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A possible phagocytic role for folliculo-stellate cells of anterior pituitary following estrogen withdrawal from primed male rats

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Summary. Ultrastructural changes suggesting a phagocytic role for the nongranular folliculo-stellate cells of the anterior pituitary are investigated in estrogen-primed male rats after withdrawal of estrogen. Morphological changes in mammotropes following the removal of a subcutaneous estradiol-containing Silastic implant include the formation of intracellular lipid bodies. These lipid bodies appear to be associated with enhanced estrogen-dependent prolactin secretion in mammotropes. Seven and 24 h after estrogen withdrawal intracellular lipid within mammotropes seems to be released into the intercellular space. Seventy-two h after estrogen withdrawal, lipid droplets are almost entirely cleared from mammotropes while folliculo-stellate cells become packed with lipid globules. Folliculo-stellate cells also undergo dramatic hypertrophy 7 and 24 h after the removal of E2-containing implants. Extensive intercellular junctions including zonulae adhaerentes, desmosomes, and putative gap junctions are formed. Intercellular junctions delineate extravascular channels into which numerous microvilli project. Folliculo-stellate cells appear capable of accumulating many lipid droplets, presumably related to mammotrope metabolism. What appear to be large secondary lysosomes as well as the lipid droplets are observed within folliculo-stellate cells; lipid, therefore, may be degraded through a lysosomal pathway in folliculo-stellate cells.

Key words: Anterior pituitary gland – Estrogen treatment – Intercellular junctions – Folliculo-stellate cells – Phagocytosis – Rat

The nongranular folliculo-stellate (FS) cells of the pituitary were first described by Farquhar in 1957, and since then their physiological role has been the subject of both controversy and speculation. This cell has been variously ascribed sustentacular (Salazar 1968), secretory (Schechter 1969), transport (Forbes 1972), stem cell (Yoshimura et al. 1977), and phagocytic (Dingemans and Feltkamp 1972) roles. The FS cell has recently been the subject of renewed interest following the immunohistochemical demonstration of brain-specific S-100 protein which may imply a glia-like function (Nakajima et al. 1980; Shirasawa et al. 1983).

Recent descriptions of FS cells have been given for a wide variety of species including fish (Yamamoto et al. 1982), amphibians (Gracia-Navarro et al. 1983; Perryman 1983), reptiles (Forbes 1972), birds (Harrisson 1978), and mammals (Shiotani 1980; Leatherland and Renfree 1982; Shirasawa et al. 1984). One common observation across species lines is the presence of characteristic intracellular lipid droplets (Dingemans and Feltkamp 1972; Perryman 1983). Previous work in our laboratories demonstrates the presence of intracellular lipid bodies (ILB) in mammotropes which appear related to estradiol-enhanced secretory activity in these prolactin-containing cells (unpublished observation). The incidence of ILB in mammotropes is shown to increase with estradiol priming and to increase further following estradiol withdrawal (this report). Therefore, the present study was undertaken to determine a possible relationship between the lipid droplets in FS cells and the ILB in mammotropes formed after estrogen withdrawal. FS cell activation is also reported to be related to estrogen and to enhanced mammotrope secretory activity in the postpartum and lactating rabbit (Shiotani 1980); this facet of FS cell metabolism also warrants investigation.

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Materials and methods

Male Sprague-Dawley rats (CD) were purchased from the Canadian Breeding Farms and Laboratories (Charles River, Montreal, Quebec). Rats weighing between 325 and 375 g were housed in a sound-proofed, temperature ($25 \pm 1°$ C), and light (0600–2000 h)-controlled environment. Purina rat chow and tap water were provided ad libitum.

Animals were divided into 8 experimental groups consisting of 3 animals each. Group 1 was comprised of 3 normal, untreated control animals; groups 2 through 5 all received subcutaneous Silastic-capsule implants (Dow Corning, 1.57 mm inner diameter, 3.18 mm outer diameter, 3 cm long) containing 50–60 mg of 17β estradiol (E2) (Sigma); groups 6 through 8 received similar but empty implants and served as experimental controls. The Silastic tubing was sealed at both ends with Silastic medical grade elastomer (Dow Corning) as previously described (Shin and Reifel 1981). Following the priming for 4 weeks, the E2-containing implants were removed from groups 3 through 5; these animals were sacrificed by decapitation 7, 24, and 72 h later respectively. Likewise, the empty implants were removed from groups 6 through 8 and animals were sacrificed 7, 24, and 72 h later respectively. In group 2, implants remained in place at the time of decapitation to serve as

E2 controls. All implants were administered and removed under light ether anesthesia.

