

Sexually mature female Sprague Dawley rats were tested by vaginal smear to establish the duration of their cycles, and were kept under standard conditions with not more than fourteen hours of light.

Hypophysectomy was carried out towards the end of oestrus when the vaginal smear showed falling off of oestrogen influence in four-day cycle rats.

It may occasion some surprise that the animals in

this experiment were kept in light conditions for fourteen hours when it was stated by other workers that a time of less than twelve hours is more appropriate for inducing four-day cycles in the female rat. This however is only one factor. The rats used in these experiments had been kept by the breeder from birth in an environment providing fourteen hours of light and it was felt that since the majority already exhibited four-day cycles there was no point in making a change and possibly upsetting other factors such as times of feeding and activity.

THE GONADAL EFFECTS OF HYPOPHYSECTOMY IN THE ADULT FEMALE RAT

In recent times most studies on the gonadal effects of hypophysectomy in the rat have been carried out on immature animals (e.g., Lane, 1935; Payne and Hellbaum, 1955 and Louvet et al., 1975a). The reason of course is obvious. In the immature animal there are no apparent cyclical changes in the genital tract and ovulation does not take place. The number of variables in an experiment are therefore reduced. Equally most of the studies have been short-term and there are few accounts of the long-term changes in the ovary following removal of the pituitary.

All are agreed that removal of the pituitary results in atrophy of the ovaries but accounts of the structural changes have consisted of isolated observations on individual structures. According to Swezy (1933) and later to Burkl and Kellner (1954) there is an increase in oogenesis after hypophysectomy. However, Ingram (1953) and Jones and Krohn (1961) showed beyond any doubt that oocytes decrease at a slower rate after hypophysectomy: in other words, atresia was slower. Complementing this observation, Williams (1956) showed that administration of gonadotrophins to hypophysectomized immature rats hastened atresia. In this present study while no attempt was made to count the oocyte population in

the ovaries it seemed that degenerative changes occurring in a regular fashion affecting the largest follicles first, followed by medium sized and small antral follicles and at a relatively late date, the pre-antral follicles. Although pre-antral follicles presisted there was no evidence of larger follicles so it is assumed that they were not growing. This may agree with the statement of Lunenfeld and Eshkol (1968) that follicular growth is retarded by the lack of gonadotrophins. It has been stated that follicles can actually develop to the pre-antral stage in the absence of gonadotrophins (Young, 1961; Mauleon, 1969 and Nakano et al., 1975) but most of the evidence for this is indirect and deduced or derived from experiments other than direct observation of the hypophysectomized animal. The small pre-antral follicles are virtually the only follicular structure to be seen seven days after hypophysectomy and even in those there were no morphologically evident mitotic figures in the granulosa cell layers. There were occasionally one or two small antral follicles. The presence of these occasional antral follicles can be explained by the slowing of the process of atresia described by Ingram (1962).

The degenerative changes in the animals in this experiment appeared first in the oocyte followed closely by disruption of the granulosa with the

theca persisting for some time thereafter but eventually undergoing a fatty degeneration.

The cells of the interstitial tissue very quickly lost RNA, became bloated with large lipid globules, and lost the ability to form 3β steroid dehydrogenase. Lipid later disappeared, the cells became small and atrophied. In 1959, Levy et al. drew attention to this rapid loss of 3β steroid dehydrogenase from the interstitial cells of the hypophysectomized rat, and in 1954 Nishizuka described atrophy of the interstitial tissue following removal of the pituitary from the mature rat. In an ultrastructural study, Flerko et al. (1967) demonstrated the disappearance of the organelles of steroidogenesis from the interstitial cells after hypophysectomy. Contrariwise, Carithers and Green (1972) showed that the administration of gonadotrophins to hypophysectomized female rats stimulated the interstitial tissue.

Apart from pre-antral follicles, the only other structure in this series that usually retained its histological structure seven days after hypophysectomy was the corpus luteum. This persistence of the corpus luteum and lack of luteolysis was described by Selye (1948) and Malven and Sawyer (1966). In this series, the corpus luteum in the hypophysectomized animals was not only structurally intact but the cells had retained part of their content of RNA and 3β steroid

dehydrogenase. Levy et al. (1959) also observed the persistence of 3β steroid dehydrogenase activity in the corpus luteum of the hypophysectomized rat and contrasted it with the rapid disappearance of the enzyme from the interstitial tissue.

The process of luteolysis is not clearly understood. Pituitary grafts will maintain the corpus luteum of hypophysectomized rats for a long time in an active condition. According to Everett (1956) this is due to prolactin secretion by the graft. Rothchild (1965a) however pointed out that it might be just as much due to the absence of LH secretion by the graft since he had found that LH administered to hypophysectomized animals induced luteolysis (Rothchild 1965b). As early as 1936 Lahr and Riddle had shown that administration of prolactin to intact adult female rats caused mucification of the vaginal smear and abolition of the oestrous cycle. This suggested that persistent progesterone secretion had been induced and by inference the corpus luteum had been maintained. Astwood (1941) showed that prolactin given to hypophysectomized rats prevented the cornifying action of oestrogen by maintaining progesterone secretion. The position appears to be that LH is necessary for ovulation and luteinization and perhaps helps to initiate progesterone production but prolactin is necessary for maintenance of the corpus luteum and its

continued progesterone secretion.

Obviously in hypophysectomized animals the luteolytic process whatever it may be ceased to operate and the corpus luteum remained relatively intact for at least two weeks and only showed marked degenerative changes after three weeks.

THE INITIATION OF FOLLICLE GROWTH AFTER HYPOPHYSECTOMY

The various substances such as FSH, LH, prolactin, oestrogen, progesterone and androgen which all influence ovarian activity, form an interlocking system. The difficulty in determining the position of any substance in this system is that each one appears to have several actions and may act on more than one type of ovarian tissue. It is particularly difficult to interpret morphological studies of the adult ovary since growth and degeneration take place side by side.

Kinetic analysis of the follicle growth (Pedersen, 1970) and sequential measurements (Hirschfield and Kortiz, 1966) indicate that some primordial follicles begin to grow each day but little is known of the mechanisms which initiate this process in the intact animal. According to Young (1961) the first stages in the growth of the primordial follicle does not require the presence of gonadotrophins, and Mauleon (1969) and Nakano et al. (1975) both claimed that the follicle can develop to the pre-antral stage in hypophysectomized rats. In the present study of the ovaries from hypophysectomized rats it was impossible to substantiate or deny this claim. It can however be conceded that small pre-antral follicles persisted in an apparently healthy state for many days following hypophysectomy. Growth was slow in this

initial stage and was confined to the granulosa (Robinson, 1918) and mitoses only became obvious when an antrum was formed (Bullough, 1942). Mitotic figures were not observed in the pre-antral follicles in untreated hypophysectomized animals in this study. It must be admitted that the number of animals studied was small but more sophisticated experiments involving possibly the use of tritiated thymidine and hormone analysis would be required to settle this point.

Much more evidence is available regarding those substances which positively stimulate growth of follicles. Oestrogens are an example of such a substance. According to Louvet et al. (1975a)if oestrogen is given to a hypophysectomized immature female rat large pre-antral follicles are formed. As long ago as 1935, Lane showed that small doses of oestrogen given to intact animals increased the number of developing follicles. Payne and Hellbaum (1955) have gone so far as to state that oestrogen is necessary to stimulate pre-antral growth of the follicles. Recently, Richards and Midgley (1976) demonstrated nuclear receptors for oestrogen in granulosa cells. It has been shown that oestrogen increases the number of FSH receptors in the granulosa of developing follicles, but it does so by inducing granulosa proliferation (Louvet and Vaitukaitis, 1976). Previously, Zeleznik et al.

(1974) claimed that the granulosa of follicles was the only site where FSH receptors were found and this was also stated by Rajameni and Midgley (1975) working with the rat. If true, this is an important point in view of the results in the present experiments involving the use of FSH. There are a number of reports scattered throughout the literature indicating that oestrogen modifies the follicular response to gonadotrophins, among them Pencharz (1940); Williams (1940) and Smith and Bradbury (1961). The latter authors also pointed out that oestrogen sensitises the ovary to FSH but not to LH.

After the elapse of seven days following hypophysectomy the animals in this study possessed ovaries in which the only really viable structures were small pre-antral follicles and corpora lutea. All other structures including the interstitial cells had undergone atrophy. If, as has been claimed, oestrogen is necessary for the initial stages of follicular growth and this must operate before FSH can act, the possible source of oestrogen in these ovaries must be considered since the administration of FSH, even in relatively small doses, was followed within a few hours by granulosa proliferation and follicular development. This growth of follicles was shown to be dose dependent and these changes were statistically significant. Although Young (1961) claimed that primordial follicles can develop to

the primary and early pre-antral stages he could find no evidence that these follicles produced steroids. In other words there could be no question of a self-stimulatory steroidal process operating in the ovaries of the hypophysectomized animals in this study and therefore no question of FSH receptor production prior to the administration of FSH itself.

It was necessary at this point to consider the sources of oestrogen production within the normal ovary. During the follicular or proliferative phase of the human menstrual cycle there is a rising curve of oestrogen production which reaches a peak at or near ovulation. The same pattern of events has been shown to exist in other primates and in the rat. According to Baird and Fraser (1974) 95% of the oestrogen produced is derived from developing follicles or the corpus luteum according to the stage of the cycle. This has been confirmed by Makris and Ryan (1975). Both structures have been shown to be capable of producing oestrogen in the rat and also in humans. The only statement to the contrary is that of Baird et al. (1976) who stated that in the sheep only the follicles seem capable of oestrogen production, the corpus luteum being entirely concerned with the formation of progesterone. The cellular source of oestrogen production by the follicle is still a matter of great controversy. Innumerable studies have been made to try to

elucidate this problem. Initially, morphological studies provided the main evidence and from these it seemed that the theca cell was bound to be the main source in the follicle. Thecal cell growth during the menstrual and oestrous cycles parallelsthe output of oestrogen and with the demonstration of 3β steroid dehydrogenase in these cells (Deane, Lobel and Romney, 1962) it seemed reasonable to assume that the case was proven. As recently as 1972, Besch and Buttram made the blunt statement that oestrogen was the steroid produced in the follicular phase and it was derived from thecal cells. Balboni (1970, 1973 and 1976) in successive morphological studies was also convinced that thecal cells were the source of ovarian oestrogen.

Experimental studies throw some doubt on this. In 1959, Falck reported his classical studies of tissue implants. Granulosa and thecal tissues were separated and implanted separately or together in the anterior chamber of the eye of castrated experimental animals. A strip of vaginal mucosa was implanted at the same time to act as an indicator of oestrogen production. Granulosa cells alone gave no indication of oestrogen formation, theca cells provided some oestrogen but the most marked effect was produced when both thecal and granulosa cells were present. These experiments have given rise to the so called

"two cell" theory of oestrogen production.

