STUDIES ON PITUITARY GONADOTROPHS IN PREPUBERTAL AND PUBERTAL FEMALE RATS: EFFECT OF CASTRATION AND LHRH TREATMENT

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ABSTRACT

The present studies were undertaken to elucidate ultrastructural changes in normal, ovariectomized, luteinizing hormone releasing hormone (LHRH) treated and ovariectomized-LHRH treated, prepubertal and pubertal female rats. Ultrastructural changes in pituitary gonadotrophs producing follicle stimulating hormone (FSH) and luteinizing hormone (LH) were carried out using the standard procedures. In some cases parallel studies were also carried out with light microscopy using Gomori's aldehyde fuchsin and periodic acid schiff procedures. In each physiological state, the parameters studied included: (a) cell size, (b) granule size, (c) granule population, (d) mitochondria, (e) Golgi complex, (f) the nuclear size and shape, (g) endoplasmic reticulum, and (h) vesiculation of the cytoplasm. In Prepubertal animals gonadotrophs of 5,10,20 and 30 days of age showed progressive changes towards maturity past day 30 which was found to be the most critical period in transformation from immature to mature stage. Conspicuous changes were observed in granules population (day 5,112±10; day 30, 370±20) and granules size (day 5, 1508±110 A°; day 30, 1810±113 A°). The cell size during this period increased from 9.5±0.2 μm to 13.3±0.45 μm. The cell shape, the mitochondria and the nuclear size and shape remained unchanged. Endoplasmic reticulum was prominent at all stages of development. However the Golgi complex became increasingly prominent by days 20 and 30. The appearance of major

changes by day 30 indicated the preparation of gonadotrophs for entering into maturity stage. No prominent changes were observed in thyrotrophs during the same period.

Changes in ovariectomized prepubertal rats (day 30) were not as dramatic as in day 60, mature cycling rats. Although vesiculation of cytoplasm was apparent in 30 day old animals, yet the change was neither as putative as in ovariectomized mature animals nor were signs of "castrate cell" formation or intiation of indentation of the nuclear membrane observed. However, in animals of comparable age (30 days old) LHRH treatment (1 μg/day, for 5 days) of intact animals resulted in transformation of majority of gonadotrophs into castrate cells with high vesiculation of the cytoplasm. This condition has been termed "pseudocastration", since the gonadotrophs mimic the same transformation as in adult ovariectomized female cycling rat. The implications of pseudocastration have been discussed in parallel with the effects of castration. The phenomenon of "pseudocastration" induced by LHRH alone is the first of its kind described in the present studies.

Changes in 60 day old animals castrated for 30 days reported in this thesis were similar to those documented earlier. The appearance of castrate cells with vesiculated cytoplasm and indentation of nuclear membrane was clearly observed. However, as reported by others, typical "signet ring" cells were not seen. Unlike 30 day old LHRH-treated

animals the 60 day old intact animals given similar LHRH treatment did not respond to LHRH challenge in the same manner as 30 day old animals; the extent of change in gonadotrophs was far less dramatic as seen in "pseudo-castrated" animals (30 day old, ovaries intact, LHRH treatment 1μg/day for 5 days). Interestingly enough, however, 60 days old ovariectomized female rats injected with LHRH did not show as much change in gonadotrophs as 60 day ovariectomized animals which did not receive LHRH. This has been attributed to inhibitory effect of LHRH at this physiological state. It has been maintained in the thesis that on the ultrastructural basis it is difficult to differentiate between gonadotrophs producing luteinizing hormone (LHG) and follicle stimulating hormone (FSHG) only on the basis of granules, though opinion of other workers describing separate FSH and LH granules has also been discussed.

INTRODUCTION

Pituitary gonadotrophs are known to synthesize FSH and LH (Pierce & Parsons, 1981). Both the hormones are glycoproteins (Monroe & Midgley, 1969; Nakane, 1970; Baker & Yu, 1971; Baker et al., 1972; Herbert, 1975; Moriarty, 1976) with testis as the target tissue in the male and ovaries in the female (Greep et al., 1941; Fevold, 1943). As early as 1940s, it was demonstrated that FSH is related with the maturation of Graaffian follicles in the ovaries and spermatogenesis in the testis (Wilfred et al., 1973; Page, 1988). Similarly, whereas LH stimulates testosterone production from Leydig cells in the testis, it induces ovulation in cyclic females (Wilfred et al., 1973; Page, 1988). The two hormones have a subunit structure, the alpha and beta subunits (De la Llosa, & Jutisz, 1969; Papkoff & Samy, 1967; Pierce et al., 1971). The alpha subunit is identical in both the hormones (Pierce & Parsons, 1981) while the beta subunit is hormone specific (Midgley & Beals, 1971; Midgley et al., 1971; Papkoff et al., 1971; Rathnam & Saxena, 1971; Saxena & Rathnam, 1971; pierce & parsons, 1981). The identity of alpha subunit of gonadotrophs with alpha subunit of thyrotrophic hormones (TSH) has also been established (Pierce & Parsons, 1981). The two subunits in gonadotrophs are held by noncovalent binding (Pierce, 1988) and dissociate or recombine in vitro when placed in proper environment (Pierce, 1988).

The synthesis and release of both hormones are regulated by luteinizing hormone releasing hormone (LHRH) synthesized in hypothalamic nuclei (Mittler et al., 1970; Redding et al., 1972; Vale et al., 1972; Labrie et al., 1973; Liu et al., 1976; Fink, 1979a; Khar & Jutisz, 1980). Changes in LHRH release from hypothalamus are related to circulating levels of gonadal steroids through a feed back mechanism (Sarkar et al., 1976; Sarkar & Fink, 1979a,b; Sherwood et al., 1980; Ching, 1982). Whether a single cell type or two distinct populations of gonadotrophs produce LH and FSH has been a subject of sustained controversy (Moriarty, 1976). Earlier studies with light microscopy using periodic acid schiff (PAS) staining or Gomori staining procedure which stain glycoproteins, failed to resolve the issue (Purves & Griesbach, 1954; Rennels, 1957,1963; Herlant, 1964; Hildebrand et al., 1957; Hellbaum et al., 1961). However, recent immunocytochemical studies using specific LH and FSH antisera do indicate the presence of two distinct populations of gonadotrophs, one synthesizing LH (Barnes, 1963; Costoff, 1973; Moriarty, 1976; Garner & Black, 1979), and the other synthesizing FSH (Moriarty, 1976; Garner & Blake, 1981). Yet, both cell types operate under the influence of LHRH. However, evidence has also been provided that the same gonadotrophs synthesize both LH and FSH (Nakane, 1970,1975; Tougard et al., 1973; Rhifer et al., 1973; Tixier-Vidal et al., 1975; Herbert, 1975 ; Moriarty, 1976; Daucheux, 1978; Batten & Hopkins, 1978; Tougard, 1980; Yoshimura et al., 1981).

Evidence for the presence of two distinct types of gonadotrophs, one producing FSH (FSHG) and the other LH (LHG) has also been provided through electron microscopic studies (Costoff, 1973); (see also Barnes, 1963). The FSHG is the largest cell found in the male pituitary with a population of 30% of total cells. While in the female, the population is only 10%. There are several Golgi areas. Mitochondria are rod shaped with a dense matrix. The endoplasmic reticulum is not well developed but is more extensive than in thyroid stimulating hormone (TSH) cells. On the other hand LHGs are more common in female, constituting as much as 20% of the total cells, than in the male rat, where there are 5-10% of these cells. These cells are larger than thyrotrophs and are polygonal in shape with eccentrically positioned nuclei. The secretory granules are denser than those of FSH. This gonadotroph does not contain the large amorphous bodies present in FSH cells.

Male and female rat pituitaries respond differently to gonadectomy. The effect of castration is more dramatic in female than in male (Costoff, 1973). Following castration, the FSH cells show increase in granulation but are devoid of large amorphous bodies. The nuclei become irregular and cytoplasm is more vacuolated. Similar changes, though more pronounced, occur in LHG. The cells are hypertrophied and have more developed ER. The Golgi apparatus is enlarged. In some cells vesicular ER is dilated and enlarged; the vacuoles containing colloidal material. In later stage of

castration (30-60 days) numerous LHG with one large vacuole are present. Such cells have been designated as "Signet ring" cells (Costoff, 1973).

Ultrastructural studies combined with immunocytochemistry have supported the earlier findings. For instance, a distinct LHG has been identified undergoing typical "Castration" changes after gonadectomy (Garner & Blake, 1981). On the basis of this study three morphological types of LHGs were identified in animals following prolonged castration: (a) cells with homogeneous cytoplasm; (b) vesiculated cells, and (c) "Signet ring" cells.

Further, though the effect of gonadectomy on pituitary gonadotroph of female rats has been adequately documented (Catchpole, 1949; Purves & Griesbach, 1952, 1954a. ; Costoff, 1973; Dullaart, 1981; Garner & Blake, 1981; King & Letourneau, 1994), yet only equivocal data are available about the effect of gonadectomy on pituitary gonadotrophs of female rats at various stages of development. Also, the effect of LHRH alone on pituitary gonadotrophs has not been adequately studied in neonatal, immature and mature female rats. Only a few studies have been directed to elucidating the changes in neonatal and prepubertal rats leading to maturity. Immunocytochemical studies using anti-LHß serum and anti- FSHß serum have shown the presence of LH and FSH immunopositive cells in 5 day neonatal and 15 day immature animals (Inoue & Hagino, 1984). Also, it has been demonstrated that exposure

of prepubertal, ovariectomized rat pituitary (days 10-35) to LHRH *in vitro* increased release of LH and FSH, though the effects of ovariectomy were less distinct than those observed *in vivo* and were generally absent in rats of less than 20 days of age. Further, only after day 20 did absence of ovaries in the donor animals showed a marked stimulatory effect on the release of gonadotrophins *in vitro* (Dullaart, 1981).

If gonadectomy, for instance, acts through continuous release of LHRH from intact hypothalamo-hypophyseal system causing hypertrophy of gonadotrophs when typical "castration cells" appear, then by the same token, LHRH treatment of prepubertal animals should be able to mimic the conditions produced by gonadectomy in adult.

The present thesis addresses these questions and focuses attention on (a) ultrastructural changes in gonadotrophs during various stages of development; (b) effect of ovariectomy on changes in pituitary gonadotrophs in immature and mature animals; (c) effect of LHRH on pituitary gonadotrophs in intact rats at various stages of development, and (d) effect of LHRH on ovariectomized female rats. The data reported here, it is hoped, will enhance our understanding of reproductive physiology of prepubertal and Pubertal female rats.

REVIEW OF LITERATURE

Organization of the Hypophysis

Organization of the hypophyseal complex comprising pars tuberalis (PT), pars distalis (PD), pars intermedia (PI) and pars nervosa (PN) is known from the studies carried out in early ninteenth century (Cushing, 1912, 1930; Wislocki & King, 1936). The erroneous concepts postulated by Cushing (1930) that the secretions of PD, PI and PN are directly or indirectly poured into the third ventricle of the brain were substantially modified (Wislocki & King ,1936; Rioch et al., 1940; Fisher et al., 1935 a, b, and scharrer & scharrer, 1940, 1944). Pituitary portal system around median eminence and pituitary gland provided information about the nonneural contact of hypothalamus with PD (Xuereb et al., 1954 a,b). The hypothalamic regulation of PD secretion through "releasing factors" delivered via the portal vessels was established in a series of studies (Guillemin, 1955; Saffran & Schally, 1955; Porter & Jones, 1956; M cCann et al., 1960 ; Guillemin et al., 1962; McCann, 1962; Schreiber et al., 1962; Talwaker et al., 1963; Deuben & Meites, 1964; Igarashi & McCann, 1964; Schally & Bowers, 1964; Dhariwal et al., 1965; Gala & Reece, 1965; Garcia & Geschwind, 1966, and Porter et al., 1967).

Structuraly, the PD, after completion of its development is composed of glandular epithelial cells, a

connective tissue stroma and many capillaries. Nerves do not terminate on or near PD cells (Green, 1966). There is no blood-brain barrier in adenohypophysis (Wislocki & King, 1936; Dempsey & Wislocki, 1955). The capillaries in PD are not sinusoid because of the absence of phagocytic elements or large gaps in their walls. Interposed between the epithelial cells and fenestrated capillaries is a double basement membrane which sometimes may be split (Farquhar, 1961). The epithelial cells arranged in cords are united by desmosomes and gap junctions (Fletcher et al., 1975; Herbert, 1979; Soji & Herbert, 1989, 1990).

This anatomical organization has the obvious consequence that hormones released into the extracellular space of the adenohypophysis from any epithelial cell can reach nearby capillaries by diffusion or bulk flow and enter them through fenestrations in their endothelial tubes. However, three other events can also be foreseen: (a) hormones can reach adenohypophyseal cells from the brain as easily as those from distant glands such as the thyroid, gonads, adrenal cortex, or adrenal medulla; (b) hormones released by one epithelial cell are free to interact with neighbouring cells that contain appropriate receptors in their cell membranes (Denef & Andries, 1983) and (c) intercellular communication between adjacent cells is possible by electrical means through gap junctions (Fletcher et al., 1975).

The storage site (Moriarty et al., 1973; Moriarty, 1976) and structure (Pierce & Parsons, 1981) of the pituitary glycoprotein hormones: thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH) and Polypeptide hormones: growth hormone (GH) (Li et al., 1976; Lewis et al., 1980) and adrenocorticotrophic hormone (ACTH) (Howard et al., 1955; Li, 1959) were identified and isolated from pars distalis of pituitary (Dada et al., 1984). Each hormone secreted by the pituitary gland is synthesized in and released from a functionally specific group of cells (ie., TSH by thyrotrophs, GH by somatotrophs, etc.). This hypothesis was initally explored by light microscopic examination (Purves & Griesbach, 1951a,b) fortified by such techniques as histochemistry (Pearse & Noorden, 1963), immunohistochemistry (Nakane, 1968) and transmission electron microscopy (Kurosumi, 1968).

Cytological Studies

Cytological studies of pituitary glands had been undertaken following castration and during pregnancy long before gonadotrophins were known. Comte (1898) reported changes in the proportion of acidophils and basophils during pregnancy. Further changes occured in pituitary after castration, and the changes were reversed by injections of extracts of gonads. Schleidt (1914) observed many vacuolated basophils following castration in humans and

designated these as "signet ring" cells. Castration cells have also been described in the rat (Addison, 1917).

Evans and Simpson (1929) and Engle (1929) reported that gonadotrophic activity was elevated post castration. Engle (1929) linked the increased gonadotrophic activity with basophils. Importance of hypophysis in maintaining gonadal function was convincingly demonstrated by the data obtained by Smith (1930) using hypophysectomized rats. After hypophysectomy the gonads atrophied but restored the normal state when pituitary extracts were injected or implants of the gland were made.

Following Smith's work, attempts were made by other workers to separate gonadotrophins from other hormones of the anterior pituitary gland. Fevold et al (1931) were the first to accomplish partial separation of FSH and LH. Fevold et al., (1931, 1933) studied the biological effects of these gonadotrophic preparations in rats and rabbits. Additional descriptions of the effects produced by follicle stimulating hormone fractions were reported by Chow (1943) and Fraenkel-Conrat et al. (1943). The action of these two hormones was to promote the growth of ovarian follicles and affect the germinal epithelium of the testes. Greep et al. (1941) and Fevold (1943) reported that luteinizing hormone stimulated luteinization of the ovaries and maintained the interstitial cells of the testes.

Guyer and Claus (1937) described vacuolation that occurred in certain basophils after castration. Severinghaus (1937) postulated that basophils secrete FSH and acidophils LH. Smelser (1944) and Giroud and Martinet (1948) corrected the erroneous hypothesis of Severinghaus by administering extracts of acidophilic and basophilic areas of ox and pig pituitaries to immature rats and mice and observed that both FSH and LH effects were elicited by extracts of basophilic parts of the pituitary glands. Wolfe and Brown (1942) found that certain basophils increased in size and number following castration.

Pituitary thyrotrophs were first identified by light microscopy of cellular changes in the pituitary gland that resulted from hyperthyroidism and thyroidectomy. These changes were described by Hohlweg and Junkman (1933), Severinghaus et al. (1934), Severinghaus (1935), and Zechwer et al. (1935). The problem arose, however, concerning which cell type produced TSH because degranulation of acidophils and hypertrophy of certain basophils occurred after thyroidectomy. Severinghaus (1937) concluded that TSH is secreted by acidophils, but Zechwer et al. (1935) and Griesbach (1941) reported that TSH is associated with basophils. Guyer and Claus (1937) postulated that TSH basophils were distinct from castration cells. Herlant (1943) used cytochemical methods to study the problem of pituitary cell specificity. Mucoproteins were known to stain with toluidine blue. When Herlant employed this staining

method, he found that grnules in TSH, FSH and LH cells stained positively. This indicated that these hormones were mucoproteins as compared with the serous proteins, luteotrophic hormone (LTH) and somatotrophic hormone (growth hormone) (STH).

Griesbach and Purves (1945) noted changes in certain acidophils and basophils after thyroidectomy. They determined the normal thyroxine requirement of thyroidectomized rats and maintained them on slightly subnormal levels. Hyperplasia and increased activity occurred in certain basophils while acidophils remained unaltered. It was concluded that TSH was secreted by basophils.

The attempt to differentiate various cell types in rat anterior pituitary began with two valuable staining procedures. The MCManus (1946) periodic acid-schiff (PAS) technique, first applied to the pituitary by Catchpole (1949) and Gomori's aldehyde fuchsin stain (1950) which has been extensively employed by Halmi (1950, 1952). With the aid of these techniques, Purves and Griesbach (1951a,b) amplified the concept of Romeis (1940) and Halmi (1950), and distinguished two types of basophils, the thyrotrophs and gonadotrophs, primarily on the basis of their response to hormone treatments (Purves & Griesbach, 1951a,b). The localization of specific hormones within specific cell types was defined by histochemical staining. The three

glycoprotein containing hormones and connected to the PAS-positive basophils, of which the gonadotrophs would be responsible for FSH and LH. The acidophils, of which there might be two groups would be the site for the production of growth hormone and luteotrophic hormone. Confusion arose because of the lack of knowledge of the chemical reaction between the dyes and the cellular or hormone elements and species differences in staining patterns.

The subject was studied in detail by Smith and Smith (1923a,b), Rasmussen (1929), Romeis (1940a,b), Pearse (1952), Herlant (1960), Pearse and Noorden (1963), and Purves (1966). From the light microscopic examination of stained pituitary sections, using acid dyes, eosin or orang G to stain acidophils and of PAS reaction to stain basophils, they concluded that: (a) hormones are stored in granules in pituitary epithelial cells; (b) hormone (or hormones) responsible for growth and lactation are found in eosinophils; (c) the eonsinophils are localized predominantly in the lateral regions of the gland; (d) basophilic cells can be thyrotrophic, gonadotrophic, or adrenocorticotrophic, and they lie predominantly in the middle of the gland; (e) the positive PAS reaction that characterizes basophils indicates glycosylation of the protein hormone molecule in these cells.

Purves and Griesbach (1951) described gonadotrophs as oval cells which stain with PAS and are located in the

dorsal and ventral parts of the anterior pituitary gland. The PAS reaction was correlated with the gonadotrophic hormone content of the gland. These cells were involuted after estrogen treatment and became hypertrophied as a result of castration.

Using PAS stain Purves and Griesbach (1952) described two types of gonadotrophs. The central gonadotroph which stained red were prominent just before the onest of sexual maturity in the female rat and degranulated just before ovulation. During pregnancy and estrogen injection these central gonadotrophs degranulated, hypertrophied, and showed morphological evidence of increased activity. After castration these cells showed a marked accumulation of glycoprotein, and assays of the glands made at this time showed them to have a high LH content. On the basis of this evidence, it was concluded that the central gonadotrophs secreted LH. These workers also defined a population of purple stained basophils as peripheral gonadotrophs. Estrogen injection caused an increase of hormone in pituitary gland and increased granulation of these cells. During pregnancy they also exhibited increased granulation. Peripheral basophils were well granulated during early castration and assays conducted at this time showed high FSH activity, indicating that these peripheral gonadotrophs secrete FSH.

Purves and Griesbach (1954) presented more data to support their views on gonadotrophs. Administration of testosterone propionate caused an increase in FSH and a decrease in LH content of rat pituitaries. They correlated this elevated pituitary FSH with an increase in granulation in peripheral gonadotrophs, and the decreased LH with a reduced number of granules in the central gonadotrophs. This work agreed with the earlier findings of Greep and Jones (1950).

Barnett et al. (1956) reported that a 2.5% solution of tricholoracetic acid extracted PAS positive FSH and TSH but not LH as shown by assays. The authors believed this to be a useful histochemical method to demonstrate LH secreting cells. They reported that LH activity was distributed throughout the gland in contrast to the earlier report of Purves and Griesbach (1954) who stated that LH cells were in the central part of the pituitary. Rennels (1957, 1963), Hildebrand et al. (1957), Hellbaum et al. (1961), and Chowdhury et al. (1971) also questioned the findings of Purves and Griesbach. Their evidence provided that the central PAS purple cells produce FSH, and the peripheral PAS red gonadotrophs produced LH. However, Kracht (1957), Hartley (1959), Herlant (1964), and Vanha-Perttula (1966) are among the investigators who agree with the postulations of Purves and Griesbach (1954). Physiological and electron microscopic data seem to support the Purves and Griesbach postulation.

Observation of adenohypophyseal cells with electron microscope revealed that all the glandular cells, with exception of follicular cells, contain electron-dense granules in their cytoplasm (Kurosumi, 1968; Farquhar, 1971; Baker, 1974). These cells can be separated on the basis of their granular shape, size, and the development of their endoplasmic reticulum. There have also been numerous electron microscopic studies of gonadotrophs by Farquhar and Rinehart (1954) and Yoshimura and Harumiya (1965) in the rat; Yamada and Sano (1960) and Barnes (1963) in the mouse; and Girod and Dubois (1965) and Dekker (1967) in the hamster. Only the study by Barnes (1963) has distinguished between FSH and LH cells.

The earliest electron microscopic study of gonadotrophs and the changes they undergo after castration was made by Farquhar and Rinehart (1954b) and Farquhar (1955). They described that FSH gonadotrophs undergo vacuolation and that the Golgi apparatus enlarges 6 days after castration. The smaller LH cells are found on capillaries and appear filigreed. These cells undergo castration changes 60 days after gonadectomy. Their report did not provide enough definitive data to distinguish the two cells types and it has been suggested by Kurosumi and Oota (1966) that the cells described by Farquhar and Rinehart are two different forms of the same cell.

Farquhar and Rinehart (1954b) and Yoshimura and Harumiya (1965) in their long-term castration studies estimated granules in the gonadotrophs to be about 200 mμ in diameter and less electron dense than granules in acidophils. The latter workers have shown FSH granules to be smaller, 150-200 mμ, and LH granules larger, 200-250 mμ in diameter. Fractionation of rat pituitary granules by Hartley et al. (1960), Perdue and McShan (1962), and Hymer and McShan (1963) have shown that gonadotrophic activity was associated with granules 150-200 mμ in diameter.

In electron microscopic study by Kurosumi and Oota (1968) it was found that in persistent estrous rats, LH cells were well granulated, indicating a storage of LH, but FSH cells were sparsely granulated. LH cells in persistent diestrous rats were slightly activated and FSH cells were atrophic. Prior to 1968 the identification of these two gonadotrophs was inconclusive. However, more definite identification of these cells has been made possible with the use of new research techniques, for example immunocytochemistry.

Costoff (1973) has reported that the follicle stimulating gonadotroph is the largest cell found in the male rat pituitary gland. In the female it is smaller and less abundant. This cell type was abundant in most sections of male rat pituitary glands. The FSH cell is the second most frequently observed type found in the male rat

pituitary, with perhaps 30% of all the cells secreting FSH; in the female it may be about 10%. These cells are round and usually found on a capillary. The nucleus is round or indented on one side. The granules are small and vary in density and are distributed throughout the cell. The granule diameter ranges from 75-200 mμ with a mean of 126 mμ. There are also large amorphous bodies throughout the cells which are unique to the FSH cell. These opaque bodies are not always seen in FSH cells. They have been observed by others (Farquhar & Rinehart, 1954; Cardel, 1961; Kurosumi & Oota, 1968). These light-staining bodies range from 0.7-1.2 μ in diameter and can be seen being formed along with the granules in the Golgi complex. Depending upon the fixation these large bodies may be as electron dense as the secretory granules. There are several Golgi areas encircling the nucleus in FSH cells, and they usually appear hypertrophied and possess a well developed network of dilated sacs and small vesicles. The mitochondria of FSH cells are filamentous rods of various shapes usually exhibiting a dense matrix. Cristae are more continuous and parallel to one another as compared to those observed in other pituitary cell types. The endoplasmic reticulum is not as well developed in the FSH gonadotroph as in the acidophils but is more extensive than in TSH cells. In hypertrophied FSH cells the endoplasmic reticulum is more irregular and has dilated sacs. In an inactive cell the endoplasmic reticulum is more vesicular with few lamellae, and ribosomes are not always

attached to the membranes. Lysosomes, multivesicular bodies, centrioles, and occasionally a cilium are also observed.

Costoff (1973) has also reported that luteinizing hormone cells are more common in the female rat, constituting as much as 20% of the total cells, than in the male, where there are about 5-10% of these cells. They are more usually found anteromedially in the pituitary. The LH cells are larger than thyrotrophs and are often located on a capillary. They are usually polygonal in shape with eccentrically positioned nuclei. The secretory granules of these gonadotrophs are more electron dense than those of FSH cells but less dense than those of acidophils. Sections of luteinizing hormone granules average 145 mμ in diameter with a range of 75-235 mμ. This gonadotroph does not contain the large amorphous bodies that are present in the FSH cells.

When the LH cells are in an inactive state, the Golgi apparatus and endoplasmic reticulum are rather inconspicuous and poorly developed. In actively stimulated cells there are extensive Golgi areas with granules in different stages of formation. However, even in the stimulated LH cell the Golgi complex is less extensive than in the FSH cell. The endoplasmic reticulum consists of scattered vesicular areas. These membranes are intermittently dotted with ribosomes but some are free in the cytoplasm. Both short, rodlike and round mitochondria are found in these cells. Few lysosomes, cilia, or centrioles are present.

The thyrotroph was the first pituitary cell type to be definitively identified with electron microscopy (Costoff, 1973). Farquhar and Rinehart (1954a) studied rat pituitary glands before and at different times after thyroidectomy and observed that only cells postulated to be the source of TSH hypertrophied. Studies by Kurossumi and Oota (1966) distinguished thyrotrophs from gonadotrophs and corticotrophs. Greatly stimulated TSH cells have been found in naturally occurring and experimentally induced tumors by Farquhar and Furth (1959), Theret and Renault (1964), Feltkamp and Kwa (1965), and Messier (1965).

Immunocytochemical Studies

The pituitary glycoprotein hormones (LH,FSH, and TSH) are molecules of about 26,000-32,000 molecular weight and consist of two subunits (Moriarty, 1976), the alpha (α) and beta (β). The α chain is common to TSH, LH, and FSH; the β chain is hormone specific, (Midgley & Beals, 1971; Midgley et al., 1971; Papkoff et al., 1971; Rathnam & Saxena, 1971; Saxena & Rathnam, 1971; Pierce & Parsons, 1981). TSH containing cells stain with PAS (Romeis, 1940a,b; Phifer et al., 1973; Girod & Trouillas, 1980). Small angular cells in the rat's pituitary gland, which contained granules with a maximal diameter of 140 nm, were found on transmission electron microscopic (TEM) examination by Farquhar and Rinehart (1954a,b) to exhibit a depletion of cytoplasmic dense granules and a marked increase in the number of lucent

cytoplasmic vesicles following thyroidectomy. The earliest immunocytochemical studies, by Phifer et al. (1972) in humans and Nakane (1970) in rats, showed that LH and FSH were present in the same cells. Nakane, in addition, found that some centrally located cells in the rat pituitary contained only one of the gonadotrophins. Furthermore, he reported that the Kurosumi-Oota FSH cell contained both FSH and LH. In addition, a third type of cell stained for FSH alone, termed as "type B" cell, was angular and contained peripherally located secretion granules (Nakane, 1970). Advancements in the field produced antisera to the specific beta chain sequence of FSH and LH, thereby eliminating potentially cross-reactive antibodies to alpha chain sequences shared by the gonadotrophins and TSH (Odell, 1979 a,b). Baker et al. (1972) pioneered the use of these antisera for the immunocytochemical localization of beta chains of LH and TSH. Tougard et al. (1971,1973) and Tixier-Vidal et al. (1975) reported that all gonadotrophs in the rat pituitary contained immunoreactive sequences to antibodies against the beta chains and whole molecules of both gonadotrophins. Similar findings were reported in 1975 by Herbert, in rats, and by Batten and Hopkins (1978), in dissociated cells from pig pituitaries. However, a second group of workers agreed with Nakane's earlier finding and reported that a small percentage (usually 10%) of the gonadotrophs contained only one of the hormones (usually FSH). This was reported for a variety of species, including rats (Purandare, et al., 1978), dogs (El Etreby & Fath El

Bab,1977), pigs (Dacheux, 1978), and the humans (Pelletier et al., 1976). In agreement with Nakane, Moriarty and associates (1980) found that a certain percentage of the gonadotrophs contained only one of the gonadotrophins (Moriarty, 1975, 1976; Moriarty & Garner, 1977). Furthermore, FSH β was found in a cell type which resembled both Nakane's type B FSH cell and the cells producing adrenocorticotrophin (ACTH) (Siperstein & Miller, 1970; Moriarty & Halmi, 1972). Subsequent studies of serially sectioned cells showed that some of these cells contained ACTH immunoreactivity as well (Moriarty & Garner, 1977). However, since these ACTH-FSH cells represented less than 10% of the gonadotroph population, no conclusions were drawn about their biological significance. Using antibodies to TSH, preabsorbed with β-human chorionic gonadotrophin and without cross- reaction with FSH or LH, Baker et al. (1972) were able to localize immunoreactive TSH cells in the rat pituitary which corresponded to thyroidectomy cells (Farquhar & Rinehart, 1954a). Moriarty and Tobin (1976a,b) employed antibodies to the β chain of TSH and obtained similar results. They (Moriarty & Tobin, 1976) found granules in the cytoplasm of TSH containing cells to be of the same size as those in the presumptive thyrotrophs of Farquhar and Rinehart (1954a). Immunoreactive TSH has been localized within a specific cell population in the rat pituitary (Kawarai, 1980); however, the cytoplasmic granules observed were larger than those described by either Farquhar and Rinehart (1954a) or Kurosumi (1968).

Changes in FSH and LH cells during the estrous cycle

Barnes (1962) traced the ultrastructure of the mouse pituitary gland through the estrous cycle. She designated the cells that were degranulated early in proestrus as FSH and those which degranulated later as LH cells. Roos (1968) traced gonadotrophs through the estrous cycle in the rat. FSH cells were degranulated during the height of diestrus, which is related to the wave of follicular growth that takes place at this time of the cycle. The FSH cells were also degranulated during the afternoon of proestrus. Degranulation of LH cells occurred during the morning of proestrus and reached a peak during the critical period of proestrus at 4.00 p.m. The second surge of FSH occurred during the critical period at the same time as LH. Roos also found LH cells to be centrally located, thereby agreeing with the work of Purves and Griesbach (1954). Monroe et al. (1969) used radioimmunoassays to measure LH levels in the rat during the estrous cycle. They found serum LH levels to be 25 times greater during the afternoon of early proestrus. These data seem to support the findings of Roos (1968). Garner and Blake (1981) described changes in rat anterior pituitary gland. LH secretion was correlated with morphological changes in LH gonadotrophs at different times during 4-day estrous cycle. One basic cell type was involved in LH secretion during rat estrous cycle with oval and less irregular shape during heightened LH secretion on

proestrous, and some LH cells were markedly degrnulated during the later portion of LH surge (Garner & Black, 1979).