Following decapitation the pars distalis of each rat was quickly excised and fixed in cacodylate buffered (pH 7.2–7.4) paraformaldehyde-glutaraldehyde fixative (modified from Karnovsky 1965). Tissue was post-fixed in 0.1 M cacodylate buffer containing 2% OsO_4: both fixations were carried out at room temperature. Adenohypophyses were embedded in Jembed 812 (J.B. E.M. Services, Montreal). Sections were stained with uranyl acetate and lead citrate (Reynolds 1963) and examined with an Hitachi 500 electron microscope.

To compare quantitative changes in lipid bodies between groups, random micrographs of mammotropes were used. Only micrographs of complete cells with visible nuclei and intact plasma membranes were used. At least 25 cells per gland (75 per experimental group) were employed for statistical reasons already described (Poole et al. 1980; Reifel et al. 1985). The average area of lipid per cell for each animal was quantified by use of a Zeiss MOP-3 image analyzer, and the standard error calculated. Statistical significance was assessed with Duncan's multiple range test for grouped data.

Results

I. Folliculo-stellate (FS) cells in control and estrogen-primed rats

In the normal male rat, FS cells are readily distinguished from secretory cells by the absence of hormone-containing granules. Inactive FS cells (Fig. 1) possess long, thin, presumably sheet-like cytoplasmic processes in close apposition to the granulated cells. The effect is often the physical separation of adjacent secretory cells. FS cells contain many mitochondria; lipid droplets and lysosome-like structures are often present. Perinuclear cytoplasm is scant compared to that of the granulated cells, and the Golgi complex and endoplasmic reticulum are relatively undeveloped. These observations in control (group 1) rats are similar to those in estrogen-primed (group 2) rats, although FS cells are less frequent in the latter.

Mammotropes and FS cells from animals with empty implants (groups 6–8) do not appear different from those observed in the normal control group 1. Although ether stress increases prolactin (PRL) secretion in normal and E2 primed male rats, these significantly augmented hormone levels last for only 20 min (Piercy and Shin 1980). No difference in tissue appearance compared to normal animals is observed 7, 24, or 72 h after exposure to ether and removal of the empty Silastic implant.

II. Mammotropes and folliculo-stellate cells following withdrawal of estrogen implants

After withdrawal of the E2 capsule, mammotropes contain more ILB (Fig. 2). In both the 7 and 24 h groups, ILB seem to be released from mammotropes into the intercellular space (Fig. 3). Free lipid globules in the intercellular space are presumably washed out during dehydration procedures. Concomitantly, FS cells hypertrophy dramatically; extensive intercellular junctions between them become readily apparent. These junctions, which are of the zonula adhaerens type, appear to occur only between apposed surfaces of adjacent FS cells and tend to be in the apical region in proximity to an extravascular channel (Figs. 4, 5). That these zonulae adhaerentes junctions are extensive is demonstrated by the plane of section in Fig. 5. Similar, but less extensive and smaller extravascular channels and intercellular junctions were observed in normal male rats and in E2-primed animals. In E2-withdrawn animals desmosomes occur between two adjacent FS cells or between an FS cell and a secretory cell (Fig. 5). In addition, some areas of close apposition of FS cell membranes resemble gap junctions (Fig. 6a, b). All types of intercellular junctions observed appear to contribute to the integrity of a system of extravascular channels (Figs. 4–6a).

Microvilli are a prominent feature of hypertrophied FS cells. These structures increase FS cell-surface area within the newly formed system of extravascular channels (Figs. 4–6a). Within hypertrophied FS cells lipid accumulates and is especially apparent 24 (Fig. 6a) and 72 h after E2 withdrawal, but is also seen in the 7 h group. By 24 h following E2 withdrawal, after the accumulation of many lipid droplets, some FS cells appear to lose their processes (Fig. 7) while other cells retain their stellate appearance (Fig. 8). Seventy-two h after E2 withdrawal many FS cells appear as in Fig. 7. Putative lysosomes are also evident in FS cells after withdrawal of E2 (Figs. 7, 8).

