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An Ultrastructural Study of the Gonadotrophs in Oophorectomized Rhesus (*Macaca mulatta*) Adults Treated with Estrogen[1] (38353)

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Removing the gonads increases the secretion of pituitary gonadotropins and causes a cellular hypertrophy of the gonadotrophs which leads to their signet-ring appearance (1, 2). When appropriate steroids are placed in gonadectomized animals, the elevated plasma gonadotropins decline within minutes (3, 4) and correspondingly morphologic evidence of the intracellular rearrangement of secretory granules is found within 1–2 days (5). To restore the structurally modified gonadotrophs of these animals to their intact state, however, requires extended treatment with appropriate hormones.

In oophorectomized monkeys, plasma estrogen can be restored to a physiological range of 30–300 pg/ml by varying the dimensions of estrogen-filled silastic implants (6). We have used these estrogen implants to study the structural reversibility of gonadotrophs in estrogen-primed, spayed rhesus monkeys. This paper reports such morphologic changes and their subsequent response to a sustained physiological level of estrogen.

Materials and Methods. Animal care and treatment. The six adult female rhesus monkeys (*Macaca mulatta*) used in this study had been spayed at least eight months before the experiment. Crystals of 17-β estradiol, E_2 (13, 5[10]-estratrien-3,17β-diol, Steraloids, Inc.) packed and sealed in 3 cm lengths of silastic tubing with an i.d. of 0.132 in. and an o.d. of 0.183 in. (Dow Corning Corp.) were implanted subcutaneously in the interscapular regions of four monkeys; the other two, which served as controls, received cholesterol implants.

The animals were fed Purina Monkey Chow and water *ad lib.*, caged singly, and subjected to light cycles of 12 hr dark and 12 hr light. On alternate mornings, before and after implantation, blood samples were drawn for plasma LH and estrogen analysis. Throughout the experiment, the hematocrit of the animals was monitored and found to be normal. The animals were exsanguinated 37 days after implantation.

Radioimmunoassay of plasma LH and estrogen. The blood samples were centrifuged at 2,500 rpm and the plasma was removed and stored frozen in two equal portions for LH (7) and estradiol (8) radioimmunoassays.

A partially purified preparation of rhesus adenohypophyseal gonadotropin (LER-M-907D) was used as a standard for the LH radioimmunoassay. Standard curves were based on triplicate estimates at each of eight points and 200 μl of all samples were assayed in duplicate.

Estradiol was determined with antisera from rabbits immunized with estradiol-17-β-succinyl bovine albumin. The antisera were diluted (1:1000) with 0.1% gelatin dissolved in PBS to obtain a 50% binding of ^{3}H estradiol in blank solution.

The hormonal data were analyzed by two tailed Student's *t* test. Comparisons were made between the preimplantation and postimplantation values, so that each animal served as its own control. The plasma LH and estradiol values from the initial three samples after estradiol implantation (interval of most rapid LH decline) were not utilized in the statistical analysis.

Electron microscopy. Immediately after exsanguination, the pituitary glands were removed and the anterior lobe separated from the rest of the pituitary. The interior lobes were cut into

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small pieces, doubly fixed in paraformaldehyde glutaraldehyde (9) and 1% osmic tetroxide followed by routine dehydration, and then embedded in Araldite.

Thin sections were cut on a Porter–Blum MT2 microtome with a diamond knife and stained in aqueous uranyl acetate (10) and lead citrate (11). Sections were blind coded before they were examined in a Philips 300 electron microscope.

Results. Plasma E_2, LH contents. Plasma E_2 ranged from nondetectable amounts to 37.4 pg/ml with a mean of 18.1 ± 2.1 pg/ml before the subcutaneous implantation of silastic E_2 implants. In the first week after implantation, there were surges of estrogen reaching 169.9 pg/ml, the average in this period was 123.88 ± 7.39 pg/ml. For the remainder of the experiment, estrogen stabilized to a mean of 97.62 ± 2.84 pg/ml (Fig. 1). In the cholesterol-treated controls, estrogen concentration remained extremely low (mean 12.82 ± 1.88 pg/ml) (Fig. 2).