Immunocytochemical studies by Dada et al. (1983) showed that there was no change in the numbers of LH and FSH cells during estous cycle. The ratio of LH cells to FSH cells also did not change throughout the cycle. Garner and Blake (1979,1981) found in proestrous rat that the basic LH cell shape was usually polygonal to ovoid with single population of granules scattered throughout the cell or concentrated at one pole; the nucleus being generally ovoid. Golgi complexes were not commonly observed. At 7-19 days of postovariectomy, same basic LH cell was present except that Golgi areas became enlarged and prominent (Garner & Blake, 1981) and LHRH infused rats also exhibited a LH cell type with a similar shape and granule size as in control proestrous rats. LHRH infusion appeared to result in LH cells becoming less irregular in shape (Garner & Blake, 1979).

Distribution of Gonadotrophs during Prepubertal age

During sexual maturation of female rats changes in pituitary content of LH (Lisk, 1968; Spona and Luger, 1973b; Dullaart, 1976,1981) and follicle stimulating hormone (FSH) (Kragt and Ganong, 1967; Dullaart, 1976,1981) have been reported. Jansen (1982) reported changes in localization and number of LH and FSH containing cells in relation with pituitary content of LH and FSH in prepubertal female rats,

the gonadotrophic cells were regularly distributed throughout the pars distalis but at 5 and 10 days of age fewer LH and FSH cells were found in lateral regions. The number of LH and FSH cells per pituitary gland increased with age (Matsumura & Daikoku, 1977). The number of LH cells per unit volume of pituitary tissue reached a maximum at 20 days; the number of FSH cells reached a maximum at 15 days and then decreased with increasing age. At all ages except 5 days, more LH than FSH cells were counted per unit volume. Some cells reacting with both anti rat LHβ than anti-rat FSHβ were detected (Jansen, 1982). The LH and FSH immunopositive cells were ovoid elements, ranging in size and intensity of staining angular cells, in which only the cell reacted with anti LHβ serum, were observed in neonatal and immature rats; however these cells were not stained with either anti FSHβ serum or anti-ACTH serum (Inoue & Hagino, 1984).

Campbell et al. (1987) reported cells containing FSH or LH were distributed throughout the entire adenohypophysis of 3, 10, and 20 day old female rats. Clusters of these cells were observed in the ventral regions of adenohypophysis of 3 days old females. The percentages of adenohypophyseal cells containing FSH increased significantly from 9% in 3 day old rats to 17% in 10 day old rats and then decreased to 14% in 20 day old animals. At all ages the percentages of adenohyphyseal cells containing FSH were similar to the percentages of cells containing LH. At 10 days of age all

cells containing LH also contained FSH. These data suggested that the increase in serum FSH in the juvenile female rat is associated with an increase in the percentage of adenohypophyseal cells containing FSH and that at this time all cells containing FSH also contain LH (Campbell et al., 1987).

Effect of Castration on Gonadotrophs

Large cells of abnormal appearance in the pituitary of the castrated rats were apparently first observed by Zacherl (1913) who interpreted these cells as being transformed acidophils. Schleidt (1914) used the term "signet ring" cell to describe the vacuolated castration cell in the rat pituitary. Addison (1917) found that castration cells in the rat were enlarged basophils. The presence of glycoprotein in castration cells in the rat was first demonstrated by Catchpole (1949). Purves and Griesbach (1952, 1954a) described two independent types of "castration cells" in rat pituitary which come into existence after gonadectomy. In their opinion these cells are derived from the two types of gonadotrophs as peripheral and central cells (Purves & Griesbach, 1954). Peripheral cells contained some coarse, dark granules but not as many as under normal conditions. The central cells were poor in glycoprotein. The gonadotrophs of both types were slightly increased in number and size 10 days after gonadectomy and vesiculated cells leading to signet ring cells were observed

16 weeks after castration. Based on light microscopic observation and bioassay data Purves and Griesbach (1952), Farquhar and Rinehart (1954a) identified the first type as cells that secrete follicle stimulating hormone (FSH) and the other type, which appeared later after ovariectomy as cells that secrete luteinizing hormone (LH).

Fernandez-Moran and Luft (1949) reported the first electron microscopic observation on the pituitary gland but their observations were restricted because, adequate sectioning techniques had not been developed. In a classical electron microscopic study Farquhar and Rinehart (1954b) described the morphology of rat gonadotrophs at different times after ovariectomy. They identified two principle types of gonadotrophs. One type appeared early after castration and showed progressive changes as time after ovariectomy increased. The second type became prominent later, by 75 days after ovariectomy. Cytological changes occuring in the rat pituitary FSH and LH cells following castration have also been described (Costoff, 1973). Seven days after gonadetomy the most noticeable changes that occured were in the gonadotrophs. The FSH cells showed an increase in granulation but were devoid of the large amorphous bodies which were usually found in intact male FSH cells. There was an increase in lysosomes. Whether the large amorphous bodies are antecedents of lysosomes or are engulfed or absorbed by the latter is not clear. The nucleus of the FSH cells was irregular and the cytoplasm was more vacuolated

but the changes were not as great as those found in LH cells. Luteinizing hormone cells, rather inconspicuous in the intact male rat, were enlarged and apparently increased in number by 7 days after castration. The hypertrophied cells had a well-developed endoplasmic reticulumn, an enlarged Golgi apparatus and many granules. In some cells the vesicular endoplasmic reticulum was dilated and enlarged and there was colloidal material in some of the vacuoles of the endoplasmic reticulum. Between 14 and 60 days following castration the FSH cells became more vacuolated and the number of lysosomes increased. The endoplasmic reticulum became smooth, and the Golgi apparatus became more distinct; the number of granules decreased. The mitochondria changed from the filamentous to the short rodlike type.

The LH cell hypertrophied greatly after long-term castration. There was an increase in granulation up to 14 days after castration, and thereafter granulation tended to decrease. The Golgi area also remained distinct up to 30 days following castration but then became more inconspicuous. The endoplasmic reticulum of these LH cells hypertrophied and became more vacuolated. The vesicles of the endoplasmic reticulum coalesced to form vacuoles, and 30-60 days after castration there were numerous LH cells with one large vacuole or "lake" in the middle of the cell . Such cells are called "signet ring" cells which can be further characterized by having the nucleus and cytoplasm oriented to one side near the cell membrane. This large

vacuole often contained some colloidlike substance. Mitochondria by this stage of castration were somewhat hypertrophied with the round type predominating. These observations indicated that LH cells undergo more profound changes in ultrastructure after castration than FSH cells (Costoff, 1973).

Dullaart (1981) on the basis of bioassy showed that in immature rats ovariectomy may change the secretory characteristics of the gonadotrophic cells but such changes were largely restricted to immature rats older than 20 days. Pituitary concentration of LH did not significantly rise after ovariectomy and only after day 20 did ovariectomy raise pituitary FSH concentration.

An ultrastructural and immunocytochemical study of LH secreting cell of rat anterior pituitary after ovariectomy by Garner and Blake (1981) showed morphological changes in LH gonadotrophs correlated with changes in rat anterior pituitary LH content. Their results suggested: (i) an increase in LH concentration in individual cell and an increase in the release rate of LH from these cells, (ii) three cytoplasmic types of LH cells become prominent: homogeneous, vesiculated and signet ring; (iii) vesiculated cells arise from homogeneous cells and signet ring cells arise from either homogeneous or vesiculated cells. King and Letourneau (1994), using immunocytochemistry and electron microscopy, have correlated rise in LH level after

minimal importance in the differentiation of LH containing cells.

Other investigators (Horacek et al., 1987a,b, 1988; Gertz et al., 1987; Hoeffler & Frawley, 1987) have described that the hypothalamic factor controls the release of adenohypophyseal hormone and can modulate the size and proportion of specific cell types. Gregerson and associates (1982, 1983) identified that LHRH treatment increased the numbers of LH and FSH containing cells but in the absence of LHRH the induction of FSH in gonadotrophs appeared to be restricted. Horacek et al. (1987b) have also observed that LHRH plays an important role in the maintenance of FSH immunoreactivity in gonadotrophs in adult hamsters. Horacek et al. (1989) reported that exogenous LHRH plays an important role in stimulating the formation of immunoreactive FSH in the pituitary gland and that, it can increase the number of gonadotrophs that develop during the neonatal period. Kudo et al. (1994), using the rat pituitary primordium in organ culture, have reported that rat pituitary primordia at 13.5 days of gestation is capable of responding to LHRH, and that LHRH is effective in stimulating the responsiveness of gonadotrophs to it during early pituitary cytodifferentiation.

Depending on the pattern of administration, LHRH either stimulates or inhibits the release of gonadotrophins. Thus, intermittent exposure of the pituitary gland to LHRH

enhances the release of LH and FSH in response to subsequent stimulation (Belchetz et al., 1978; Crowley & McArthur, 1980). The self-priming effect of LHRH, (Aiyer et al., 1974; Edwardson & Gilbert, 1976; Pickering & Fink, 1976; Koiter et al., 1982) after prolonged continuous exposure to LHRH desensitized the pituitary gland (Schuiling & Gnodde, 1976; Davies et al., 1977; Sandow et al., 1979,1980) so that response to acute increments of LHRH was strongly reduced (Koiter et al., 1981 ; Badger et al., 1983).

Though desensitization is generally accompanied by Partial depletion of the pituitary gonadotrophin stores (Schuiling et al., 1983), the state of desensitization cannot be explained by depletion of these stores only (Schuiling & Gnodde, 1976; Sandow et al., 1979; Schuiling et al., 1983). Down regulation of the LHRH receptors also plays a role in the genesis of the phenomenon (Catt and Dufau, 1977; Clayton, 1982). Not only LHRH may have stimulatory or depressing effects on pituitary gonadotrophin secretion, but estrogens may also sensitize (Positive effect: Schuiling & Gnodde, 1976) or desensitize (negative effects: Blake et al., 1974; Schuiling & Gnodde, 1977) the pituitary gland to the effect of LHRH. During first 9h after administration of estradiol benzoate to the ovariectomized rat the LHRH responsiveness of the pituitary gland depressed (Schuiling & Gnodde, 1976). Thereafter it increased and exceeded the LHRH responsiveness of

ovariectomized rat not exposed to estrogen (Schuiling & Gnodde, 1976).

Debeljuk et al. (1973) reported an acute injection of LHRH is more effective in elevating the level of circulating LH than circulating FSH. In contrast, multiple injections or prolonged infusion of LHRH are more effective in terms of elevation of serum FSH level and increasing the ratio of FSH to LH concentration in circulating blood (Debeljuk et al., 1973; Johnson & Mallampati, 1975; MC Neilly et al., 1979; Gregerson & Campbell, 1984; campbell et al., 1987). Attempts to explain differential effects of LHRH on LH and FSH release are complicated by the fact that both hormones often have been localized in the same cell (Tougard, 1980; Yoshimura et al., 1981; Dada et al., 1983). Dada et al. (1983) reported that all gonadotrophs of adult male rats contained LH, and about 89% of these cells contained FSH as well. In contrast Garner et al. (1990) explained that exogenous LHRH can increase number of gonadotrophins in the anterior pituitary gland, synthesis of FSH in gonadotrophs, and basal serum LH and FSH concentration in adult rat.

In the light of present review of the literature it is evident that a lot remains to be worked on the changes in the gonadotrophs during prepubertal age at verious developmental stages.

MATERIALS AND METHODS

ANIMALS

Female Sprague Dawley rats were obtained from the departmental colony. They were kept in a temperature controlled room (24-27°C) with daily lighting (13L:11D). The rats had free access to laboratory rat chow and water. The day of mating was designated as first day of pregnancy and the first day of birth of the pups was taken day one of postnatal life. For adult rats vaginal smears were prepared by saline lavage. Rats which displayed two or more consective 4-day cycles were used for experiments.

CHEMICALS

All general chemicals used in this study were of analytical grade and were obtained from Merck, [illegible] Germany, Sigma Chemical Co., USA, and Fluka, Switzerland. Luteinizing hormone releasing hormone(LHRH) and Osmium tetraoxide were purchased from Sigma Chemical Co., USA. Durcupan resin was the product of Fluka,Switzerland. Glutaraldehyde was obtained from Merck, [illegible] Germany. Orange G and Basic fuchsin were obtained from BDH England.

ANIMAL PROTOCOL

In experiments involving drug treatment, (LHRH 1μg/ 100μl of 0.9% Nacl) three groups of five animals each were

given intravenous (tail vein) injections once daily (~10.00-12.00h) for 5 days. The animals were weighed before and after last injection. The animals were killed on day 6 after the final injection. The pituitaries were removed, weighed on a Mettler balance and immediately fixed in an appropriate fixative as described below.

Castration

Virgin rats were weighed, lightly anaesthetized with ether and gonads were removed under sterile conditions (~10.00-12.00h). Antibiotic injections (i.m.) were given for 3 days to avoid infection. Animals were kept in separate cages until the time of experiment.

HISTOLOGICAL PROCEDURES

Technique for pituitary gland fixation: The pituitary glands from normal and treated groups of rats were dissected out following sacrifice, weighed to the nearest mg and immersed immediately in Sera fixative (absolute ethanol 60 ml, formaldehyde 30 ml and glacial acetic acid 10 ml) for 3-4h for histological examination. The fixed pituitaries, were dehydrated in ascending propanol series, then immersed in 3 changes of methyl benzoate for 24h, cleared in benzene and embedded in paraffin wax. Tissue blocks were sectioned sagittaly with a cambridge microtome at a thickness of 3-4μ. The sections were affixed to precleaned albuminized glass slides and each

alternate slide was stained with aldehyde fuchsin, orange G (Halmi, 1952; Gomori, 1952) and periodic acid schiff (PAS) (McManus & Mowry, 1960), and Herlant's stain II (Herlant, 1962). Microscopic examination was carried out under a research microscope (Optiphot Research Microscope, Olympus). Measurements of glycoprotein containing cells and their nucleay diameters were made by a precalibrated ocular micrometer in order to obtain their mean size.

ELECTRON MICROSCOPY

For electron microscopy of the pituitary glands, the rats were subjected to the following experimental procedures.

Group A: Female rats aged 5, 10, 20, 30, 45, and 60 days were anesthetized with ether. The pituitary glands were removed and the separated anterior portion was immersed in 2.5% cold Glutaraldehyde prepared in 0.1M (pH 7.2) phosphate buffer. Tissues were cut into cubes approximately 1mm and left for 2h in the fixative. After fixation, the tissues were rinsed thrice in the buffer and post fixed in 1% cold osmium tetraoxide bufferd with 0.4M phosphate buffer (pH 7.2) for 1-2h. The osmicated tissues were rinsed in cold phosphate buffer (pH 7.2), dehydrated in graded series of acetone, rinsed thrice with 100% acetone and immersed in one change of 1/2h of absolute propyleneoxide before embedding in Durcupan resin according to standard procedures.

Semithin and ultrathin sections were made with glass knives on a LKB ultratome V. Semithin section of 0.5-1μ were stained with 1% toluidine blue. The ultrathin sections were transfered to 150 or 200 mesh copper grids. These were contrasted using uranyl acetate and lead citrate. The sections were examined and photographed on a Joel Sx-100 Transmission Electron Microscope (TEM). In order to quantify the granular changes in cells of the anterior pituitary, the population of granules per cell in selected sections were counted and mean values were calculated for each cell.

Group B: Female rats of 20, 30, and 60 days of age were injected via tail vein with 1μg LHRH once daily for 5 days. Animals were decapitated on day 6 and the pituitaries were removed, weighed and processed for ultrastructural studies in the same manner as described earlier.

Group C: Female rats of 30 and 60 days age were anesthetized with ether and castrated. The animals were kept for 30 days. Following this period, animals were injected i.v. with 1μg LHRH once daily for 5 days prior to sacrifice. The rats were sacrificed on day 6 after final injection at 66 and 96 days of age respectively. Intact, age-matched animals were used as untreated controls. The pituitary glands were removed, immersed in the fixative and processed for ultrastructural analysis as described previously under electron microscopy procedures.

RESULTS AND DISCUSSIONS

1. PITUITARY CELL TYPES AT VARIOUS STAGES OF DEVELOPMENT

Changes in Gonadotrophs (day 5, 10, 20, 30, 45 and 60 of Development)

Identification of pituitary cell types is essentially based on size and shape of secretory granules. The following Table1indicates the established criterion of granule size for various cell types.

Table 1

Granule size in various Pituitary Cell types

Cell type	Granule size (A°)
Mammotroph	6000 - 9000
Somatotroph	3000 - 3500
Corticotroph	2000
Gonadotroph	1000 - 3000
Thyrotroph	1000 - 1500

Data from Rhodin (1974)

Accordingly, in the present work data based on granule size have been used as a guideline for identifying anterior pituitary cell types and changes in gonadotrophs in 5, 10, 20, 30, 45 and 60 day old female rats. The observations reported here are based on electron microscopic studies. However, to a limited extent information has also been drawn from histochemical data.

Electron micrographs of Pituitary gonadotrophs from female rats of various ages are shown in Figs. 4-11. It may be seen that in gonadotrophs of all ages granules show identical size (range: 1333 - 2748 A°; mean: 1890 A°; Table 2, Fig.1). It is essentially on the basis of this parameter that gonadotrophs have been distinguished from other cell types; the other characteristics used included: (a) cell shape and size, (b) nuclear shape and size, (c) granular endoplasmic reticulum, (d) Golgi complex and (e) mitochondria. Data pertaining to these parameters in gonadotrophs of various ages are recorded in Table 2 and may be compared with those in Tables 3, 4 and 5 for other cell types.

In gonadotrophs of day 5 and day 10 animals, neither the granule size nor the population of granules seems significantly different (granule size day 5: 1508±110 A°; day 10: 1333±30 A°; granule population day 5: 112±10, day 10: 126±27). A major shift in granule population occurs between days 10 and 20, showing an increase of 146% over day 10. Subsequently, however, the granule population more or less remains constant showing an average increase of 15% between day 20 and day 45. The population of granules falls by 27% by day 60 as compared to day 45.

The activity of gonadotrophs is known to be related with the secretion of FSH and LH. The gonadotrophs, accordingly contain both types of granules. This has been demonstrated by histochemical studies (Daucheux, 1978;

Batten & Hopkins, 1978; Yoshimura et. al., 1981; Dada et al., 1983). The Secretory activity of the granules is regulated by LHRH. In day 5 and 10 rats, the gonadotrophs have poorly developed Golgi complex and granular endoplasmic reticulum; the vesiculation also being absent (Figs. 4,5). These characteristics may be compared with gonadotrophs from day 45 and day 60 animals, which have well developed Golgi and more extensive granular ER (Figs. 8,11). The vesiculation of cytoplasm is also indicative of secretory activity (Fig. 11). From these observations as also from the nuclear features it may be surmised that in prepubertal female rats, the gonadotrophs may have only limited secretry activity, (this aspect is discussed more fully elsewhere in the thesis). It may also be concluded that major transformation in gonadotrophs on way to maturity occurs between days 10 and 30 (Table 2, Fig. 1).

Changes in Thyrotrophs (day 5, 10, 20, 30 45 and 60 of Development)

Thyrotrophs are known to secrete TSH, under the regulating control of Thyrotrophic Hormone Releasing Factor (TRF). Like gonadotrophs, the thyrotrophs contain secretory granules at all ages of development. Among all the trophic cells of the Pituitary the granule size of thyrotrophs is the smallest, ranging in size from 580-950 A^{o}. (Fig. 2) The granules are not evenly dispersed in the cytoplasm. The bulk of these granules are arranged along the plasma membrane in

single or multiple rows (Figs. 12-16). The mitochondria are prominent and more in number compared to gonadotrophs of corresponding ages. The nucleus is oval or polygonal in shape with a prominent nucleolus. Data of various parameters are recorded in Table 3.

The data of Table 3 and those presented in Fig. 2 demonstrate the extent of variation in thyrotrophs as they proceed to maturity from day 5 to day 60. These changes may be seen in electron micrographs (Figs. 12-16) and, compared with similar age related changes in gonadotrophs (Figs. 4-11) or in somatotrophs (Figs. 17-20), it has been observed that granular size in thyrotrophs of all ages remains within standard known limit for these cells (Table 1). The minimum size of 580±30 A° in day 5 thyrotrophs increases to 950±15 A° in day 60 thyrotrophs (Table 3, Fig. 2). The increase in size appears to be consistent with advancement in age. Similarly, there is no critical transitional time in the transformation of granule population, as is seen in gonadotrophs. The ER is well developed all along the age profile, beginning day 5. So is the case with the number of mitochondria. Not much change in nuclear shape is observed. It can been concluded on the basis of this information that thyrotrophs as early as day 5 may have demonstrable secretory activity.

Changes in Other Cell Types (Somatotrophs, Corticotrophs and Lactotrophs)

Other cell types which included somatotrophs, corticotrophs and lactotrophs (mammotrophs) from animals of different ages were studied. Age related characteristics of somatotrophs and corticotrophs are recorded in Tables 4 and 5. Electron micrographs of somatotrophs (days 5, 30, 45 and 60), corticotrophs (days 10, 20 and 60) and lactotrophs (day 20) are shown in Figs. 17-24.

Somatotrophs secrete growth hormone (STH).The cells are medium-sized, round or oval. Large sized round granules (range: 2160-3160 A°) are present in all stages of development. So is the case with endoplasmic reticulum. A more or less round nucleus occupies most of the cytoplasm. The granules are dispersed throughout the cytoplasm. The morphology of the secretory granules and endoplasmic reticulum of granular type is more clearly identified in Figs. 19,20. Granular endoplasmic reticulum with cisternae and abundance of free ribosomes can be seen in Fig. 19. A comparative analysis of various parameters of somatotrophs of different age groups is presented in Table 4 and Fig.3.

The data of Table 4 and those of Fig. 3 indicate that granule size reaches its maximum by day 60; the granule population rising from day 5 (73±13/cell) through day 60 (130±20/cell). The ER is prominent in all age groups. However, granular ER (indicating high synthetic activity) is

Table 2

Comparative data on gonadotrophs of normal female rats of various ages

Parameter	Age (days)					
	Prepubertal				Pubertal	
	5	10	20	**30**	45	60
Granule size (A^{o})	1508 ± 110	1333 ± 30	1816 ± 90	**1810 ± 113**	2147 ± 130	2748 ± 100
Granule population/ cell (No.)	112±10	126± 27	311 ± 37	**370± 20**	413 ± 30	300±20
Cell size (μm)	9.5± 0.2	10± 0.7	11.5± 0.8	**13.3± 0.45**	13.57± 0.2	15± 0.8
Cell shape	oval or Polygonal	oval or Polygonal	Oval or Polygonal	**Oval or Polygonal**	Polygonal or Irregular	Polygonal or Irregular
Nuclear size (μm)	5± 0.5	5.2± 0.2	5.25± 0.1	**5.34± 0.5**	5.4± 0.5	7.5± 0.5
Nuclear shape	Round or Oval	Round or Oval	Round or Oval	**Round or Oval**	Oval or Round	Irregular or Round
Endoplasmic reticulum (ER)	Not Prominent	Not Prominent	Not Prominent	**Prominent**	Prominent	Prominent
Golgi complex	Small	Small	Prominent	**Prominent**	Prominent	Prominent
Mitochondria	Oval or Round	Oval or Round	Oval or Round	**Oval or Round**	Oval or Round	Oval or Round

Data of 30 days old animals is indicative of critical transformation from prepubertal to pubertal stage. See also subsequent sections

Table 3

Comparative data on thyrotrophs of normal female rats of various ages.

Parameter	Age (days)					
	Prepubertal				Pubertal	
	5	10	20	30	45	60
Granule size (A^{o})	580± 30	610± 10	700± 10	710± 10	890± 20	950±15
Granule population/ cell (No.)	85± 21	90±20	103± 20	112± 23	130± 20	148± 30
Cell size (μm)	7.5± 0.5	9± 0.7	10.5± 0.5	11± 0.7	11.5± 0.5	12.4± 0.3
Cell shape	Polyhe d-ral	Polyhe d-ral	Polyhe d-ral	Polyhe d-ral	Polyhe d-ral	Polyhe d-ral
Nuclear size (μm)	6.5± 0.1	7.0± 0.1	8.0± 0.5	9.0± 0.3	9.5± 0.1	10.0± 0.4
Nuclear shape	Oval or Polygonal	Oval or Polygonal	Oval or Polygonal	Oval or Polygonal	Oval or Polygonal	Oval or Polygonal
Endoplasmic reticulum (ER)	Not Prominent	Prominent	Prominent	Prominent	Prominent	Prominent
Golgi complex	Not Prominent	Not Prominent	Not Prominent	Not Prominent	Not Prominent	Not Prominent
Mitochondria	Prominent	Prominent	Prominent	Prominent	Prominent	Prominent

Table 4

Comparative data on somatotrophs of normal female rats of various ages

Parameter	Age (days)					
	Prepubertal				Pubertal	
	5	10	20	30	45	60
Granule size (A^{o})	2212± 50	2160± 100	2250± 100	2450± 112	2220± 112	3160±200
Granule population/ cell (No.)	73± 13	74± 14	99± 11	116± 20	130± 10	130±20
Cell size (μm)	7.0± 0.5	7.5± 0.3	8.0± 0.5	8.5± 0.5	9.25± 0.25	11±0.75
Cell shape	Round or oval	Round or oval	Round or oval	Round or oval	Oval	Oval
Nuclear size (μm)	5.0± 0.2	5.2± 0.3	5.3± 0.3	5.5± 0.5	5.7± 0.2	6.8±0.4
Nuclear shape	Round or oval	Round or oval	Round or oval	Round or oval	oval or Round	Oval or Round
Endoplasmic reticulum (ER)	Prominent	Prominent	Prominent	Prominent	Very Prominent	Very Prominet
Golgi complex	None	Small	Small	Small	Small	Small
Mitochondria	Few	Few	Few	Few	Few	Few

Table 5

Comparative data on corticotrophs of normal female rats of various ages

Parameter	Age (days)					
	Prepubertal				Pubertal	
	5	10	20	30	45	60
Granule size (A^{o})	1135± 25	1480± 30	1600± 20	1610± 10	1635± 20	1666±20
Granule population/ cell (No.)	43± 22	40± 15	44± 10	40± 5	40± 5	58±8
Cell shape	Elongated or polygonal	Elongated or polygonal	Elongated or polygonal	Elongated or polygonal	Elongated or polygonal	Elongated or polygonal
Endoplasmic reticulum (ER)	Less	Less	Less	Less	Less	Less
Golgi complex	Not Prominent	Not Prominent	Not Prominent	Not Prominent	Not Prominent	Poorly developed
Mitochondria	Prominent	Prominent	Prominent	Prominent	Prominent	Prominent

observed on day 30, but more prominently in days 45 and 60 somatotrophs. It is concluded from the study of somatotrophs of various age groups that: (a) no significant variation take place in granule size of somatotrophs between 5-45 days of age but there is 42% increase on day 60 ; (b) the cells appear to maintain hormonal synthesis and secretory activity between day 10 and day 45 onwards.

Changes in Corticotrophs (day 5,10,20,30,45 and 60 of Development)

The electron micrographs of corticotrophs from 10,20 and 60 day old rats may be seen in Figs. 21-23. Comparison of various parameters in different age groups is shown in Table 5. In contrast to the other cell types of the anterior pituitary, the corticotrophs are characterized by: (a) elongated cell shape; (b) granules ranging between 1135-1666 A°; (c) lack of significant variation in granule size and population in various age groups; (d) relatively inconspicuous Golgi complex and poorly developed endoplasmic reticulum; (e) abundance of mitochondria, and (f) exceptionally pale and electron-lucent cytoplasm.

Discussion

Using the same criteria as employed by others (Rennels, 1962; Matsumura & Daikoku, 1977; Garner& Blake, 1979; Garner, 1981; Jafri, 1985; Campbell et al., 1987) we have

identified various types of pituitary cells in all stages of development beginning day 5. In gonadotrophs, based on electron microscopic studies, it was not possible to differentiate between LH and FSH secreting cells. However, in other studies using cytochemical methods distinction has been made between two types of gonadotrophs (Moriarty, 1976; Jansen, 1982; Campbell et al .,1987). Prepubertal rat pituitaries have also been shown to secrete LH and FSH when challenged with LHRH in vivo (Garner et al.,1987, 1990) or during incubation in vitro (Spona & Luger,1973a,b; Redding et al.,1972). These studies have, by and large, demonstrated that (a) gonadotrophs are differentiated at an early stage after (day 4) during prepubertal development, (Matsumura & Daikoku, 1977; Inoue & Hagino, 1984), (b) secretory granules with identical characteristics as in the adult are invariably present in gonadotrophs, (Moriarty,1976; Garner & Blake, 1979), (c) in the absence of the activity of pituitary hypothalamic axis, the hormonal secretion by the gonadotrophs is negligible (Dullaart, 1981), and (d) the gonadotrophs through all the stages of development react to LHRH by secretion of FSH and LH. Our electron microscopic studies confirm these conclusions to a varifing degree, though the evidence, at best, remains circumstantial.

In gonadotrophs during prepubertal development beginning day 5, we have observed noticeable changes in granule size, granule population and the extent of differentiation of Golgi complex. Though not well developed in early stages,

the ER becomes more prominent in later stages. This indicates hormonal synthesis activity. Yet, on the basis of electron microscopic studies, or even histochemical, and hormonal assay studies (immunoassays), it is difficult to speculate about the nature of hormonal molecules. Both FSH and LH are glycoproteins (Protein is synthesized in the ER and the sugar moiety is synthesized in the Golgi complex) (Farquhar & Wellings, 1957; Purves, 1961; Farquhar, 1961b; Redman et al., 1966; Beams & Kessel, 1968; Neutra & Leblond, 1969; Rambourg et al., 1969). It has been extensively documented that all glycosidases associated with the synthesis of sugar moieties for α and β subunits of FSH and LH are located in the Golgi complex (Smith & Farquhar,1970).

From our studies which show progressive development of Golgi, it can be suggested that the nature of the two hormonal molecules synthesized during early and later stages of prepubertal development may differ in the extent of glycosylation. The immunoassays measure only immunological activity. Thus, these cannot be presented as an evidence for glycosylation; the immunological activity being related only to the protein part (Samli & Geschwind, 1967). Fragments of protein parts of these hormones have been shown to have substantial immunological activity (Moriarty,1976; Dada et al.,1983; Campbell et al., 1987). Either studies on half lives of the molecules synthesized at various stages (deglycosylated FSH and LH are known to have very short half life) or receptor binding biological assays (which require glycosylation) can give clue as to the prepubertal stage at

which fully glycosylated molecules are synthesized. Obviously, more work is required to be done in this regard using *in vitro* microbioassays which are as sensitive as immunoassays.

The extent of development of Golgi is also an indication of the secretory activity of the cells. In gonadotrophs of various ages there is a progressive differentiation of the Golgi. This indicates low secretion or perhaps none during early stages of prepubertal development. Sham-operated or ovariectomized prepubertal animals showed very little secretory activity during *in vitro* culture (Dullaart,1981). Synthesis and secretion are sequentially related categories, dictated by intact hypothalamo-hypophyseal axis. However, such does not appear to be the case in prepubertal animals, at least in early stages. There is little difference in granule size, morphology and population in day5 and day10 animals. This may be attributed to poor development of Golgi.