Quantitation of ILB area per mammotrope in different experimental groups suggests the clearance of lipid over the 72 h following E2 withdrawal. Clearance appears to occur after an initial increase in lipid formation 7 h after implant removal followed by subsequent decline 24 and 72 h later (Fig. 9). Significant differences between the 24 h group and the E2 control group attain a level of significance of $p < 0.05$. Differences between lipid area are significant ($p < 0.01$) between all other groups except the E2 control and 72 h group as determined by Duncan's multiple range test.

Discussion

It is suggested that ILB in mammotropes are correlated with enhanced PRL secretion as monitored by PRL radioimmunoassay (unpublished observation). ILB are cleared from mammotropes over the 3 days following E2 withdrawal, and large accumulations of lipid appear in FS cells. Exposure and subsequent withdrawal of E2, a major mammotrope mitogen and PRL secretagogue (Lloyd et al. 1975; Shin 1979), thus provides a useful model to demonstrate the possible relationship between FS cell metabolism as it relates to estrogen enhanced secretory activity and ILB formation in mammotropes. Evidence of phagocytosis by FS cells under a number of experimentally induced endocrine conditions has been reported (Dingemans and Feltkamp 1972; Shiotani 1980) although the stimuli employed in vivo do not provide the morphological changes required to document specific functions in FS cells.

Dingemans and Feltkamp (1972) noted the relative abundance of lipid droplets in FS cells compared to other adenohypophyseal cell types and suggested that this lipid, in conjunction with extensive junctional complexes, provides the "most prominent identifying mark" of FS cells. This observation is clearly reinforced in Fig. 6. We have demonstrated a possible relationship between the ILB in mammotropes and the massive increase in lipid content of FS cells in the pars distalis. Furthermore, lysosomal degra-