Before estrogen treatment, plasma LH concentrations varied considerably within and between monkeys, ranging from 1.03 μg/ml to 3.55 μg/ml (mean 2.23 ± 0.14 μg LER-M-907D/ml). After E_2 implantation, plasma LH concentrations declined steadily and stabilized at levels substantially lower than those of the preimplantation control levels. Plasma estrogen concentrations in monkey 5292 were lower than those of the other three and the LH pattern displayed several peaks in the postimplantation period. The average LH values after implantation for animals 5735, 3583, 3587 and 5292 were 0.46 ± 0.07, 0.37 ± 0.03, 0.69 ± 0.05 and 1.14 ± 0.12 μg/ml, respectively (Fig. 1). The P values were less than 0.005 in all animals

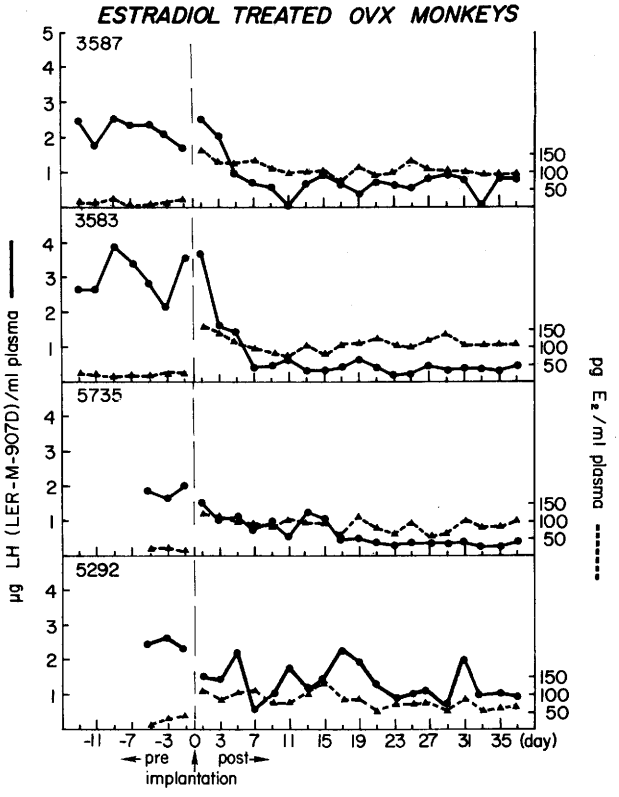

FIG. 1. LH and E_2 contents in estrogen-primed, ovariectomized (OVX) monkeys. Animal numbers are indicated on the upper left-hand corners. The solid lines indicate the variation in LH contents, and the dotted lines indicate the E_2 fluctuations.

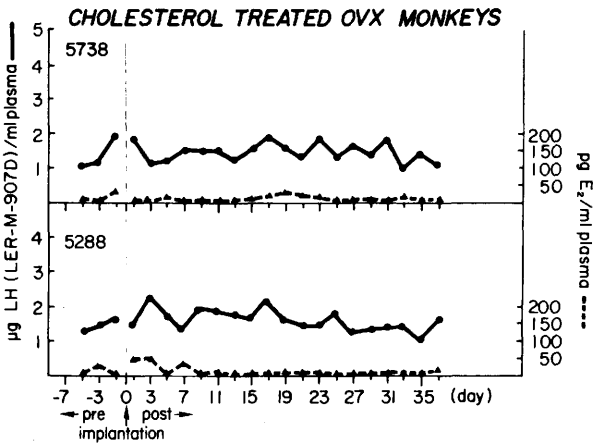

FIG. 2. LH and E_2 contents in control animals. Animal numbers are indicated on the upper left-hand corners. The solid lines indicate the LH levels and the dotted lines indicate the E_2 content. Unlike those in E_2-treated animals, the two lines remained separate throughout the experiment.

except for 5292 which was less than 0.01. There were no statistical differences in plasma LH before and after cholesterol implantation, the averages in these animals being 1.48 ± 0.12 and 1.54 ± 0.07 μg/ml, respectively.

Ultrastructural morphology of the pars distalis. The gonadotrophs in the cholesterol-treated controls were the largest cells of the anterior pituitary gland (Fig. 3). Most of the hypertrophy was due to the ballooning of the cisternae of the granular reticulum. These dilated endoplasmic reticula (ER) were distended with mater-

