Electron Microscopic Study of the Localization of Labeled Gonadotropins in the Sertoli and Leydig Cells of the Rat Testis* (34522)

A. E. Castro,[1] A. C. Seiguer,[1] and R. E. Mancini
(Introduced by A. L. Gimeno)

Centro de Investigaciones sobre Reproduccion, Facultad de Medicina, Paraguay 2155, piso 10, Buenos Aires, Argentina

Ferritin molecules were used to label antibodies in immunoelectron microscopical studies (1). In previous experiments, thyrotropin (TSH) was similarly labeled, and its subcellular distribution in the thyroid gland after injection was described (2). We have recently studied, at the optical level, the localization of ferritin-labeled follicle stimulating (FSH) and luteinizing (LH) hormones (3). Lack of data on the ultrastructural sites of localization of the gonadotropins in the rat testis prompted us to extend our studies at the electron microscopical level, using ferritin-labeled FSH and LH.

Material and Methods. Two lots of male adult Wistar rats, each comprising 12 animals maintained on a standard diet, were intravenously injected with a single dose of 5 or 10 mg of the same preparations reported elsewhere (3): (a) luteinizing hormone (NIH-LH-S3) labeled with ferritin (LH-Fe) and (b) follicle stimulating hormone (NIH-FSH-S1) similarly labeled (FSH-Fe). The biological activity was controlled by the ovarian ascorbic acid depletion test for LH (4) and the ovarian weight method for FSH (5). The relative potency (RP) of each labeled preparation was expressed in terms of unlabeled hormones, being 0.36 for labeled LH and 0.52 for FSH (3). Six lots of control rats, comprising three animals each, were respectively injected by the same route with a single dose of one of the following: (a) denatured FSH-Fe or LH-Fe, obtained by heating for 1 hr in an acid medium; (b) unlabeled LH or FSH (10 mg) plus ferritin (20 mg); (c) ferritin (20 mg); and (d) rat serum albumin labeled with ferritin (10 mg) (6). In all the groups rats were killed at 30 min, at 3 hr, and at 12 hr after the injection. Small pieces of testis were fixed for 2 hr in 1% buffered osmium tetroxide (7), dehydrated, and embedded in Epon (8); thin sections were stained with uranyl acetate (9) or lead citrate (10). Micrographs were taken with a Siemens Elmiskop I at the original magnifications of 20,000 to 60,000.

Results. Concerning the dose of LH or FSH, better results were obtained with 10 mg than with 5 mg. As early as 30 min after the injection of LH-Fe, the testicle showed dense molecules of ferritin in the intertubular spaces around the collagen fibrils and in the cytoplasm of cells which have been postulated as an immature type of Leydig cell (11) (Fig. 3) The particles were localized in cytoplasmic vesicles adjacent to the plasma membrane as well as in many others unconnected with this membrane. In rats injected 3 or 12 hr prior to the sacrifice, the particles were observed inside vacuoles and bodies of different size, shape, and density. Also transitional stages between the vacuoles and the dense bodies were observed (Fig. 4). While in these bodies the majority of ferritin particles remained until the last period of observation, they tended to disappear from vesicles and vacuoles after 3 hr. In no case were ferritin molecules found in the so-called mature Leydig cells (11). Scant accumulation of particles was detected within 30 min to 12 hr in similar bodies of some peritubular cells and in the basal lamina of the seminiferous

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[1] Present address: Institute of Molecular Evolution, University of Miami, 521 Anastasia, Coral Gables, Florida 33134.