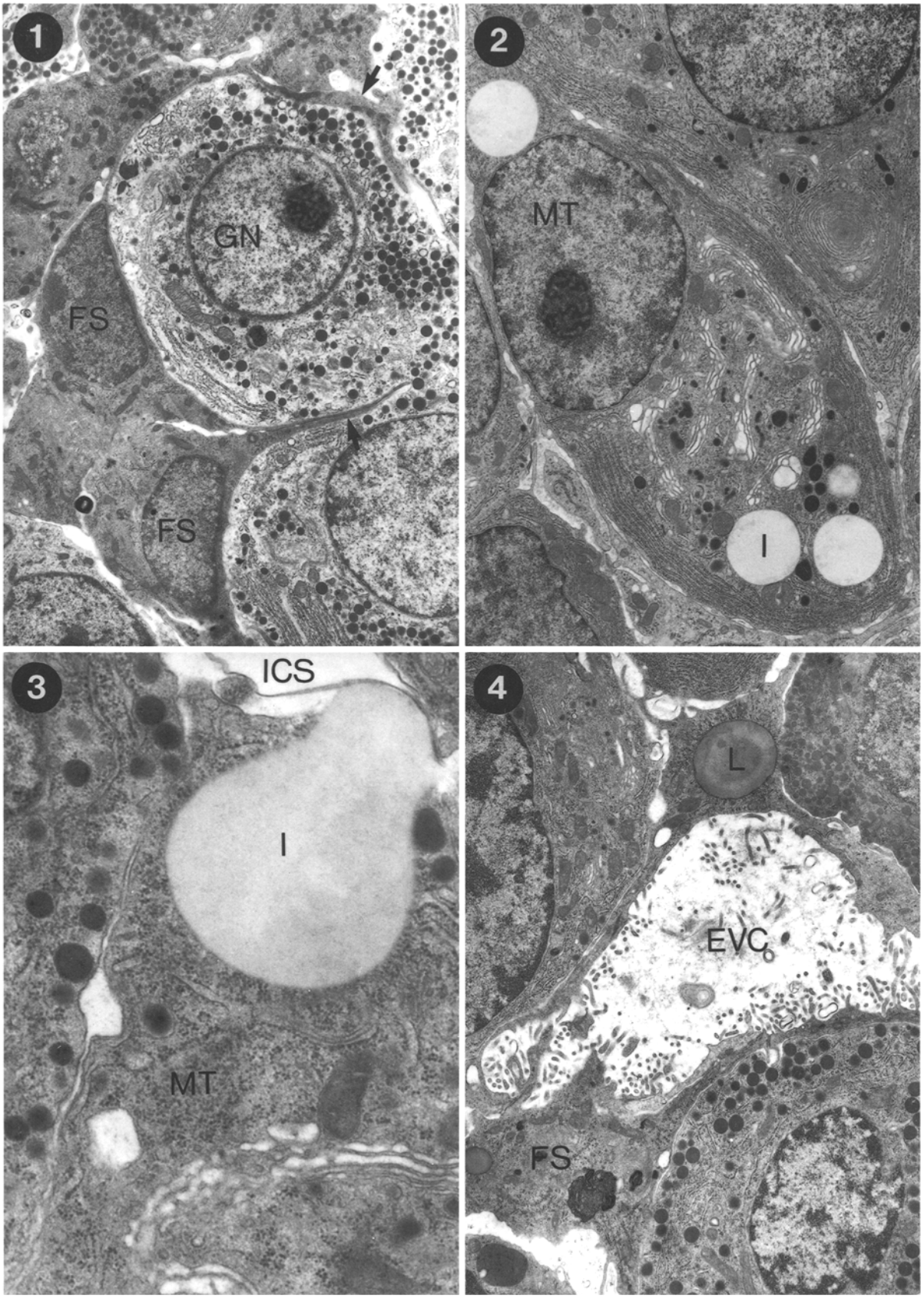