It seems reasonably certain that granulosa cells do not produce oestrogen from basic substances such as cholesterol or acetate. Ryan et al. (1968) could find no evidence of oestrogen production by granulosa cells maintained *in vitro*. Channing and Coudert (1976) showed that removal of granulosa cells and fluid from follicles did not alter the oestrogen level of ovarian vein blood. According to Flerko et al. (1967) electron microscope studies of rat ovaries showed that the organelles usually found in steroid producing cells are only found in granulosa cells derived from follicles about to ovulate. Despite this there are a number of *in vitro* studies which demonstrate that granulosa cells can produce, if not oestrogen, at least steroidal substances (Ryan and Petro, 1966 and Ryan et al., 1968). Ryan et al. (1968) demonstrated some progesterone production by granulosa cells from antral follicles cultured *in vitro*. Hillier et al. (1977) found that granulosa cells of pre-antral follicles from immature hypophysectomized rats pre-treated with oestrogen produced small amounts of progesterone. Again, the importance of oestrogen in this matter should be noted. In addition, Lucky et al. (1977) showed that if testosterone or 5α dihydrotestosterone were added to the culture medium, the formation of progesterone was greatly facilitated. This effect

was neutralised if an anti-androgen was added (Hillier et al., 1977). Granulosa cells appear to have receptor sites for androgens as well as oestrogens (Schreiber and Ross, 1976). The same stimulatory effect of androgens on progesterone production was noted in granulosa cells cultured from mature antral follicles (Nimrod and Lindner, 1976 and Schomberg et al., 1976). The importance of all this is that granulosa cells are capable of performing some of the biochemical transformations in the steroid pathway but only after initial stimulation by oestrogen. Under certain circumstances they can even form oestrogens from androgenic precursors. Armstrong and Papkoff (1976) showed that the blood oestrogen of immature hypophysectomized rats increased following the administration of testosterone provided antral formation was stimulated. Giving 5α dihydrotestosterone in these circumstances had no effect, the difference being that testosterone can be aromatized and 5α dihydrotestosterone cannot. According to Fortune and Armstrong (1977) the theca secretes aromatizable androgens and Baird and his associates (1976) stated that androstenedione was the main steroid produced by the ovarian stroma.

Seven days after hypophysectomy the animals in this study had no intact antral follicles which could have produced oestrogen to start the initial

growth of primary follicles. Granulosa cells had disappeared from the antral follicles so that even if the theca had produced aromatizable androgen it would have remained an androgen and was likely to intensify the atretic process. The gross accumulation of lipid combined with the absence of RNA and 3β steroid dehydrogenase in the theca makes it unlikely however that androgens were being formed. For the same reason and also because mitotic activity was absent from the surviving small pre-antral follicles the theca could not have been producing oestrogen. Nevertheless FSH produced marked follicular activity in the ovaries of the hypophysectomized animals in this experiment and there must have been at least a potential source of oestrogen in these ovaries which would cause the initial granulosa cell growth and induce the formation of FSH receptors.

Two other tissues in the rat ovary must be considered in this matter - the interstitial tissue and the corpora lutea. Functional studies of the rat interstitial tissue is difficult because of its diffuse distribution. Several morphological studies have been carried out. Flerko et al. (1967) in an electron microscope study found that the interstitial cells were the first in the ovary to develop the organelles of steroid producing cells. These appeared around the eighteen day of life. At a later

date they formed in the thecal cells of vesicular follicles. Flerko and his colleagues were of the opinion that the interstitial and thecal tissues were the source of ovarian oestrogen. Guraya (1974) using histochemical techniques is also of the opinion that interstitial cells have the characters of steroidogenic cells and demonstrated the presence of cytoplasmic lipid and 3β steroid dehydrogenase. Deane, Lobel and Romney (1962) in morphological studies were of a similar opinion. The existence of an interstitial tissue in other species, such as is seen in the rat, is a matter for debate but its equivalent in the human, the ovarian cortical stroma, is also thought to be steroidogenic (Dubreuil, 1945, 1946, 1948; Mossman et al., 1964; Motta, 1966 and Balboni, 1974). It is said to undergo changes during the different phases of the menstrual cycle (Duke, 1947; Catchpole et al., 1950 and Balboni, 1959, 1962). Balboni (1970, 1973, 1976) is of the opinion that it produces oestrogen. Morphological changes also occur in the rat interstitial tissue during the oestrous cycle. According to Motta and Bourneva (1970) activity is most marked during pro-oestrus and oestrus. Since the blood oestrogen level is high during pro-oestrus and oestrus it would seem natural to conclude that this activity of the interstitial tissue is related to oestrogen production. The question is - in what

capacity? In addition, in the present experiments, assuming that the interstitial tissue cells are capable of producing oestrogens, were they able to do so seven days after hypophysectomy? Or failing this, could they be induced to do so by the administration of gonadotrophins and thereby initiate the beginning of granulosa cell proliferation?

There is considerable evidence to indicate that gonadotrophins influence the stromal endocrine compartments (Claesson et al., 1954; Balboni, 1960; Savard et al., 1965; Armstrong, 1968; Merker and Diaz Encinas, 1969 and Guraya, 1971, 1973). Nishizuka (1954) showed that hypophysectomy in the adult rat caused atrophy of the interstitial tissue of the ovary. According to this observer degeneration with pyknosis of nuclei begins within five days and there is a marked decrease in the volume of the tissue by fifteen days post-hypophysectomy. In the present experiments the appearance of nuclear pyknosis was slower in appearing, round about the twelfth day post-hypophysectomy, but shrinkage of the tissue was apparent almost at the same time as in Nishizuka's study - sixteenth day. By twenty-two days post-hypophysectomy recognisable interstitial tissue had almost disappeared from the ovaries of the animals in the present experiments. Functional changes however appeared very quickly after hypophysectomy.

Cytoplasmic RNA diminished steadily while, initially, lipid accumulated, suggesting storage and lack of steroidogenesis. Most striking was the diminution of 3β steroid dehydrogenase. Within a few days this enzyme had disappeared entirely from the cytoplasm of the interstitial cells. This agreed with the findings of Levy et al. (1959). At the time when experiments with gonadotrophins were commenced, i.e., seven days post-hypophysectomy, the interstitial tissue cells possessed no RNA, contained large amounts of the lipid which had accumulated in the earlier days, and gave a negative reaction for 3β steroid dehydrogenase. It seems highly unlikely therefore that these cells could have been producing oestrogen to initiate granulosa cell growth. In any case, as has been seen, there was no evidence of mitotic activity in the granulosa cells of the persisting pre-antral follicles.

The other possibility mentioned, that gonadotrophins might stimulate this tissue to produce oestrogens is worth considering. Carithers and Green (1972), studying the ultra-structure of rat ovarian interstitial cells, found evidence of stimulation of these cells by gonadotrophins after hypophysectomy. Rice and Savard (1966) and Leymarie and Savard (1968), studying human stromal cells in vitro, found that the function of the cells was augmented by gonadotrophins and Baird (1976) stated that a minimal level of LH

is necessary for the maintenance of steroid synthesis by the stroma. In the present experiments the interstitial cells morphologically showed evidence of stimulation by both FSH and LH. In the case of LH however there was no sign of follicular growth and indeed there was evidence to the contrary which will be considered later. With FSH there was growth of pre-antral follicles which proceeded to mature into antral follicles: the question is one of timing. If the oestrogen responsible for starting granulosa cell growth was formed by the interstitial cells under the influence of FSH, signs of returning function in these cells would become apparent before the onset of granulosa cell growth, i.e., return of cytoplasmic RNA, decrease in lipid and the re-emergence of 3β steroid dehydrogenase. This did not seem to be so in the present experiments. While these changes did take place in the interstitial cells they lagged behind granulosa cell proliferation which was well established by 24 hours after an adequate dose of FSH. This fits in with the findings of Carithers and Green (1972) who gave pregnant mare's serum to hypophysectomized rats. The first signs of stimulation seen with the electron microscope occurred in the nuclei of the interstitial cells and were only marked 36 hours after treatment. Cytoplasmic changes occurred later. Despite these arguments the

possibility does remain that the function of the interstitial tissue cells might return sufficiently to form a little oestrogen.

At the same time there are the corpora lutea which were structurally intact in seven day post-hypophysectomy animals. The persistence of corpora lutea has been commented upon by Selye (1948) and Malven and Sawyer (1966) and would indicate that the pituitary secretions are luteolytic. This has been suspected for a considerable time (Rothchild and Quilligan, 1960) and LH has been incriminated (Rothchild and Schwartz, 1965).

The corpora lutea in the present animals retained considerable cytoplasmic RNA, showed only a moderate accumulation of lipid and still possessed large amounts of 3β steroid dehydrogenase. The latter observation confirms the findings of Levy et al. (1959). Only after twelve days was there a marked increase in lipid in the luteal cells and slowly progressive degenerative changes thereafter. In other words at seven days post-hypophysectomy, the corpora lutea possessed the characteristics of steroid producing cells. Since it was not possible to see any sign of growth of the granulosa cells in the small pre-antral follicles it must be assumed that this steroidogenic activity was more potential than actual so far as oestrogen production was concerned.

Following the administration of FSH however there appeared to be an increase of RNA, a reduction in the amount of intracellular lipid and an intensification of the reaction for 3β steroid dehydrogenase in the luteal cells. This was noted with the smallest dose of FSH and occurred before there was any sign of follicular activity. This change was dose and time related. Follicular growth was also dose and time related but it lagged behind the histochemical changes in the corpora lutea. This contrasts with the relationship between the alterations in interstitial cell activity and follicular growth. Granulosa cell growth was always in advance of interstitial cell change. These findings suggest that the corpora lutea may have been forming oestrogen under the influence of FSH.

No reference could be found in the literature relating directly to this possibility. In vitro experiments with corpora lutea involving incubation with suitable substrates have shown that they can form both progesterone and oestrone, chiefly progesterone (Zander, 1958; Zander et al.,1959; Hammerstein et al., 1964 and Savard et al., 1965), and LH stimulated the process (Rice et al., 1964). Under normal conditions the rat corpora lutea secrete small quantities of progesterone and then only for a short space of time (Bogdanove, 1972). McDonald et al. (1966) showed that if LH was given to hypophysectomized rats

oestrogen was increased but only if corpora lutea were present. This at least shows that the corpora lutea are responsive to stimulation. On the other hand, Louvet et al. (1975a and b) found if LH was administered to hypophysectomized rats there was a fall in ovarian weight even if the animals had been pre-treated with oestrogen. Histologically there was increased atresia of follicular tissue. This was also the experience in the present experiments.

The only report which may have some bearing on the findings in the present experiments is that of Hori et al. (1968). These workers found that while small doses of FSH given to hypophysectomized rats had no effect, large doses resulted in oestrogen secretion. Hypophysectomy however was carried out in dioestrus and little time had elapsed between hypophysectomy and the beginning of treatment.

The suggestion that FSH stimulates the corpora lutea to produce oestrogen seems a reasonable hypothesis in the light of these findings. The rise in FSH at or near the time of ovulation has never been explained (McClintock and Schwartz, 1968 and Gay et al., 1970b). FSH is known to stimulate the formation of 3β steroid dehydrogenase by granulosa cells (Zeleznik et al., 1974), and there is no evidence that it does not still have an influence when these cells become luteinized.

THE ACTION OF FSH ON OVARIAN STRUCTURE AND FUNCTION

Reference has already been made to the changes found in corpora lutea following administration of FSH. This section deals with changes in other ovarian structures.

In the present experiments it was shown that with FSH there was a production of antral follicles with doses above 25 µg. The phases of growth with time were: 1. Growth of pre-antral follicles, 2. Appearance of antral follicles with diminution of pre-antral follicles, and 3. Reappearance of pre-antral follicles, suggesting a continuing mechanism. From available evidence, the initial growth of pre-antral follicles appears to be due to oestrogen stimulation and as has been suggested this oestrogen could come from the corpus luteum stimulated by FSH.