We have observed that dramatic changes take place in granule size and granule population in transition from day10 to day 30; the granule size increasing by 36% and granule population by 203%. Similar observations, though, in a different type of study, have been reported earlier. For instance, it has been reported that, the number of LH cells per unit volume of pituitary reached a maximum by day 20

(Jansen, 1982). Though there are no other similar electron microscopic studies on prepubertal female rat pituitaries, yet on the basis of present studies we are inclined to suggest that pituitary gonadotrophs show progressive changes throughout prepubertal development. The most critical changes take place between days10 and 30 leading upto maturity. Further, as discussed in a subsequent section, the responsiveness of 20 day prepubertal pituitary is evident when challenged with LHRH. The electron microscopic studies in this physiological state show the emergence of castrate cells under the influence of LHRH. This also shows that in 20 day old female prepubertal rats, there is yet no activation of hypothalamo-pituitary axis, since the cells at this stage can respond only to exogenous treatment with LHRH.

Little information is available about functional differentiation of thyrotrophs during prepubertal development. As described by others (costoff,1973) thyrotrophs in mature animals, are the smallest in size, as are the granules (range:1000-1500 A°) . The endoplasmic reticulum is poorly developed ,the mitochondria are oval or rod shaped. The Golgi complex is small. The nuclear cytoplasmic ratio is 0.80, which is significantly different from other cell types for example gonadotrophs (0.50). However the most significant difference which distinguishes this cell type from others is not only the smaller size of granules, but also the arrangement of granules along the inner surface of plas-

ma membrane (Figs. 12,13). This arrangement perhaps permits the association of granules with capillary vessels. This cell type synthesizes and secrets TSH which is a glycoprotein. There is general agreement that enzyme for the synthesis of sugar moiety of glycoproteins is located in the Golgi (Farquhar & willings, 1957; Farquhar, 1961b;Smith & Farquhar,1970). Accordingly, one should expect a similar mode of synthesis of the glycoprotein in this cell type. Interestingly enough, however, the Golgi in thyrotrophs is not as well developed as in other glycoprotein synthesizing pituitary cells, the gonadotrophs. What implications this has for the overall activity of the cell, remains uncertain. Yet, there are physiological explanations which can be advanced to support this line of evidence. The difference lies in the nature of target tissues (gonads in the case of gonadotrophs and thyroid in the case of TSH). Ovary, for example,has cyclical activity in the process of ovulation. This places an acute demand for gonadotrophs in the critical phases of ovulation cycle. This being so, the synthesis and storage of FSH and LH in substantial quantities only can fulfil physiological need for the ovulation cycle. Such is however not the case with thyroid as target organ, which does not require large bursts of TSH under normal physiological state for the release of thyroxine. The thyroidectomy cells which appear after thyroidectomy indicate the severe changes in thyrotrophs which take place under this acute condition.Thus,a less developed ER, a smaller Golgi, lesser number of granules, smaller size of granules are morphologi-

cal features which are consistent with the physiological role of this cell type compared to gonadotrophs. Certainly more experimental data are needed to seek a fuller explanation of this concept.

Table 6

Comparison of nuclear-cell ratio in gonadotrophs and thyrotrophs of various ages

Age (days)	Nuclear - Cell Ratio		P value
	Gonadotrophs	Thyrotrophs	
5	0.52	0.86	P<0.001
10	0.52	0.77	P<0.001
20	0.43	0.76	P<0.01
30	0.40	0.81	P<0.001
45	0.39	0.82	P<0.001
60	0.50	0.80	P<0.001

In prepubertal rats (days 5-30), the thyrotrophs on morphological grounds appear as functional as thyrotrophs in adult animals (day60). However, whether in the presence of a poorly developed Golgi, the cells do secrete TSH during early prepubertal stages remains to be elucidated. Huge amounts of TSH have been detected in neonatal pituitaries by RIA: 10233±1056ng/pituitary, Compared to 399±72ng/pituitary of LH (Gash et al., 1982). In these studies neither the 12 nor 15 day post coitus anlagen grafted in kidney capsules showed similar built up of TSH (15day anlagen: 169 ng/pituitary; 12 day anlagen: 212 ng/pituitary). Similarly,

antiserum to TRH administered to 5 day old animals did not affect the serum TSH levels (Theodoropoulos et al.,1979;Oliver et al.,1981). Based on these observations and encephalectomy studies (Jost et al.,1970; Tonooka&Greer,1978), it has been concluded that hypothalamic control of TSH secretion is minimal until after birth, though TSH synthesis remains maximal and is essentially independent of release of the hormone. In our studies the morphological features of thyrotrophs described during prepubertal development, to a varying degree, support the experimental findings (op. cit.). If morphological differentiation as related to Golgi and ER between days 5-45 are any indication (Figs. 12-16) then, it can be suggested that day 30 thyrotrophs do present a stage of hypothalamo-Pituitary axis activation with TRH playing a role both for stimulating synthesis and release of TSH. In this respect thyrotrophs apprear to be different from gonadotrophs during prepubertal development. Another feature which distinguishes thyrotrophs is the ratio of nuclear to cells size during development. In our studies it has been observed, that this ratio is high on day 5 and is maintained throughout prepubertal development. In the case of gonadotrophs, on the average, the ratio is lower and shows significant decline between days 20-45.

FIGURES

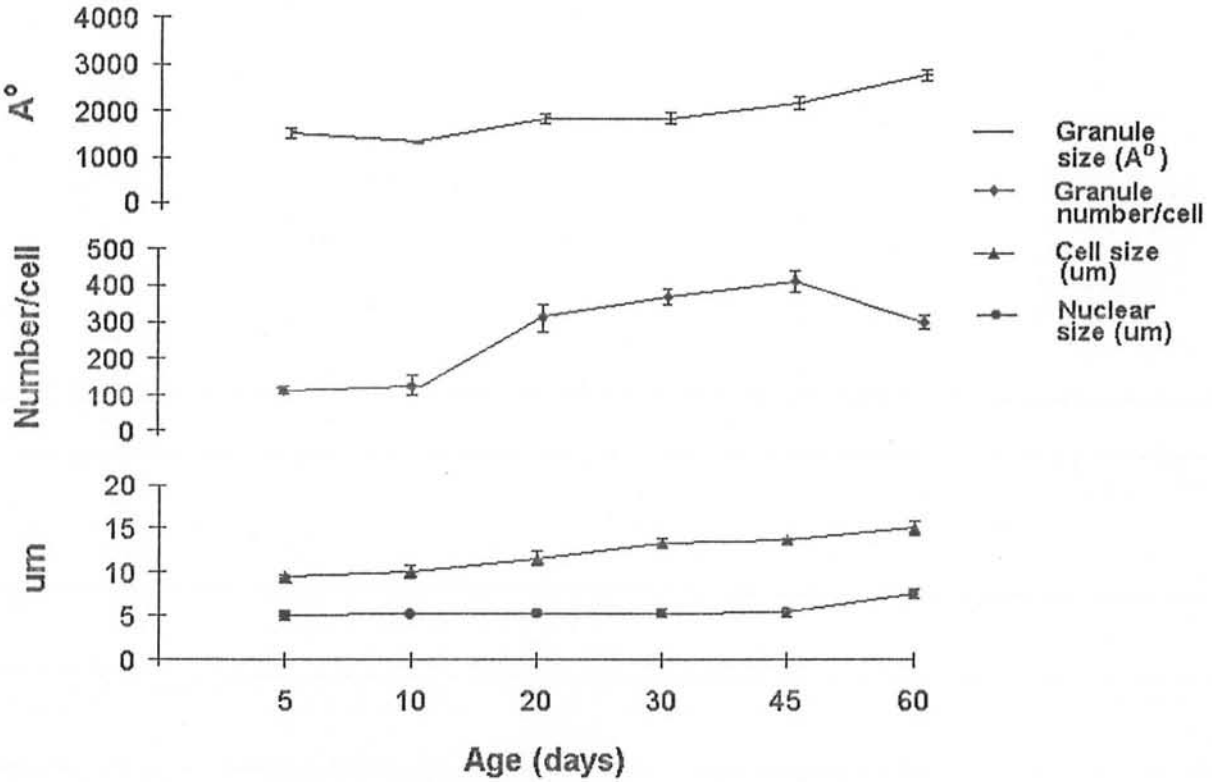


Fig. 1. Comparison of various parameters in gonadotrophs of normal female rats of various ages. The cycling rats (45 & 60 days old)were killed on the day of metestrous.

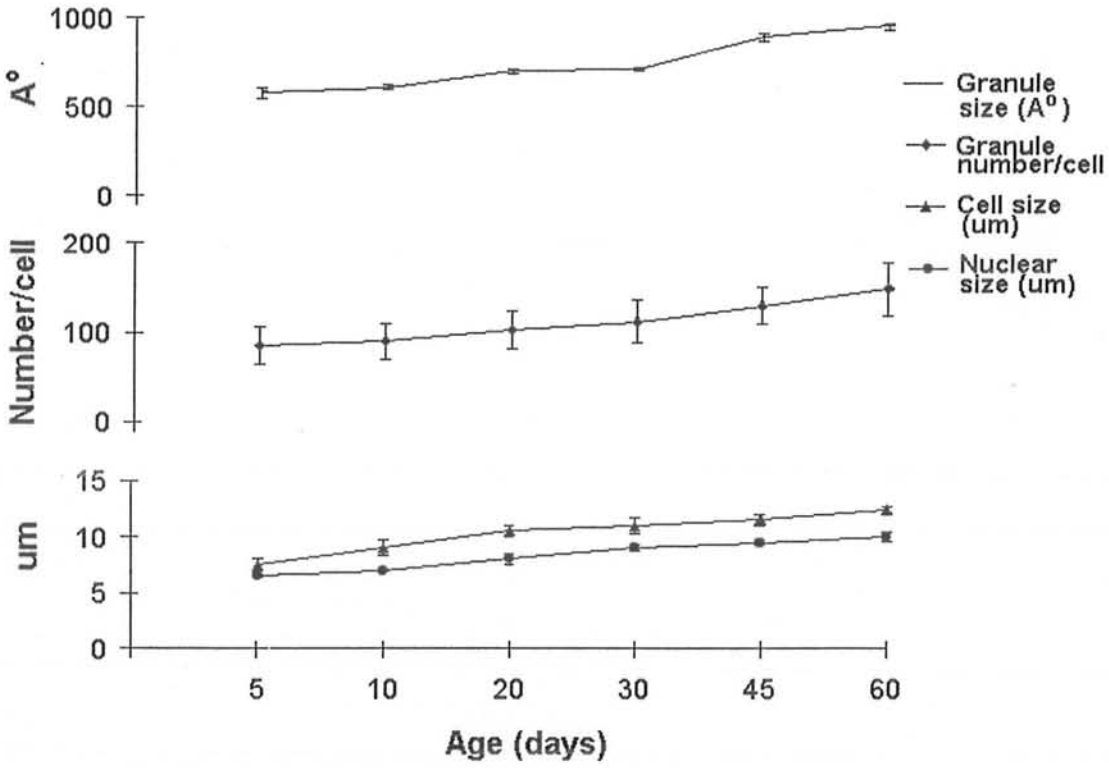


Fig. 2. Comparison of various parameters in thyrotrophs of normal female rats of various ages. The cycling rats (45 & 60 days old)were killed on the day of metestrous.

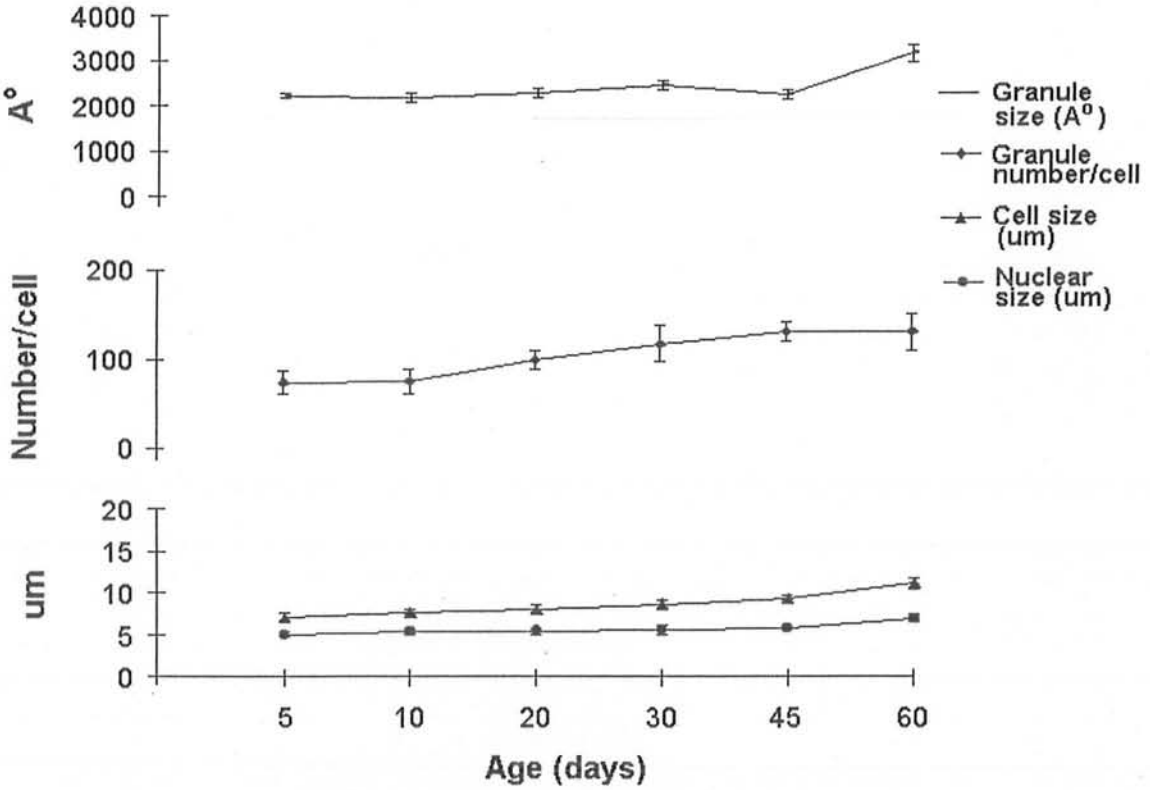


Fig. 3. Comparison of various parameters in somatotrophs of normal female rats of various ages. The cycling rats (45 & 60 days old)were killed on the day of metestrous.

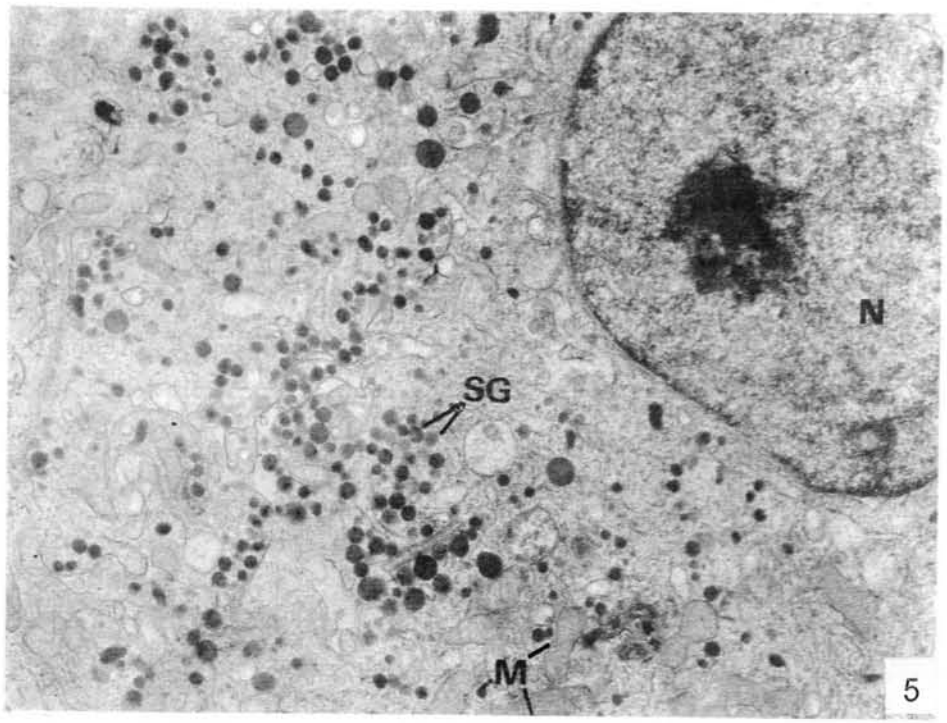
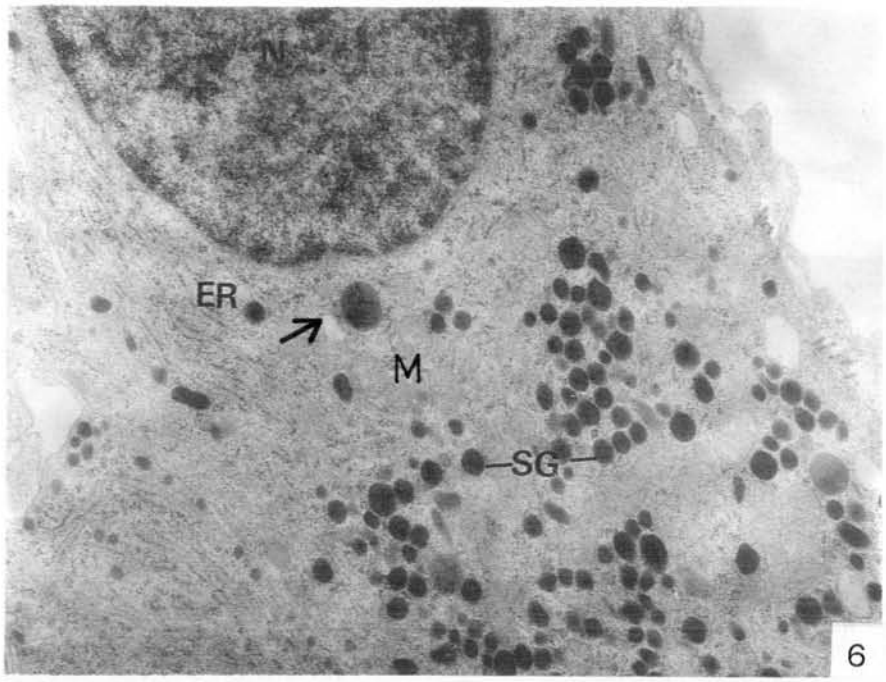


Fig. 5. Electron micrograph of a gonadotroph from 10 days old prepubertal female rat. Mitochondria (M), nucleus (N), secretory granules (SG) which are dense and vary in size (mean: 1333 A^{o}). x 18000

Fig. 6. Electron micrograph of a gonadotroph from 20 days old prepubertal female rat. Endoplasmic reticulum (ER) is more developed with prominent ribosomes, mitochondria (M), nucleus (N). Secretory granules (SG) are dense and of varying size (mean: 1816 A^{O}). Large bodies of different density (—>) are shown. x 18000



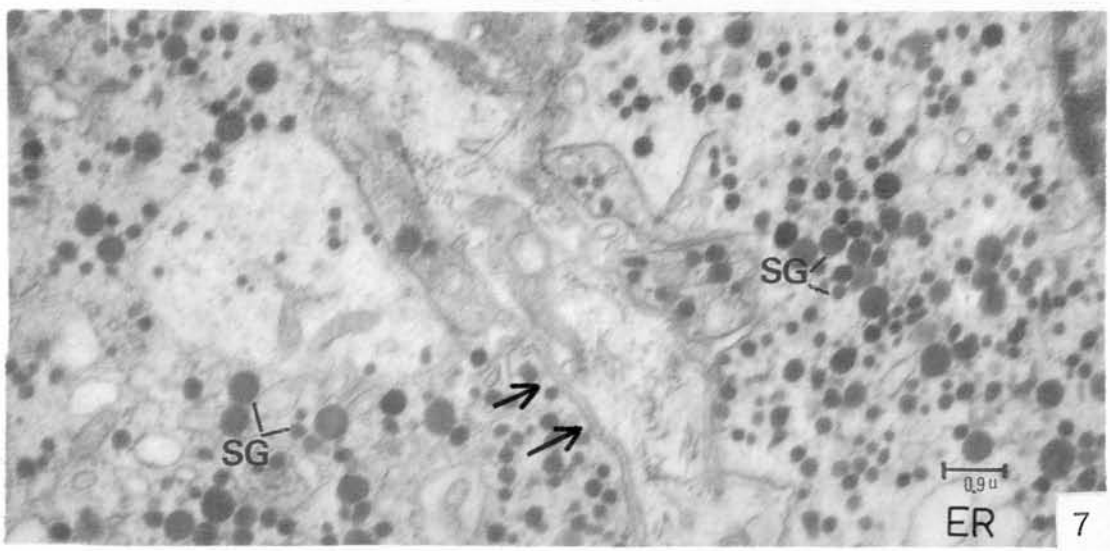


Fig. 7. Electron micrograph of adjacently placed gonadotrophs (→). There is an increase in granules population (mean: 370). Vesicles of endoplasmic reticulum (ER), Secretory granules vary in size (mean: 1810). The mean size of granules is not significantly different from 20 days old animals. Changes in ER and other subcellular components indicate a critical stage in prepubertal development tending towards puberty on day 30. x 18000

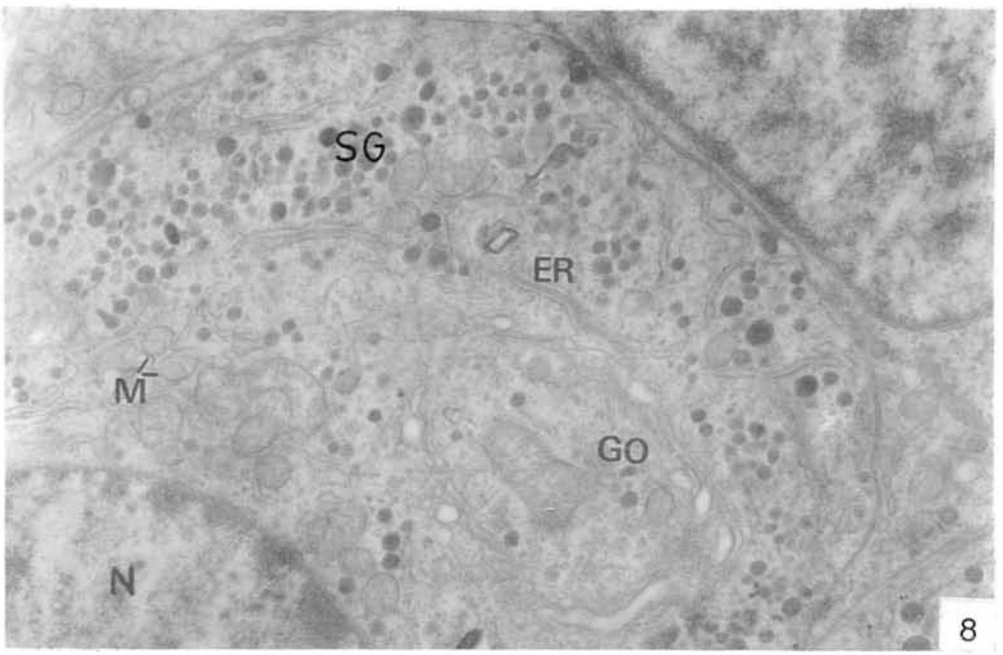


Fig. 8. Electron micrograph of a gonadotroph from 45 days old rat. The animals show clear sign of achieving puberty by age and cytoplasmic structures. Granular endoplasmic reticulum (ER) is well developed, Golgi complex (GO) is indicating initiation of secretory activity, mitochondria (M) are compact and numerous, nucleus (N), secretory granules (SG) are evenly dispersed and vary in size (mean: 2147 A^{o}); mean population of granules increase to 413/cell. x 18000

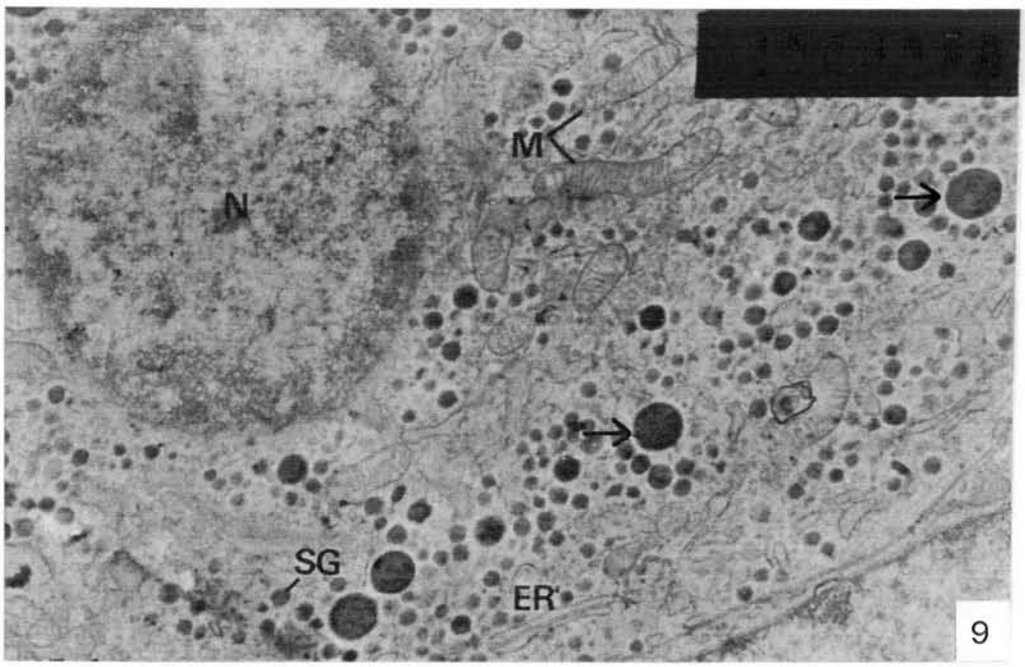


Fig. 9. Electron micrograph of a gonadotroph from 45 days old rat. Large dense bodies (—>), scattered granular endoplasmic reticulum (ER), mitochondria (M) with cristae, nucleus (N) and secretory granules (SG) are shown.

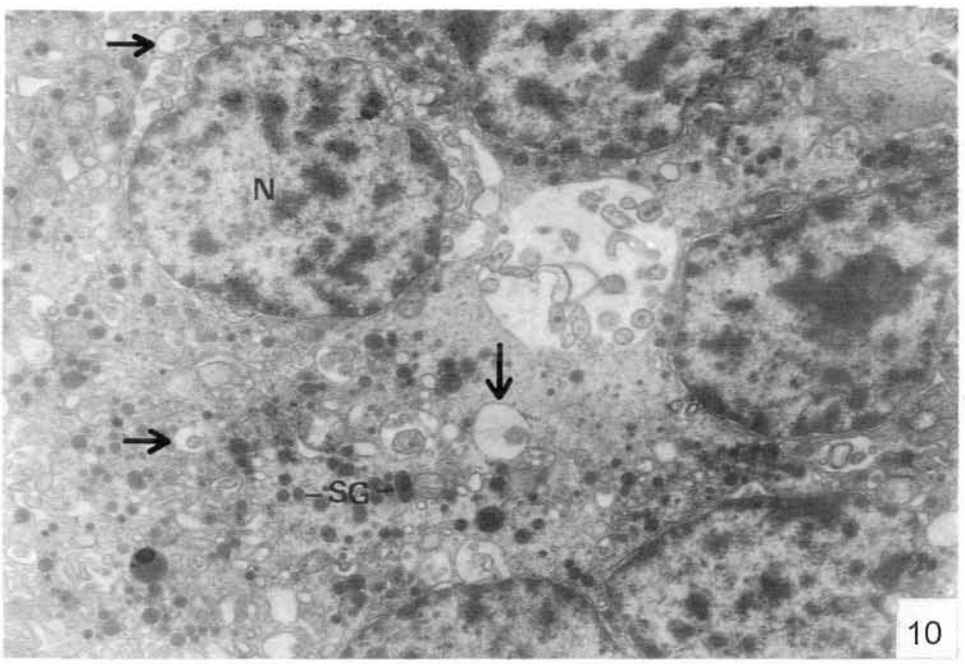


Fig. 10. Electron micrograph of a group of gonadotrophs from 60 days old rat. The animals as indicated by age and cytoplasmic structures achieved maturity. Nucleus (N), with dispersed chromatin, secretory granules (SG) though vary in size (mean: 2748 A°) show decrease in population (mean: 300/cell), empty granules (—>) are also visible. x 7200

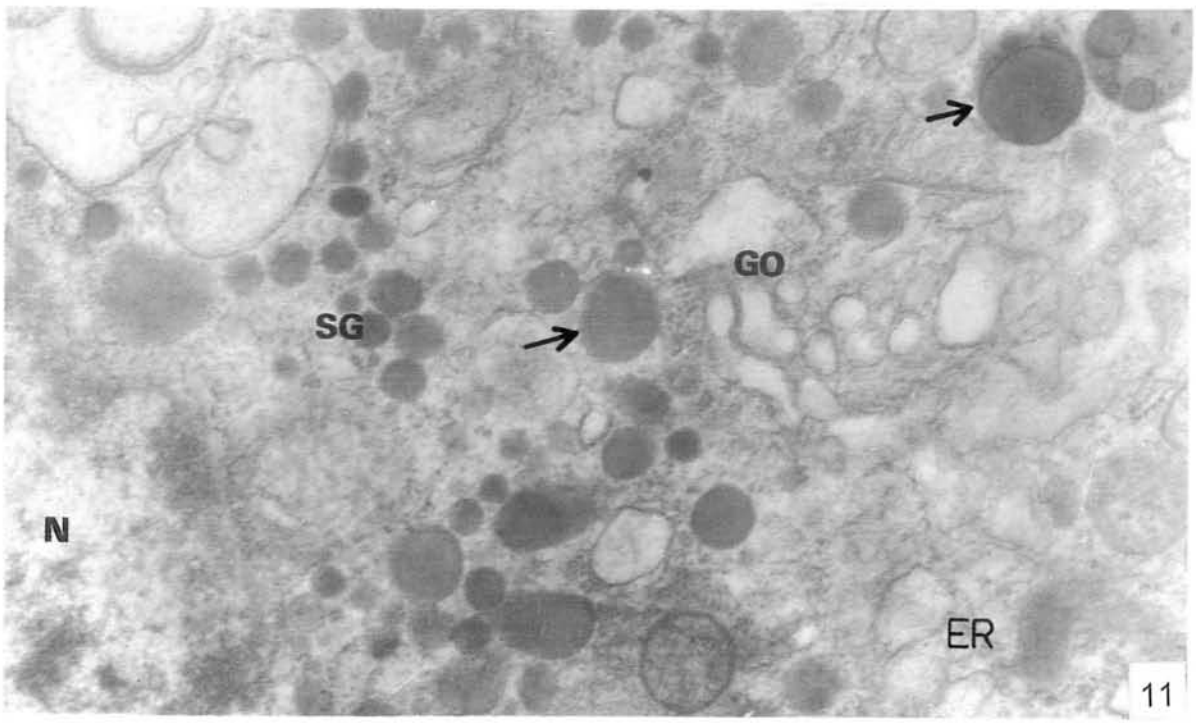
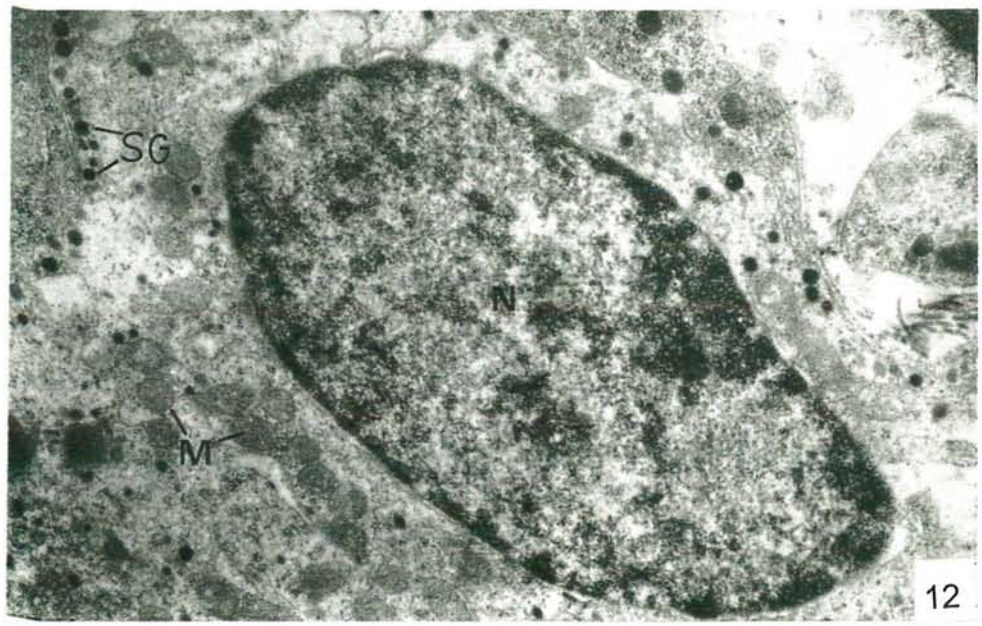