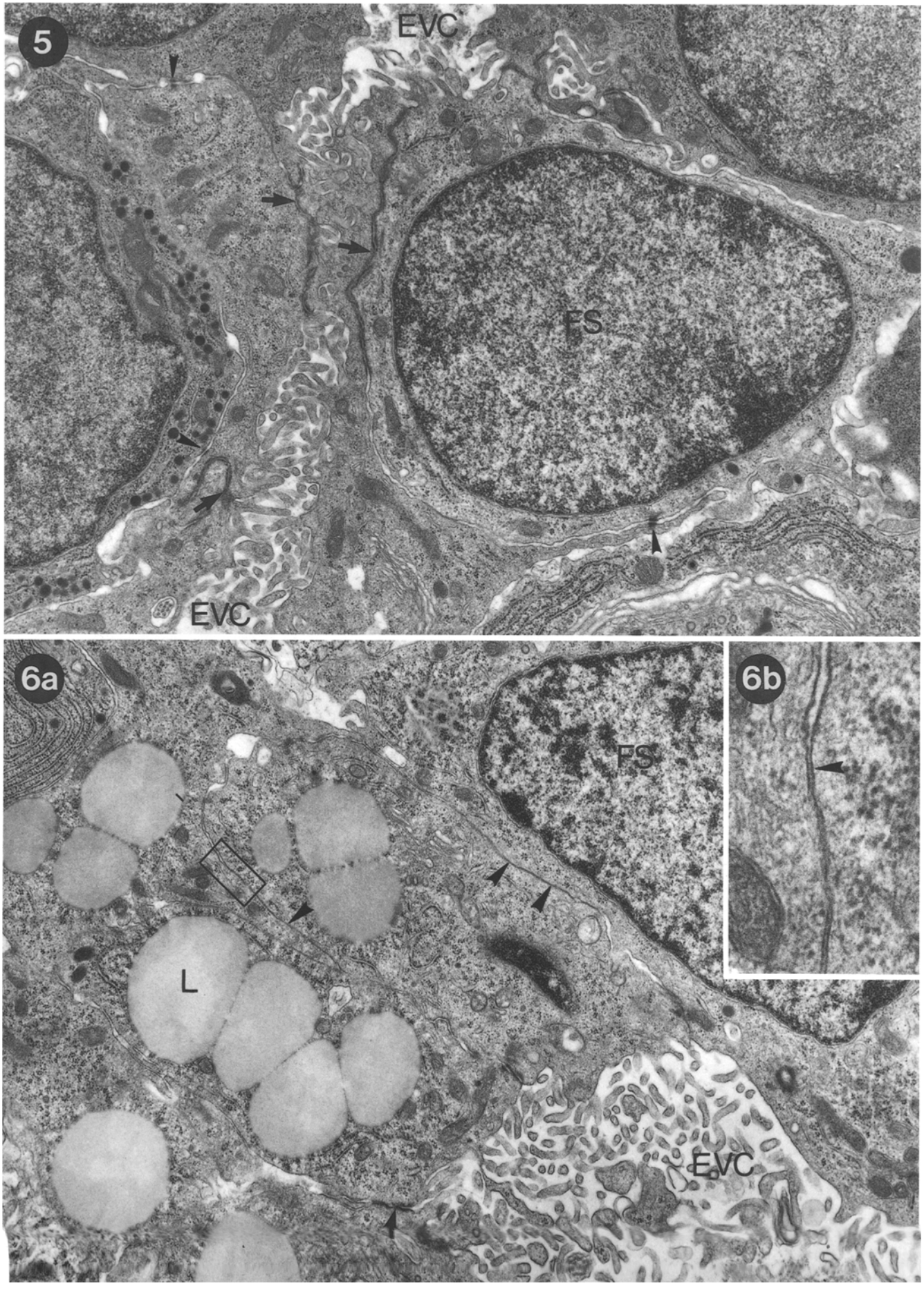