The reappearance of pre-antral follicles would indicate a persisting secretion of oestrogen. At this stage, in addition to the corpus luteum as a possible source of oestrogen there is the regenerated interstitial tissue and the theca around the growing follicles. Granulosa cells are thought to be the exclusive site for FSH receptors (Zeleznik et al., 1974). If this is true then the stimulation of interstitial tissue and theca would not appear to be due to a direct action of FSH but rather to local factors. The fact that the growth of these tissues

was a relatively late feature in the FSH experiments also supports this idea.

The appearance of thecal tissue around the follicles could not have been expected. In addition it was interesting to note that RNA and 3β steroid dehydrogenase appeared in both thecal and interstitial tissue. Zeleznik et al. (1974) stated that granulosal 3β steroid dehydrogenase activity was stimulated in immature rats given FSH. It may be that this is one of the actions of FSH which is not confined to one particular cell type.

There was no evidence that oestrogen was being secreted in large quantities in the FSH experiments in this study since the uterus was only slightly increased in size and this was not statistically significant. The task was to try to relate morphology to function and this raised questions in regard to both thecal and interstitial cells. Despite large doses of FSH the thecal cells retained their simple spindle shape and neither they nor the interstitial cells showed any sign of luteinization. There is a considerable amount of evidence to show that thecal and interstitial cells can form androgens in certain conditions. Dorrington et al. (1975) showed that granulosa cells incubated with testosterone and stimulated by FSH produce oestrogen, and *in vivo* studies Armstrong and Papkoff (1976) found the blood oestrogen increased following the administration

of testosterone and FSH. This effect was not produced if a non-aromatizable androgen was used instead of testosterone, indicating that FSH induced the ability in granulosa cells to aromatize androgens. It might therefore be argued that the theca and interstitial tissue in the experimental animal model were producing androgens which were aromatized by granulosa cells under the influence of FSH. Fortune and Armstrong (1977) have shown however that thecal cells will produce aromatizable androgen but only when stimulated by LH. It therefore seems unlikely that androgens were being produced by the theca in the present animals. In addition, androgens can actually prevent the stimulatory effect which oestrogen has on follicular growth and may produce atresia (Louvet et al., 1975b). There was no sign of inhibition of follicular growth or early atresia in the experiments with FSH alone. From this it seems reasonable to suggest that the morphological forms assumed by the thecal and interstitial cells following FSH administration are not associated with production of androgen in any quantity.

Antral formation occurred quickly after the administration of FSH and with the higher doses antra formed prematurely in very small follicles. Instead of a single space forming, numerous small spaces appeared among the granulosa cells all around

the circumference of these follicles. The exact mechanism of antrum formation is not clearly understood (Zachariae, 1957) but it has been shown that FSH stimulates granulosa cells to secrete large molecule proteoglycans containing sulphate radicles both in vivo and in culture (Mueller et al., 1978). These large molecules must alter the osmotic equilibrium within the follicle and induce a flow of fluid from the surrounding tissues into the granulosa cell tissue. In Mueller's work the effect was dose dependent and the appearances in the ovaries of FSH treated hypophysectomized animals in the present experiments would support this.

The Graafian follicles produced in the ovaries of FSH treated hypophysectomized rats were not larger than medium sized antral follicles. In the lower dose experiments there was a tailing off in the proliferation of granulosa cells 48 hours after FSH administration and an occasional follicle showed early degenerative changes. With higher doses there was no evidence of cessation of growth at 48 hours. This would indicate either that the FSH had been "used up" at the lower dose range or that an insufficient number of FSH receptors had been formed. There was no evidence to allow a decision as to which of these possibilities was operating.

At no time was there any evidence of ovulation.

According to Harrington and Elton (1969) and Harrington et al. (1970) FSH can cause ovulation if the follicles are at the proper stage of development. Aron et al. (1969) stated that pure FSH caused luteinization in follicles but Schwartz and Ely (1970) while not disagreeing, found no evidence of ovulation. It may be that the maximum time of observation in the present experiments, 48 hours, was too short. Lostroh and Johnson (1966) found that ovulation could be produced in hypophysectomized rats by exogenous gonadotrophins after an interval of 2½ days.

Ying and Greep (1971) using a combination of FSH and LH in an intact female rat, observed that follicles were ready to respond to exogenous gonadotrophins and to ovulate 24 and 48 hours ahead of schedule in four and five day cyclic rats if gonadotrophins were given on the second day of dioestrus. As stated previously, hypophysectomized animals were given a single injection of FSH alone, so it is probable that this dose of FSH was used to promote follicular growth and probably another dose of FSH or FSH and LH was needed to induce ovulation. It was not however the purpose of the experiments to induce ovulation but to study the effect of FSH on the existing corpora lutea.

THE INFLUENCE OF LH ON THE OVARIAN STRUCTURE IN HYPOPHYSECTOMIZED ADULT FEMALE RATS

There have been a number of studies, with conflicting results, on the action of LH on the ovaries. For instance, Lostroh and Johnson (1966) stated that pure LH had no effect on the ovary. Other observers noted considerable changes following the administration of LH. Much depends on the animal used in experiments and the condition of the ovaries at the time.

Many workers have used immature, intact or hypophysectomized animals. No doubt this was with the idea of reducing the number of variables in their experiments by eliminating the factor of cyclical activity in the ovaries, pituitary and hypothalamus. Fewer experiments have been carried out on adult animals. Another important factor in dealing with hypophysectomized adult animals is the timing of hypophysectomy and the interval between the operation and the beginning of experimentation.

As already stated, the animals in the present experiments were adult female rats hypophysectomized in the late oestrous phase and further experimentation was delayed for a period of seven days. At this stage the ovaries contained corpora lutea which were morphologically intact, but all follicles had disappeared or were advanced in atresia apart from small pre-antral types (Stages 1, 2 and 3). The

interstitial tissue was fatty and inactive with nuclei showing clumping of chromatin.

The earliest change observed in the ovaries following the administration of LH was hyperaemia in and around the corpora lutea. This confirms the findings of Kupperman et al. (1948); Albert and Berkson (1951) and Wurtman (1964). McCracken et al.(1971) showed that LH increased the ovarian blood flow by 25-50% within 30 minutes. The changes noted in the present study were an increase in cytoplasmic RNA, the presence of lipid only in the form of fine globules and an apparent increase in 3β steroid dehydrogenase activity in the cells of the corpora lutea. All of these changes would suggest functional activity in the corpora lutea.

McDonald et al. (1966) administered LH to hypophysectomized rats and found that the blood oestrogen increased considerably but only if the ovaries contained corpora lutea. If this occurred in the LH treated hypophysectomized animals in the present study, and the activity noted in the corpora lutea would suggest that it might have, it would be reasonable to expect some growth stimulation of the small primary follicles still remaining in the ovaries. This was not found. Although there was no direct evidence of oestrogen production in these animals it is interesting to speculate on why follicular growth did not occur if oestrogen was present.

Delforge et al. (1972) found that LH actually diminished mitotic activity in the granulosa layer. Louvet et al. (1975a and b), in a series of experiments with hypophysectomized rats, demonstrated that LH caused a fall in ovarian weight. In addition, if the animals were treated with oestrogen the stimulatory growth effect of oestrogen was abolished by LH. Histologically they were able to show that LH stimulated the interstitial tissue and the theca in these animals, but caused atresia of follicles. Previously Wallach and Noriega (1970) found that LH administered to animals with oestrogen implants increased the number of atretic follicles. The present experiments show that LH stimulates the interstitial tissue, reducing the intercellular lipid, increasing cytoplasmic RNA, inducing 3β steroid dehydrogenase activity and reversing the clumping of chromatin in nuclei. This agrees with the statements of Rennel (1951) and Ryle (1972). The interstitial cells were partially luteinized. These observations and those of the authors quoted are in line with the findings of Hillier et al. (1978) who demonstrated that granulosa cells of hypophysectomized rats possessed no receptors for LH, thus indicating that LH can only manifest its effects in tissues outside the basement membrane of the follicle, i.e., thecal and interstitial cells. Binding sites for LH were found in these tissues by Zeleznik et al. (1974).

Previously many authors had claimed that interstitial cells were sensitive to gonadotrophins (Claesson et al., 1954; Balboni, 1960; Savard et al., 1965; Armstrong, 1968; Merker and Diaz Encinas, 1969; Guraya, 1971, 1973 and Carithers and Green, 1972). Marsh et al. (1976) were able to show that LH increased steroidogenesis in rat interstitial tissue and subsequently Savard and Leymarie (1976); Armstrong and Dorrington (1977) and Richards (1978) demonstrated that the steroids produced were mainly androgens.

From this type of evidence it has been adduced that the lack of stimulation by oestrogen and atresia of follicles following the administration of LH was due to the relatively large quantities of androgen produced by the interstitial tissue. Louvet et al. (1975a and b) found that if LH was administered to an oestrogen implanted hypophysectomized rat and an anti-androgen was subsequently given, the atretic effects of the LH were prevented, a finding subsequently substantiated by Hillier and Ross (1979).

In the experiments with LH in this series it can be postulated that while LH stimulated the corpora lutea to produce oestrogen, its action on the interstitial tissue was such that quantities of androgen were secreted sufficient to overcome the stimulatory effect of the oestrogen on the granulosa cells.

In these experiments it was noted that in some animals Graafian follicles were still present seven

days after the operation of hypophysectomy. LH given to these animals resulted in stimulation of the interstitial cells and growth of the theca with some degree of luteinization of both. The granulosa cells of these follicles were uniformly degenerate. This would tend to support the above postulate.

If this suggestion is true then it follows that the appearance of luteinization in the interstitial tissues and theca is an indication of androgen production.

THE COMBINED ACTION OF FSH AND LH ON THE OVARIAN STRUCTURE OF HYPOPHYSECTOMIZED ADULT RATS

This section is an extension of the experiments with LH and to some extent FSH. Follicle growth and luteinization can be induced by FSH plus LH and if the timing and dosage are correct ovulation will occur in hypophysectomized animals. According to Ying and Greep (1971) follicles almost ready for ovulation are present by the second day of the oestrous cycle in intact rats. In other words, it requires at least another two days from this stage for ovulation to occur. Lostroh and Johnson (1966) stated that it requires 2½ days to produce ovulation by administration of gonadotrophins to hypophysectomized rats and futher that only a small amount of LH is required. It is not surprising therefore that in the present experiments where observation was deliberately limited to a maximum period of 48 hours, ovulation was not observed.

One of the most striking effects of the administration of FSH plus LH to the hypophysectomized animals in this series was the increase in size of the corpora lutea. These were obviously pre-existing and not new corpora lutea since the centres were occupied by quite dense fibrous tissue. There was a marked increase in vascularity and in other signs of activity - cytoplasmic RNA and 3β steroid dehydrogenase. The increase in the size of these bodies was much more than was observed

with LH alone even in large doses and indeed in the latter instance the corpora lutea actually became smaller with the passage of time. This suggests that FSH was still exerting an influence on the corpora lutea either directly or indirectly, and to some extent would support the contention that FSH in itself has an influence on the corpus luteum.

The FSH-LH mixture resulted in follicular growth. Around the follicles there was a well defined thecal zone with swollen spindle cells containing plump nuclei even at the lowest dosage of LH. Fine lipid droplets, cytoplasmic RNA and 3β steroid dehydrogenase made their appearance in the thecal cells. Similar changes occurred in the interstitial cells. It was interesting to see that the interstitial cell changes shown at a combined dose level of 25 μg FSH and 25 μg LH were the same as those obtained with higher doses of LH alone. Again this would tend to confirm the impression in previous experiments that FSH by itself has a stimulatory effect on interstitial cells.