Fig. 11. Electron micrograph of an active gonadotroph from 60 days old female rat. A dilated Golgi complex (GO), Nucleus (N) large amorphous bodies with different density (—>), probably lysosomes are shown. Secretory granules (SG) vary in size. Several vesicles of endoplasmic reticulum (ER) are present. x 36000

Fig. 12. Electron micrograph of a thyrotroph from 5 days old prepubertal female rat. Endoplasmic reticulum (ER), mitochondria (M), nucleus (N) secretory granules (SG). Noted that in the thyrotroph as early as day 5,SG are arranged along the inside of the cytoplasmic membrane, a feature which distinguishes this cell type from other trophic cell of the pituitary. x 27000



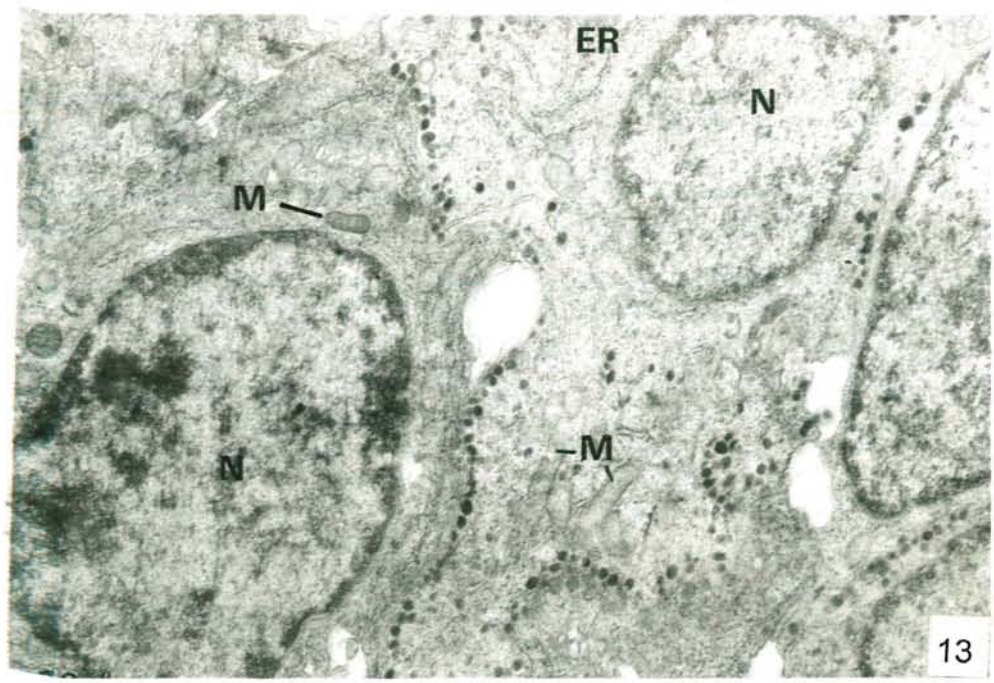
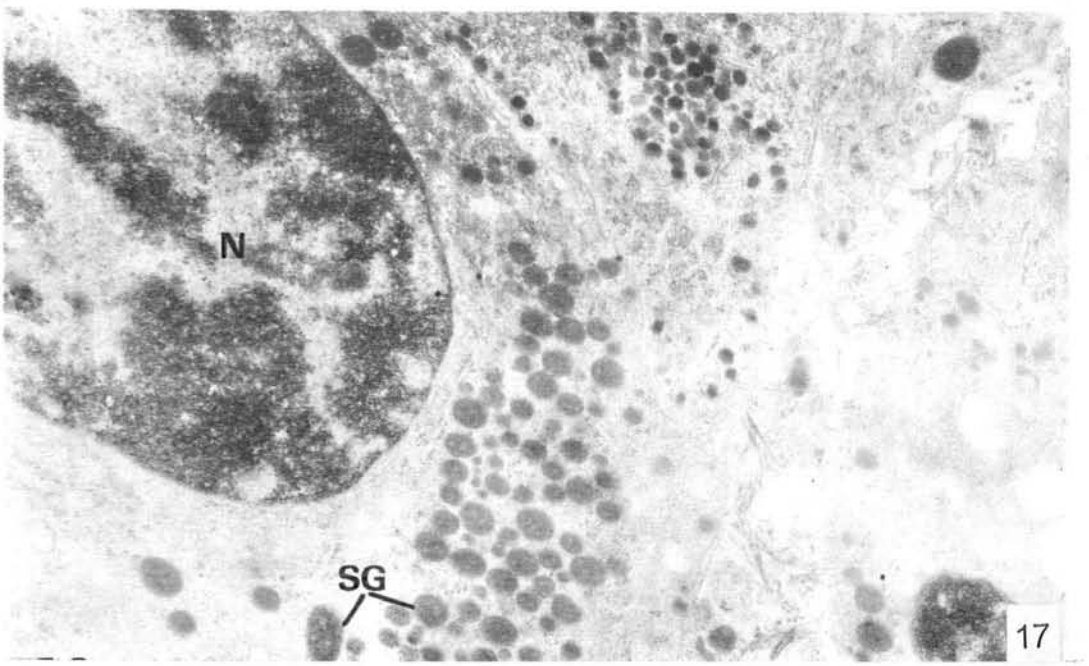


Fig.13. Electron micrograph of a thyrotroph from 10 days old prepubertal female rat (centre). Description is same as in legend to Fig. 12. x 18000

Fig. 17. Electron micrograph of a typical somatotroph from 5 days old prepubertal female rat. Secretory granules (SG) are mostly oval and as well developed as in animals of advanced age. This cell type has been distinguished from lactotroph on the bases of granules size, number and distribution of granules in the cytoplasm, (compare with Fig. 24). x 18000



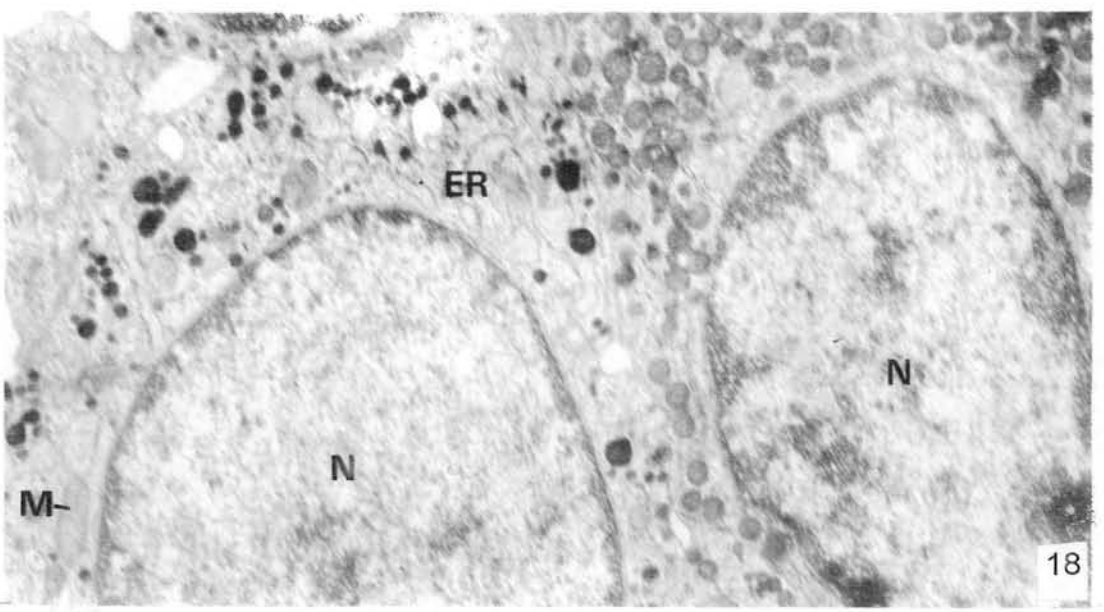


Fig. 18. Electron micrograph of pituitary cells from 30 days old prepubertal female rat. A gonadotroph (left) and a somatotroph (right) are compared. Differences in secretory granules(SG) size,shape and distribution in cytoplasm may be [illegible] this stage the cytoplasm of somatotroph is almost granules. x 18000

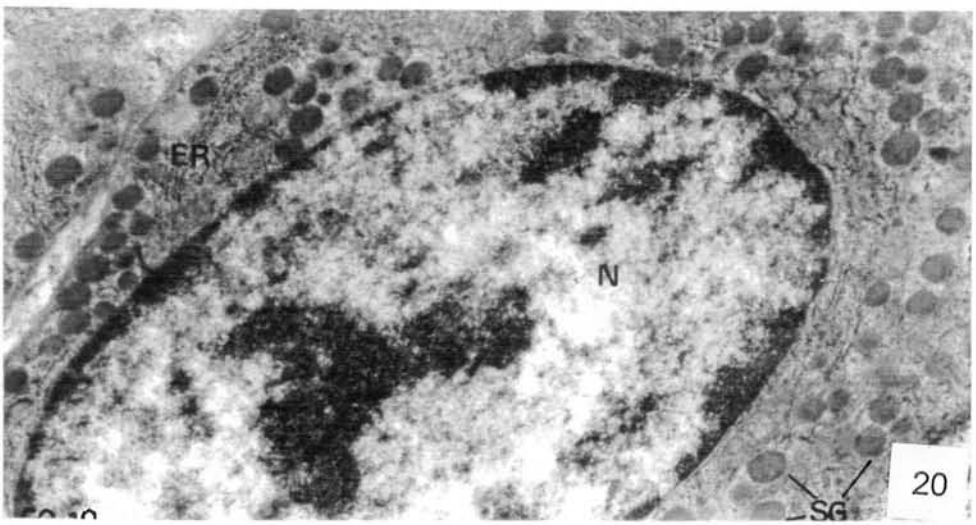


Fig. 20. Electron micrograph of a somatotroph from 60 days old female rat. A large nucleus (N) in the center of the cell. Secretory granules (SG), granular endoplasmic reticulum (ER) is scattered in the cytoplasm. x 18000

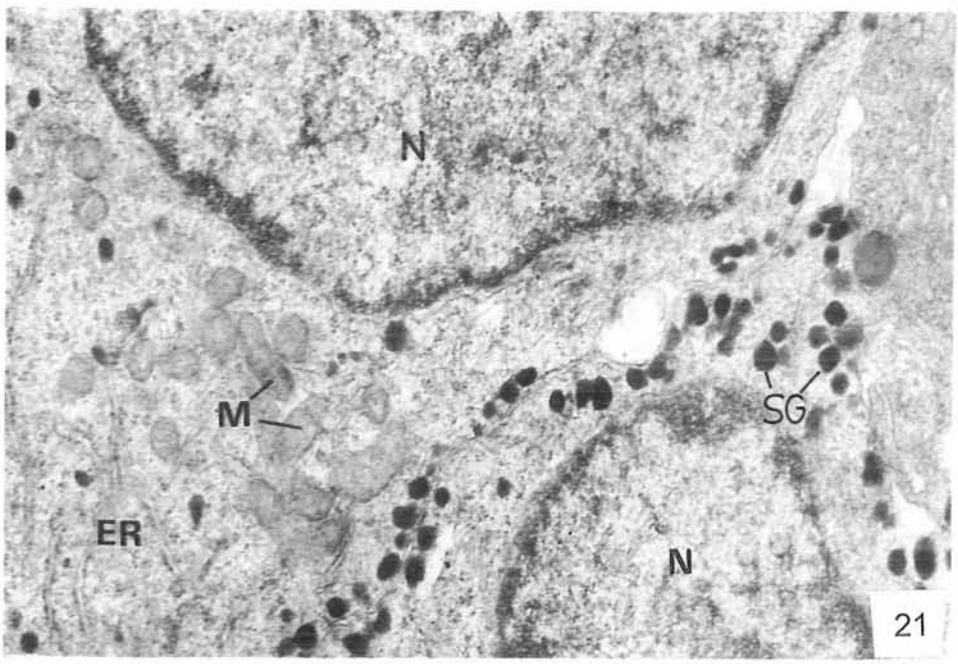


Fig. 21. Electron micrograph of a corticotroph from 10 days old prepubertal female rat. Endoplasmic reticulum (ER), mitochondria (M) nucleus (N), secretory granules (SG). The SG are larger in size compared to thyrotrophs and are arranged along the inside of cytoplasmic membrane. In all major features the 10 days old cell resembles those of advanced age. x 27000

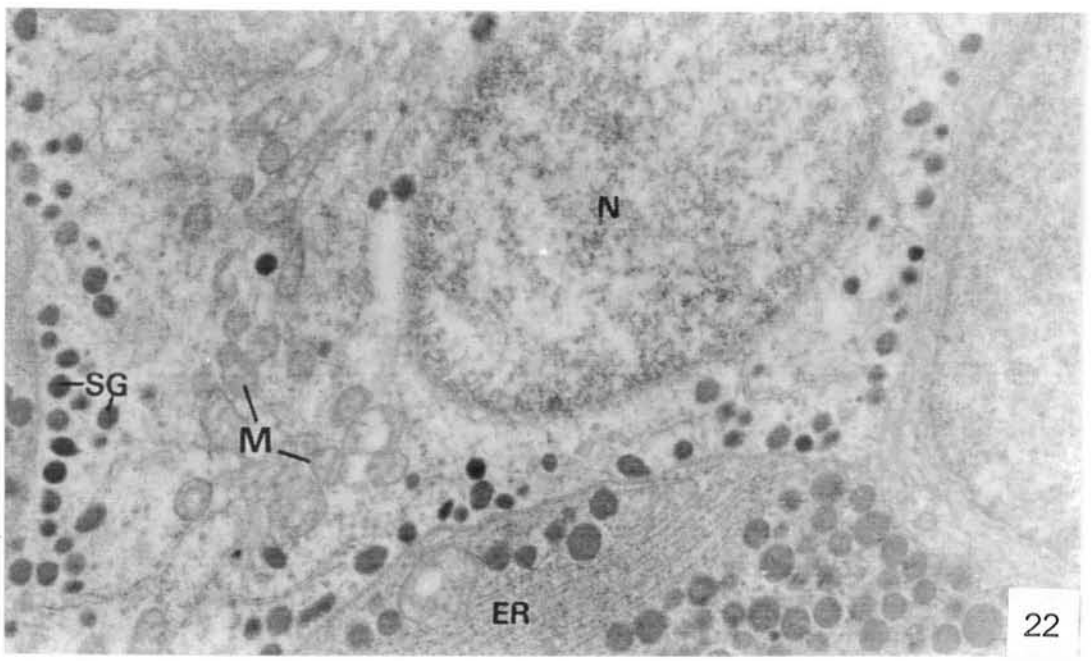
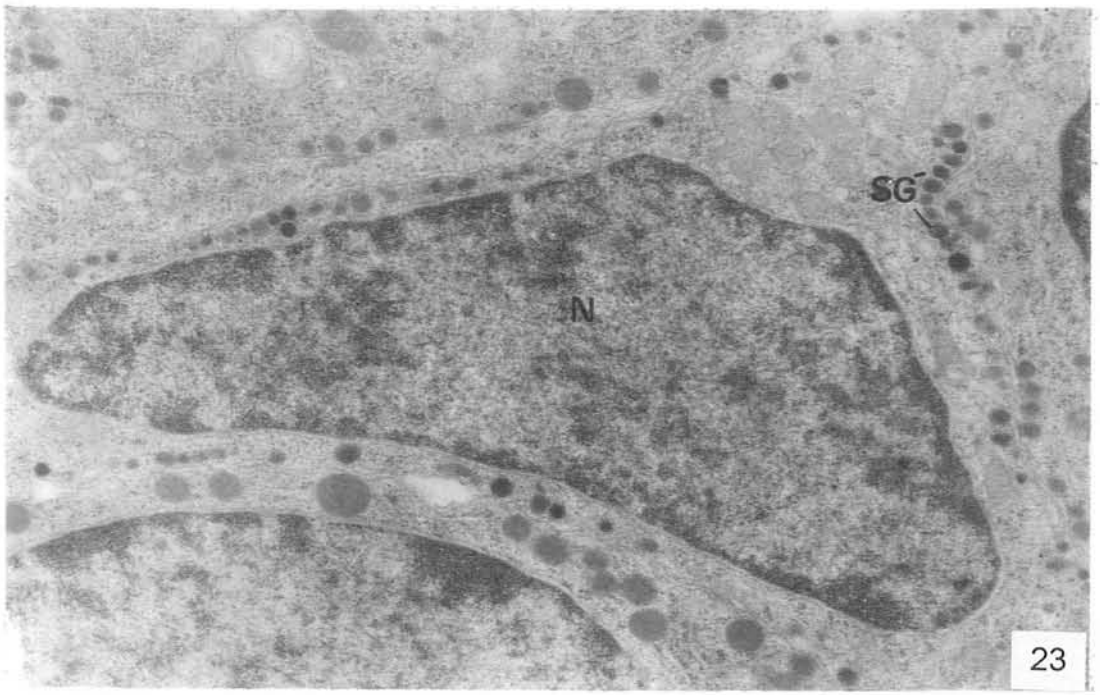


Fig. 22. Electron micrograph of a corticotroph from 60 days old female rat. The legend same as to Fig. 21, except the secretory granules (SG) are increased in number. x 18000

Fig. 23. Electron micrograph of a corticotroph from 20 days old prepubertal female rat. Description same as in Fig. 21. Note the elongated cell and nucleus. x 18000



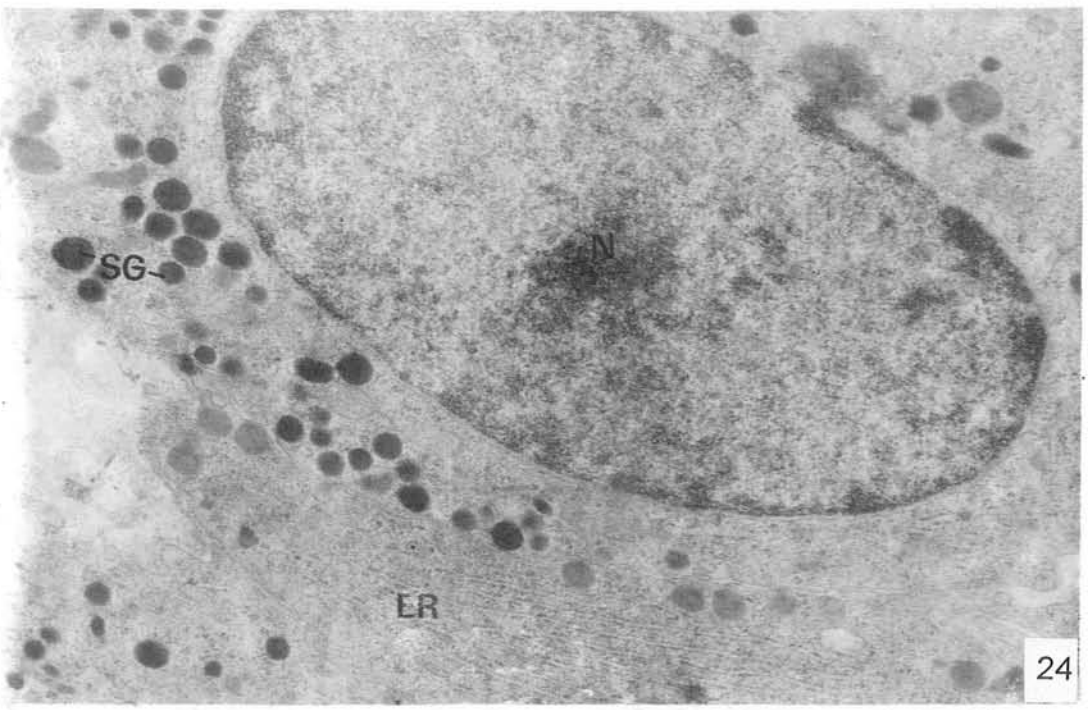


Fig. 24. Electron micrograph of a lactotroph (mammotroph) from 20 days old prepubertal female rat. This may be compared with 5 and 3[illegible] days old somatotrophs in Figs. 17,18. Secretory granules (SG) are larger in size and lesser in number, elongated nucleus (N) in the center of the cell are shown. x 18000

2. EFFECT OF OVARIECTOMY ON GONADOTROPHS

Thirty and sixty day old animals were ovariectomized as described in materials and methods. In each group, animals were killed 30 days after ovariectomy. Pituitaries were taken out and processed for electron microscopy. The purpose of this experiment was to find out changes, if any, in gonadotrophs following ovariectomy of immature and mature animals as compared to normal groups with intact ovaries. The results obtained are described separately for 30 and 60 days old animals.

Thirty day old animals

In 30 day old animals, the parameters studied included: (a) cell size, (b) nuclear size and shape, (c) endoplastmic reticulum, (d) Golgi complex, (e) mitochondria, (f) granule size and population and (g) changes in cytoplasm. A comparison of these parameters is recorded in Table. 7.

It can be concluded from the data of Table 7 and Fig.25 that no major changes in most of the parameters studied are apparent in 30 day old ovariectomized immature animals as compared to normal animals of the same age with intact ovaries. However, the effect of ovariectomy even in immature animals is clearly demonstrated by the emergence of cells

with vesiculated cytoplasm ("castrate cells") which are conspicuous by their absence in pituitaries of normal rats. In a large population of cells studied under electron microscope, 33% cells had vesiculated cytoplasm and 67% cell had homogeneous cytoplasm (compare Fig. 7 with Figs. 27-29) There was no apparent difference in the size and population of granules, but obvious differences were seen in the dispersion of granules in both cell types, that is, those with homogeneous cytoplasm and those with vesiculated cytoplasm, as compared to normal pituitary gonadotrophs of the same age in which only homogeneous cells are present. The granules in normal cells are more or less evenly dispersed. In pituitary gonadotrophs of overiectomized animals, the granules tend to aggregate and give polar appearance especially in cells with homogeneous cytoplasm (Fig. 27). In "castrate cells", the areas with vesicles generally have a low population of granules. No signet ring cells have been observed. The Golgi is poorly developed indicating low secretory activity. No nuclear indentation has been observed.

Sixty day old animals

More intensive changes in gonadotroph morphology were observed in 60 day old castrated animals, as compared to normal animals of the same age or 30 day castrated animals. The data are recorded in Table 8. It may be observed that leading changes continue in the same direction as initiated in 30 day old animals.

Table 7

Comparison of changes in gonadotrophs in 30 day old castrated and normal rats

Parameter	Normal	Castrated
Granule size (A^{o})	1810± 113	1800± 100
Granule population/ cell (No.)	370± 20	313± 10
Cell size (μm)	13.3± 0.45	13.75± 0.5
Cell shape	Oval or Polygonal	Polygonal or Oval
Nuclear size (μm)	5.34± 0.5	6.5± 0.6
Nuclear shape	Round or Oval	Polygonal
Endoplasmic reticulum (ER)	Prominent	Prominent, many vesicles
Golgi complex	Prominent	Prominent
Mitochondria	Oval or Round	Oval or Round
Cytoplasm	Homogeneous	Homogeneous or vesiculated

Table 8

Comparison of changes in gonadotrophs in 60 day old castrated and normal rats

Parameter	Normal	Castrated
Granule size (A^{o})	2748± 100	2079± 70
Granule population/ cell (No.)	300± 20	345± 20
Cell size (μm)	15± 0.8	15.5± 0.8
Cell shape	Polygonal or Irregular	Polygonal or Irregular
Nuclear size (μm)	7.5± 0.5	7± 0.3
Nuclear shape	Irregular or Oval	Irregular or Oval
Endoplasmic reticulum (ER)	Prominent	Prominent, many vesicles
Golgi complex	Prominent	Prominent
Mitochondria	Oval or Round	Oval or Round
Cytoplasm	Homogeneous	Homogeneous or Vesiculated

As shown in Figs. 30,31 vesiculation not only becomes more extensive but also the number of castrate cells shows a marked increase; there are 60% vesiculated cells (Figs. 30,31 & 33) and 40% cells with homogeneous cytoplasm (Fig. 32). Most marked difference was observed in the Golgi complex which increases in size. Also the number of granules as seen in the proximity of the Golgi is increased (Fig. 33). This organelle appeared to have achieved the stage of high secretory activity (Figs. 33,34). The granules, unlike the normal cells are not evenly distributed. Most of the granules are aggregated in pockets enmeshed between the vesicles in "castrate cells". No significant change in granule population was observed, though the size of granules was decreased (compare Fig. 10 with Figs. 30, 31 & 35). In cells with homogeneous cytoplasm (Fig. 32), the granules were dispersed in polar aggregation. The remarkable feature of castrate cells is related to the indentation of nuclear memberane. This was clearly initiated in this cell type as observed under the electron microscope (Fig. 32). For comparison of 60 day old castrated animals with normal rats of the same age, reference may be made to Table 8, Fig. 26.

Discussion

Changes have been reported in pituitary gonadotrophs following gonadectomy in rats (Farquhar & Rinehart,1954*b*; Purves & Griesbach,1954*b*;Herlant,1964; Yoshimura & Harumiya, 1965; Kurosumi & Oota,1968; Rennels et al., 1971; Garner &

Blake,1981). In general, these changes are related to increase in cell size (Costoff,1973; Garner & Blake,1981), increase in granules with the passage of time after gonadectomy (Garner & Blake,1981), enlargement of the Golgi apparatus (Farquhar & Rinehart, 1954; Farquhar, 1955), increased secretion of gonadotrophins (Dullaart, 1981), changes in dense bodies (Garner & Blake, 1981) and vesiculation of the ER; the vesicles appearing as large vacuoles after coalesence (Costoff, 1973). It has been difficult to distinguish between LH and FSH cell types amongst gonadotrophs in earlier studies based on light microscope (Rennels,1957, 1963; Hildebrand et al., 1957; Hellbaum et al., 1961; Vanhaperttula,1966). However in subsequent studies distinct FSH and LH cell populations have been reported (Barnes, 1963; Costoff, 1973; Moriarty, 1976); the FSH population predominating (Moriarty, 1976). In persistent estrous rats, Graafian follicles in the intact ovary were observed, though LH cells were also present, indicating a suppression of LH secretion (Kurosumi & Oota, 1968). Recent immunocytochemical studies supported by bioassay of FSH and LH have provided conclusive evidence for the presence of distinct FSH and LH populations in the pituitary of rats (Garner & Blake, 1981).

In our studies we have made no attempt to distinguish between FSH secreting and LH secreting cells. However, it is now well established, that following ovariectomy major changes are initiated in the LH secreting cells (Costoff, 1973).

Though the data reported in our studies is stated to be related to gonadot. rophs, yet, these may indicate changes in LH secreting cells. Many of the changes we are reporting in pituitary gonadotrophs of 30 and 60 day ovariectomized rats are in general agreement with those reported by others (Farquhar & Rinehart, 1954b; Purves & Griesbach, 1954; Costoff, 1973; Garner & Blake, 1981). However, we have observed more significant changes in the extent of development of the Golgi and ER vesicles and/or vacuoles in 30 day and 60 days ovariectomized animals. Evidence of increased secretion of gonadotrophins occurs following ovariectomy (Dullaart, 1981; Garner & Blake, 1981; Elias & Blake, 1983) is commensurate with rapid development of the Golgi area in 60 day but not in 30 day ovariectomized animals (compare Fig. 11 with Fig. 34). Similar is the case with cytoplasmic vacuoles which appear as a result of coalesence of vesicles of ER (Costoff, 1973). As a result of these cytoplasmic changes three types of LH secreting cells have been described on the basis of immunocytochemical studies: (a) cells with homogeneous cytoplasm; (b) cells with vesiculated cytoplasm, and (c) signet ring cells in which nucleus is pushed to one side, the cytoplasm may be vesiculated or homogeneous (Garner & Blake,1981). The vesiculated cell though identified during various phases of the estrous cycle in rats (Costoff, 1973) resembles a typical "ovariectomy cell". The prevalence of these cells in ovariectomized animals is known since the pioneering studies of Purves and Griesbach (1951b) with light microscope.

Our studies with immature female rats ovariectomized on day 30 show substantial vesiculation but little development in the Golgi.There are no previous studies of ultrastructural changes in ovariectomized immature rats. However, increase in circulating levels of LH and FSH in ovariectomized immature female rats (days 5, 15,20,25,30 and 35) have been taken to mean that even day5 and 10 prepubertal pituitaries are sensitive to ovariectomy (Dullaart,1981). These studies are consistent with in vitro studies in which increase in release of gonadotrophins was observed when compared with sham-operated animals or when challenged with LHRH. In any case LH release compared to FSH was negligible without LHRH at the ages of 25, 30 and 35 days. These interesting observations have not been pursued by studying simultaneous ultrastructural changes in gonadotrophs. Our studies on 30 day old animals support these findings to the extent that gonadotrophs of immature animals (30 day) are responsive to ovariectomy, since vesiculation,a manifestation of ovariectomy, is clearly seen in immature ovariectomized animals (Fig. 28). Sensitization of hypothalamo-hypophyseal axis, early in prepubertal stage is also known (Dullaart, 1981). It appears that the changes we have observed in ovariectomized prepubertal animals document the evidence that in the absence of the ovary perhaps increased LHRH environment results in the hypertrophy of gonadotrophs.

There is a wide morphological departure in the extent of responsiveness of gonadotrophs to ovariectomy in 6o day

old animals compared with 30 day old animals. The acuteness of response is understandable, since in mature animals the circulating levels of ovarian steroids, by and large, exercise regulatory control over LHRH release. Thus, once the source of steroids is eliminated (high sensitization of hypothalamus prevailing) the gonadotrophs are exposed to a sustained high environment of LHRH resulting in rapid hypertrophy; this is excellently revealed in Fig. 33, in the advancement of Golgi and vesiculation. These cells provide a picture of increased synthesis and release of gonadotrophins as reported from in vivo bioassay data of circulating level of FSH in mature ovariectomized rats (Elias & Blake,1983). Interestingly enough, we were unable to locate typical signet ring cells as observed by other workers. It appears that longer duration of ovariectomy is necessary before this cell type can be observed (Purves & Griesbach, 1954b; Costoff, 1973; Garner & Blake, 1981).