Fig. 1. Folliculo-stellate cells (*FS*) surrounding gonadotrope (*GN*); *arrows* show cytoplasmic processes. × 5540

Fig. 2. Mammotrope (*MT*) 7 h after E2 withdrawal; intracellular lipid body (*I*). × 5400

Fig. 3. Release of intracellular body (*I*) from mammotrope (*MT*) into intercellular space (*ICS*) 24 h after E2 withdrawal. × 22200

Fig. 4. Extravascular channel (*EVC*) delineated by FS cells (*FS*) 24 h after E2 withdrawal; lipid globule (*L*). × 11880


5
EVC
FS
EVC
6a
FS
L
EVC
6b

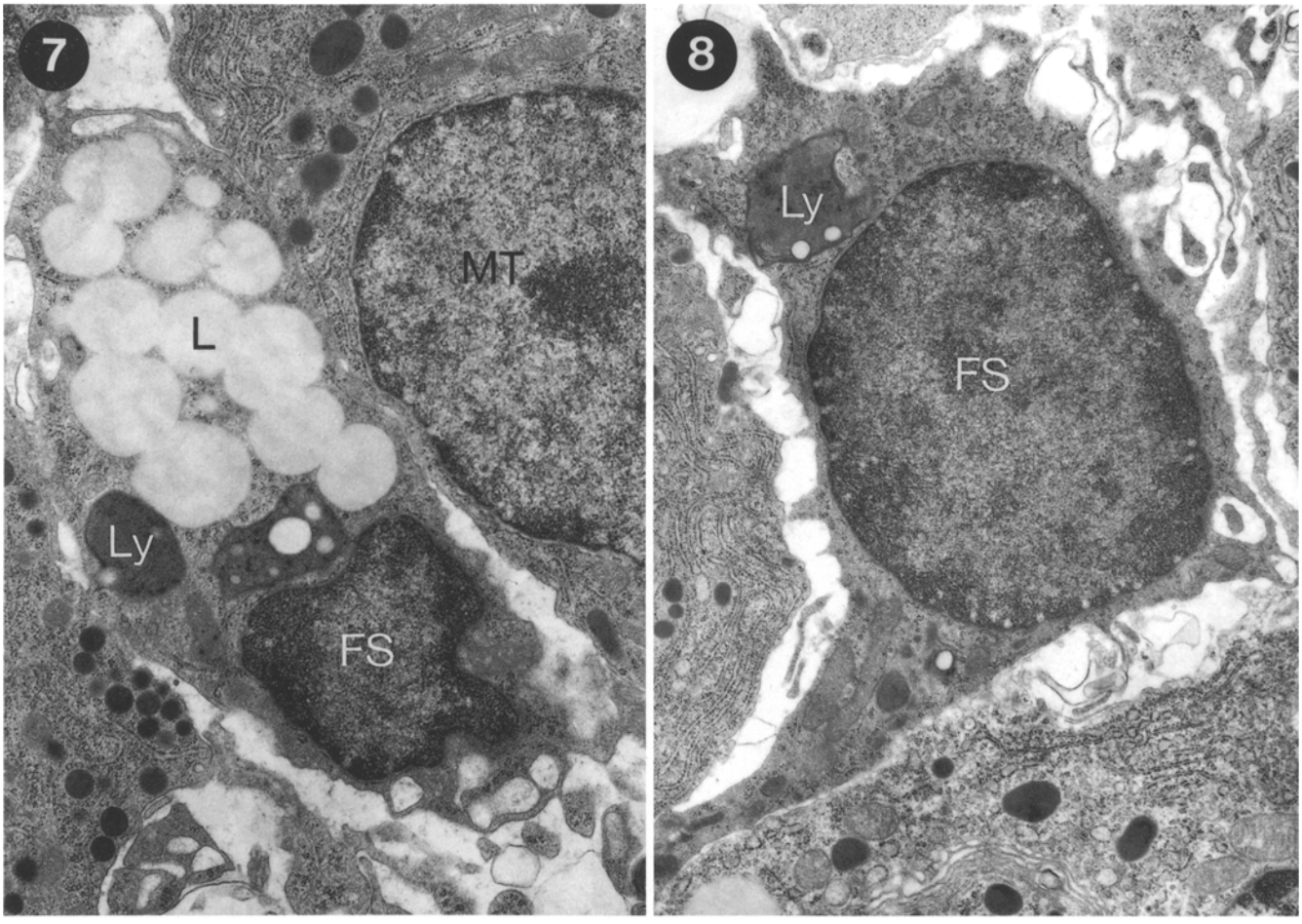


Fig. 7. FS cell (*FS*) 24 h after E2 withdrawal; lipid body (*L*); putative lysosome (*Ly*); Mammotrope (*MT*). ×11875

Fig. 8. FS cell (*FS*) 7 h after E2 withdrawal; lysosome (*Ly*); mammotrope (*MT*). ×9100

dation of lipid droplets appears to occur in FS cells (Figs. 7, 8).

As to the formation of lipid droplets in mammotropes, Smith and Farquhar (1966) proposed a scheme for the disposal of secretory granules in E2-primed mammotropes by crinophagy; inhibited or slowed secretion was required for granule degradation (Farquhar 1977). In this model, PRL granules were digested in lysosomes by a multistep process which resulted in the formation of a residual free lipid globule and an electron dense digested body. A means for either the disposal or reuse of this crinophagy-related lipid in mammotropes has not been reported. It is possible that FS cells are also involved in the metabolism of this residual material.

A physiological correlate of the experimental E2 withdrawal in our experiments occurs during the period immediately following parturition. At this time enhanced estrogen levels are known to decline both rapidly and dramatically. Interestingly, Shiotani (1980) noted that lysosomes and lipid droplets are common in the FS cells of postpartum and lactating rabbits. Likewise, during lactation, a process in which PRL and estrogen play vital regulatory roles relating to the initiation and maintenance of milk production, an increase in lysosomes and lipid droplets was observed in FS cells (Shiotani 1980). It is apparent from our own findings that withdrawal of E2 implants from rats produces dramatic ultrastructural changes in FS cells which are associated with altered estrogen-dependent PRL secretion in mammotropes. These observations, however, do not preclude a phagocytic function for FS cells under other endocrine conditions (Dingemans and Feltkamp 1972; Shiotani 1980) and their phagocytic activity probably represents a general feature of adenohypophyseal metabolism.