With the appearance of stimulated thecal cells the mitotic activity in the granulosa cells diminished. This became obvious when the dose of LH was raised to 50 μg. Further increase in the dose of LH caused full luteinization of the thecal and interstitial cells but degenerative changes occurred in the granulosa cells until a stage was reached where the

follicles were converted into atretic cysts surrounded by a thick zone of luteinized theca. These changes were reflected in the results of the follicle counts which confirmed the histological impression of an initial increase in the follicle growth followed by arrest of growth with higher doses of LH. Statistical analysis showed that the increase in the follicle numbers was significant. The changes were reminiscent of the histological changes noted in the polycystic ovarian syndrome in the human subject (Morris and Scully, 1958). It should be noted that in that syndrome FSH levels are generally normal or slightly low whereas LH values tend to be at a constant high level (Keettel et al., 1957; McArthur et al., 1958b; Yen et al., 1970 and Givens et al., 1974).

The degeneration and disappearance of the granulosa layer was directly related to the increase in the dose of LH. This subject has already been discussed in the previous section. It seems logical from all the observations made by various workers (already quoted) that the action of LH is to increase the secretion of androgens by thecal and interstitial tissue. It would appear that the androgen is in excess of the amount which can be dealt with by the granulosa cells. The stimulatory influence of oestrogen which is necessary for the continuing action of FSH and the maintenance of the granulosa cells is opposed by the excess androgen and granulosa growth

ceases. At the same time there was a statistically significant increase in the uterine weight of these animals following the administration of FSH + LH indicating possibly that the level of circulating oestrogen had increased. There may therefore be a distinct difference between the circulating level of oestrogen and the intra-follicular level.

In view of the above certain questions must be posed regarding the high levels of gonadotrophins which are attained prior to ovulation. Why does the surge of LH at mid-cycle not result in atresia of follicles, particularly when it has been found that the level of blood oestrogen tends to fall at this time, and why is there an increase in FSH at the same time?

FSH appears to have several roles to play in relation to steroidogenesis. In the discussion of the initiation of follicle growth the observation of many workers was quoted which appeared to show that in the early stages of follicle growth androgen could be converted to oestrogen by a process of aromatization in the granulosa cells under the influence of FSH. (Dorrington et al., 1975 and Armstrong and Papkoff, 1976). There is considerable evidence however to suggest that an equally important action of FSH is to stimulate the production of progesterone. Armstrong and Dorrington (1976) carried out three sets of <u>in vitro</u> experiments with granulosa cells from the follicles of immature rats. Incubation of

the cells in a suitable substrate containing FSH resulted in the production of progesterone. Similar cells incubated with androgens but without FSH produced very small amounts of progesterone but if the cells were incubated with both FSH and androgens the production of progesterone was increased many times over. Lucky et al. (1977) cultured granulosa cells from hypophysectomized immature rats with testosterone and demonstrated the secretion of progesterone. Hillier et al. (1977) carried out a similar experiment but found that the addition of an anti-androgen prevented progesterone production. Similar observations of the action of FSH and androgens have been made with granulosa cells of mature follicles from intact rats (Nimrod and Lindner, 1976 and Schomberg et al., 1976).

On the other hand when LH is added to a granulosa cell culture in a suitable substrate no progesterone is produced. An additional point which can be made is that pure FSH alone will cause luteinization of the granulosa of large follicles but does not cause ovulation (Aron et al., 1969).

The following hypothesis is put forward for consideration. During the period just before ovulation the theca produces large quantities of androgen under the influence of LH. If this were unopposed it would result in atresia of follicles. The coincidental surge of FSH stimulates the ability

of granulosa cells to produce progesterone as well as intra-follicular oestrogen from the thecal androgens and thus avoids their anti-oestrogen atretic action. The FSH may also be responsible for initiating the luteinization of the granulosa cells.

If the arguments regarding the action of LH and androgens on follicular development are acceptable the progressive enlargement of the uterus and hyperplasia of endometrium with FSH plus LH have to be explained. This can only be explained on the basis of an increase of circulating oestrogens. It would seem reasonable therefore to postulate that there are two mechanisms of oestrogen production. One is the oestrogen produced by the ovarian stroma, including theca and possibly interstitial cells which is responsible for maintaining the blood level. Two is the intra-follicular oestrogen produced by the interaction of theca and granulosa cells.

BIBLIOGRAPHY

ADLERCREUTZ, H. & LUUKKAINEN, T. (1968) Gas phase chromatographic methods for estrogens in biological fluids. In Gas Phase Chromatography of Steroids (Eik-nes, K.B. & Horning, E.C. Eds.) New York: Springer-Verlag.

ALBERT, A. & BERKSON, J. (1951) Clinical bioassay for chorionic gonadotrophins. Journal of Clinical Endocrinology, 11, 805-812.

ALLEN, E., PRATT, J.P., NEWELL, Q.U. & BLAND, L.J. (1930) Human ova from large follicles; including a search for maturation divisions and observations on atresia. American Journal of Anatomy, 46, 1-43.

ARMSTRONG, D.T. (1968) Hormonal control of uterine lumen fluid retention in the rat. American Journal of Physiology, 214, 764.

ARMSTRONG, D.T. & DORRINGTON, J.H. (1976) Androgens augment FSH - induced progesterone secretion by cultured rat granulosa cells. Endocrinology, 99, 1411-1414.

ARMSTRONG, D.T. & DORRINGTON, J.H. (1977) Estrogen biosynthesis in the ovaries and testes. Advances in Sex Hormone Research, 3, 217-258.

ARMSTRONG, D.T. & PAPKOFF, H. (1976) Stimulation of aromatization of exogenous and endogenous androgens in ovaries of hypophysectomized rats in vivo, by follicle stimulating hormone. Endocrinology, 99, 1144-1151.

ARON, C., ASCH, G., ASCH, L., ROOS, J. & HAXEMBOURGER, M. (1965) Donrees nouvelles sar les facteurs neuro-hormonaux de la luteinization chez ovulation du coit au cours du cycle oestral. Pathologie Biologie, 13, 603.

ARON, C., ROOS, J. & ASCH, G. (1969) Action luteinisante d'une preparation follicule stimulante (FSH) chez la ratte mune. Comptes Rendus Hebdoma-adaires des Seances de l'academie des Sciences (Paris), 268, 830.

ASTWOOD, E.B. (1941) The regulation of corpus luteum function by hypophyseal luteotrophin. Endocrinology, 28, 309.

BAIRD, D.T. & FRASER, I.S. (1974) Blood production and ovarian secretion rates of estradiol and estrone in women throughout the menstrual cycle. Journal of Clinical Endocrinology and Metabolism, 38, 1009-1017.

BAIRD, D.T., SWANTON, I. & SCARAMUZZI, R.J. (1976) Palsatile release of LH and secretion of ovarian steroids in sheep during luteal phase of estrous cycle. Endocrinology, 98, 1490.

BAKER, B.L. & KLAPPER, Z.F. (1961) Oxidative enzymes in the foreign body giant cells. Journal of Histochemistry, 9, 713.

BAKER, T.G. & WAI SUM, O. (1976) Development of the ovary and oogenesis. Clinics in Obstetrics and Gynaecology, Vol. 3, No. 1.

BALBONI, G.C. (1959) Histologic activity of the ovary in physiological and experimental conditions. Archivio Italiano di Anatomia e di Embroiologia, 64/1, 40-73.

BALBONI, G.C. (1960) Action of gonadotrophins on the ovaries in vitro. Archivio Italiano di Anatomia e di Embroiologia, 65, 115-136.

BALBONI, G.C. (1970) Observations on the ovary. Acta Analytica (Basel), 73, Suppl. 56:60+

BALBONI, G.C. (1973) The problem of the ovarian stroma. Archivio Italiano di Anatomia e di Embroiologia, 78, 37-58.

BALBONI, G.C. (1976) Histology of the ovary. In The Endocrine Function of the Human Ovary (James, V.H.T., Sevio, M. & Giusti, G. Eds) pp. 1-24, London: Academic Press.

BALBONI, G.C. & DE DOMINICIS, R. (1962) Research with electron microscope on the corpus luteum of the rat. Sperimentale, 112, 87-103.

BELL, E.T., MURKERJI, S. & LORAINE, J.A. (1966) The relationship of gonadotrophins excretion to ovulation during menstrual cycle. Acta Endocrinologica (Kobenhaven), 51, 578-590.

BESCH, P.K. & BUTTRAM, V.C.B. (1972) In Reproduction Biology, (Balin, H. & Glasser, S. Eds) p. 562, Excerpta Medica, Amsterdam.

BOGDANOVE, E.M. (1972) In The Regulation of Mammalian Production (Segal, S.J., Crozier, R., Corfman, P.A. & Condliffe, P.G. Eds) Ch. 6, Springfield, III: Charles C. Thomas.

BOGDANOVE, E.M., HILLIARD, J. & SAWYER, C.H. (1971) Serum LH patterns in the female rabbit as determined by radio-immunoassay. In The Proceedings of the Fifty Third Annual Meeting of the Endocrine Society, Abstract 68, Lippencott, Philadelphia.

BOLING, J.L., BLANDAU, R.J., SODERWALL, A.L. & YOUNG, W.C. (1941) Growth of Graafian follicle and the time of ovulation in the oestrous rat. Anatomical Records, 79, 313-331.

BRAMBELL, F.W.R. (1928) The development and morphology of the gonads of the mouse: the growth of the follicles. Proceedings of the Royal Society B, 103, 258-272.

BRAMBELL, F.W.R. (1956) Ovarian changes. In Marshall's Physiology of Reproduction (Parkes, A.S. Ed.) Vol. 1, pp. 397-542. London: Longmans.

BROWN, J.B., KLAPPER, A. & LORAINE, A.J. (1958) The urinary excretion of ostrogens, pregnandiol and gonadotrophins during the menstrual cycle. Journal of Endocrinology, 17, 401.

BROWN-GRANT, K. (1969) The induction of ovulation by ovarian steroids in the adult rat. Journal of Endocrinology, 43, 553.

BROWN-GRANT, K., EXLEY, D. & NAFTOLIN, F. (1970) Peripheral plasma oestradial and luteinizing hormone concentration during the oestrous cycle of the rat. Journal of Endocrinology, 48, 295.

BULLOUGH, W.S. (1942) The method of growth of follicle and corpus luteum in the mouse ovary. Journal of Endocrinology, 3, 150-156.

BURGER, H.G., CATT, K. & BROWN, J.B. (1968) Relationship between plasma luteinizing hormone and urinary oestrogen secretion during the menstrual cycle. Journal of Clinical Endocrinology and Metabolism, 28, 1508-1512.

BURKL, W. & KELLNER, G. (1954) Hypophysektomie und postnatale ovgenese bei ratten. Acta Anatomica, 23, 49-57.

BUTT, W.R. (1967) The purification and chemical properties of human pituitary FSH. In Reproduction in Female Mammal (Lamming, G.E. & Amoroso, E.C. Eds.) p 113. London: Butterworths.

CARGILLE, C.M., ROSS, G.T. & YOSHIMI, T. (1969) Daily variation in plasma FSH, LH and progesterone in normal menstrual cycle. Journal of Clinical Endocrinology, 29, 12.