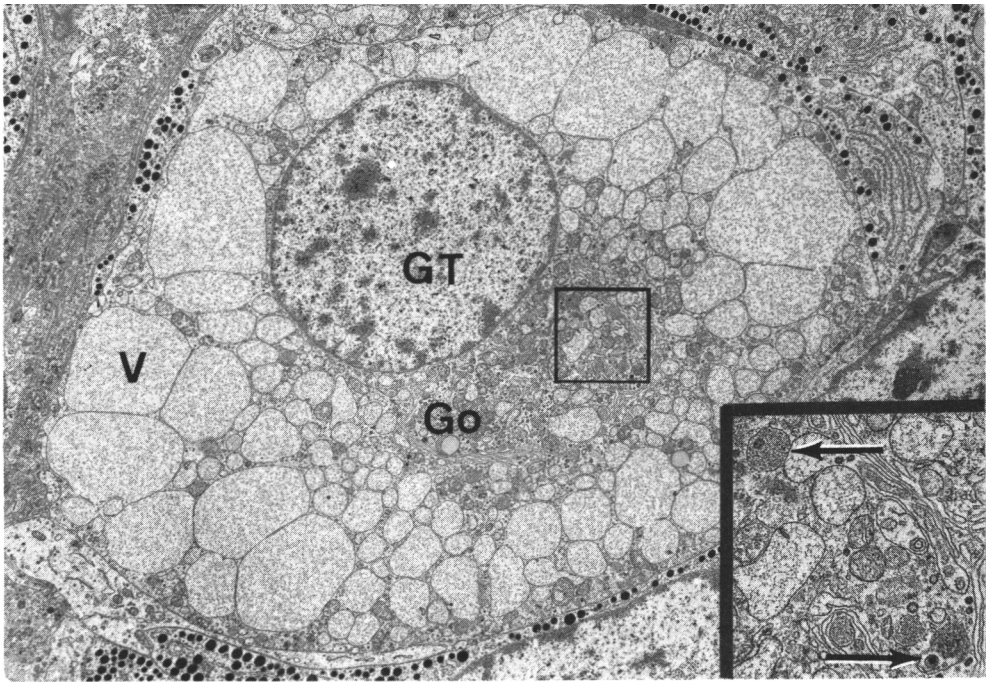

FIG. 3. "Castration cell" in spayed rhesus monkey. The hypertrophied gonadotroph (GT) contains numerous vesicles (v) and is nearly devoid of secretory granules. The prominent Golgi (Go) complex is made of several parallel cisternae. × 4,600. The insert corresponds to the area marked by the square. Arrows point to granules with an electron opaque core. × 13,400

ials of moderate density but no intracisternal granules were found. Numerous small vesicles were found in the prominent Golgi complex, but very few immature granules. Patches of heterochromatin were distributed on the round nuclei which were often located away from the center. Dispersed among the dilated endoplasmic reticula were rodlike mitochondria and secretory granules. The mitochondria had lamellar cisternae, and both the electron opacity and the diameter of the secretory granules varied considerably. A core of electron opaque material was seen in some of these granules, but most were more electron lucid than those of the other parenchymal cells. Dense bodies were occasionally seen.

After estrogen treatment, the gonadotrophs were generally reduced in cell size. Because of their characteristic vesicular remnants and scanty amounts of secretory granules, some gonadotrophs are regarded as intermediate forms. However, such cells were few (Fig. 4) and their dilated ER contained flocculent material. The Golgi complex was not prominent; only a few secretory granules and some empty vesicles were found in the Golgi region. The nucleus was pyknotic and the nuclear outline tended to be irregular. The appearance of the nucleus also suggests the possible degenerative nature of these cells. In these cells, unusually large secretory granules sometimes reached a diameter of 750 mμ; the average is 490 nm.

Most of the gonadotrophs in the estrogen-treated monkeys were characterized by somewhat round or oval cells which contained a large number of diffusely distributed secretory granules (Fig. 5). The diameter of these granules ranged from 150 to 500 nm, but most were about 240 nm. The electron opacity of the granules was quite uniform; however, some appeared more lucid and others contained a more opaque core than normal. The earlier dilated endoplasmic reticula showed a drastic involution with only a little dilation. The eccentrically situated nuclei had a fine chromatin pattern and some patches of heterochromatin. Like that of the other parenchymal cells, the nuclear outline of the gonadotroph was smooth, and the number of mitochon-