FIGURES

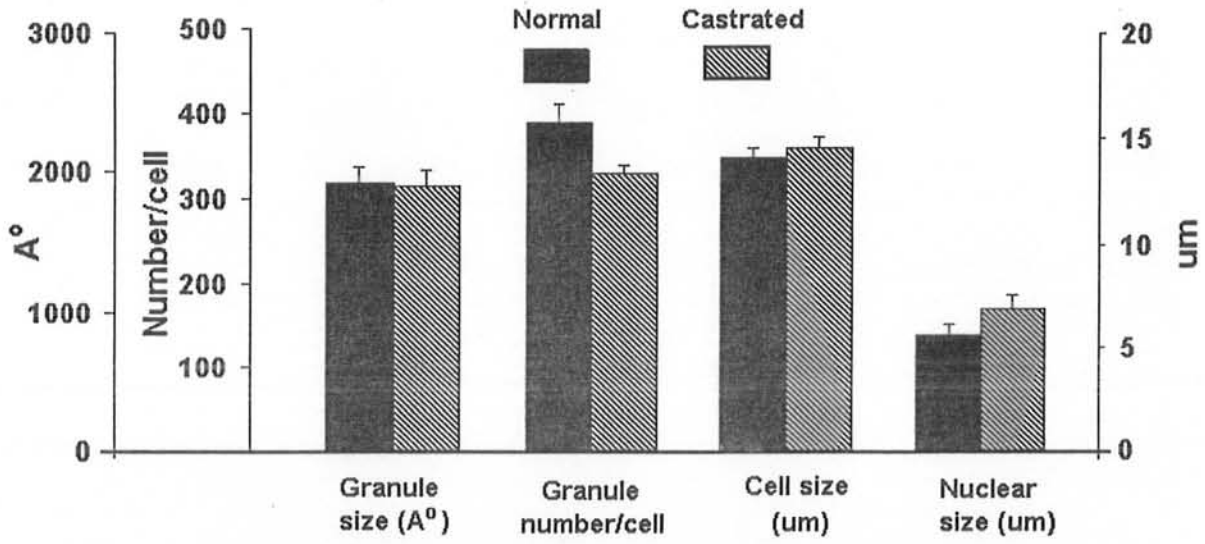


Fig. 25. Comparison of various parameters: granule size (A^0), granule number per cell, cell size (um) and nuclear size (um) in normal and castrated rats. Normal rats were 30 days old; castrated animals were examined 30 days after ovariectomy of 30 days old animals.

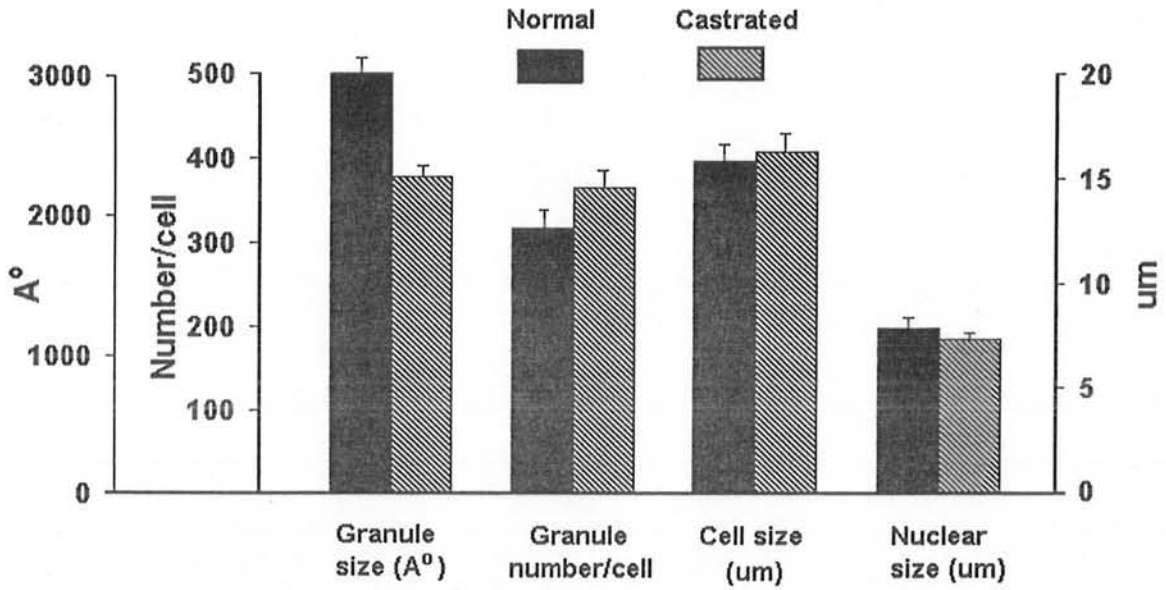


Fig.26. Comparison of various parameters: granule size (A^{o}), granule number per cell, cell size (um) and nuclear size (um) in normal and castrated rats. Normal rats were 60 days old; castrated animals were examined 30 days after ovariectomy of 60 days old animals.

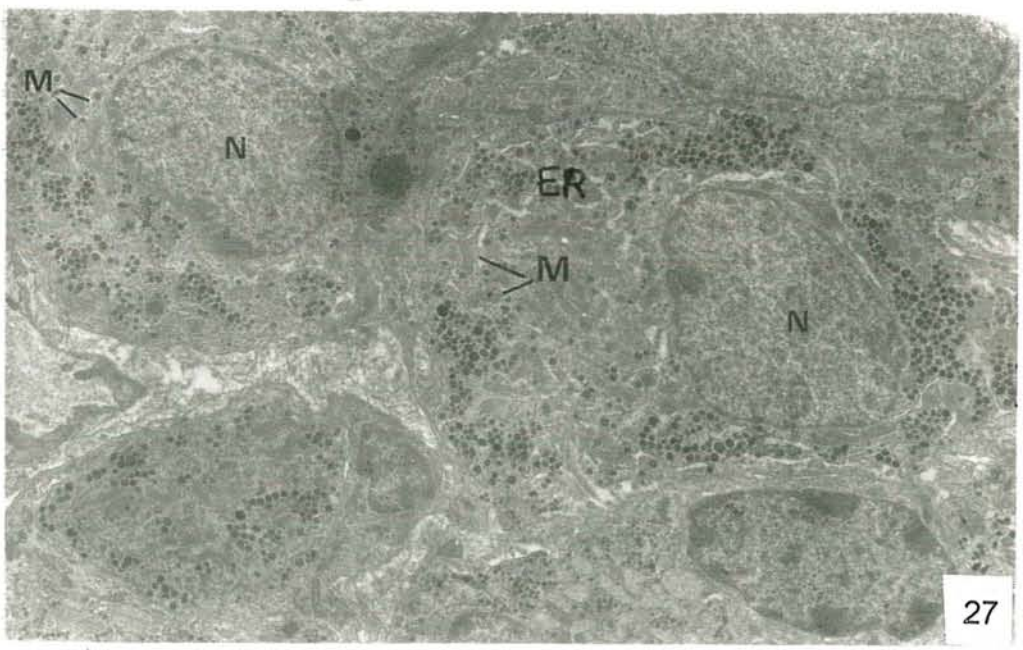
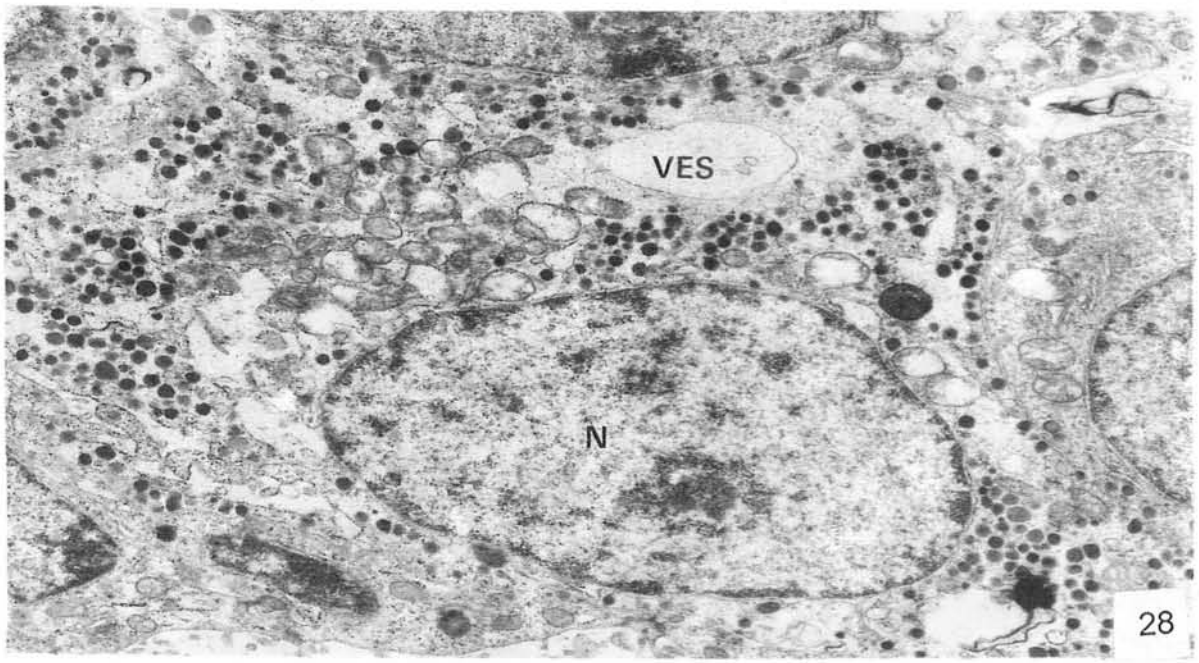


Fig. 27. Electron micrograph of a group of gonadotrophs from castrated female rat. Animals were killed 30 days after ovariectomy of 30 days old rats. Four types of cells are seen: (a) cell with homogeneous cytoplasm; (b) cell in which vesiculation of endoplasmic reticulum (ER) is clearly visible. Numerous mitochondria are seen in cells with homogeneous cytoplasm. The nucleus is pushed to one side as typically seen in gonadotrophs of castrated animals. x 5400

Fig. 28. Electron micrograph of a gonadotroph from 30 days old prepubertal rat, castrated for 30 days. Note the appearance of vesicles (VES), the shape of the nucleus (N) which is pushed to one side and distribution of granules in regions of the cytoplasm without vesicles. The Golgi is not prominent. x 10800



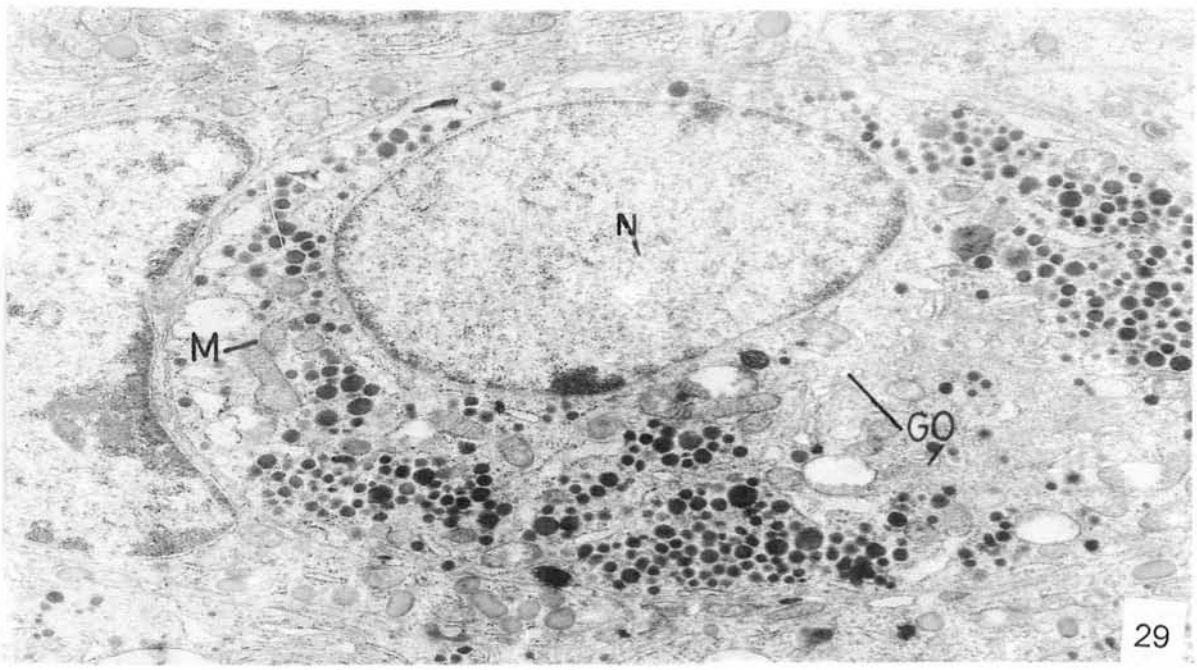
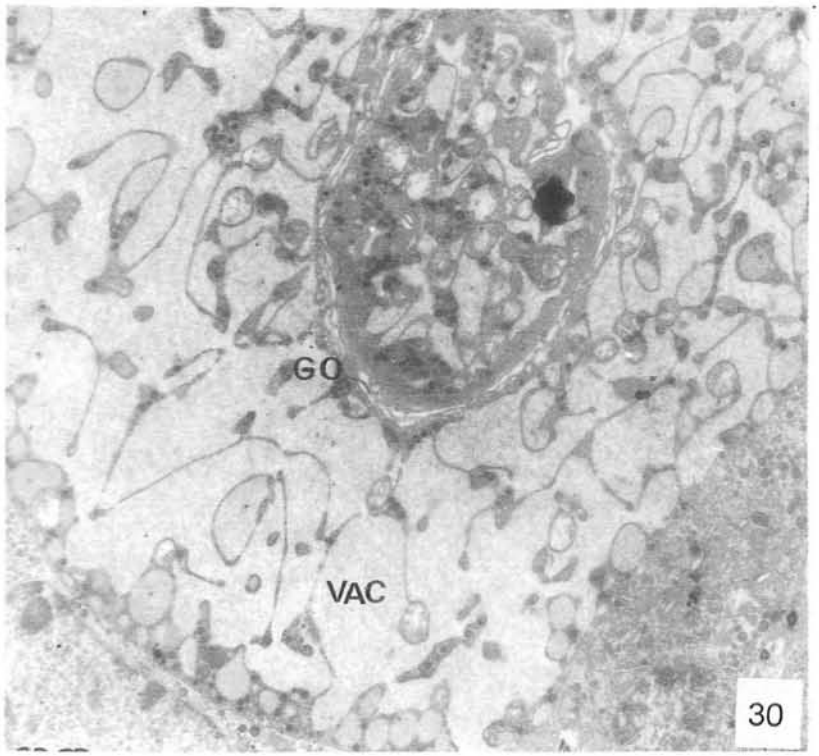
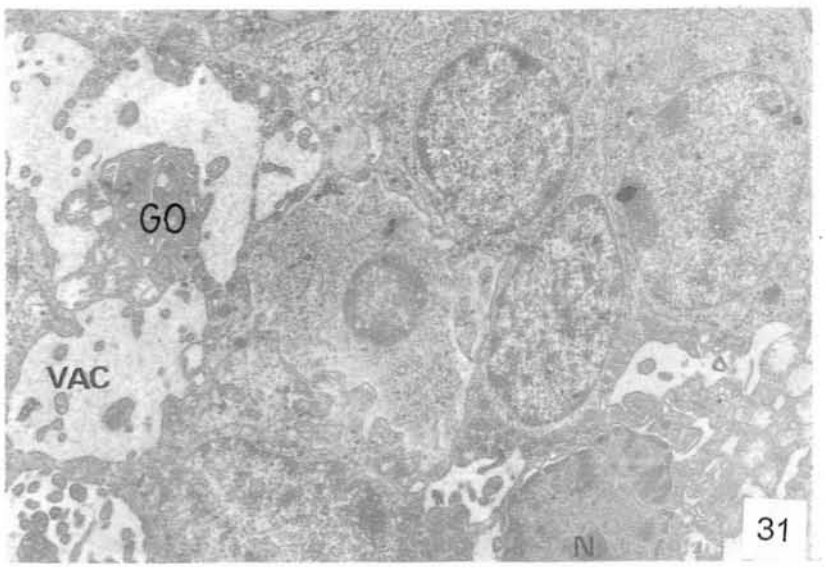


Fig.29. Electron micrograph of a gonadotroph from 30 days old prepubertal rat, castrated for 30 days. Legend same as in Fig. 28. However, appearances of a well developed Golgi complex (GO) may be noted. x 10800

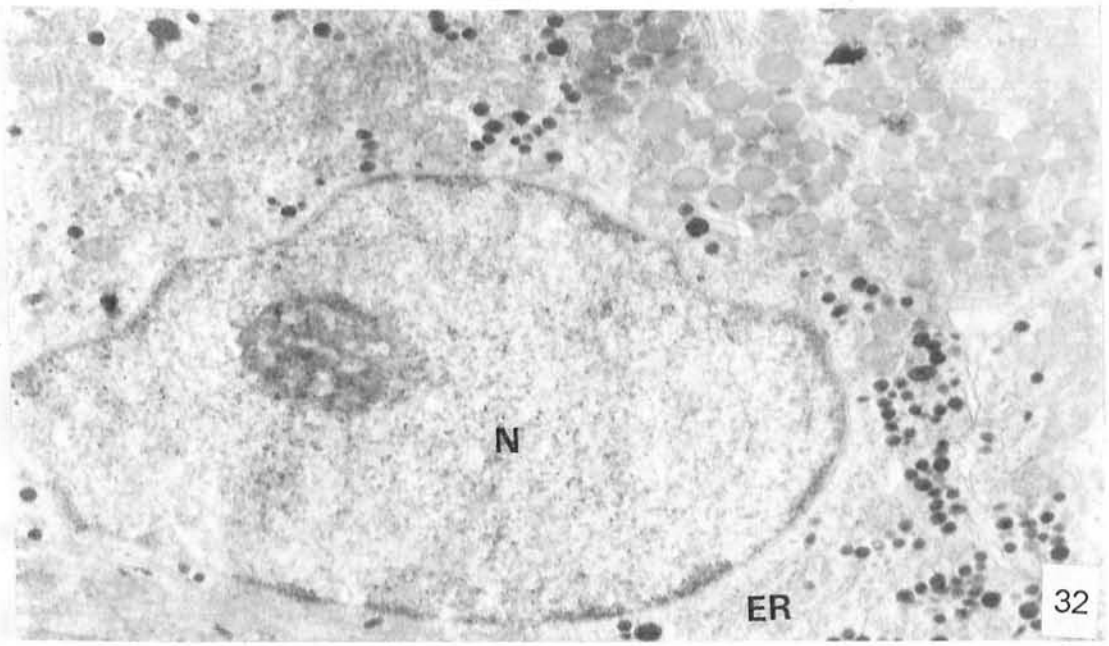
Fig.30. Electron micrograph of a gonadotroph from 60 days old cyclic rat castrated for 30 days. The cell presents all of typical feature of a "castrate cell". Note the extensive vacuolation (VAC) of cytoplasm. Ring shaped Golgi area (GO) is well developed enclosing a portion of cytoplasm containing vesicles around granules. x 7200





micrograph of a gonadotroph from 60 days
for 30 days. The highly
complex
gives

Fig. 32. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days. Two features may be noted: (a) this gonadotroph has homogeneous cytoplasm; and (b) the nuclear membrane ,as expected,is irregular. x 18000



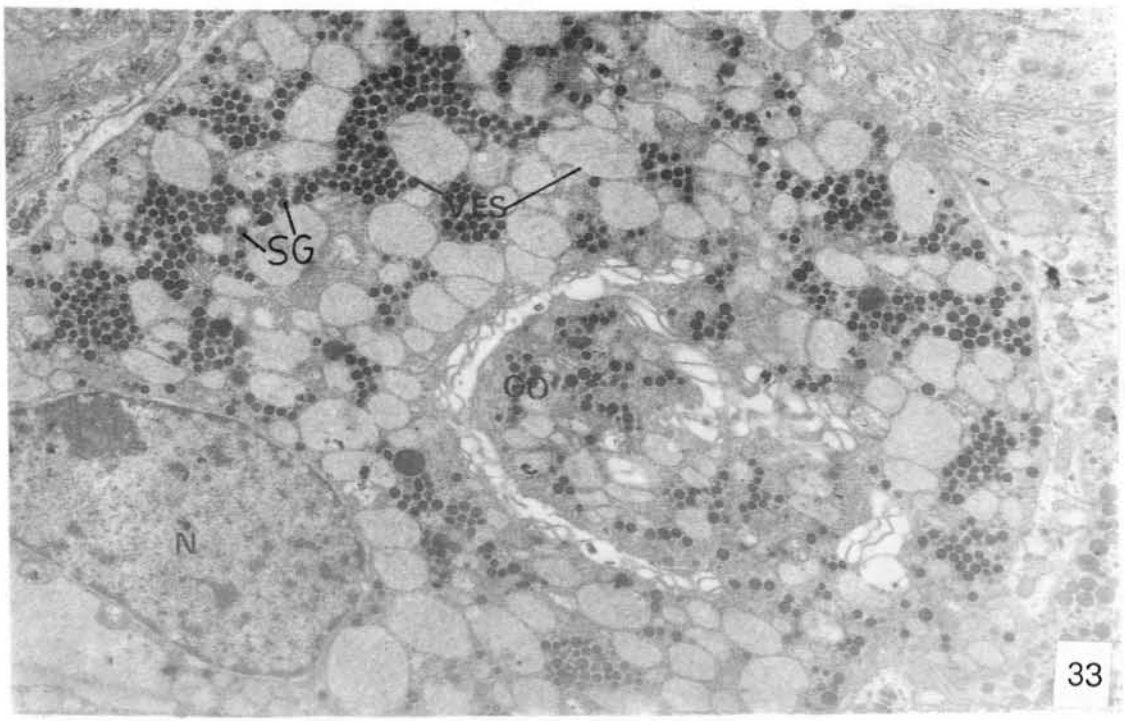
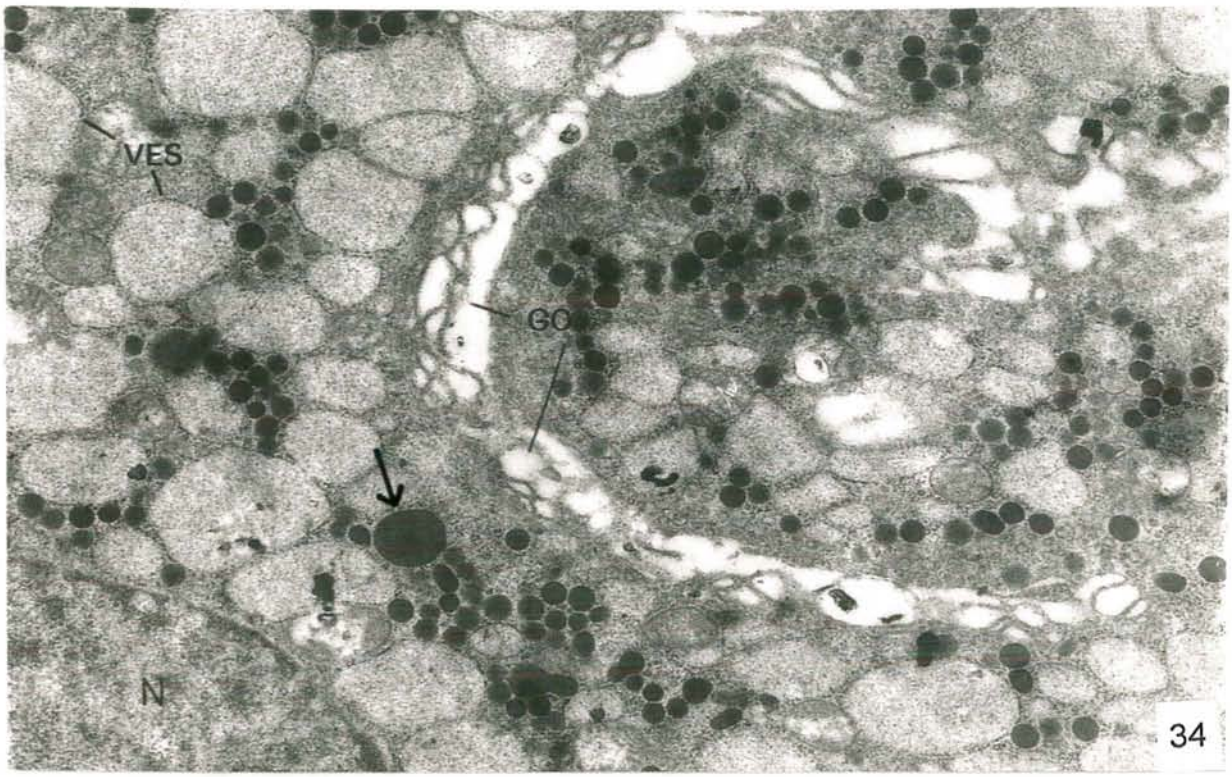


Fig. 33. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days. The cytoplasm is heavily vesiculated but vacuolation as seen in the castrate cell (Fig. 30), is not apparent. The secretory granules (SG), larger in number and are distributed differently. The Golgi complex (GO) is well developed with the same features as seen in castrate cell (Fig. 30). The nucleus (N) is pushed to one side (extreme lower left). x 7200

Fig. 34. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days. All the features described same as legend to Fig. 33, are amplified. Especially note the development of Golgi complex (GO) and presence of dense body (—>). x 18000



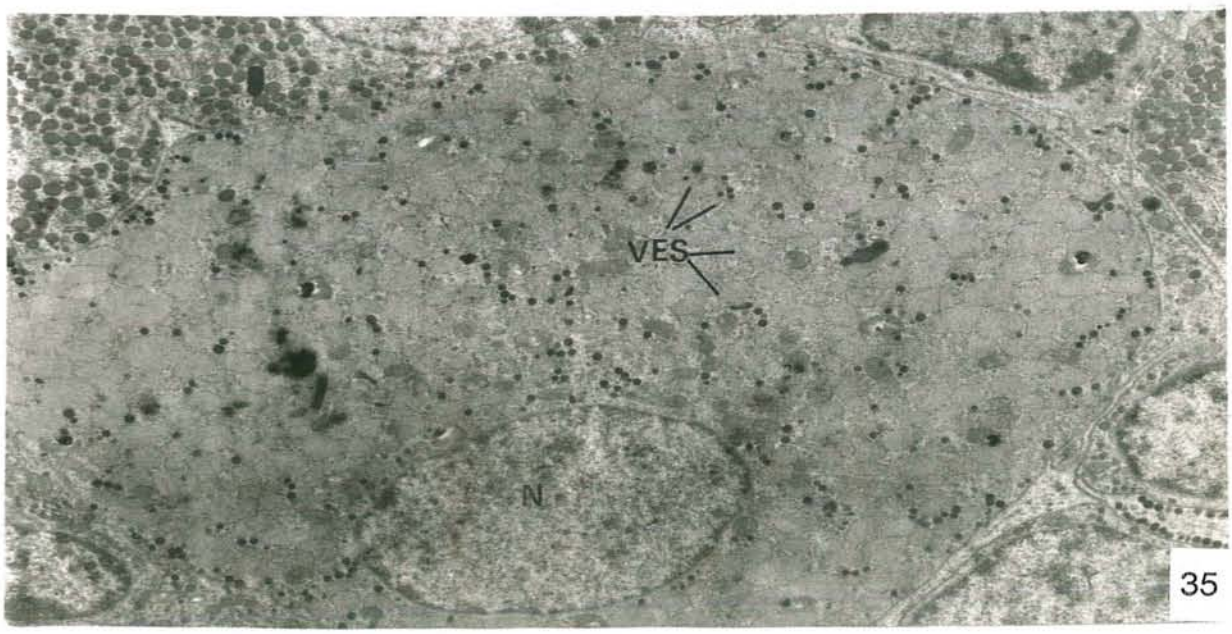


Fig. 35. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days. The gonadotroph type is same as in Fig. 33. However, the cytoplasm is fully vesiculated and the granules are sparsely distributed. x 7200

3. CHANGES IN GONADOTROPHS FOLLOWING LHRH TREATMENT

Experiments were undertaken to find out the effect of LHRH treatment on pituitary gonadotrophs of "immature" and "mature" animals. Whereas the mechanism of negative feed back on the release of gonadotrophin is well known, little information is available with regard to the effect of LHRH on gonadotrophs in immature animals which theoretically are in a state of persistent negative feed back but apparent "desensitization" of hypothalamus. The question we raised was: whether LHRH would produce the same changes in immature gonadotrophs as are produced by gonadectomy in gonadotrophs of mature rats. For this purpose animals of various ages, 20, 30, 60 days were injected i.v. with 1μg LHRH daily, for 5 consecutive days and killed on day 6. The pituitaries from these animals were processed for light microscopy and electron microscopic studies as described under Materials and Methods. In this section the results of these experimental studies are described.

Effect of the LHRH Treatment on Gonadotrophs

Comparative effect of LHRH treatment in 20, 30, and 60 day animals is recorded in Table 9, Fig. 36. Morphological changes accompanying LHRH treatment are shown in Figs. 37-53.

As shown in Table 9 and Figs. 28-41 comparison of 20 day old LHRH treated animals with normal animals showed significant changes in cytoplasm. The treated and untreated gonadotrophs could be easily identified by the presence of vesiculated cells in the former category and none in the latter category in which the whole cell population has homogeneous cytoplasm (compare Figs. 37, 38 with Fig. 6). In treated animals 10-20% cells had vesiculated cytoplasm. The vesiculated cytoplasm was indicative of appearance of "Castrate cells", as one would see in ovariectomized animals. The Golgi, though better developed in LHRH treated gonadotrophs compared to normal animals of the same age was nowhere closer to the 60 day ovariectomized animals in its extent of develoment. Indentation of the nuclear membrane as seen in castrate cells was also initiated (Figs. 37, 39). In LHRH treated castrate cells, the granules are dispersed in between the vesicles.

LHRH treatment of 30 day old animals induces changes which, though more acute, are similar to those in 20 day old animals. Endoplasmic reticulum is more extensive, granule size and granule population do not differ markedly, the Golgi is prominent, the nuclear membrane is more indented, the mitochondria are more prominent and the population of vesiculated cells is markedly increased (40%) (Compare Figs. 42, 43 & 47 with Figs. 37,38). It has also been observed that in "Castrate cells", the number of granules decreases and the size of granules does not go beyond 1762±50 A°. In

Table 9

Comparative data on gonadotrophs following LHRH treatment in female rats (day 20, 30 and 60)

Parameter	Age (days)		
	Prepubertal		Pubertal
	20	30	60
Granule size (A^{o})	1351±60	1762±50	1850±40
Granule population/ cell (No.)	300±14	233±20	174±30
Cell size (μm)	10.8±0.8.	12.67±0.6	14.5±0.5
Cell shape	Polygonal or irregular	Polygonal or irregular	Polygonal or irregular
Nuclear size (μm)	5.5±0.5	5.5±0.6	6.5±0.5
Nuclear shape	Irregular or Oval	Polygonal or Oval	Polygonal or Oval
Endoplasmic reticulum(ER)	Prominent	Prominent	Prominent
Golgi complex	Prominent	Prominent	Prominent
Mitochondria	Many, Round	Many, Round or Oval	Many, Round or Oval
Cytoplasm	Homogenous or Vesiculated	Homogenous or Vesiculated	Homogenous or Vesiculated

cells with homogeneous cytoplasm, the size of granules varies in normal range (Table 9, Fig. 36) However, the nucleus is highly indented (comparable to 60 day castrated animals). The granules show polar aggregation (Figs. 44,45).