CARITHERS, J.R. & GREEN, J.A. (1972) Ultrastructure of rat ovarian interstitial cells: Response to gonadotrophins. Journal of Ultrastructure Research, 39/3-4, 251-261.

CATCHPOLE, H.R., GERSH, I. & PAN, S.C. (1950) Some properties of ovarian connective tissue in relation to parenchymatous changes. Journal of Endocrinology, 6, 277-281.

CHANNING, C.P. (1970) Effect of stage of the menstrual cycle and gonadotrophins on luteinization of rhesus monkey granulosa cells in culture. Endocrinology, 87, 49-60.

CHANNING, C.P. & COUDERT, S.P. (1976) The contribution of granulosa cells and follicular fluid to ovarian estrogen secretion in the rhesus monkey in vivo. Endocrinology, 79, 590-597.

CLAESSON, L., HILLARP, N.A. & HOGBERG, B. (1954) Lipid changes in interstitial gland of rabbit ovary at oestrogen formation. Acta Physiologica, Scandinavia, 29, 329-339.

CLARK, E.B. (1923) Observations on the ova and ovaries of the guinea pig. Anatomical Records, 25, 313-331.

COLE, H.H. & CUPPS, P.T. (1969) Reproduction in domestic animals. New York & London: Academic Press.

CORNER, G.W. (1938) The site of formation of oestrogenic substances in the animal body. Physiological Review, 18, 154-172.

COURRIER, R. (1950) Interactions between estrogens and progesterone. Vitamins and Hormones, 8, 179.

CROFT, P.G. (1960) Ed. An introduction to the anaesthesia of the laboratory animals. The Universities Federation for Animal Welfare, London.

DEANE, H.W. & ANDREWS, J.S. (1953) A comparison of the staining of lipid droplets in the mouse ovary by the Schiff reaction and by the Ashbel-Seligman Carbonyl reaction. Journal of Histochemistry, 1, 283-295.

DEANE, H.W., LOBEL, B.L. & ROMNEY, S.L. (1962) Enzymatic histochemistry of normal human ovaries of the menstrual cycle, pregnancy and early puerperium. American Journal of Obstetrics and Gynaecology, 83, 281-294.

DE GRAAF, R.E.G. (1672) In De Mulierum Organis Generationi Inservientivus Tarctatus Novus. Published in Layden, Ex. Off. Hackiona.

DELFORGE, J.P., THOMAS, R., ROUX, F., CARREIRO DE SEQUEIRA & FERIN, J. (1972) Time relationships between granulosa cell growth and luteinization and plasma luteinizing hormone discharge in human. Fertility and Sterility, 23, 1-11.

DEMPSEY, E.W. & SEARLES, H.F. (1943) Environmental modification of certain endocrine pehnomena. Endocrinology, 32, 119.

DONALDSON, H.H. (1924) Ed. In The Rat, Data and Reference Tables, Philadelphia.

DONINI, P., PUZZUOLI, D., D'ALESSIO, I. & DONINI, S. (1969a) Purification and separation of follicles stimulating hormone from human pituitary gonadotrophins of male urine. Farmaco (Pratica), 23, 325-330.

DONINI, P., PUZZUOLI, D., D'ALESSIO, I. & DONINI, S. (1969b) Purification of urinary luteinizing hormone from human menopausal gonadotrophins. Farmaco (Pratica), 23, 316-324.

DORRINGTON, J.H., MOON, Y.S. & ARMSTRONG, D.T. (1975) Estradiol-17β biosynthesis in cultured granulosa cells from hypophysectomized immature rats; stimulation by follicle stimulating hormone. Endocrinology, 97, 1328-1331.

DUBREUIL, G. (1945) La glande interstitielle, thecale on oestrogene de l'ovaire feminin. Comptes Rendus de Seances de la Societe de Biologie et de Ses Filiales (Paris), 850-852.

DUBREUIL, G. (1948) Incidents in development of Graafian follicles in the human ovary. Gynecologie et Obstetrique, Paris 47/5, 628-630.

DUBREUIL, G. (1953) Notions tres simples sur la constition des ovaries de grossesse molaire et de chorioepitheliome. Annales d'Endocrinologie (Paris), 14, 223-224.

DUBREUIL, G. & RIVIERE, M. (1946) Morphology and histology of the human progestational and gestational corpus luteum. La Gynecologie, 43, 13, 65-82.

DUFAU, M.L., DULMANIS, A., CATT, K.J. & HUDSON, B. (1970) Measurement of plasma estradiol by competitive binding assay employing pregnancy plasma. Journal of Clinical Endocrinology, 30, 351.

DUKE, K.L. (1947) Fibrous connective tissue of rabbit ovary from sex differentiation to maturity. Anatomical Records, 98, 507-525.

DUPON, G., HOSSEINIAN, A. & KIM, M.H. (1973) Simultaneous determination of plasma estrogens, androgens and progesterone during the human menstrual cycle. Steroids, 22, 47-61.

ESHKOL, A., LUNENFELD, B. & PETERS, H. (1970) In Gonadotrophins and Ovarian Development (Butt, W.R., Crooke, A.C. & Ryle, M. Eds.) p 249. Edinburgh: E. & S. Livingstone.

EVANS, H.M. & COLE, H.H. (1930) An introduction to the study of the oestrous cycle in the dog. Memoirs of the University of California, 9, 66-118.

EVERETT, J.W. (1956) Functional corpora lutea maintained for months by autografts of rat hypophysis. Endocrinology, 58, 786.

EVERETT, J.W. (1969) Neuroendocrine aspect of mammalian reproduction. Annual Review of Physiology, 383-416.

EVERETT, J.W. & NICHOLS, D.C. (1968) The timing of ovulatory release of gonadotrophin induced by oestrogen in pseudopregnant and diestrous cycling rat. Anatomical Records, 160-346.

FAIMAN, C. & RYAN, R.J. (1967) Serum follicle stimulating hormone and luteinizing hormone concentration during the menstrual cycles as determined by radio-immunoassays. Journal of Clinical Endocrinology, 27, 1711.

FALCK, B. (1959) Site of production of oestrogen in the rat ovary as studied in microtransplants. Acta Physiologica, Scandinavica, 47, Suppl. 163, 1-101.

FEVOLD, H.L., HISAW, F.L. & LEONARD, S.L. (1931) The gonad stimulating and luteinizing hormones of the anterior lobe of the hypophysis. American Journal of Physiology, 97, 291.

FLERKO, B., HAJOS, F., SETALO, G. (1967) Electron microscopic observation on the rat ovaries in different stages of development and steroidogenesis. Acta Morphologica, Academiae Scientiarum, Hungaricae, 15, 163-183.

FORTUNE, J.E. & ARMSTRONG, D.T. (1977) Androgen production by theca and granulosa isolated from proestrous rat follicle. Endocrinology, 100, 1341-1347.

FRANCHIMONT, P. (1967) Immunological properties of gonadotrophins and their clinical applications. Acta Allergol, 22, 231-252.

FUKUSHIMA, M., STEVENS, V., GANTT, C. & VORYS, N. (1964) Urinary FSH and LH excretion during the normal menstrual cycle. Journal of Clinical Endocrinology, 24, 205.

GAY, V.L., MIDGLEY, A.R., Jr. & NISWENDER, G.D. (1970b) Patterns of gonadotrophins secretion associated with ovulation. Federation Proceedings, 29, 1880.

GIVENS, J.R., ANDERSON, N.R., UMSTOT, E.S. & WISER, W.L. (1974) Clinical findings and hormonal responses in patients with polycystic ovarian disease with normal versus elevated LH levels. Obstetrics and Gynaecology, 47, 388-394.

GOEBELSMANN, U., MIDGLEY, A.R. & JAFFE, R.B. (1969) Regulation of human gonadotrophins. Journal of Clinical Endocrinology and Metabolism, 29, 1222-1230.

GOLDENBERG, R.I., VAITUKAITIS, J.L. & ROSS, G.T. (1972) Oestrogen and FSH interaction on follicle growth in rats. Endocrinology, 90, 1492-1498.

GREEN, S.H., MANDL, A.M. & ZUCKERMAN, S. (1951) The proportion of ovarian follicles in different stages of development in rats and monkeys. Journal of Anatomy, London, 85, 325-329.

GUILLEMIN, R. & SCHALLY, A.V. (1963) Recent advances in the chemistry of neuroendocrine mediators originating in the central nervous system. Advances in Neuroendocrinology, p. 314-344.

GURAYA, S.S. (1971) Morphology, histochemistry and biochemistry of human ovarian compartments and steroid hormone synthesis. Physiological Reviews, 51, 785-807.

GURAYA, S.S. (1974) Morphology, histochemistry and biochemistry of human oogenesis and ovulation. International Review of Cytology, 37, 121-151.

GURAYA, S.S., STEGNER, H.E. & PAPE, C. (1973) Morphology, histochemistry and biochemistry of human oogenesis and ovulation. International Review of Cytology, 37, 121-147.

HAMBERGER, C. (1931) Hormone of anterior hypophyseal lobe in urine of male castrates. Ugeskrist Fur Laeger, 93, 27-30.

HAMMERSTEIN, J., RICE, B.F. & SAVARD, K. (1964) Steroid hormone formation in the human ovary. Journal of Clinical Endocrinology and Metabolism, 24, 597-605.

HARRINGTON, F.E., BEX, F.J., ELTON, R.L. & ROACH, J.B. (1970) The ovulatory effects of follicle stimulating hormone treated with chymotrypsin in chlorpromazine blocked rats. Acta Endocrinologica, Kobenhaven, 65, 222.

HARRINGTON, F.E. & ELTON, R.L. (1969) Induction of ovulation in adult rats with follicle stimulating hormone. Proceedings of the Society for Experimental Biology and Medicine, New York, 132, 841.

HARRISON, R.J. (1948) The changes occurring in the ovary of the goat during the oestrous cycle. Journal of Anatomy, London, 82, 21-48.

HARRISON, R.J. (1962) The structure of the ovary. In The Ovary (Zuckerman, S., Mandl, A.M. & Eckstein, P. Eds.) Vol. 1, p 143, Academic Press, New York and London.

HARTREE, A.S. (1966) Separation and partial purification of protein hormones from human pituitary glands. Biochemical Journal, 100, 754-766.

HILLIER, S.G., KNAZEK, R.A. & ROSS, G.T. (1977) Androgenic stimulation of progesterone production by granulosa cells from preantral and ovarian follicles; further in vitro studies using replicate cell cultures. Endocrinology, 100, 1539-1549.

HILLIER, S.G., ZELEZNIK, A.J. & ROSS, G.T. (1978) Independence of steroidogenic capacity and luteinizing hormone receptor induction in developing granulosa cells. Endocrinology, 102; 937-946.

HILLIER, S.G. & ROSS, G.T. (1979) Effect of exogenous testesterone on ovarian weight, follicular morphology and intraovarian progesterone concentration in estrogen-primed hypophysectomized immature female rats. Biology of Reproduction, 20, Part II, p 261.

HIRSHFIELD, I.N. & KORITZ, S.B. (1966) Pregnenolone synthesis stimulation in large particles from bovine adrenal cortex and bovine corpus luteum. Journal of Endocrinology, 78,165-168.

HOFFMANN, J.C. (1970) Light and reproduction in the rat. Biology of Reproduction, 2, 255.