Several important points about the complex organization of the pars distalis suggested are the dynamic nature of the intercellular junctions formed in response to some

Fig. 5. FS cell (*FS*) 7 h after E2 withdrawal; extravascular channel (*EVC*); desmosomes (*arrowheads*); zonula adhaerens junctions (*arrows*). ×13200

Fig. 6a FS cells (*FS*) 24 h after E2 withdrawal; extravascular channel (*EVC*); lipid droplet (*L*); zonula adhaerens junctions (*arrows*); very close apposition of cell membranes and possible gap junction (*arrowheads*). ×13250. **b** Part of Fig. 6a showing putative gap junction (*arrowhead*). ×66250

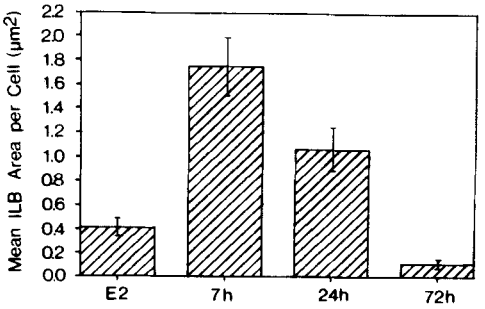


Fig. 9. Quantitative analysis of intracellular lipid body area in mammotrope cells from different experimental groups. E2 is group 2; 7 h is group 3; 24 h is group 4; 72 h is group 5 (see materials and methods). Bars depict the standard error of the mean

stimulus or, possibly, to the removal of one. The adhaerens junctions delineate the extravascular channel in apical regions and are reinforced distal to the channel by desmosomes (Figs. 4, 5). The dynamic nature of these extravascular channels might facilitate or retard the access of various hypothalamic releasing or inhibiting factors in the vascular filtrate to the intercellular space. Gap junctions between these cells could aid in the flow of ionic information or modulate the ionic environment (MacVicar 1984) around secretory cells by an ion-shunting mechanism. However, more work is required to prove the existence of gap junctions between FS cells and to establish their possible significance. At any rate, it is possible that FS cells play a vital role in ensuring the access of hypothalamic factors to their target organ. These cells may "mop up" the detritus of cellular metabolism including apparently large amounts of lipid. This lipid could otherwise clog the intercellular spaces and interfere with the fine regulation of endocrine function at the level of the membrane receptor.

The apparent phagocytic activity of FS cells, their sustentacular function, as well as the localization of brain-specific S-100 protein in these cells has prompted a comparison of FS cells to glial elements (Nakajima et al. 1980; Shirasawa et al. 1983). That FS cell morphology is reminiscent of that of ependymal cells of the central nervous system may also be of import. FS cells exhibit microvilli, intercellular junctions, and occasional cilia. These are also properties of ependymal cells (Watson 1974) and especially obvious features of tanycytes (specialized ependyma) of the median eminence (ME) (Ugrumov and Mitskevich 1980; Merchant and Dollar 1981; Brawer and Walsh 1982; Brion et al. 1982).

Brawer and Walsh (1982) reported that lipid droplets occur in "large grape-like clusters" in the perikaryon and distant processes of tanycytes. These workers considered lipid droplets to be diagnostic of tanycytes of the ME as they are considered diagnostic of FS cells of the adenohypophysis (Dingemans and Feltkamp 1972). It has also been demonstrated that the presence of lipid bodies and lysosomes in ependymal tanycytes is closely related to estrogen metabolism (Merchant and Dollar 1981; Brawer and Walsh 1982). Furthermore, Zimmermann (1982) employing cytometric techniques suggested that tanycytes play a role in the sequestration of biological agents in cases of E2 withdrawal effected by ovariectomy. Apparent sequesetration of lipid occurred after adrenalectomy (Brion et al. 1982) in the ME tanycytes and has also been reported in pituicytes of the neurohypophysis of dehydrated, postpartum, and lactating rats (Tweedle and Hatton 1980, 1982). Lipid droplets and lysosomes in tanycytes and pituicytes may be associated with conditions of increased secretion in the ME and neurohypophysis respectively.

Our work has demonstrated the accumulation of lipid and the appearance of putative secondary lysosomes in the FS cells of the adenohypophysis under conditions of altered PRL secretion. This accumulation of lipid and apparent degradatory activity in FS cells occurred after the formation of spectacular and extensive extravascular channels within the parenchyma of the pars distalis. It is well known that the hypothalamic-hypophyseal axis through neurohemal contact at the ME represents a highly integrated network, and it may be that integrated glia-like activity within the anterior pituitary is also operative. The possibility of ionic communication between glia (MacVicar 1984) also raises questions concerning the intrapituitary regulation of endocrine function by glia-like FS cells.

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