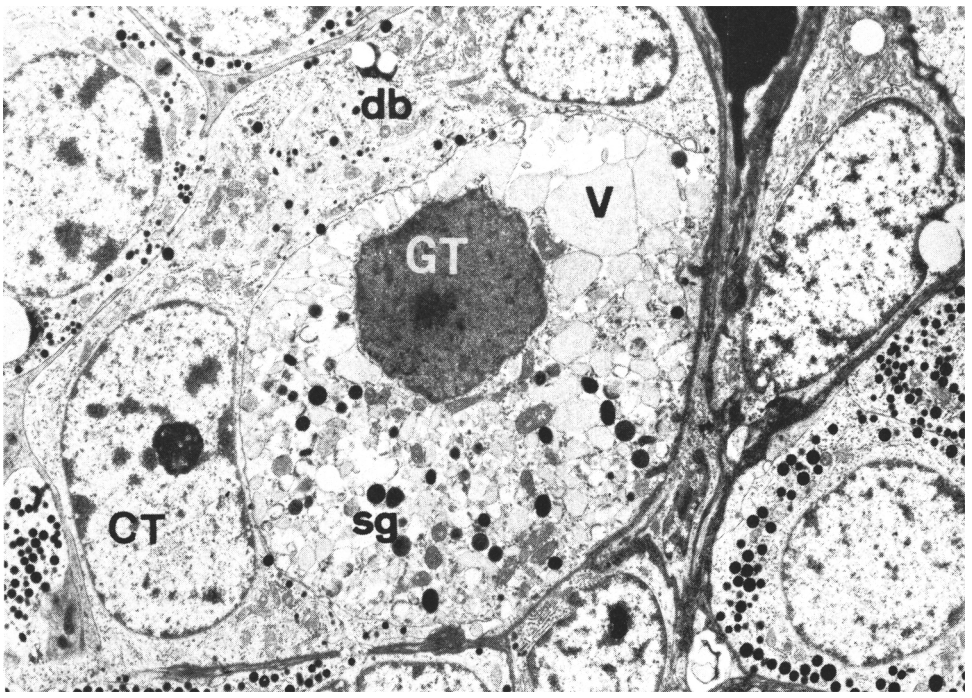

FIG. 4. The intermediate forms of gonadotrophs in E_2-treated animals. The cell size is similar to that of other parenchymal cells. The irregularly outlined nucleus is more electron opaque than other cell types. Vesicles (v) of different sizes are present, and the secretory granules (sg) vary considerably in size and electron density. A large dense body (db) is shown in the adjacent corticotroph (CT). × 4,600

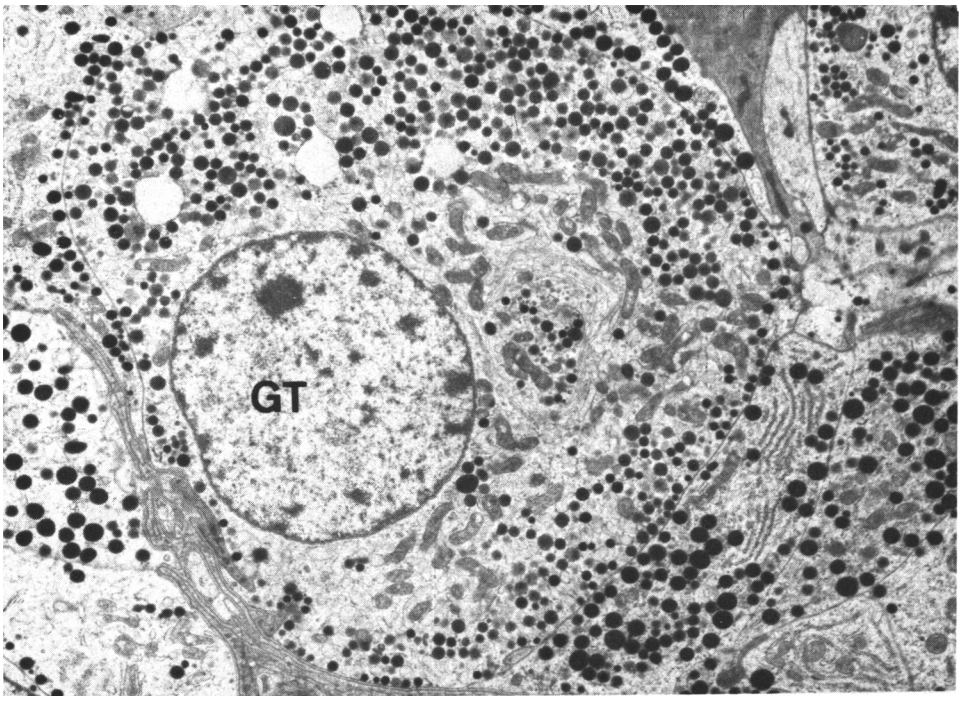

FIG. 5. A fully responded gonadotroph in an E_2-treated OVX monkey. Numerous secretory granules and some dilation of the ER are present. The Golgi complex with a few immature secretory granules is situated near the nucleus. × 6,000.

dria appeared to have increased.