HORI, T., IDE, M. & MIYAKE, T. (1968) Ovarian estrogen secretion during estrous cycle and under the influence of exogenous gonadotrophins in rat. Endocrinologica Japanica, 15, 215.

INGRAM, D.L. (1953) The effect of hypophysectomy on the number of oocytes in the adult albino rat. Journal of Endocrinology, 9, 307-311.

INGRAM, D.L. (1963) Atresia. In The Ovary (Zuckerman, S. Ed.) Vol. 1, 247, New York: Academic Press.

JAFFE, R.B., LEE, P.A. & MIDGLEY, A.R. (1969) Serum gonadotrophins before, at the inception of, and following human pregnancy. Journal of Clinical Endocrinology and Metabolism, 29, 1281-1283.

JOHANNSON, E.D.B. & WIDE, L. (1969) Pre-ovulatory levels of plasma progesterone and luteinizing hormone in women. Acta Endocrinologica, 62, 82-88.

JONES, E.C. & KROHN, P.L. (1961) Effect of hypophysectomy on age changes in ovaries of mice. Journal of Endocrinology, 21, 497-509.

KEETTEL, W.C., BRADBURY, J.T. & STODDARD, F.J. (1957) Observations on the polycystic ovary syndrome. American Journal of Obstetrics and Gynaecology, 73, 954-962.

KONIG, P.A. (1965) Histopochemie von enzymen immenschlichen ovarium. Bibliotheca Gynaecologica, 34, 77-203.

KRAMER, M.M., HARMAN, M.T. and BRILL, A.K. (1933) Disturbances of reproduction and ovarian changes in the guinea pig in relation to vitamin C deficiency. American Journal of Physiology, 106, 611-622.

KREY, L.C. & EVERETT, J.W. (1971) Ovulation after estrogen treatment in the rat oestrous cycle, significance of induced progesterone secretion. Federation Proceedings, 300-310.

KUPPERMAN, H.S., McSHANE, W.H. & MEYER, R.K. (1948) Gonadotrophinc hormones and ovarian hyperaemia in the rat. Endocrinology, 43, 275-285.

LAHR, E.L. & RIDDLE, O. (1936) Temporary suppression of estrous cycle in the rat by prolactin. Proceedings of the Society of Experimental Biology, 34, 880.

LANE, C.E. (1935) Some influences of oestrin on the hypophysis-gonad complex of the immature female rat. American Journal of Physiology, 110, 681.

LEVY, H., DEANE, H.W. & RUBIN, B.L. (1959) Visualization of steroid 3β dehydrogenase activity in tissues of intact and hypophysectomized rats. Journal of Endocrinology, 65, 932-943.

LEYMARIE, P. & SAVARD, K. (1968) Steroid hormone formation in the human ovary. Journal of Clinical Endocrinology and Metabolism, 28, 1547-1554.

LILLIE, R.D. & ASHBURN, L.L. (1943) Supersaturated solutions of fat stains in dilute isopropanyl for demonstration of acute fatty degeneration not shown by Herxheimer technique. Archives of Pathology, 36, 432.

LINDNER, H.R., TSAFRIRI, A., LIEBERMAN, M.E., ZOR, Y., KOCH, Y., BAUMINGER, S. & BARNEA, A. (1974) Gonadotrophin action on cultured Graafian follicle. Recent Progress in Hormone Research, 30, 79.

LINTERN-MOORE, S., PETERS, H., MOORE, G.P.M. & FABER, M. (1974) Follicular development in the infant human ovary. Journal of Reproduction and Fertility, 39, 53-64.

LONG, J.A. & EVANS, H.M. (1922) The oestrous cycle of the rat and its associated phenomena. Memoirs of the University of California, Vol. VI, University of California Press, Berkeley.

LOSTROH, A.J. & JOHNSON, R.E. (1966) Amount of ICSH and FSH required for follicle development, uterine growth and ovulation in hypophysectomized rat. Endocrinology, 79, 991.

LOUVET, J.P., HARMAN, S.M. & ROSS, G.T. (1975a) Effect of human chorionic gonadotrophin, human ICSH and human FSH on ovarian weights in oestrogen-primed hypophysectomized immature female rats. Endocrinology, 96, 1179-1186.

LOUVET, J.P., HARMAN, S.M., SCHREIBER, J.R. & ROSS, G.T. (1975b) Evidence for a role of androgens in follicular maturation. Endocrinology, 97, 366-372.

LOUVET, J.P. & VAITUKAITIS, J.L. (1976) Induction of FSH receptors in rat ovaries by estrogen priming. Endocrinology, 99, 758-764.

LUCKY, A.W., SCHREIBER, J.R., HILLIER, S.G., SCHULMAN, J.D. & ROSS, G.T. (1977) Progesterone production by cultured pre-antral rat granulosa cells: stimulation by androgen. Endocrinology, 100, 128-133.

LUNENFELD, B. & ESHKOL, A. (1968) The role of gonad-stimulating hormone on the development of the infantile ovary. In Gonadotrophins (Rosenberg, E. Ed.) Geron-X, Los Altos, California.

McARTHUR, J.W., INGERSOL, F.M. & WORCESTER, J. (1958) The urinary excretion of interstitial cell and follicle stimulating hormone activity during the normal menstrual cycle. Journal of Clinical Endocrinology and Metabolism, 18, 1186.

McARTHUR, J.W., INGERSOL, F.M. & WORCESTER, J. (1958b) The urinary excretion of interstitial cell and follicle stimulating hormone activity by women with diseases of the reproductive system. Journal of Clinical Endocrinology and Metabolism, 18, 1192

McCLINTOCK, J.A. & SCHWARTZ, N.B. (1968) Changes in pituitary and plasma follicle stimulating hormone concentrations during the rat estrous cycle. Endocrinology, 83, 433.

McCRACKEN, J.A., BAIRD, D.T. & GODING, J.R. (1971) Factors affecting the secretion of steroids from the transplanted ovary in the sheep. Recent Progress in Hormone Research, 27, 537-582.

McDONALD, G.J., ARMSTRONG, D.T. & GREEP, R.O. (1966) Stimulation of estrogen secretion from normal rat corpora lutea by luteinizing hormone. Endocrinology, 79, 289.

McDONALD, G.J. & GREEP, R.O. (1972) Ability of luteinizing hormone to acutely increase serum progesterone levels during the secretory phase of the rhesus menstrual cycle. Fertility and Sterility, 23, 466-470.

McDONALD, G.J., KEYES, P.L. & GREEP, R.O. (1969) Steroid secreting capacity of X-irradiated rat ovaries. Endocrinology, 84, 1004.

MAKRIS, A. & RYAN, K.J. (1975) Progesterone, androstenedione, testosterone, estrone and estradial synthesis in hamster ovarian follicle cells. Endocrinology, 96, 694-701.

MALVEN, P.V. & SAWYER, C.H. (1966) Forms of new corpora lutea in mature hypophysectomized rat. Endocrinology, 78, 1259.

MANDL, A.M. & ZUCKERMAN, S. (1950) The number of normal and atretic ova in the mature rat. Journal of Endocrinology, 6, 426-435.

MANDL, A.M. & ZUCKERMAN, S. (1951a) Number of normal and atretic oocytes in unilaterally spayed rats. Journal of Endocrinology, 7, 112-119.

MANDL, A.M. & ZUCKERMAN, S. (1951b) The relation of age to numbers of oocytes. Journal of Endocrinology, 7, 190-193.

MANDL, A.M. & ZUCKERMAN, S. (1951c) The growth of the oocytes and follicle in the adult rat. Journal of Endocrinology, 7,

MARSH, J.M. (1976) The role of cyclic AMP in gonadal steroidogenesis. Biological Reproduction, 14, 30-53.

MATHEWS, L.H. (1941) Reproduction in the spotted hyaena crocuta. Phil. Trans. B, 230, 1-78.

MAULEON, P. (1969) Oogenesis and folliculogenesis. In Reproduction in Domestic Animals (Cole, H.H., Cupps, P.T. Eds) Second Edn., pp. 187-215. New York:Academic Press.

MERKER, H.J. & DIAZ-ENCINAS, J. (1969) Das elektronen-mikroskopische bild des ovars juveniler ratten und kaninchen nach stimulierung mit PMS and HCG. 1. theka und stroma. Zeitschrift fur Zellforschung und Mikroskopische Anatomie, Berlin, 94, 605-623.

MIDGLEY, A.R., Jnr.,& JAFFE, R.B. (1966) Human luteinizing hormone in serum during the menstrual cycle, determination by radio-immunoassay. Journal of Clinical Endocrinology and Metabolism, 26, 1375.

MIDGLEY, A.R., Jnr. & JAFFE, R.B. (1968) Regulation of human gonadotrophins. Journal of Clinical Endocrinology and Metabolism, 28, 1699-1703.

MORRIS, J. McL. & SCULLY, R.E. (1958) In Endocrine Pathology of the Ovary. p. 120-142. St Louis, Mosby.

MOSSMAN, H.W., KOERING, J.M. & FERRY, D. (1964) Cyclic changes of interstitial gland tissue of the human ovary. American Journal of Anatomy, 115, 235-256.

MOTTA, P. (1966) Osservazioni salla fine shutlura delle cellule interstiziali dell'ovaio. Biol. Lat. (Milano), 19, 107-137.

MOTTA, P. & BOURNEVA, V. (1970) A comparative histochemical study of 5 Δ 3β hydroxysteroid dehydrogenase and lipid control in the rat ovary with special reference to the interstitial cells. Acta Histochemica, 38, 340-351.

MUELLER, P.L., SCHREIBER, J.R., LUCKY, A.W., SCHULMAN, D.J., RODBARD, D. & ROSS, G.T. (1978) Follicle stimulating hormone stimulates ovarian synthesis of proteoglycans in the oestrogen-stimulated hypophysectomized immature female rat. Endocrinology, 102, 824-831.

NAKANO, R., MIZUNO, T., KATAYAMA, K. & TOJO, S. (1975) Growth of ovarian follicles in rats in the absence of gonadotrophins. Journal of Reproduction and Fertility, 45, 545.

NEILL, J.D., JOHANNSON, E.D.B. & KNOBIL, E. (1969) Failure of hysterectomy to influence the normal pattern of cyclic progesterone secretion in the rhesus monkey. Journal of Endocrinology, 84, 464-465.

NIMROD, A. & LINDNER, H.R. (1976) A synergistic effect of androgen on the stimulation of progesterone secretion by FSH in cultured rat granulosa cells. Molecular Cell Endocrinology, 5, 315-320.

NISHIZUKA, Y. (1954) Histological studies on ovaries: mainly histochemical observations. Acta Scholae Medicinalis Universitatis in Kioto, 31, 215-257.

PAPANICOLAOU, G.N. & BRIDGES, E.L. (1942) New procedures for staining vaginal smears. Science, 95, 438-439.

PAPANICOLAOU, G.N. & BRIDGES, E.L. (1957) Simple methods for protecting fresh smears from dying and deteriorating during mailing. Journal of the American Medical Association, 164, 1330-1331.

PARLOW, A.F. (1958) A rapid method for LH and factors stimulating LH secretion. Federal Proceedings, 17, 402.

PARLOW, A.F. (1964) Differential action of small doses of estradiol on gonadotrophins in the rat. Endocrinology, 75, 1.

PAYNE, R.W. & HELLBAUM, A.A. (1955) The effect of estrogens on the ovary of the hypophysectomized rat. Endocrinology, 57, 193-199.