In 60 day old animals treated with LHRH on the day of metestrous, these changes were not as prominent as in 60 days ovariectomized animals or immature rats treated with LHRH. Most of the cell population had homogeneous cytoplasm, though endoplasmic reticulum was highly developed. The granule size remained within normal range, but the number of granules was markedly depleted (Figs. 50, 51 & 53 and Fig. 36). The Golgi was well developed, indicating substantial secretory activity, yet, it was not as extensive as in 60 days old ovariectomized animals. Mild indentation comparable with 20 day old animals was observed. There was no trace of castrate cells, abundantly seen in 60 day old ovariectomized animals. On the basis of this information, it can be concluded that the nature of changes induced after LHRH treatment of 60 day old animals are less extensive compared to 20 day or 30 day old animals. These changes are less extensive than in post ovariectomized animals of the same age.

Discussion

The effect of LHRH on pituitary gonadotrophs has been the subject of a large number of in vivo (Corbin & Milmore,1971; Garner & Blake,1979; Watanabe,1981) and in vitro

studies (Redding et al.,1972; Spona & Luger, 1973a,b; Root et al., 1975; Dullaart,1976). In general, LHRH has been shown to increase the synthesis (Garner & Blake, 1979) and release (Aiyer et al., 1974; Watanabe et al.,1985; Garner et al., 1990; Evans et al., 1991) of LH and FSH. There are conflicting reports about the extent of granulation and degranulation in gonadotrophs under the influence of LHRH (Garner & Blake, 1979). Age related responsiveness of gonadotrophs to LHRH has also been reported (Spona & Luger, 1973a); day 30 prepubertal pituitaries showing highest responsiveness during in vitro incubation of pituitary in the presence of LHRH. Despite these extensive studies, only limited information is available on correlated morphological studies. The works of Garner et al. (1990), Watanabe (1981) and Watanabe and his associates (1985) are only indicative and, at best, describe changes in cell size, cell shape, nuclear shape and granulation (size and number) (Garner & Blake, 1979) based on immunohistochemical (Garner et al.,1990) and ultrastructural analysis. To the best of our knowledge solid data are not available on ultrastructural studies of gonadotrophs following LHRH treatment in immature and mature female rats. The present study, in which 20, 30 and 60 day old normal animals have been used for studying the effect of LHRH, provides substantial new information about ultrastructural changes in gonadotrophs.

Although attempts have been made to classify gonadotrophs into FSH secreting, LH secreting or FSH/LH

secreting cells on the basis of immunohistochemistry using LHβ anti serum (Moriarty, 1976; Garner & Black, 1979). Yet, there is equivocal evidence to show that castrate cell which appears following ovariectomy is a modified LH secreting cell (Inoue & Kurosumi,1981; Dada et al., 1983). This be so, the electron micrographs we are presenting for LHRH-treated 20, 30 and 60 day old animals may be considered at the same status as gonadotrophin secreting cells in immunohistochemical and ultrastructural studies (Rennels et al., 1971; Garner & Blake, 1979; Watanab et al., 1985; Garner et al., 1990).

As would be expected, chronic in vivo LHRH treatment in the presence of intact pituitary- ovarian axis is likely to mimic a state of ovariectomy in which sustained additional quanta of LHRH are released from the hypothalamus. Similar state prevails in the persistent estrous rats (Kurosumi & Oota, 1968). It is under such a condition that the castrate cell appears. This is now documented from our data in Figs. 28-35 and 37-53 which compare ovariectomized animals with LHRH treated intact immature and mature animals. The LHRH treatment produces the same condition as if the animals were ovariectomized (see also preceding section). In view of these findings, we are inclined to term the effect of LHRH alone as pseudocastration. This process has not been described before but provides an easy means of studying castration-like effects in animals treated with LHRH alone,

that is, without invasive ablation of gonads in males and females.

Marked differences observed in the gonadotrophs at 20, 30 and 60 day old animals challenged with LHRH are understandable as each age group presents a distinct stage of "physiological" development. Responsiveness of prepubertal animals, day zero onward to LHRH (Spona & Luger, 1973a) supports this contention. Day 30, in our opinion, is the most critical period in transformation of animals from prepubertal physiological state to achievement of puberty. This is supported by our data as well as those of others (Spona & Luger 1973a; Dullaart, 1981). The day 30 prepubertal animals respond to LHRH with greater avidity as compared to day 20 animals (compare Figs. 42-47 with Figs. 37, 38 & 41). For example, the presence of a large number of vesiculated cells with crenated nuclear membrane is characteristic of day 30-animals. Interestingly enough, unlike others we were unable to locate a substantial number of vesiculated cells with nuclear features of castrate cells or "signet ring" cells in ovariectomized 30 day old animals. Yet, such cells are abundantly present in 30 day LHRH treated animals with intact ovaries. Similarities between LHRH treated 30 day prepubertal animals and 60 day old ovariectomized animals can be explained on the basis of reasons discussed in preceding sections. Much of this has to do with exposure of pituitaries to increased levels of LHRH. In one case the mechanism operates through hypothalamic-ovarian axis, and in

the other case by direct effect of exogenous LHRH on pituitary gonadotrophs. The model of pseudocastrated animals, we believe, could be effectively used for *in vitro* study of the biology of "castrate cells", signet ring cells, and the mechanism of action of LHRH, particularly with reference to release and synthesis of LH and FSH. Further, use of such animals prepared by administering LHRH could provide useful information about the endocrinology of prepubertal development.

FIGURES

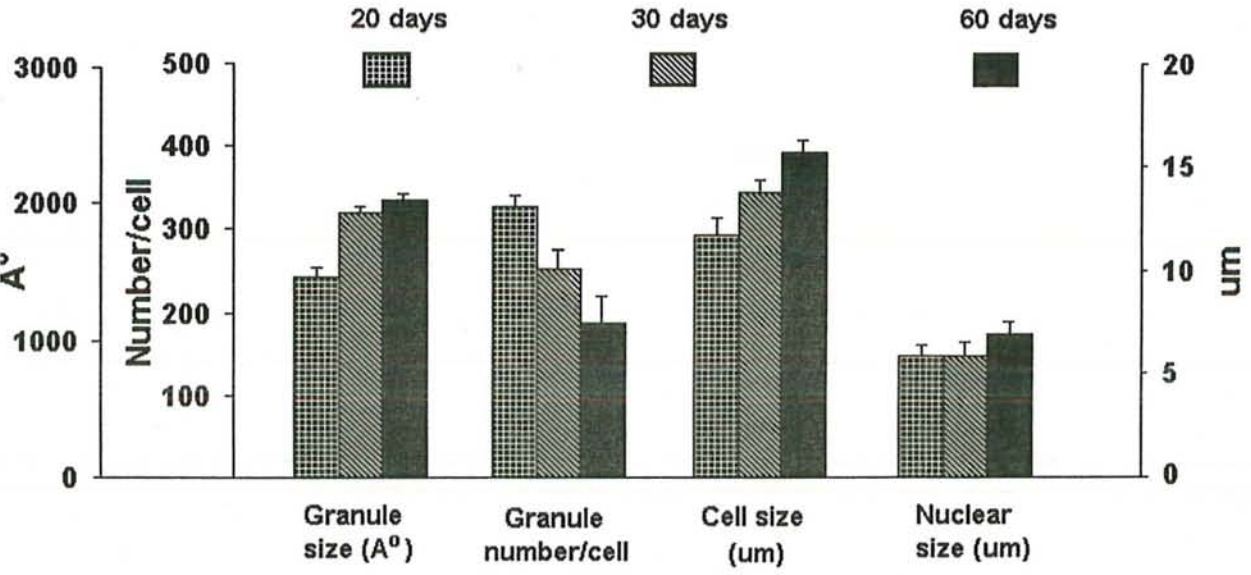
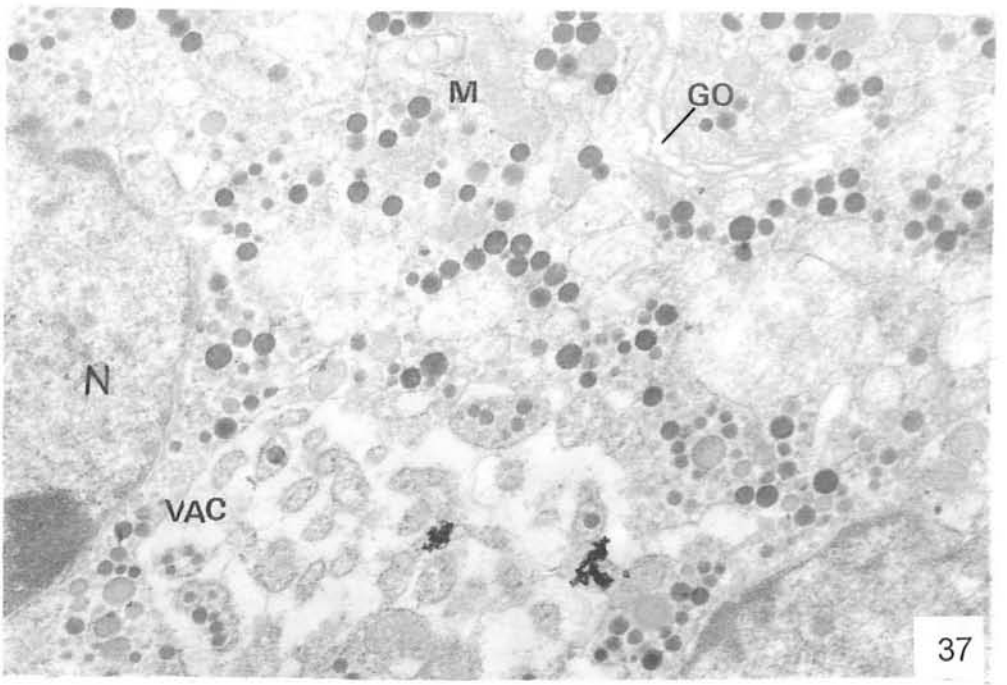


Fig. 36. Comparison of various parameters: granule size (A^{o}), granule number per cell, cell size (um) and nuclear size (um). Intact animals of 20, 30 and 60 days old were injected with 1ug/day LHRH for 5 days, the animals were examined on day 6.

Fig. 37. Electron micrograph of a gonadotroph from 20 days old prepubertal rat, treated with LHRH. LHRH 1 ug/day was injected for 5 days and animals were killed on day 6. Note the vacuolation (VAC) of the cytoplasm and prominence of Golgi complex (GO). This be compared with 20 days old animals without LHRH (Fig. 6), which have neither vesiculation nor a prominent Golgi complex. x 18000



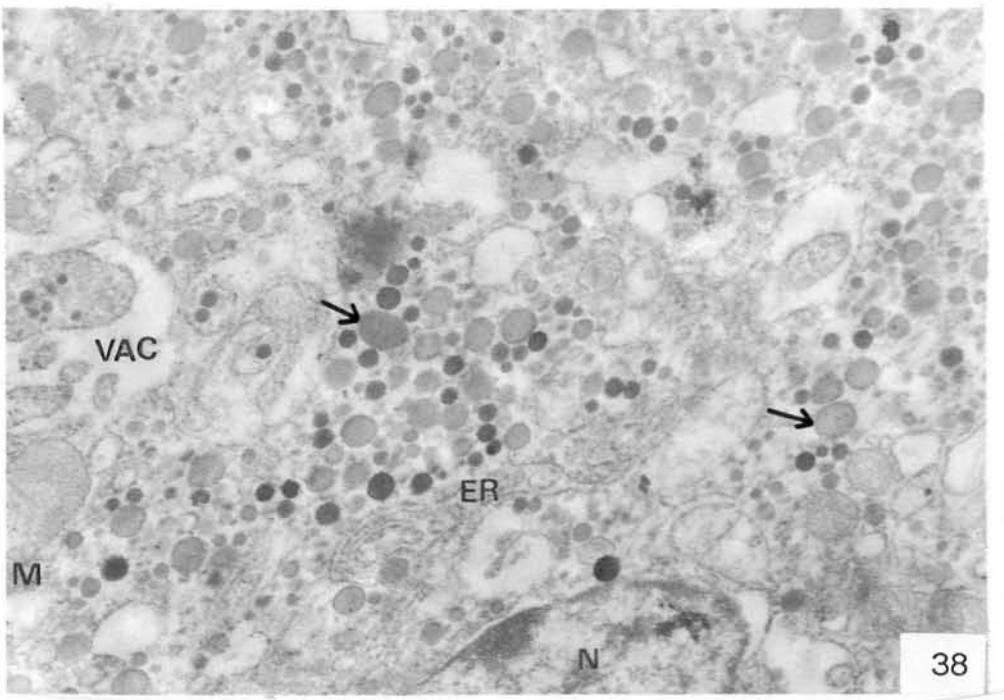
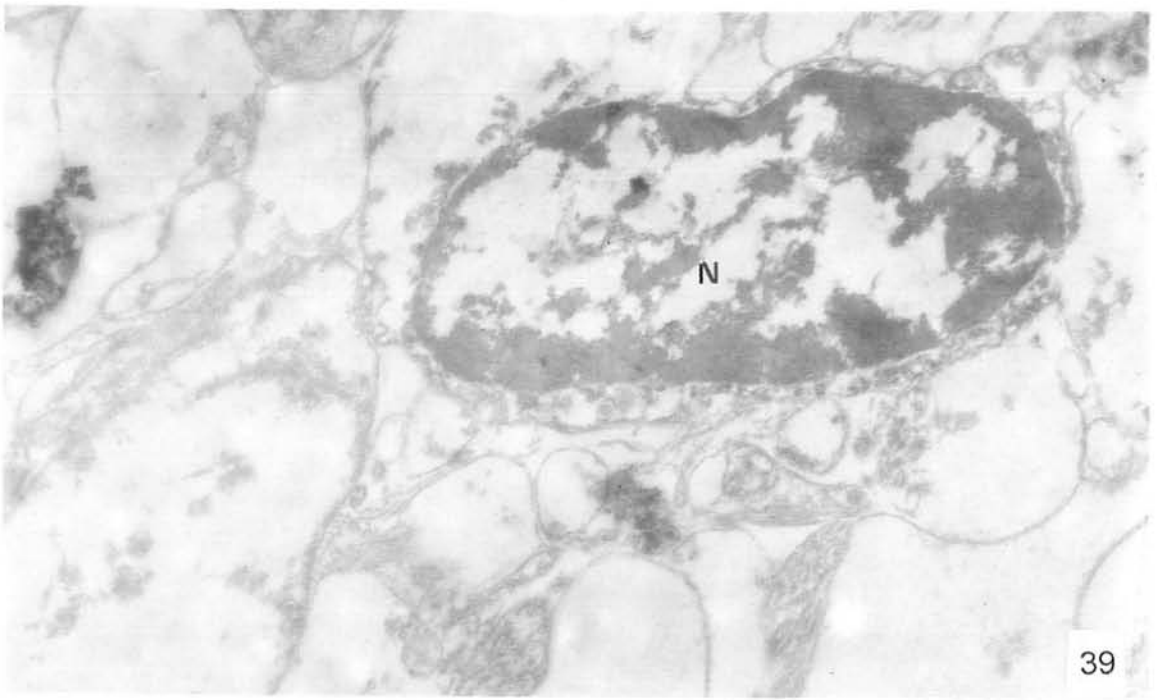
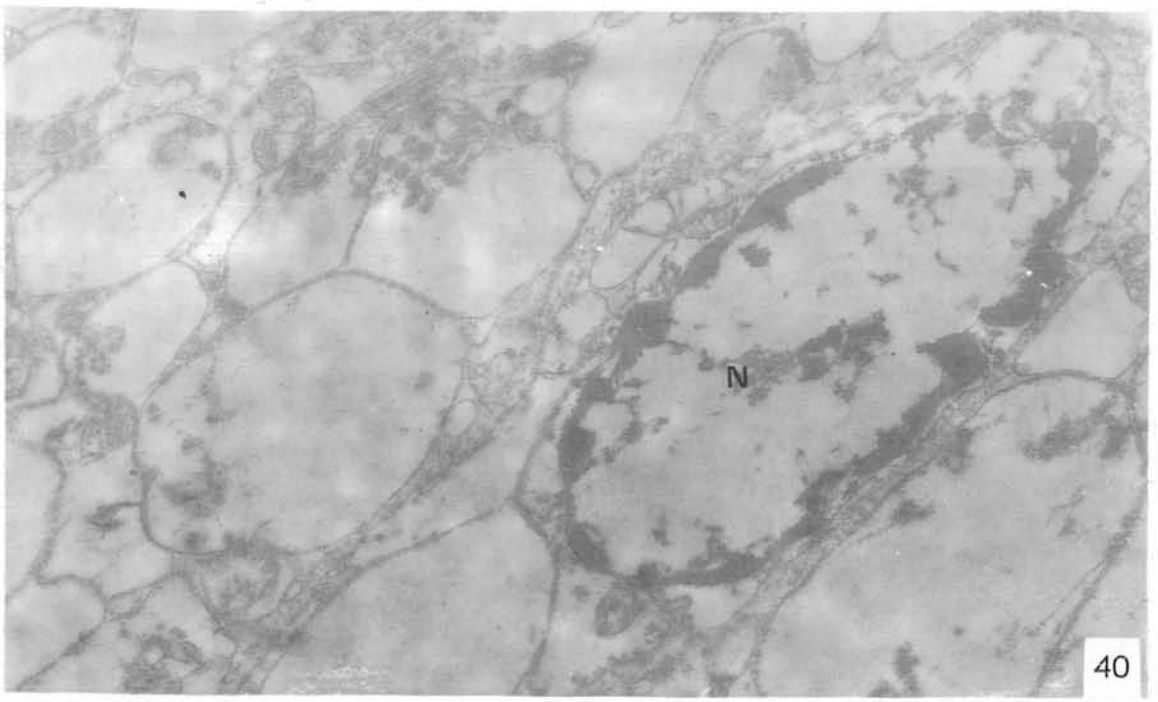


Fig. 38. Electron micrograph of a gonadotroph from
prepubertal rat, treated with LHRH. Regimen of LHRH
as described under legend to Fig. 37. Different c
which along with high vacuolation (VAC), a
endoplasmic reticulum (ER) is seen. Note also
large amorphous bodies dens).





Figs. 39,40. Electron micrographs of gonadotrope from 20 days old prepubertal rat, treated with LHRH. Enlarge of LHRH treatment same as described under legend to x 1800. Showing vacuolation. The indentation of the nucleus initiated (Fig. 39). x 18000

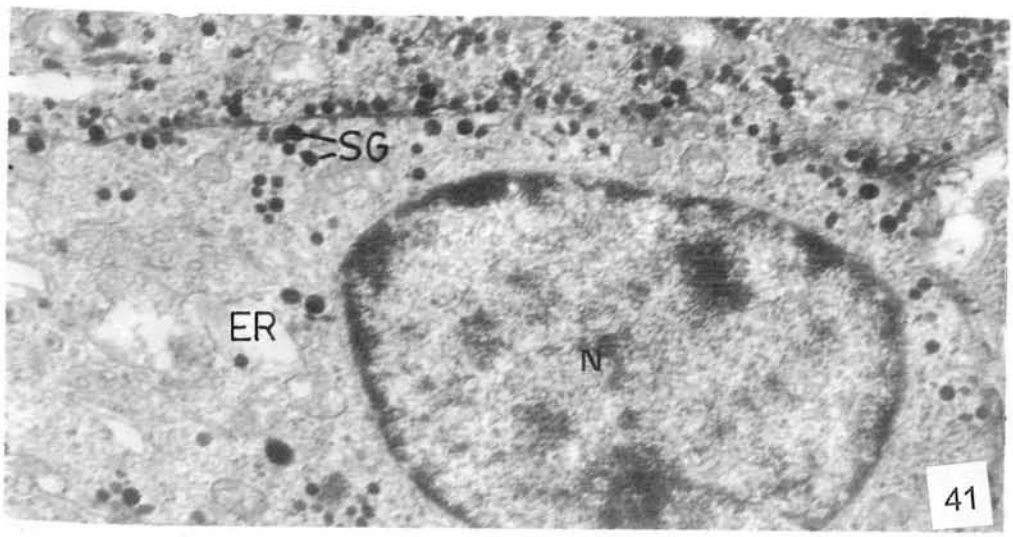
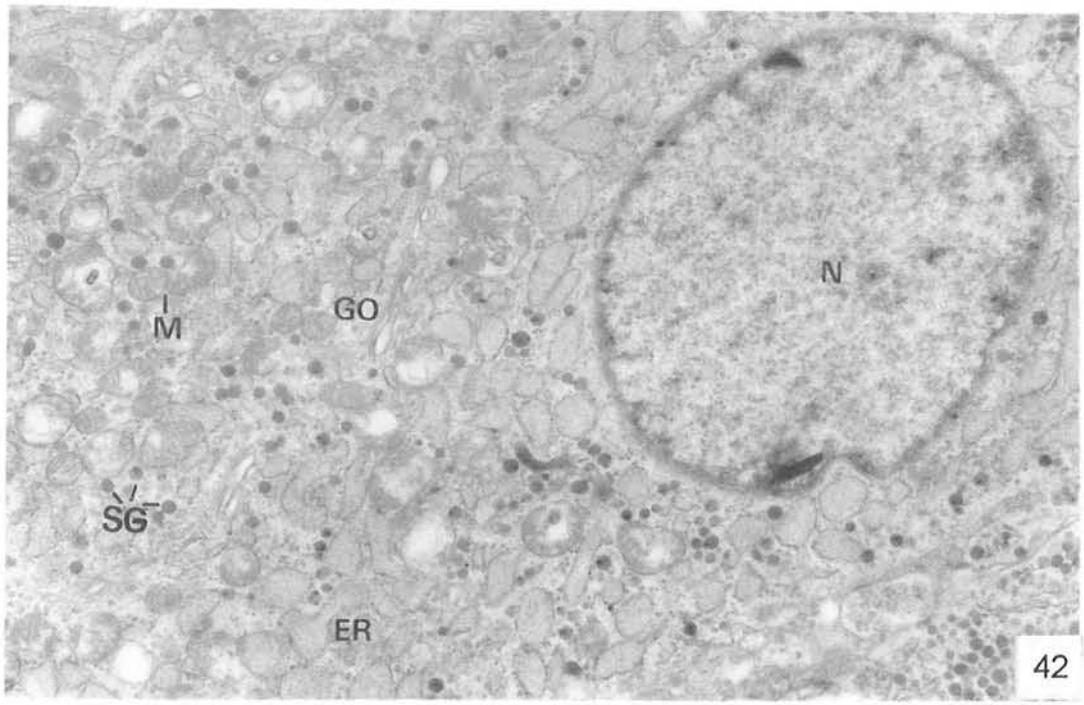


Fig. 41. Electron micrograph of a gonadotroph from 20 days old prepubertal rat, treated with LHRH. Regimen of LHRH treatment same as described under legend to Fig. 37. The gonadotroph has homogeneous cytoplasm. The endoplasmic reticulum (ER) is dilated. The nuclear membrane is regular, the secretory granules (SG) are sparsely distributed. x 18000

Fig. 42. Electron micrograph of a gonadotroph from 30 days old prepubertal rat, treated with LHRH. Regimen of LHRH treatment same as described under legend to Fig. 37. Appearance of vesicles are clearly seen following LHRH treatment. The nucleus is slightly indented. The granules are sparsed compare with gonadotrophs in Figs. 37, 38. x 10800



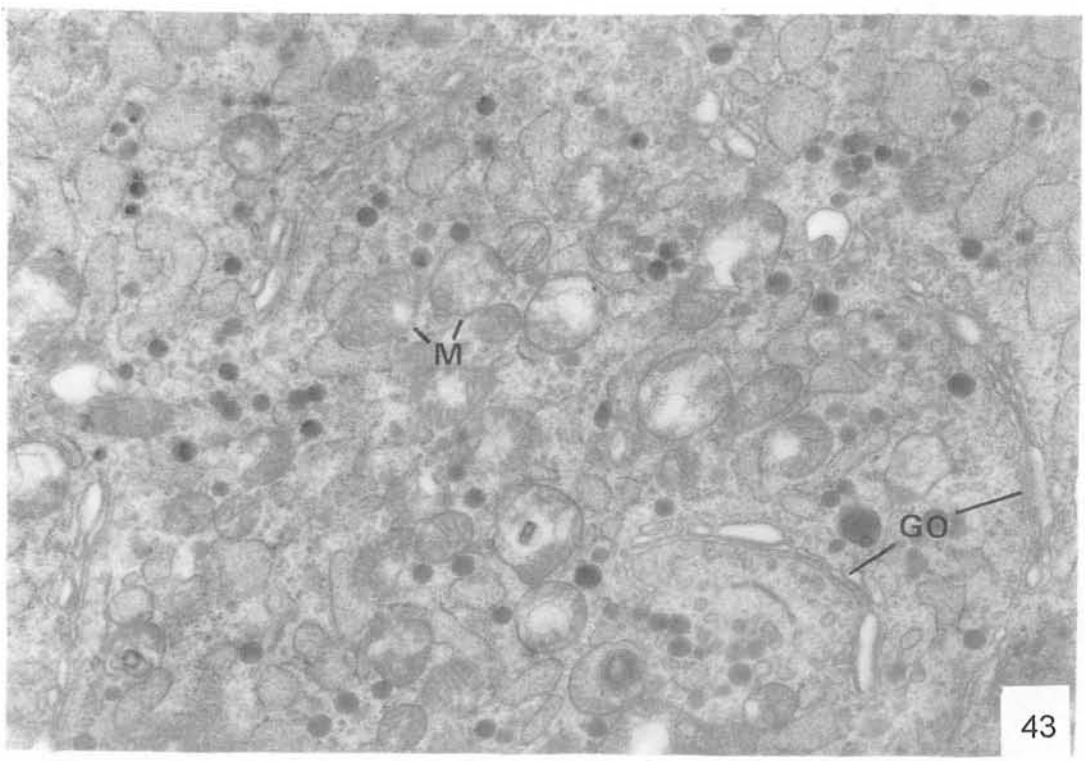
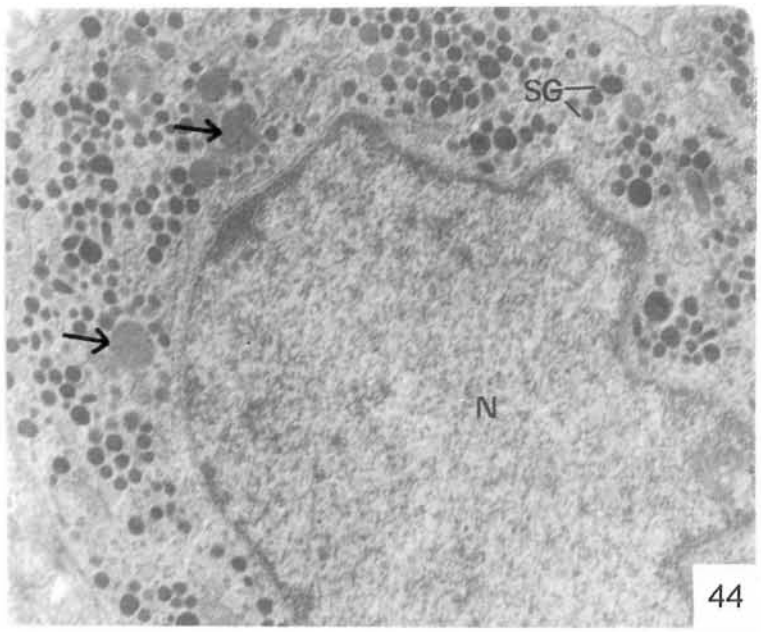
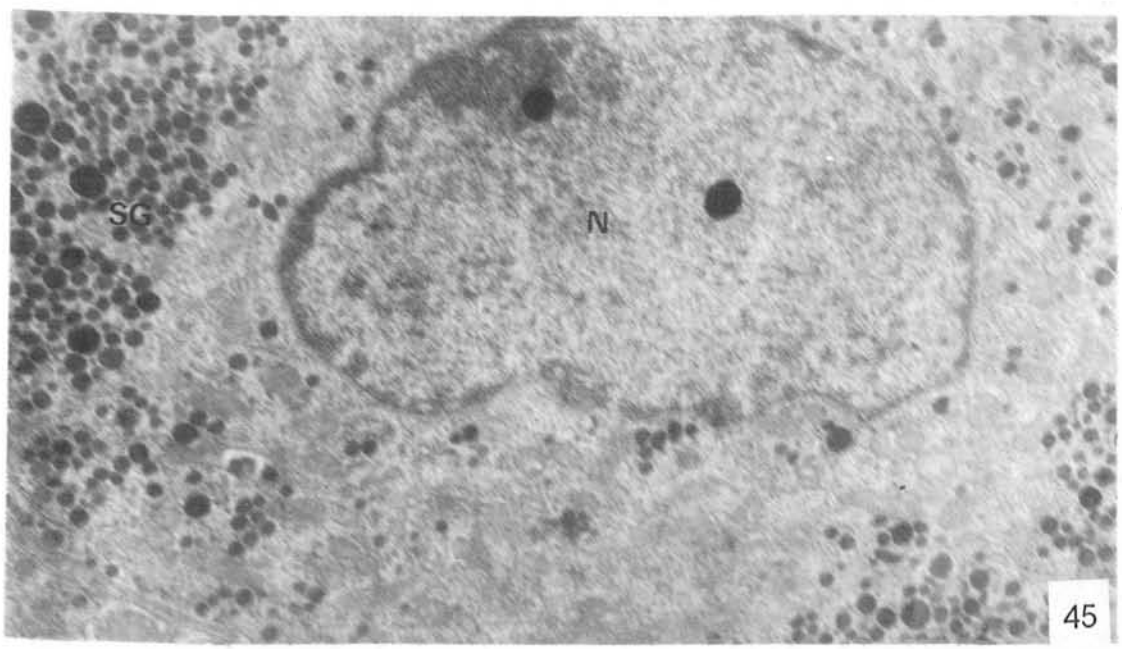
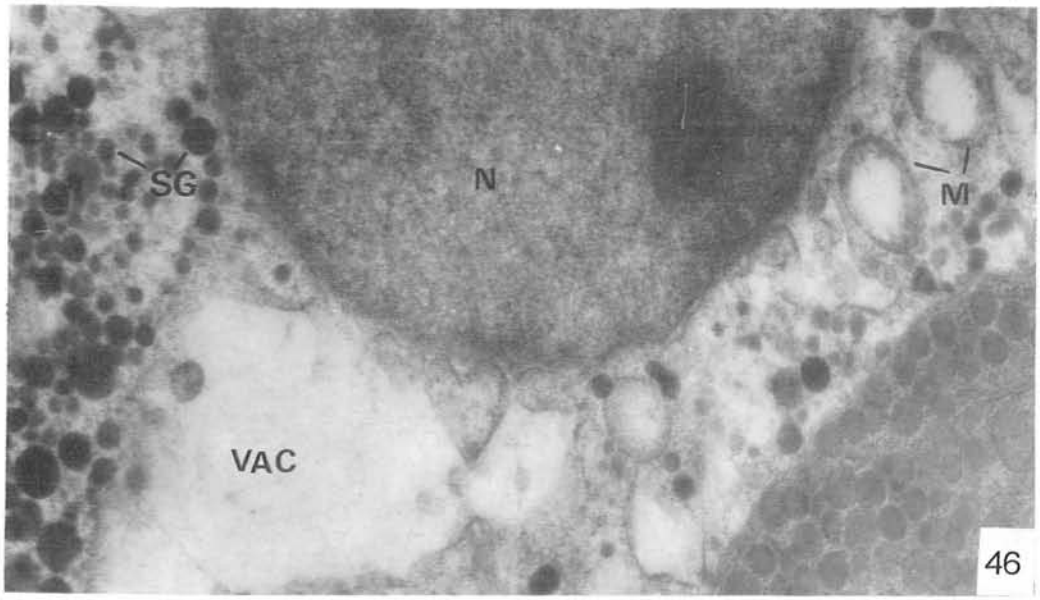