Other parenchymal cells showed nonspecific changes characterized by a random appearance of dense bodies (Fig. 4). The granules of these cells did not differ appreciably in the cholesterol or estrogen-treated spayed animals.

Discussion. "Castration cells" were earlier reported in the pituitary glands of gonadectomized rodents (1, 2), but they have not been demonstrated ultrastructurally in subhuman primates. At light microscopic levels, the "castration cells" had a signet-ring appearance. These cells are highly active in synthesis and secretion of the gonadotropins; and are probably responsible for the elevation of circulating gonadotropins in the gonadectomized animal. Ultrastructural morphology of the "castration cell" corroborate the functional changes, since the endoplasmic reticula are dilated, the Golgi bodies are prominent and there are but a few secretory granules remaining in these cells. Our observations of these cells in long-term spayed animals provide morphologic evidence of a negative feedback system for estrogen in rhesus monkeys. Moreover, the present study demonstrates the reversibility of the structural modifications in chronically stimulated gonadotrophs in E_2-treated spayed monkeys.

The plasma LH values (Fig. 1) of the estrogen-treated animals corroborate the previously reported fact that the administration of physiologic levels of estrogen prevents LH elevation in oophorectomized monkeys (6). We have been, however, unable to ascertain whether the numerical increase in secretory granules and the involution of the gonadotrophs in E_2-treated animals were mediated through hypothalamic hormones. We can, however, infer from the diminished ER that estrogen plays an important role in regulating the cisternal contractility of the reticulum.

The sensitivity of the feedback mechanism seems to be directly related to the estrogen levels. The residual amount of estrogen in cholesterol-treated animals, which is probably of adrenal origin, does not prevent the elevation LH. Moreover, the degree and rapidity of LH suppression after estrogen administration are related to the amount of circulating estrogen. The mean estrogen levels in E_2 animals vary from

75.94 ± 5.38 to 103.35 ± 3.77 pg/ml. In animals with mean estrogen concentrations above 100 pg/ml (Nos. 3583, 3787), LH concentrations were readily suppressed; correspondingly, their pituitary gonadotrophs showed uniformity in granule accumulation. After E_2 implantation, LH concentrations declined more slowly in monkeys 5735 and 5292 and animal 5292 displayed LH fluctuations as late as 31 days after implantation. The latter two animals had circulating estrogen titers of 81.73 ± 4.94 and 75.94 ± 5.38 pg/ml, respectively. Moreover, many intermediate forms of gonadotrophs were present in their pituitary glands.

Although pyknotic nuclei are associated with cell degeneration, the closely adhered nuclear membrane, the intact mitochondria, the involution of cytoplasmic vesicles and the reaccumulation of secretory granules indicate that these cells are more likely to be an intermediate form of gonadotroph. The abnormal accumulation of large secretory granules in the intermediate form of gonadotrophs observed in our animals has also been seen in the pituitary glands of castrated rats (2). The significance of this finding is not certain, but it suggests that the larger secretory granules are formed earlier than the small ones; alternatively, the latter may be released at a faster rate to create the appearance of an accumulation of larger secretory granules. Whatever the controlling mechanism for the synthesis and release of secretory granules in gonadotrophs, it is clear that given appropriate amounts of estrogen for an extended duration, both the structure and function of gonadotrophs can be modified from a highly active to a less active state.

Summary. Adult rhesus monkeys, spayed for at least eight months before experimentation, were given subcutaneous implants of estrogen or cholesterol. Before treatment, estrogen in these animals ranged from nondetectable levels to 37 pg/ml. After estrogen treatment a physiological level of 75–103 pg/ml was reached; no change in estrogen content was observed in controls given cholesterol. After estrogen administration, plasma LH, which was relatively high in spayed monkeys, declined significantly; cholesterol treatment, however, did not alter the LH contents. An ultrastructural examination of the pituitary in control animals showed that castration cells were present; these hypertrophic gonadotrophs contain numerous large vesicles but few secretory granules. After estrogen treatment, two different forms of gonadotrophs emerged. One, which is thought to represent an intermediate form, had vesicular remnants, a pyknotic nucleus, and an accumulation of large secretory granules. Another, by far the most numerous, contained a large number of secretory granules, showed little or no dilation of ER, and returned to a size comparable to the other parenchymal cells. Our results in spayed rhesus monkeys indicate that both the structure and function of the gonadotrophs are reversed after estrogen treatment.

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