PEDERSEN, T. (1970) Follicle kinetics in the ovary of the cyclic mouse. Acta Endocrinologica, Copenhagen, 64, 304-323.

PENCHARZ, R.I. (1940) Effects of estrogens and androgens alone and in combination with chorionic gonadotrophin on the ovary of the hypophysectomized rat. Science, 91, 554-555.

PFLUGER, E.F.W. (1863) Ueber die eierstocke der saugethiere und des menschen, Leipzig.

PHIFER, R.F., MIDGLEY, A.R. & SPICER, S.S. (1972) Histology of the human hypophyseal gonadotrophin secreting cells. In Gonadotrophins (Saxena, B.B., Boling, C.G. & Candy, H.M. Eds) p. 9-25, New York: Wiley-Interscience.

PHILLIPP, E. (1930) Die bildungsstatte des hypophysenvor der lappenhormons. Zentral Blatt Fur Gynakologie, 54, 1858-1866.

RAJAMEMI, H.J. & MIDGLEY, A.R. (1975) In Methods in Enzymology, (Hardman, J.G. & O'Malley, B.W. Eds) Vol. 37, Part B, p. 145. New York:Academic Press.

RAMIREZ, V.D. & SAWYER, C.H. (1965) Fluctuations in hypothalamic LH-RF during the rat estrous cycle. Endocrinology, 76, 282-289.

REICHERT, L.E. & PARLOW, A.F. (1964) Partial purification and separation of urinary gonadotrophins of non-pregnant humans. Journal of Clinical Endocrinology and Metabolism, 24, 1040.

RENNEL, E.G. (1951) Influence of hormones on the histochemistry of ovarian interstitial tissue in the immature rat. American Journal of Anatomy, 88, 63-107.

RICE, B.F., HAMMERSTEIN, J. & SAVARD, K. (1964) Steroid hormone formation in the human ovary. Journal of Clinical Endocrinology and Metabolism, 24, 606-615.

RICE, B.F. & SAVARD, K. (1966) Steroid hormone formation in the human ovary. Journal of Clinical Endocrinology and Metabolism, 26, 593-609.

RICHARDS, J.S. (1975) Estradiol receptor content in rat granulosa cells during follicular development, modification by estradiol and gonadotrophins. Endocrinology, 97, 1174.

RICHARDS, J.S. (1978) Hormonal control of ovarian follicular development: A 1978 perspective. Recent Progress in Hormone Research.

RICHARDS, J.S. & MIDGLEY, A.R., Jnr. (1976) Protein hormone action: A key to understanding ovarian follicular and luteal cell development. Biology of Reproduction, 14, 87-94.

ROBINSON, A. (1918) The formation, rupture and closure of ovarian follicles in ferrets and some associated phenomena. Transactions of the Royal Society, Edinburgh, 52, 302-362.

ROTHCHILD, I. (1958) Progesterone and the menstrual cycle. Obstetrics and Gynaecology, 11/5, 564-572.

ROTHCHILD, I. (1965a) Inter-relations between progesterone and the ovary, pituitary and CNS in the control of ovulation and regulation of progesterone secretion. Vitamins and Hormones, 23, 209-305.

ROTHCHILD, I. (1965b) The corpus luteum hypophysis relationship: The luteolytic effect of luteinizing hormone. Acta Endocrinologica (Kobenhaven) 49, 107-119.

ROTHCHILD, I. & QUILLIGAN, E.J. (1960) The corpus luteum pituitary relationship. Endocrinology, 67, 122.

ROTHCHILD, I. & SCHWARTZ, N.B. (1965) The corpus luteum-hypophysis relationship. The effects of progesterone and oestrogen on the secretion of luteotrophin and luteinizing hormone in the rat. Acta Endocrinologica (Kobenhaven), 49, 120-137.

RYAN, K.J. (1968) On obtaining luteinizing and follicle stimulating hormones from human pituitaries. Journal of Clinical Endocrinology, 28, 886-896.

RYAN, K.J. & PETRO, Z. (1966) Steroid biosynthesis by human ovarian granulosa and thecal cells. Journal of Clinical Endocrinology and Metabolism, 26, 46-52.

RYAN, K.J., PETRO, Z. & KAISER, J. (1968) Steroid formation by isolated and recombined ovarian, granulosa and thecal cells. Journal of Clinical Endocrinology and Metabolism, 28, 355-359.

RYLE, M. (1972) The growth (in vitro) of mouse ovarian follicles of different sizes in response to purified gonadotrophins. Journal of Reproduction and Fertility, 30, 395-405.

SAVARD, K., MARSH, J.M. & RICE, B.F. (1965) Gonadotrophins and ovarian steroidogenesis. Recent Progress in Hormone Research, 21, 285-365.

SAVARD, K. & LEYMAIRE, W.J. (1976) Steroidogenic capacities of the different compartments of the human ovary. In The Endocrine Function of the Human Ovary (James, V.H.T., Said, M. & Guisti, G. eds.) pp. 37-45. London: Academic Press.

SAWYERS, C.H., MARKEE, J.E. & TOWNSEND, B.F. (1949) Cholinergic and adrenergic components in the neurohumeral control of the release of LH in rabbits. Endocrinology, 44, 18-37.

SAXENA, B.B., DEMURA, H., CANDY, H.M. & PETERSON, R.E. (1968) Radio-immunoassay of human follicle stimulating and luteinizing hormones in plasma. Journal of Clinical Endocrinology and Metabolism, 28, 511.

SCHOMBERG, D.W., STOUFFER, R.L. & TYREY, L. (1976) Modulation of progestin secretion in ovarian cells by dihydrotestosterone. Biochemical and Biophysiological Research Communications, 68, 77-85.

SCHREIBER, J.R. & ROSS, G.T. (1976) Further characterization of a rat ovarian testosterone receptor with evidence for nuclear translocation. Endocrinology, 99, 590-596.

SCHWARTZ, N.B. & BARTOSIK, D. (1962) Changes in pituitary LH content during the rat oestrous cycle. Endocrinology, 71, 756.

SCHWARTZ, N.B. & ELY, C.A. (1970) Comparison of effect of hypophysectomy, anti-serum to ovine LH and ovariectomy on oestrogen secretion during the rat oestrous cycle. Endocrinology, 86, 1420.

SCHWARTZ, N.B. & GOLD, J.J. (1967) Effect of a single dose of anti-LH serum at pro-oestrus on the rat oestrous cycle. Antatomical Records, 157, 137.

SELYE, H. (1948) In textbook of Endocrinology, Fourth printing, p. 234. Acta Endocrinologica, Universitie De Montreal, Montreal, Canada.

SHAIKH, A.A. (1971) Estrone and estradiol levels in the ovarian venous blood from rats during the estrous cycle and pregnancy. Biological Reproduction, 5, 297-307.

SMITH, B.D. & BRADBURY, J.T. (1961) Ovarian weight response to varying doses of oestrogens in intact and hypophysectomized rats. Proceedings of the Society for Experimental Biology and Medicine, 107, 946-949.

SMITH, P.E. & ENGLE, E.T. (1926) Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system. American Journal of Anatomy, 40, 159.

STEELMAN, S.L. & POHLEY, F.M. (1953) Assay of the follicle stimulating hormone based on the augmentation with human chorionic gonadotrophin. Endocrinology, 53, 604.

STEVENS, V.C. & VORYS, N. (1967) The regulation of pituitary function by sex steroids. Obstetrics and Gynaecological Survey, 22, 781.

STOCKARD, C.R. & PAPANICOLAOU, G.N. (1917) The existence of a typical oestrous cycle in the guinea pig with a study of its histological and physiological changes. American Journal of Anatomy, 22, 225-283.

SWEZY, O. (1933) Orogenesis and its relation to hypophysis. Science Press, Lancaster, Pennyslvania.

TAYMOR, M.L., AONO, T. & PHETEPLACE, C. (1968) Follicle stimulating hormone and luteinizing hormone in serum during the menstrual cycle determined by radio-immunoassay. Acta Endocrinologica, (Kobenhaven).

THORNEYCROFT, I.H., MISHEL, D.R., Jnr., STONE, S.C. et al. (1971) The relation of serum 17-hydroxyprogesterone and estradiol-17-beta levels during the human menstrual cycle. American Journal of Obstetrics and Gynaecology, 111, 947-951.

TREVAN, D.J. & SHARROCK, A. (1951) Methyl green-pyronin-orange G. stain for formalin-fixed tissues. Journal of Pathology and Bacteriology, 63, 326.

TURNER, C.D. & BAGNARA, J.T. (1971) In General Endocrinology, Fifth Edition, p. 517, published by W.B. Saunders, London.

VAN DYKE, K. & KATZMAN, P.A. (1968) Effect of pregnant mare's serum on RNA polymerase activity and protein synthesis in ovaries of immature rats. Endocrinology, 83, 107.

WALLACH, E.E. & NORIEGA, C. (1970) Effect of local steroids on follicular development and atresia in rabbit. Fertility and Sterility, 21, 253-267.

WEISNER, B.P. & CREW, F.A.E. (1930) The gonadotrope actions of the anterior lobe of the pituitary. Proceedings of the Royal Society, Edinburgh, 50, 79.

WILLIAMS, P.C. (1940) Effect of stilbestrol on the ovaries of hypophysectomized rats. Nature, 145, 388-389.

WILLIAMS, P.C. (1956) The history and fate of redundant follicles. In Ciba Foundation Colloquia on Aging (Wolstenholme, G.E.W. & Miller, E.C.P. eds) Vol. 2, pp. 59-68, London:Churchill.

WURTMAN, R.J. (1964) An effect of LH on the fractional perfusion of the rat ovary. Endocrinology, 75, 927-933.

YEN, S.S.C., VELA, P. & RANKIN, J. (1970) Inappropriate secretion of follicle stimulating hormone and luteinizing hormone in polycystic ovarian disease. Journal of Clinical Endocrinology and Metabolism, 30, 435-442.

YING, S.Y. & GREEP, R.O. (1971) Effect of age of rat and dose of a single injection of estradiol benzoate (Ed.) on ovulation and the facilitation of ovulation by progesterone (P) Endocrinology, 89, 785.

YOUNG, W.C. (1961) The mammalian ovary. In Sex and Internal Secretions (Young, W.C. ed.) Vol. 1, p. 449. Baltimore: Williams and Wilkins.

ZACHARIAE, F. (1957) Studies on the mechanism of ovulation. Acta Endocrinologica,(Kobenhaven) 26, 215-224.

ZANDER, J. (1958) Steroids in human ovary. Journal of Biological Chemistry, 232, 117-122.

ZANDER, J., BRENDLE, E., VON MUNSTERMANN, A.M., DICZFALUSY, E., MARTINSEN, B. & TILLINGER, K.G. (1959) Identification and estimation of oestradiol 17β and oestrone in human ovaries. Acta Obstetrica et Gynaecologica, Scandinavica, 38, 724-736.

ZELEZNIK, A.J., MIDGLEY, A.R., Jnr. & REICHERT, L.E., Jnr. (1974) Granulosa cell maturation in the rat. Endocrinology, 95, 818-825.

ZONDEK, B. & ASCHEIM, S. (1926) Uber die funktion des ovariums dentsche medizinische. Wochenschrift, 52, 343.

ZONDEK, B. & ASCHEIM, S. (1927) Hypophysenvorder lappen und ovarium. Beziehungen der endokrinen Drusen zur ovarial-funktion. Ei und Hormon Klinische Wochenschrift, 6, 1322.