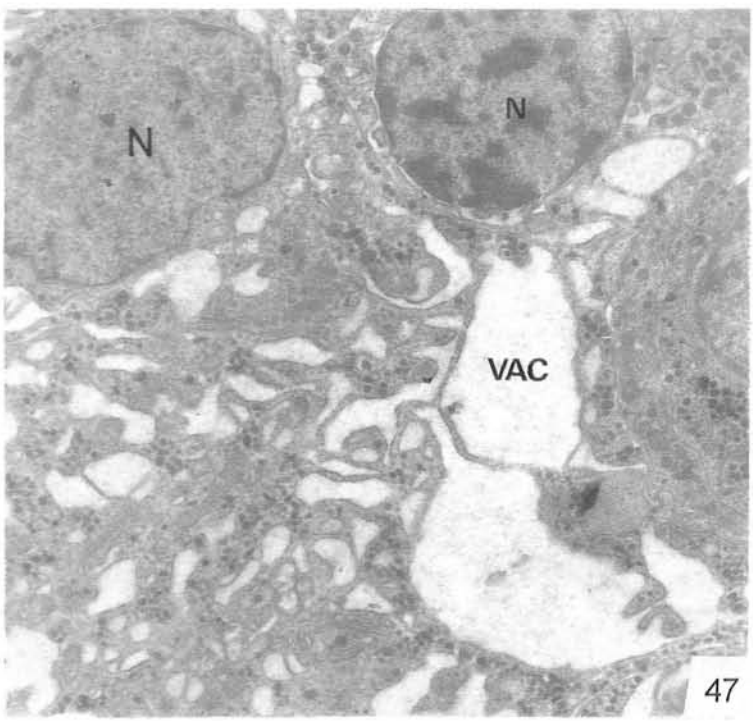
Fig. 43. Electron micrograph of a gonadotroph from 30 days old prepubertal rat, treated with LHRH. Regimen of LHRH treatment same as described under legend to Fig. 37. A prominent Golgi (GO) is clearly visible. The extent of vesiculation is same as in Fig. 42. This may be compared with 30 days castrated animal (Figs. 28,29). x 18000



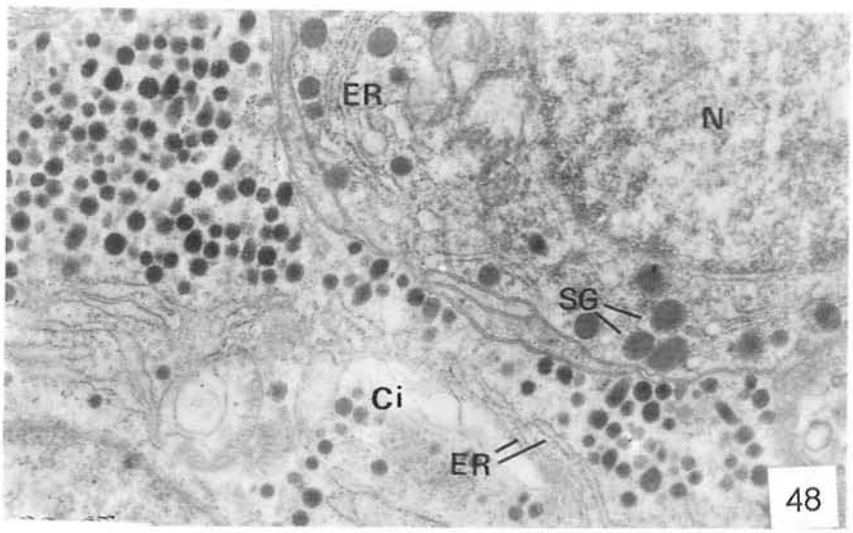


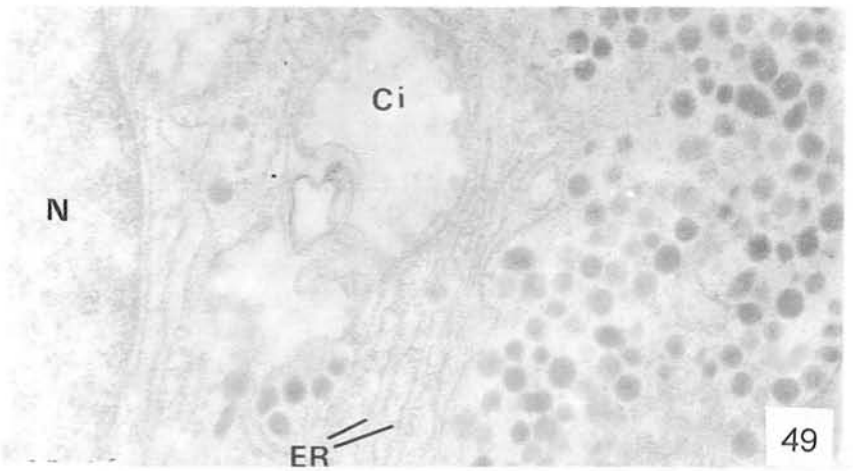
Figs. 44,45. Electron micrographs of gonadotrophs from 30 days old prepube with LHRH. Regimen of LHRH treatment same as described under legend changes in nuclear membrane as expected in a gonadotroph of cyclic castrat seen. The nuclear membrane shows notable indentations. The gonadotroph state of a "castrate cell" though the cytoplasm is not vesiculated. Large d treated with LHRH. Fig. 4





Figs. 46,47. Electron micrographs of gonadotrophs fr[illegible] 30 days old prepubertal rat, treated with LHRH. Regime[illegible] of LH treatment same as described under legend to Fig. [illegible] The cells show high degree of vacuolation (VAC), and [illegible]ens in [illegible] shows slight indentati[illegible]clear m[illegible]br[illegible]
Fig. 47 with x 7[illegible]





Figs. 48,49. Electron micrographs of gonadotrophs from 60 days old cyclic rat, treated with LHRH. Regimen of treatment same as described under legend to Fig. 37. Endoplasmic reticulum (ER) with cisternae (Ci) is clearly seen at the vicinity of nucleus (N). Fig. 48 with x 14400, Fig. 49 with x 27000

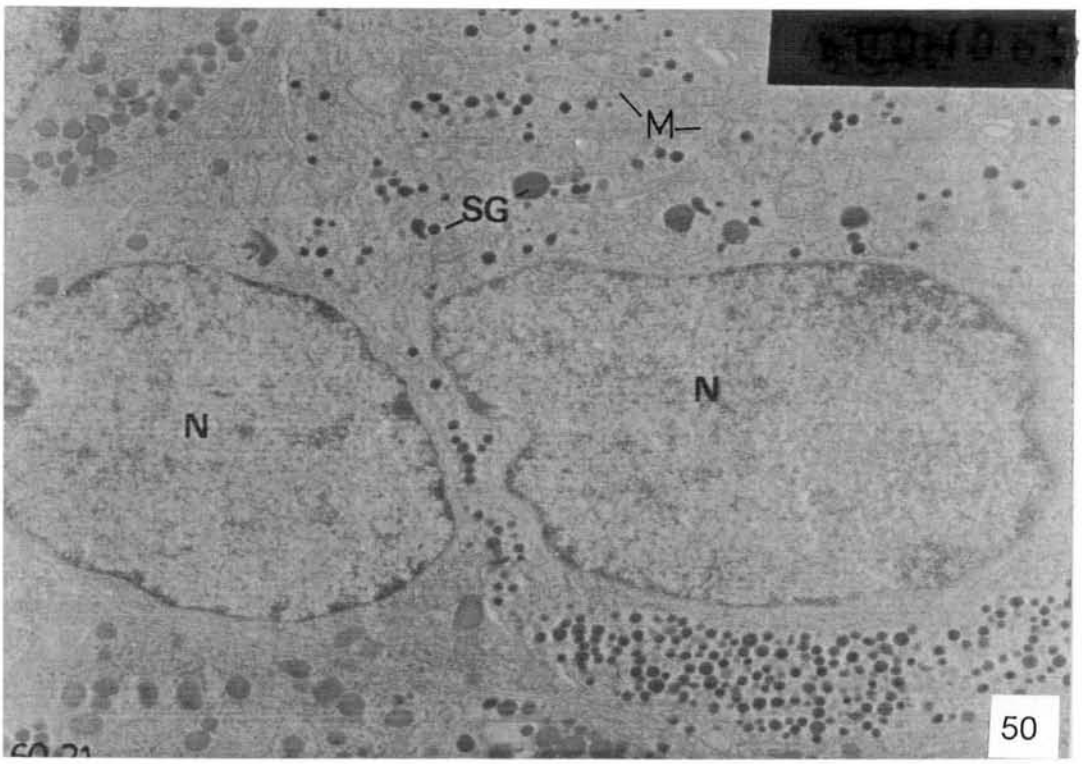


Fig. 50. Electron micrograph of gonadotrophs from 60 days old cyclic rat, treated with LHRH. Regimen of treatment same as described under legend to Fig. 37. Sparsely granulated cell, with slightly indented nucleus (N) pushed to one side the cell. Endoplasmic reticulum (ER), few electron-lucent mitochondria (M) can be clearly seen. x 10800

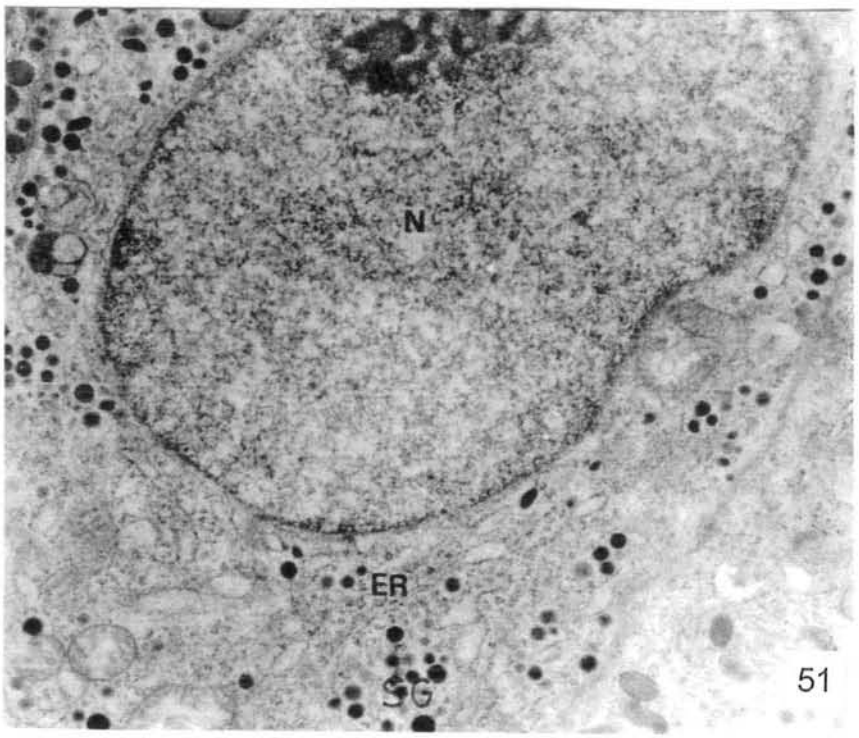


Fig. 51. Electron micrograph of a gonadotroph from 60 days old cyclic rat, treated with LHRH. Regimen of treatment same as described under legend to Fig. 37. An slightly indented nucleus (N) and secretory granules (SG) are shown. The dilated granular endoplasmic reticulum (ER) is scattered throughout the cytoplasm. x 18000

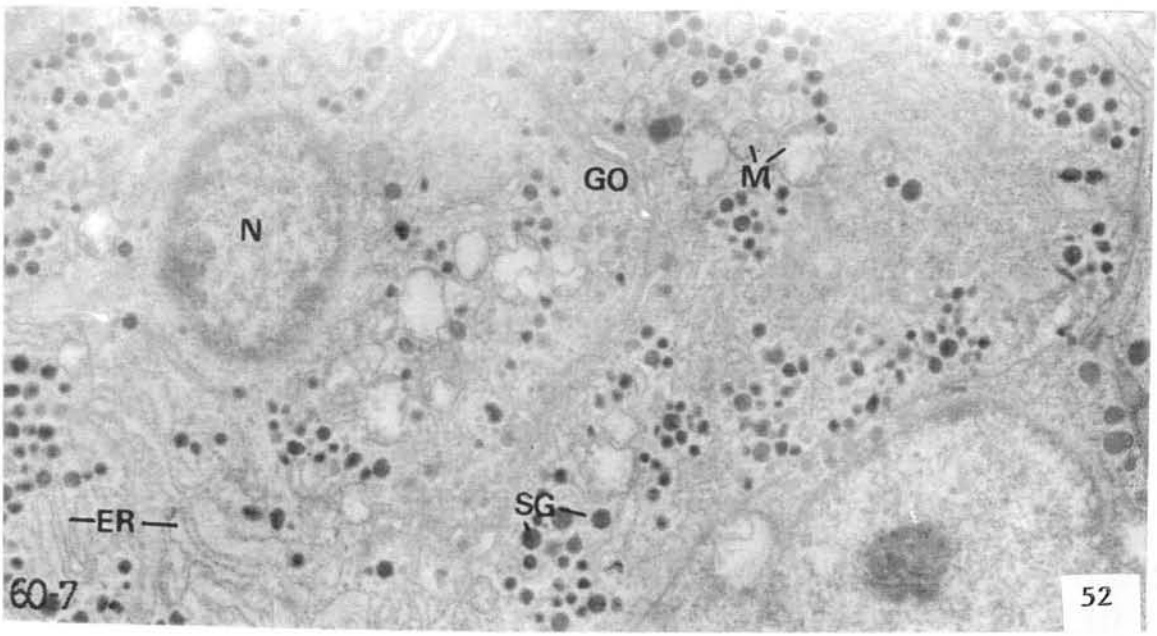


Fig. 52. Electron micrograph of a gonadotroph from 60 days old cyclic rat, treated with LHRH. Regimen of treatment same as described under legend to Fig. 37. The cell is large with small and oval nucleus (N). Dilated endoplasmic reticulum (ER) is scattered, mitochondria (M) is swollen. The extensive Golgi complex (GO) and secretory granules (SG) are shown. x 9000

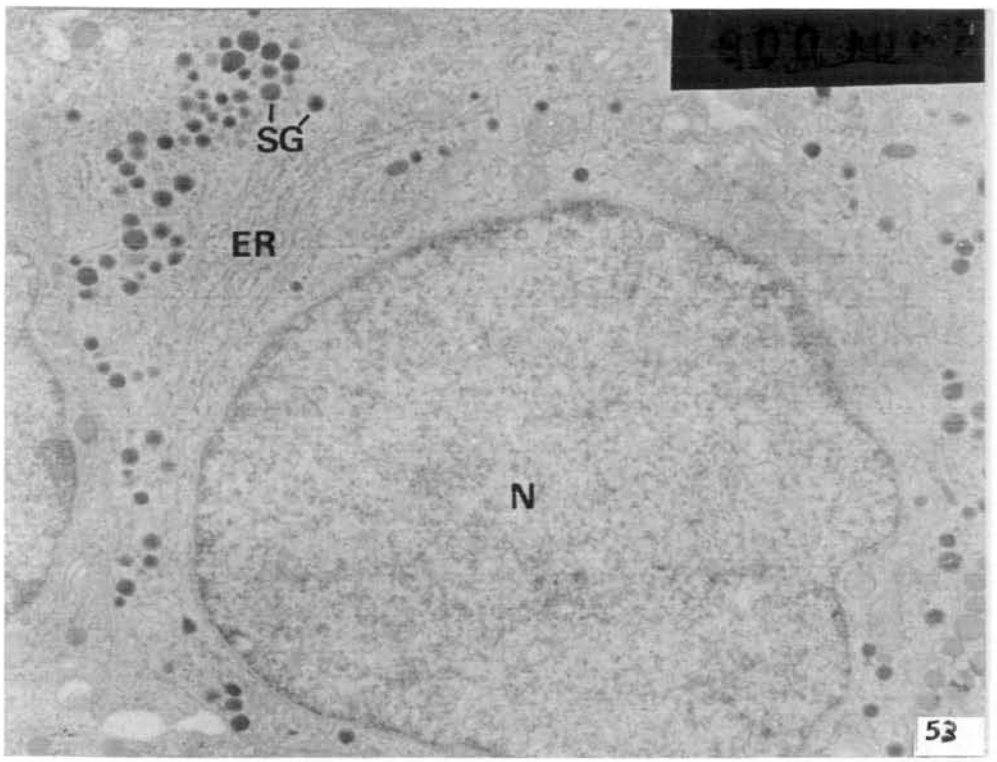


Fig. 53. Electron micrograph of a gonadotroph from 60 days old cyclic rat, treated with LHRH. Regimen of treatment same as described under legend to Fig. 37. Endoplasmic reticulum (ER) is parallel to nuclear membrane. Cell is sparsely granulated, mitochondria (M) can be seen around the indented nucleus (N). x 14400

4. EFFECT OF LHRH ON OVARIECTOMIZED RATS

Thirty days old rats

In this set of experiments changes in gonadotrophs of 30 days old ovariectomized rats were compared with 30 day old intact animals treated with LHRH. Major features of these changes are compared in Table 10, and shown in Fig.54.

Comparison of the data of Table 10 indicates some increase in granule size as well as granule population. However, most pronounced changes are seen in the shape of the nucleus and indentation of the nuclear membrane. In ovariectomized animals, the nuclei are round and,in all the cells observed,there was no initiation of the indentation of nuclear membrane which is so obvious in 30 day old intact rats treated with LHRH (compare Fig. 56 with Figs. 44, 45). The number of mitochondria is reduced. They are swollen and contain discontinous cristae (Fig. 58). The endoplasmic reticulum and the Golgi in two cases are almost at the same stage of development, both indicative of identical synthetic and secretory activity. The vesiculated cells are fewer in number in ovariectomized rats, as compared to intact animals treated with LHRH. The granules in both cases donot show polar aggregation, which are randomly distributed in the vesiculated cytoplasm.

Sixty day old rats.

Changes in 60 day old ovariectomized treated with LHRH are compared with those of 60 day old ovariectomized rats and intact rats. The changes are recorded in Table 11, Fig. 55. Marked differences are seen between the gonadotrophs of LHRH treated ovariectomized and intact LHRH treated animals in almost all the parameters studied. In the ovariectomized LHRH treated animals compared with 60 day intact-treated animals there is some increase in cell size and nuclear size of gonadotrophs. There is significant decrease in granule size and significant increase in granule population (granule size, 1850±40 A° , 1426±52 A°, granule population/cell, 174±30, 329±20). Endoplasmic reticulum is prominent in both cases but in ovariectomized animals large cisternae are also prominent. Indentation of the nuclear membrane is seen in both cases. However cells with oval and regular nuclear membrane predominate in ovariectomized animals. Cells with vesiculated cytoplasm are abundant in the ovariectomized animals. In intact animals, whereas vesiculated cells are present, cells with homogeneous cytoplasm predominate. In the ovariectomized animals not treated with LHRH, on the other hand, the granules predominate and there are significant changes in granule size and population. (Intact with LHRH: 1850±40 A°, 174±30/cell, ovariectomized without LHRH 2079±70 A°, 345±20/cell, ovariectomized with LHRH 1426±52 A°,329±20/cell). Also, vesiculated and homogeneous cells in ovariectomized animals without LHRH are dis-

Table 10

Comparative data on gonadotrophs of 30 day old rats following LHRH treatment in Intact and castrated animals

Parameter	Intact with LHRH	Castrated without LHRH	Castrated with LHRH
Granule size(A^{o})	1762±50	1800±100	1930±30
Granule population/ cell (No.)	233±20	313±10	262±30
Cell size (μm)	12.67±0.6	13.75±0.5	14±0.5
Cell shape	Polygonal or Irregular	Polygonal or Irregular	Polygonal or Irregular
Nuclear size (μm)	5.5±0.5	6.5±0.6	6.75±0.3
Nuclear shape	Polygonal	Polygonal	Round
Endoplasmic reticulum (ER)	Prominent	Prominent, many vesicles	Prominent
Golgi complex	prominent	Prominent	Prominent
Mitochondria	Round or Oval	Oval or Rod	Oval or Rod
Cytoplasm	Homogeneous	Homogeneous	Homogeneous or vesiculated

Table 11

Comparative data on gonadotrophs of 60 day old rats following LHRH treatment in intact and castrated animals

Parameter	Intact with LHRH	Castrated without LHRH	Castrated with LHRH
Granule size(A^{o})	1850 ±40	2079±70	1426±52
Granule population/ cell (No.)	174±30	345±20	329±20
Cell size (μm)	14.5±0.5	15.5±0.8	16.4±0.4
Cell shape	Polygonal or Irrigular	Polygonal or Irrigular	Polygonal or Irrigular
Nuclear size (μm)	6.5±0.5	7±0.3	7.5±0.5
Nuclear shape	Irrigular or Oval	Irrigular or Oval	Irrigular or Oval
Endoplasmic reticulum (ER)	Prominent	Prominent with vesicles	Prominent with vesicles
Golgi complex	Prominent	Prominent	Prominent
Mitochondria	Many, Round or Oval	Many, Oval or Rod	Few, Oval or Rod
Cytoplasm	Homogeneous or Vesiculated	Vesiculated or Homogeneous	Vesiculated or Homogeneous

tributed in the same ratios in ovariectomized animal with LHRH (Figs. 60-66).

Discussion

Farquhar and Rinehart (1954b) were the first in publishing an indepth study of anterior pituitary gland cells at the ultrastructural level in ovariectomized rats. They reported the appearance of two types of gonadotrophs: (a) those with vesiculated cytoplasm appearing early in ovariectomy, and (b) those with advanced degree of vesiculation which appeared 35 days after ovariectomy. The latter has elongated or folded nuclei and bizarre cytoplasmic formation resembling a filigree. On the basis of these observations, it was concluded that the former cells secreted FSH, and the latter cells secreted LH. Some of these observations have been confirmed by recent workers, for example Garner and Blake (1981), who combined immunocytochemical staining with ultrastructural studies. We have used a different approach to extend these studies. Our use of immature ovariectomized animals treated with LHRH Provided us with a means of detecting the appearance of various types of cells in non cyclic female rats. This aspect has been discussed in pervious sections. However, using a cyclic female in metestrous, ovariectomizing it and treating it with LHRH provided yet another means of comparing the combined effect of ovariectomy-LHRH treatment on mature and immature animals. Interestingly enough, we have observed only two

types of cells in 60 day ovariectomized-LHRH treated animals; the vacuolated and homogeneous cells with some indentation of nuclei in vacuolated cells. We were unable to detect signet ring cells in these animals. The absence of signet ring cells in 60 day old ovarictomized-LHRH treated animals and their presence in 30 day LHRH treated ovariectomized animals raises the issue of differential response of immature and mature animals to simultaneous manipulation of the animals with ovariectomy and LHRH treatment. It appears, as we have indicated earlier, that day thirty is a critical stage in prepubertal development and its response to LHRH alone, ovariectomy alone and ovariectomy combined with LHRH is likely to be more intense. LHRH alone or ovariectomy alone fails to evoke development of signet ring cells. It is only because of the combined effect of ovariectomy and LHRH that "signet ring" cells appear in 30 day old animals ovariectomized for 30 day and injected with LHRH. On the basis of this information we suggest that the appearance of signet ring cells is directly related to LHRH treatment, although these animals had reached the age of 60 day , including 30 day of ovariectomy. This still does not explain as to why in 60 day old animals LHRH does not produce the same response. It may also be noted that in 60 day old animals ovariectomy did not reveal signet ring cells among gonadotrophs.

Surprisingly enough, day 60, ovariectomized and LHRH treated animals lacked "signet ring" cells and cells with

indented nuclei so prominently observed in day 60 animals in which only ovaries were removed.There wasamarked decrease in granules size though the extent of development of Golgi was identical. It has already been discussed in section 3 that LHRH alone does not produce as acute a change in gonadotrophs as ovariectomy in 60 day old animals. It is difficult to develop a convincing physiological evidence to explain the absence of "signet ring" cells and cells with indented nuclei in ovariectomized-LHRH treated animals. Yet, it appears, as one would expect, increased release of LHRH from hypothalamus in ovariectomized animals promotes changes in gonadotrophs. To this extent the changes observed are comparable with those reported by others (Rennels et al., 1971; Costoff, 1973; Garner & Blake, 1981; Moriarty, 1976, 1982). However, additional exogenous source of LHRH reverses some of these changes including the decrease in granule size. The complexity of these changes becomes more evident when LHRH treatment produces opposite effects in 30 day ovariectomized animals. For instance, in 30 day ovariectomized animals neither "signet ring" cells nor cells with indented nuclei are present. But these cells appear when the same ovariectomized animals are treated with LHRH; the granule size increases and their number decreases. Whereas these changes (30 day old animals) are in agreement with the putative role of LHRH, the differences we have observed may be interpreted in terms of the functional age of hypothalamus in 30 day and 60 day old animals. In the former group the effect may be due to exogenous LHRH alone

while in the latter group, it may due to additive effect of exogenous and endogenous LHRH, assuming of course that the hypothalamus of immature animals remains desensitized despite the absence of ovary. Even so, the mechanism involved remains to be fully explained.

FIGURES

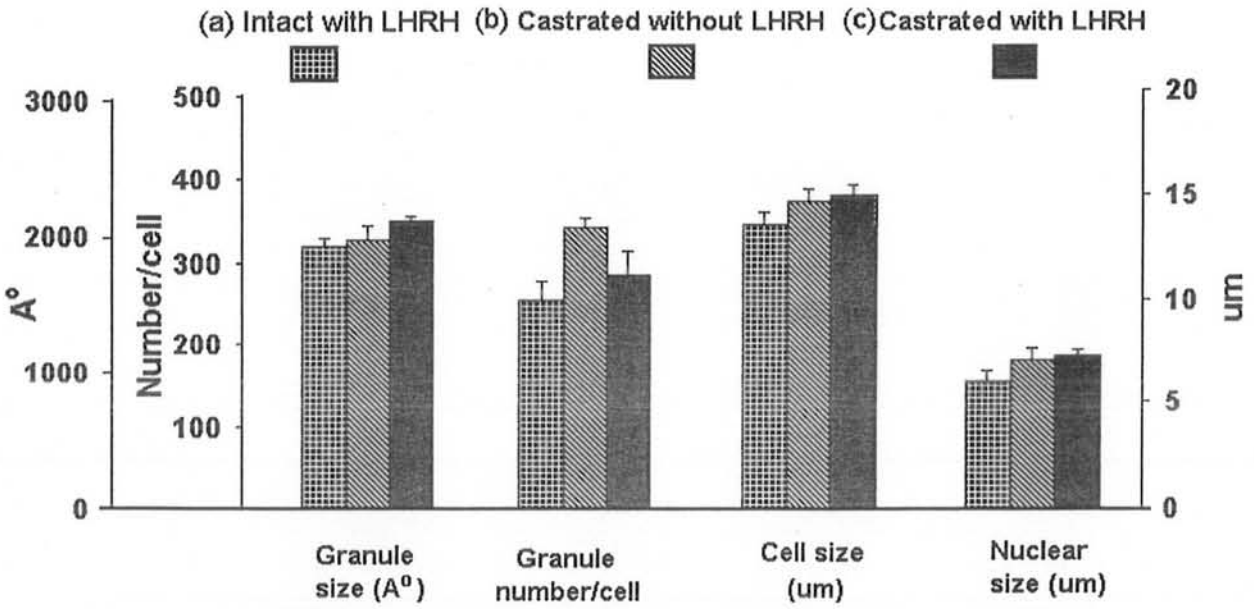


Fig. 54. Comparison of various parameters: granule size (A^0), granule number per cell, cell size (um) and nuclear size (um). (a) Intact with LHRH, (b) castrated without LHRH, and (c) castrated with LHRH. Intact animals of 30 days old were injected with LHRH 1ug/ day for 5 days, the animals were killed on day 6 and pituitary was processed for microscopic studies. Castrated animals without LHRH treatment were examined 30 days after ovariectomy of 30 days old animals. In castrated and LHRH treated rats, castration was carried out in the same way except that the animals were injected LHRH 1ug/day for 5 days and examined on day 6.

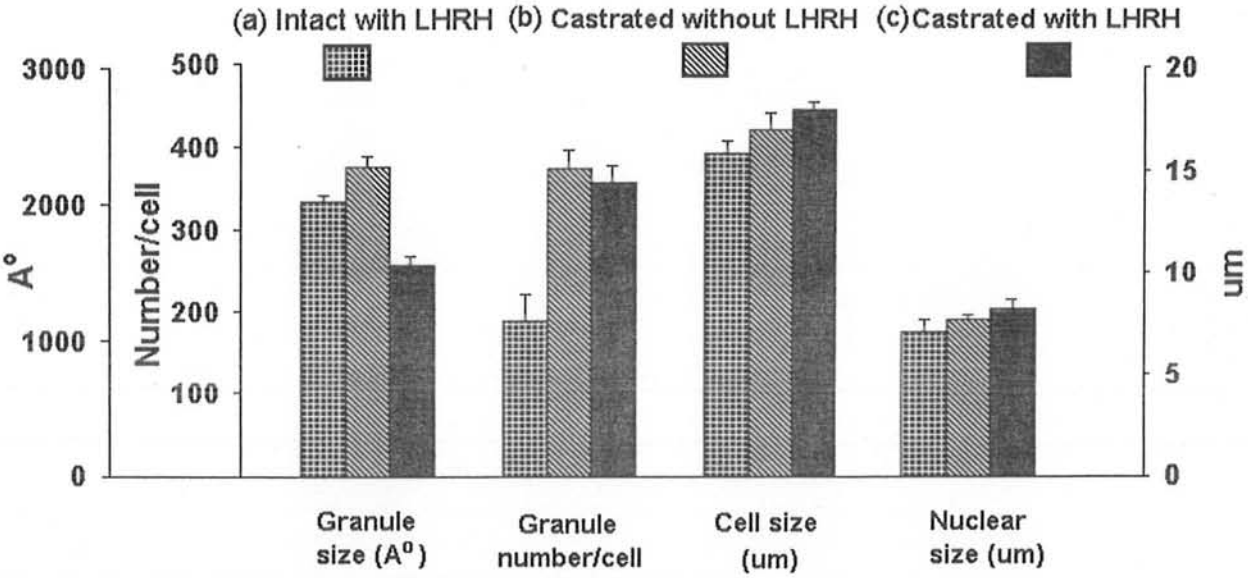


Fig. 55. Comparison of various parameters: granule size (A^{o}), granule number per cell, cell size (um) and nuclear size (um). (a) Intact with LHRH, (b) castrated without LHRH and (c) castrated with LHRH. Intact animals of 60 days old were injected with LHRH 1ug/ day for 5 days, the animals were killed on day 6 and pituitary was processed for microscopic studies. Castrated animals without LHRH treatment were examined 30 days after ovariectomy of 60 days old animals. In castrated and LHRH treated rats, castration was carried out in the same way except that the animals were injected LHRH 1ug/day for 5 days and examined on day 6.