ZUCKERMAN, S., MANDL, A.M. & ECKSTEIN, P. Eds. The Ovary, Vol. I, Academic Press: London and New York, 1962.

ZUCKERMAN, S. & PARKES, A.S. (1932) The menstrual cycle of the primates. Proceeding of the Zoological Society, London, 139-191.

APPENDIX I

Experiment	Total Body Weight				Ovarian Weight		Uterine Weight	
	At Start of Experiment		At Sacrifice					
INTACT RAT	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2
DAY 1:								
1 hour	180	182	182	184	0.0582	0.0582	0.3822	0.3824
6 hours	182	182	182	184	0.0578	0.0584	0.3684	0.3926
12 hours	180	180	182	182	0.0584	0.0582	0.3728	0.3836
18 hours	184	182	186	182	0.0576	0.0584	0.3904	0.3782
24 hours	180	182	184	184	0.0584	0.0578	0.3698	0.3814
DAY 2:								
1 hour	182	184	184	186	0.0486	0.0522	0.3862	0.3844
6 hours	180	186	182	186	0.0488	0.0498	0.3884	0.3926
12 hours	184	182	184	184	0.0502	0.0486	0.3928	0.4002
18 hours	182	180	184	184	0.0498	0.0496	0.3722	0.3668
24 hours	188	180	188	184	0.0504	0.0488	0.3928	0.3742
DAY 3:								
1 hour	182	180	184	184	0.0428	0.0426	0.3684	0.3826
6 hours	180	182	182	184	0.0442	0.0422	0.3602	0.3622
12 hours	184	180	184	182	0.0426	0.0428	0.3742	0.3602
18 hours	180	184	182	186	0.0462	0.0482	0.3586	0.3724
24 hours	186	180	186	182	0.0446	0.0448	0.3662	0.3564
DAY 4:								
1 hour	180	182	182	184	0.0486	0.0486	0.3864	0.3926
6 hours	180	180	184	182	0.0500	0.0488	0.3726	0.3848
12 hours	184	182	186	184	0.0490	0.0468	0.3922	0.3722
18 hours	182	180	186	184	0.0488	0.0492	0.3864	0.3894
24 hours	186	184	188	186	0.0498	0.0486	0.3688	0.3926
SHAM OPERATION	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2
DAY 1	182	180	174	166	0.0542	0.0582	0.3884	0.3674
DAY 2	182	182	174	174	0.0496	0.0486	0.3982	0.3872
DAY 3	186	182	172	174	0.0446	0.0406	0.3966	0.3666
DAY 4	180	183	174	168	0.0494	0.0490	0.3882	0.3886

Total body weight, ovarian weight and uterine weight of Intact and Sham Operation rats (Day 1 - 4)

APPENDIX II

Experiment	Total Body Weight				Ovarian Weight		Uterine Weight	
	At Start of Experiment		At Sacrifice					
HYPOX. CONTROL	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2	Rat 1	Rat 2
DAY 1: 1 hour	184	182	170	168	0.0542	0.0560	0.3384	0.3380
6 hours	182	180	168	166	0.0568	0.0558	0.3360	0.3364
12 hours	186	180	172	168	0.0580	0.0546	0.3362	0.3380
18 hours	182	180	168	166	0.0524	0.0522	0.3362	0.3286
24 hours	186	182	172	168	0.0492	0.0500	0.3284	0.3286
DAY 2: 30 hours	188	184	175	170	0.0402	0.0428	0.3270	0.3274
36 hours	180	182	166	166	0.0422	0.0442	0.3260	0.3286
42 hours	180	182	166	164	0.0440	0.0442	0.3088	0.3324
48 hours	182	180	168	166	0.0394	0.0392	0.3224	0.3194
DAY 3: 54 hours	188	182	172	168	0.0408	0.0400	0.3289	0.3289
60 hours	192	184	176	170	0.0388	0.0402	0.3264	0.3220
66 hours	190	180	172	166	0.0398	0.0334	0.3302	0.3214
72 hours	180	184	166	170	0.0402	0.0398	0.3104	0.3200
DAY 4: 78 hours	182	180	168	166	0.0386	0.0384	0.3168	0.3188
84 hours	186	184	172	170	0.0378	0.0390	0.3140	0.3124
90 hours	182	188	168	172	0.0394	0.0386	0.3008	0.3278
96 hours	184	184	170	172	0.0386	0.0398	0.3102	0.3094
DAY 5	182	182	168	172	0.0362	0.0360	0.3089	0.3089
6	182	180	170	168	0.0350	0.0342	0.3042	0.3006
7	180	186	166	170	0.0302	0.0302	0.2970	0.2974
8	184	180	170	166	0.0297	0.0301	0.2700	0.2744
DAY 9	186	182	172	168	0.0290	0.0288	0.2684	0.2744
10	184	180	170	168	0.0292	0.0292	0.2502	0.2502
11	185	186	172	170	0.0286	0.0296	0.2468	0.2504
12	184	182	170	168	0.0288	0.0286	0.2264	0.2260
DAY 13	186	184	172	166	0.0286	0.0278	0.2286	0.2362
14	184	182	170	168	0.0274	0.0270	0.2232	0.2250
15	180	182	170	174	0.0292	0.0266	0.2196	0.2284
16	186	186	170	172	0.0260	0.0264	0.2221	0.2211
DAY 17	188	186	170	166	0.0266	0.0274	0.2400	0.2342
18	190	180	168	164	0.0266	0.0268	0.2300	0.2304
19	180	182	170	170	0.0248	0.0256	0.2260	0.2246
20	180	180	176	166	0.0210	0.0218	0.2160	0.2189
DAY 21	182	186	172	172	0.0226	0.0214	0.2028	0.2000
22	178	186	174	172	0.0218	0.0218	0.2028	0.2028
23	184	180	172	174	0.0222	0.0262	0.2104	0.2000
24	180	182	172	174	0.0222	0.0220	0.2006	0.2018
DAY 25	180	184	176	170	0.0216	0.0224	0.2102	0.2008
26	182	180	178	172	0.0210	0.0230	0.1990	0.1988
27	184	182	172	178	0.0200	0.0232	0.2002	0.2108
28	182	180	176	176	0.0219	0.0219	0.2002	0.2036

Total body weight, ovarian weight and uterine weight of hypophysectomized controls (Day 1 - 28)

APPENDIX III

Experiment	Ovarian Weight		Uterine Weight	
	Rat 1	Rat 2	Rat 1	Rat 2
Hypophysectomized Rat + 25 µg FSH				
1 hour	0.0302	0.0300	0.3020	0.3060
6 hours	0.0310	0.0318	0.3022	0.3020
12 hours	0.0360	0.0346	0.3009	0.3019
24 hours	0.0350	0.0354	0.3084	0.3192
48 hours	0.0350	0.0350	0.3042	0.3046
Hypophysectomized Rat + 50 µg FSH				
1 hour	0.0302	0.0302	0.3038	0.3020
6 hours	0.0350	0.0355	0.3096	0.3104
12 hours	0.0402	0.0402	0.3124	0.3100
24 hours	0.0416	0.0420	0.3100	0.3188
48 hours	0.0420	0.0422	0.3210	0.3186
Hypophysectomized Rat + 75 µg FSH				
1 hour	0.0302	0.0306	0.3042	0.3046
6 hours	0.0368	0.0368	0.3050	0.3066
12 hours	0.0418	0.0436	0.3064	0.3072
24 hours	0.0432	0.0436	0.3168	0.3168
48 hours	0.0434	0.0430	0.3260	0.3264
Hypophysectomized Rat +100 µg FSH				
1 hour	0.0321	0.0325	0.3024	0.3072
6 hours	0.0364	0.0376	0.3050	0.3062
12 hours	0.0421	0.0423	0.3084	0.3080
24 hours	0.0436	0.0436	0.3186	0.3186
48 hours	0.0432	0.0434	0.3200	0.3206

Effect of FSH, B-1 on the Ovarian and Uterine weights of the hypophysectomized rat.

APPENDIX IV

Experiment	Ovarian Weight		Uterine Weight	
	Rat 1	Rat 2	Rat 1	Rat 2
Hypophysectomized Rat + 25 µg LH				
1 hour	0.0304	0.0300	0.2970	0.2974
6 hours	0.0316	0.0318	0.2880	0.2884
12 hours	0.0321	0.0342	0.3000	0.3004
24 hours	0.0322	0.0324	0.2840	0.2848
48 hours	0.0311	0.0312	0.2930	0.2966
Hypophysectomized Rat + 50 µg LH				
1 hour	0.0312	0.0312	0.2880	0.2880
6 hours	0.0320	0.0326	0.2904	0.2900
12 hours	0.0338	0.0338	0.2848	0.2834
24 hours	0.0344	0.0346	0.2862	0.2862
48 hours	0.0340	0.0340	0.2920	0.2922
Hypophysectomized Rat + 75 µg LH				
1 hour	0.0310	0.0314	0.2860	0.2860
6 hours	0.0358	0.0358	0.2886	0.2886
12 hours	0.0340	0.0349	0.2890	0.2892
24 hours	0.0354	0.0358	0.2900	0.2900
48 hours	0.0352	0.0346	0.2904	0.2894
Hypophysectomized Rat + 100 µg LH				
1 hour	0.0316	0.0318	0.2880	0.2884
6 hours	0.0338	0.0338	0.2886	0.2880
12 hours	0.0368	0.0360	0.2890	0.2894
24 hours	0.0352	0.0354	0.2900	0.2900
48 hours	0.0350	0.0344	0.2900	0.2912

Effect of LH (IRC, 24.6.69) on the Ovarian and Uterine weights of the hypophysectomized rat.

APPENDIX V

Experiment	Ovarian Weight		Uterine Weight	
	Rat 1	Rat 2	Rat 1	Rat 2
Hypophysectomized Rat + 25 µg FSH + 25 µg LH				
1 hour	0.0302	0.0306	0.2972	0.2972
6 hours	0.0345	0.0349	0.3084	0.3088
12 hours	0.0388	0.0388	0.3488	0.3482
24 hours	0.0410	0.0414	0.4577	0.4577
48 hours	0.0408	0.0400	0.4588	0.4580
Hypophysectomized Rat + 25 µg FSH + 50 µg LH				
1 hour	0.0304	0.0304	0.2880	0.2884
6 hours	0.0380	0.0388	0.3020	0.3360
12 hours	0.0460	0.0466	0.3910	0.3926
24 hours	0.0460	0.0460	0.4680	0.4682
48 hours	0.0400	0.0416	0.5180	0.5192
Hypophysectomized Rat + 25 µg FSH + 75 µg LH				
1 hour	0.0306	0.0306	0.2890	0.2898
6 hours	0.0440	0.0444	0.3026	0.3026
12 hours	0.0462	0.0466	0.3988	0.3994
24 hours	0.0464	0.0464	0.4824	0.4846
48 hours	0.0430	0.0440	0.5352	0.5292
Hypophysectomized Rat + 25 µg FSH + 100 µg LH				
1 hour	0.0304	0.0308	0.2884	0.2884
6 hours	0.0488	0.0486	0.3112	0.3116
12 hours	0.0512	0.0518	0.4078	0.4082
24 hours	0.0510	0.0524	0.4922	0.4926
48 hours	0.0484	0.0488	0.5480	0.5468

Effect of 25 µg FSH, B-1 + varying doses of LH (IRC, 24.6.69) on the Ovarian and Uterine weights of the hypophysectomized rat