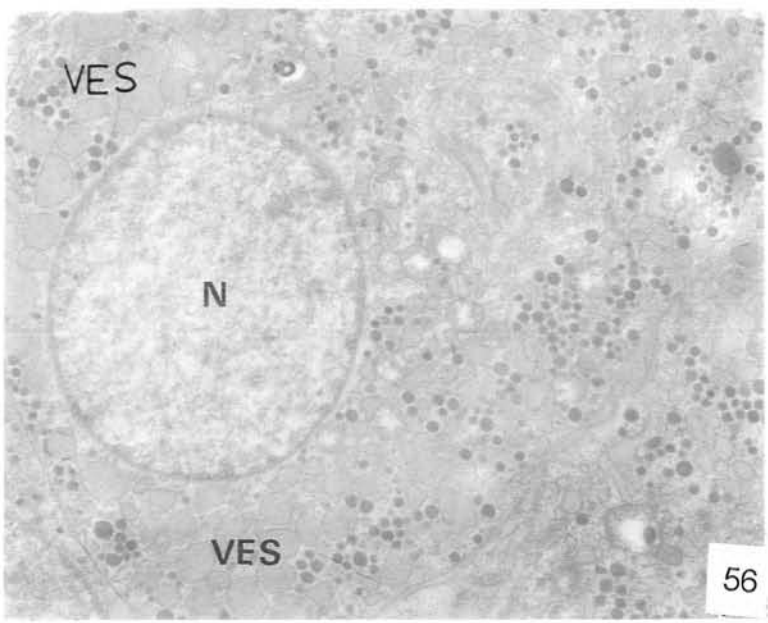
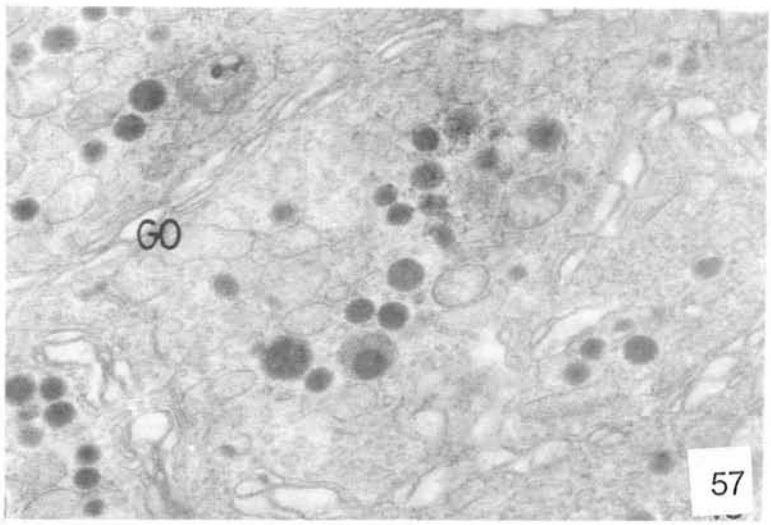
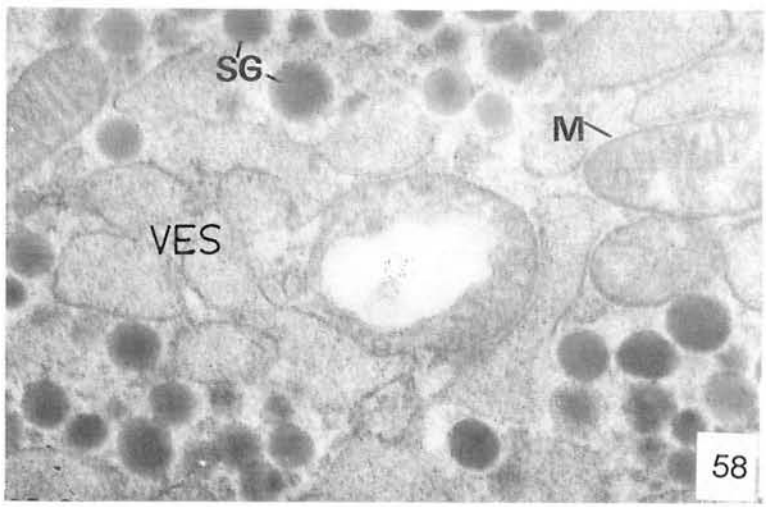


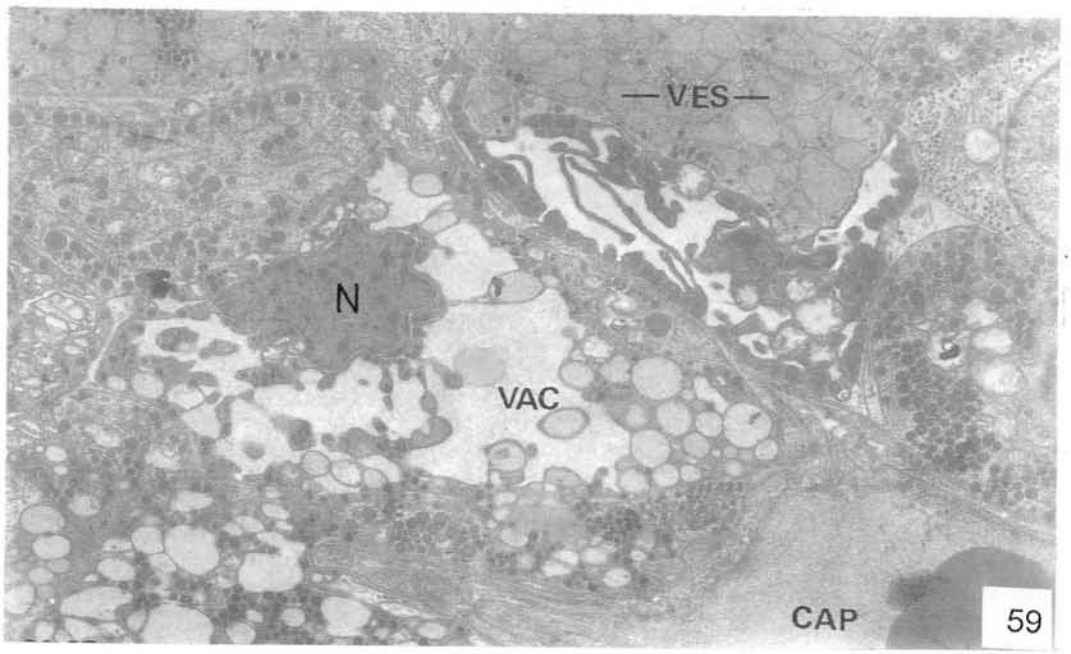
Fig. 56. Electron micrograph of a gonadotroph from 30 days old rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). The cytoplasm is highly vesiculated as in 30 days castrated and LHRH treated animals Figs. 28, 42 and 44. But in this cell there is no indentation of the nuclear membrane. x 9000





Figs. 57,58. Electron micrographs of gonadotrophs from 30 days old rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). A portion of two gonadotrophs is enlarged to show the development of Golgi complex (GO) and vesicles (VEs), mitochondria (M) with discontinued cristae in Fig. 58. Fig. 57 with x 14400 and Fig. 58 with x 36000

Fig. 59. Electron micrograph of a gonadotroph from 30 days old rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). The cytoplasm is highly vacuolated and the nuclear membrane shows multiple indentation. The nucleus (N) is pushed to one side and indentation is more intense as compared to 30 days old castrated or LHRH treated rats, propably a signet ring cell. (compare this figure with Figs. 28 and 44,45). x 5400



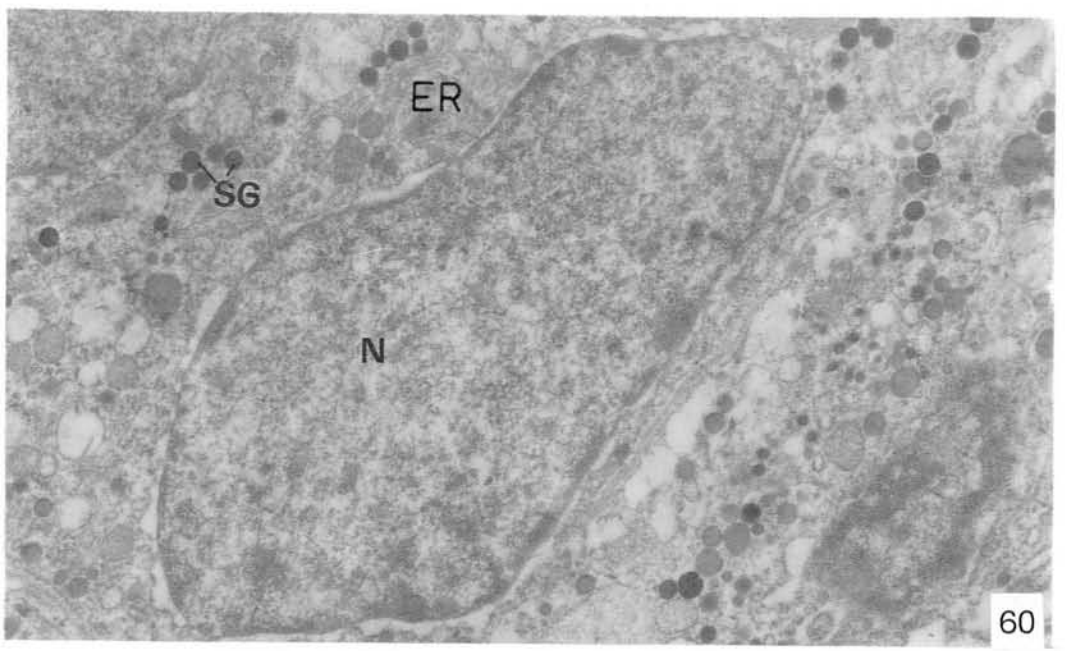
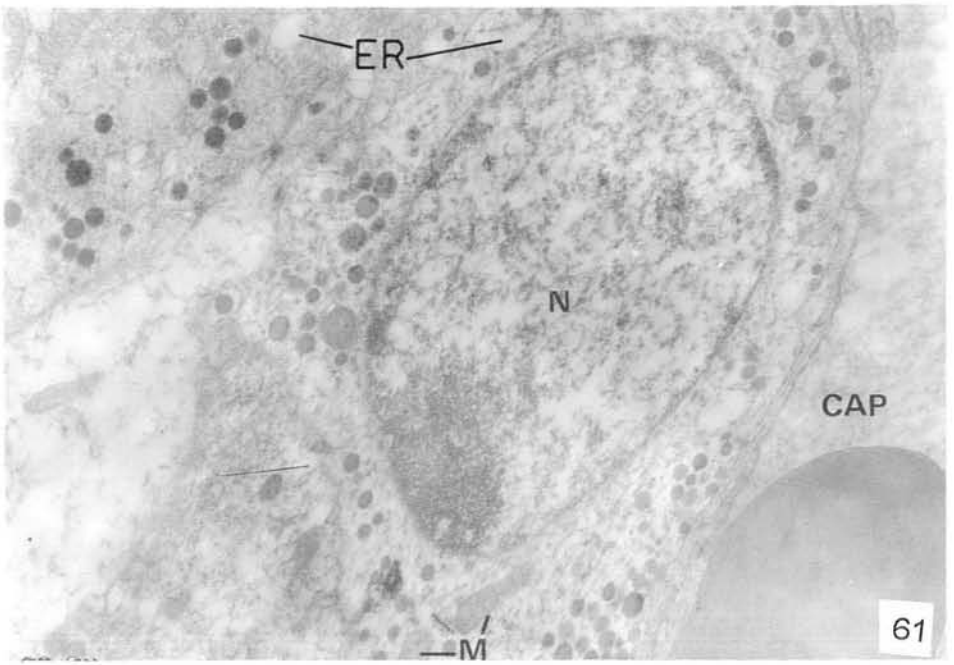


Fig. 60. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). A long nucleus (N) with irregular nuclear membrane occupies most of the cytoplasm. Secretory granules (SG) are sparsely distributed. Dilated endoplasmic reticulum (ER) are shown. x 18000

Fig. 61. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). Small cell with an oval nucleus (N) in the centre of the cell. Cell is sparsely granulated. Some large bodies (→), dilated endoplasmic reticulum (ER) and mitochondria (M) can be seen. x 18000



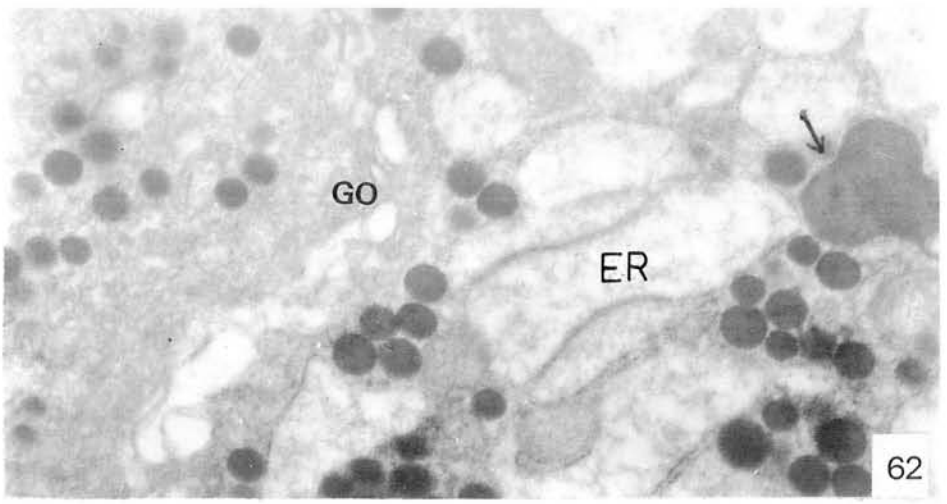
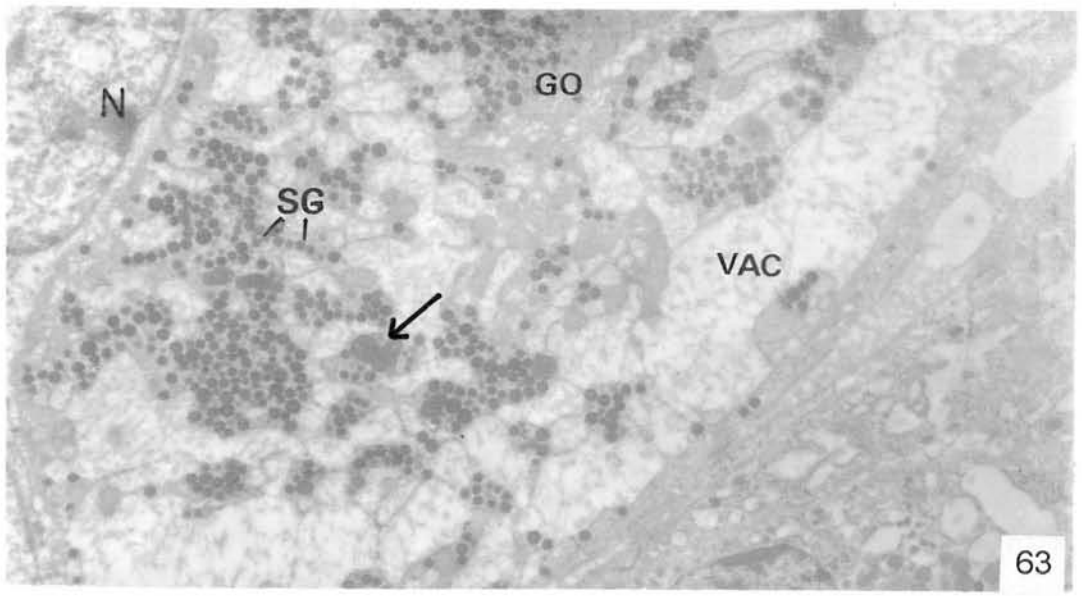


Fig. 62. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). Enlarged portion of gonadotroph from Fig. 63. An dilated Golgi area (GO), dilated endoplasmic reticulum (ER) and lysosome (⇒) are shown. x 27000

Fig. 63. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). This cell is highly vacuolated (VAC), contains secretory granules (SG) and several dense bodies (—>) probably lysosomes. Nucleus (N) is pushed to one side of the cell and Golgi complex (GO) is clearly shown. x 7200



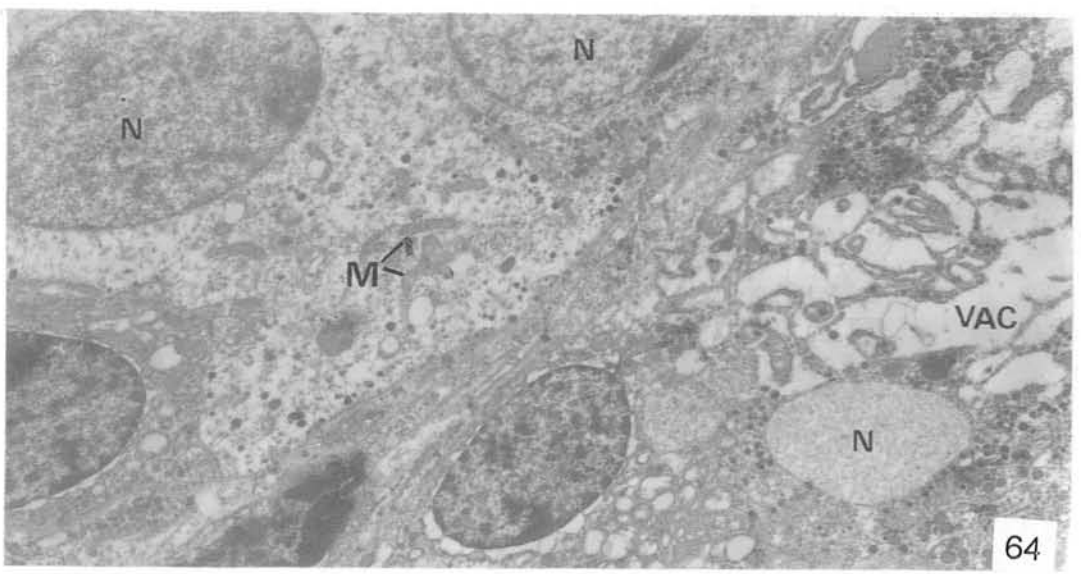
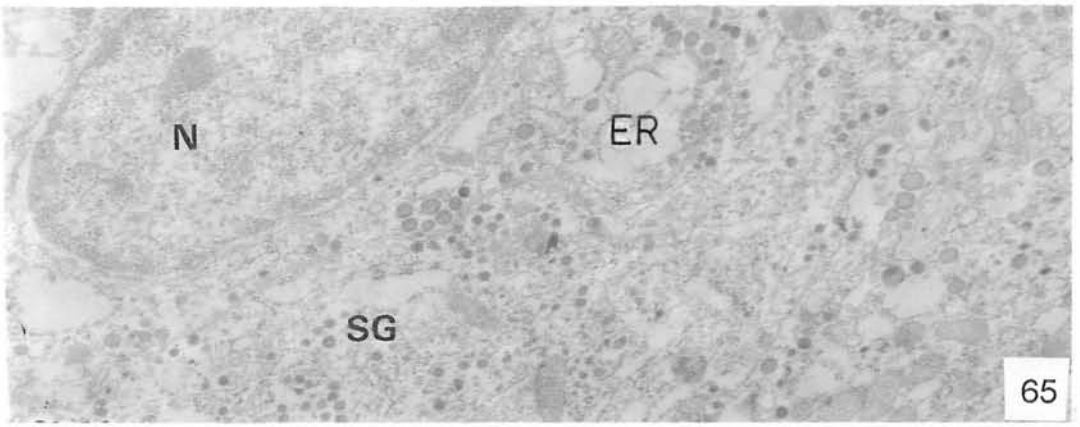


Fig. 64. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). A highly vacuolated (VAC) gonadotroph (right), secretory granules (SG) are aggregated and distributed in between vacuoles. Small cell at extreme lower right is a homogeneous gonadotroph with eccentric nucleus (N). A thyrotroph at upper left with numerous long, rod shaped mitochondria (M) and oval nucleus (N) can be seen. x 7200

Fig. 65. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). Sparsely granulated cell, dilated endoplasmic reticulum (ER), mitochondria (M) with small cristae are shown. x 18000



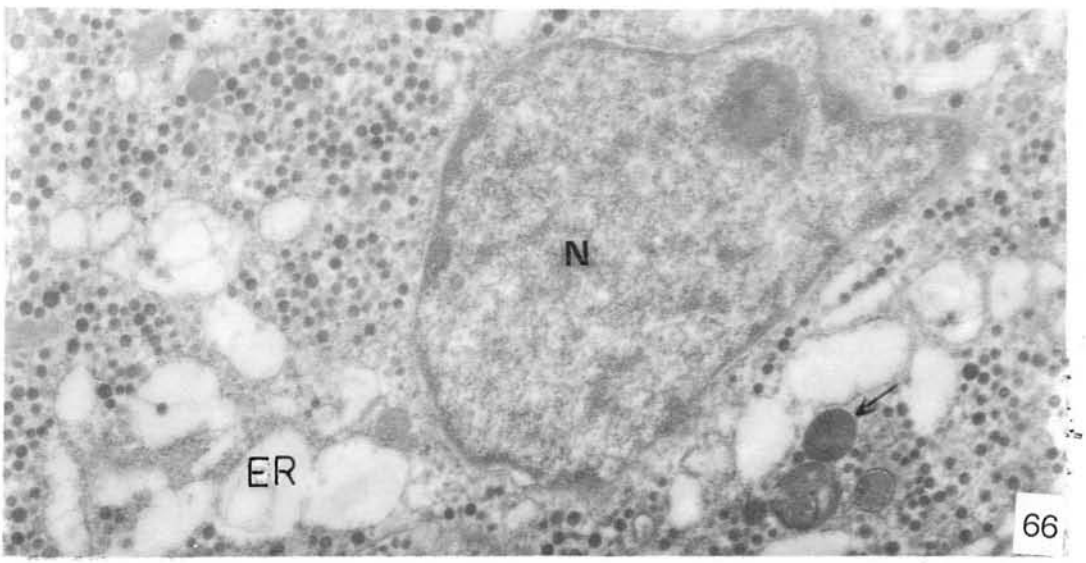


Fig. 66. Electron micrograph of a gonadotroph from 60 days old cyclic rat, castrated for 30 days and then treated with LHRH (1 ug/day for 5 days). Number of secretory granules (SG) are increased. Nuclear membrane is indented, several vacuolar endoplasmic reticulum (ER) and large dense bodies (—>) are shown. x 18000

General Discussion

This general discussion covers those areas which have received only brief consideration in the preceding sections. Further, during the course of this study and as indicated by results, a number of core issues remained unattended. These warrant fuller attention so as to bring into focus the theme of the thesis. It includes: (a) whether there is one type of gonadotroph producing both FSH and LH, or, there are two types of gonadotrophs independently synthesizing FSH and LH; (b) whether in the immature female rat LHRH alone can induce "castration like" effect in gonadotrophs (pseudo-castration) and (c) whether gonadectomy of immature and mature rats results in the same sequel of events as described in literature (changes in "castration cell" morphology). The following paragraphs present a more comprehensive dispensation on these aspects.

Gonadotrophs

Pituitary gonadotrophs have been extensively studied using various methods which include, light microscopy, immunocytochemistry (using antibodies to hormone specific β subunit of FSH and LH) and electron microscopy. In general, the earlier studies (Farquhar & Rinehart, 1954a,b; Farquhar, 1955; Barnes, 1962; Kurosumi & Oota, 1966; Nakane, 1970; Phifer et al., 1972) postulated a single gonadotroph which synthesized both FSH and LH; though the FSH and LH granules were suspected to differ in size and morphology (Kurosumi &

Oota, 1968; Costoff, 1973; Moriarty, 1976). However, with the availability of antibody to β subunits of FSH and LH, more specific identification of FSH and LH granules have been made. Yet, the data at best are conflicting. The coexistence of LH and FSH granules in one type of gonadotroph has been demonstrated (Nakane, 1970, 1975). This has been confirmed by many investigators (Tougard et al., 1973, 1980; Phifer et al., 1973; Tixier-Vidal et al., 1975; Herbert 1975; Moriarty, 1976; Daucheux, 1978; Batten & Hopkins, 1978; Yoshimura et al., 1981). Interestingly enough, ACTH cells have also been shown positive for FSH and LH using immunocytochemistry (Moriarty et al., 1976, 1977, 1980, 1982). Earlier, Moriarty (1976) was able to demonstrate three types of cells. TypeI or typeII stain for both gonadotrophic hormones whereas others (usually type II or type III) contain only FSH or LH. Furthermore, it has been suggested that LH cells are not a single type, but include a wide range of subtypes. In essence, these studies do demonstrate that gonadotrophs are ovoid in shape but some cell types while stain for FSH using anti-FSHβ are stellate in shape resembling ACTH cell in morphology. Differences in granules staining with LHβ have also been noted.

In the last decade careful ultrastructural studies based on morphological criteria have identified two types of gonadotrophs, (a)FSH gonadotroph (FSHG) and (b)LH gonadotroph (LHG). The FSHG is of more frequent occurrence in the male compared to female. The cells are round usually found

on capillaries. The granules are small, vary in density and are distributed throughout the cell. The large amorphous bodies which may not always be present in FSHG, have however been observed by several workers (Farquhar & Rinehart, 1954b; Cardel, 1961; Kurosumi & Oota, 1968; Costoff, 1973). A number of Golgi areas encircle the nucleus in FSHG and represent a network of dilated sacs and small vesicles. The mitochondria are rod like, the ER is not well developed but in hypertrophied cells it is more extensive and has dilated sacs.

LHG are more common in the female rats than in male rats (female: 20%; male 5-10%). The cells are larger than thyrotrophs and polygonal in shape. The nucleus is eccentric. The granules are electron dense compared to FSHG (granule size: 145mμ; range: 75-235mμ). The large amorphous bodies are absent. ER and Golgi complex are in an inactive state, rather inconspicuous and poorly developed. However, in the stimulated LHG the Golgi area is extensive. The mitochondria are round or rod like.

The studies reviewed in the preceding paragraphs, have attempted to provide an ultrastructural basis for identifying the two types of gonadotrophs (FSHG and LHG). Our studies however do not substantiate this view point. Firstly, it must be conceded that we have little to contest with immunological studies, since, much of the information we are providing is based on ultrastructural studies on various

stages of development: immature, prepubertal and pubertal. Thus, keeping aside the immunological data, which is at best conflicting (See vide-supra), and keeping in view only the touchstone of ultrastructural criteria described by Costoff (1973) for separating FSH gonadotrophs from LH gonadotrophs, we do not find compelling reasons to agree to the presence of two types of gonadotrophs in female rats. Our examination of gonadotrophs at all stages of development, from day 5 onwards, does not give any indication of differentiation of gonadotrophs into two distinct types. Such criteria as granule size, cell shape, Golgi complex, mitochondria, placement of nucleus and its shape have little to offer to be able to distinguish between FSH type and LH type; though the distinction of gonadotrophs from thyrotrophs and other cell types is abundantly obvious. We are also not inclined to agree (using morphological criteria) that LHG and FSHG do appear during cyclic and manipulative stages of the female rat since we failed to find evidence for the two gonadotroph types in prepubertal and pubertal ovariectomized or LHRH treated rats. Secondly, the immunological studies made with antibodies to hormone specific β subunit of two hormones, may be, by and large; suspect in view of the polyclonal nature of the antisera used and the possibility of cross contamination of the two antigens (FSH and LH). Perhaps, monoclonal antibodies against highly purified antigens may provide a better means of interpreting information.

Taken together, the current evidence and the information we have obtained from ultrastructural studies on gonadotrophs in prepubertal and pubertal rats in various physiological states do not justify a subclassification of gonadotrophs into FSHG and LHG as has been suggested by various workers.

Pseudocastration

The effect of ovariectomy on gonadotrophs in pubertal cycling female rats has provided substantial information about changes in the period following ovariectomy. The major known changes are directed towards hypertrophy of cells which results in the appearace of three types of cells: (a) those with vesicles and homogeneous cytoplasm; (b) those with vacuoles and heterogeneous cytoplasm, and (c) those with eccentric nuclei with indented nuclear membrane. The classical "signet ring" cells vesiculated gonadotrophs have also been observed during the estrous phase of normal cycling rat (Costoff, 1973, Garner & Blake, 1981). Variable changes in granule number and size have also been described. Those workers who consider FSH secreting cells and LH Secreting cells as independent entities (Garner & Blake, 1979, 1981) have attributed castration changes both to FSHG and LHG (Costoff, 1973; Moriarty, 1982). However, LHG seems to be more prone to castration effect than FSHG (Garner & Blake, 1981).

This be so, we have demonstrated for the first time, as described in previous sections (see sections 2 and 3) that castration effect in prepubertal rats can be produced either by removing the ovaries or by injecting LHRH in intact animals of the same age. The LHRH effect has been termed "Pseudocastration". In doing so we have made a few significant observations which advance our information about reproductive physiology of immature animals.

Firstly, on ultrastructural basis, both in ovariectomized and LHRH treated, aged 20 and 30 day , we have not be able to separate LHG and FSHG types. This is consistent with our observations in the pubertal rat as discussed else where in the thesis. Secondly, the day 30 animals appear to be in a state of critical response to ovariectomy as well as LHRH. Though the response to LHRH challenge is more pronounced compared to ovariectomy alone, LHRH under these conditions seems to affect pituitary gonadotrophs directly but enhanced release of LHRH from hypothalamus cannot be ruled out as a result of secondary feedback. This needs to be explored further by measuring the plasma and ovarian steroids during LHRH treatment. However the evidence is unequivocal, since 30 day LHRH treated animals present the same gonadotrophic picture as pubertal 60 day female animals castrated for thirty days. This comparison has been made in relevant sections.

Effect of LHRH on ovariectomized immature and mature rats

Interesting results were obtained when immature female rats (day 30) were ovariectomized for 30 days and then treated with 1μg LHRH (i.v.) for 5 days. Ovariectomy alone did cause hypertrophy of gonadotrophs but the extent of changes were not as acute as in animals treated with LHRH alone. This has been interpreted to mean that at this stage of development, the hypothalamo-hypophyseal-ovarian axis is as yet at an early stage of sensitization the direct effect of LHRH on Pituitary gonadotrophs can mimic the condition produced by ovariectomy of cycling animals. This interpretation was further strengthened when drastic changes, such as high vesiculation, indentation of the nuclear membrane and distribution of granules was observed in LHRH treated ovariectomized animals at 30 days of age. On the basis of this information it is possible to suggest that (a) removal of ovaries in day 30 animals has a limited "hypothalamic" effect in that the endogenous release of LHRH is only minimal, (b) that such animals, if provided with additional quanta of exogenous LHRH after 30 days of castration do exhibit changes in gonadotrophs which are similar to or as acute as those observed in 60 day old castrated animals, and (c) our findings also confirm that at this stage of development (30 day) the pituitary gonadotrophs are responsive to LHRH. It may be noted that animals less than 30 days of age do not respond to LHRH in the same manner as 30 day old intact animals or 30 day old ovariec-

tomized animals. Further, these findings throw light on the extent of maturity of hypothalamo- hypophyseal-ovarian axis in prepubertal animals. It is also interesting that ovariectomized metestrous rats show same changes in gonadotrophs (Castration cell, extensive vesiculation) as one would expect in cycling castrated animals. However, when castration of these animals is combined with LHRH, the extent of change is not as dramatic as in 60 day castraced cycling animals. Why this is so is not clear. Yet, it may be suggested that exogenous LHRH inhibits the process of change. The mechanism remains to be elucidated.

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