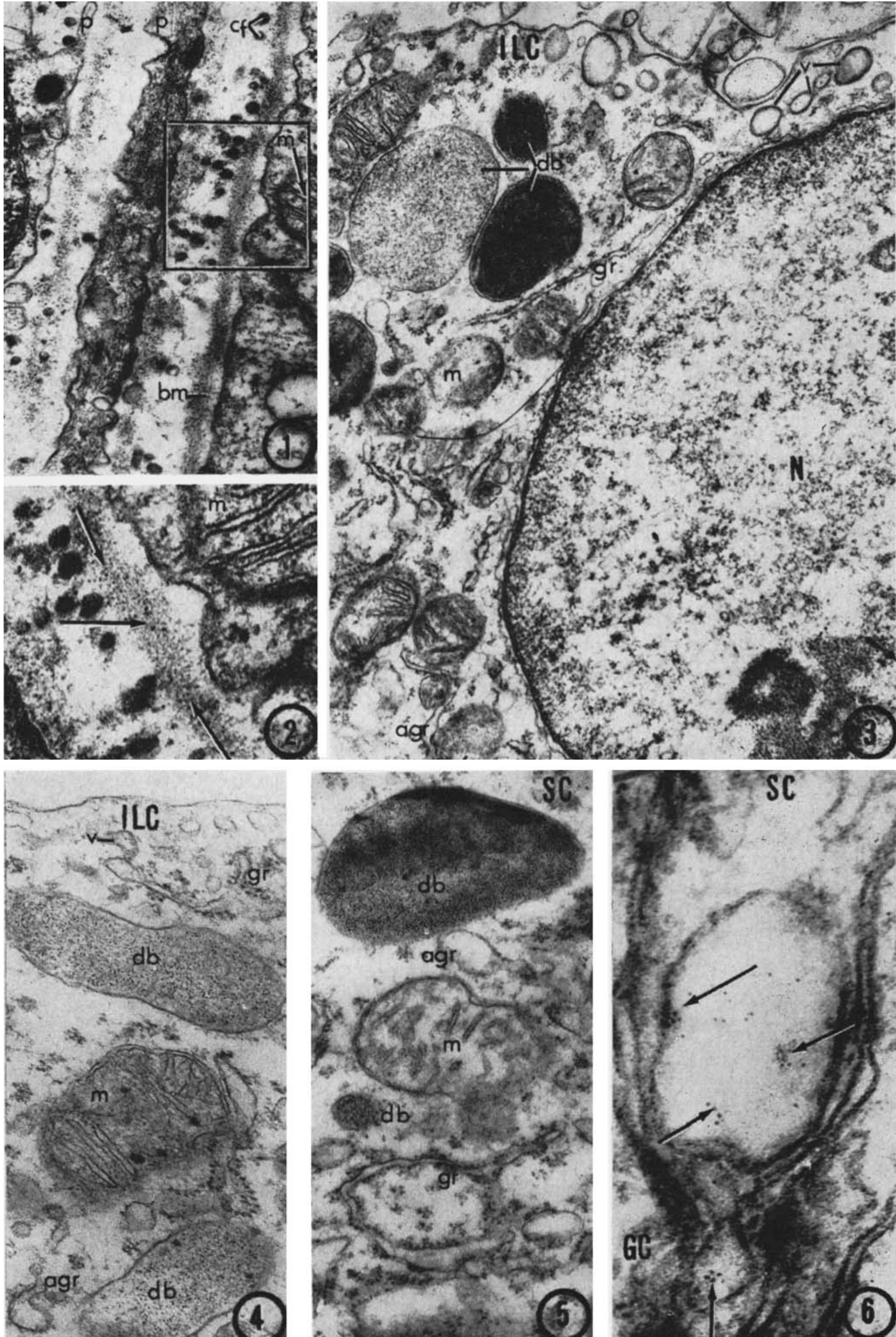
tubule wall. Very little ferritin was seen inside the cytoplasm of the Sertoli cells, and such particles were located in bodies very similar to those described above.

The testicles of the animals injected with FSH-Fe presented, from 30 min onward particles in arterioles and capillaries as well as in some areas of the basal lamina of the seminiferous tubule wall. At 30 min, the described membrane-bound vesicles and vacuoles filled with ferritin were predominantly seen in the basal portion of the Sertoli cell cytoplasm; at 3 hr these organelles had almost completely disappeared, and bodies containing a great amount of ferritin were seen irregularly distributed in the cytoplasm of this cell, reaching a maximum at 12 hr (Figs. 5 and 6). Similar particles were occasionally detected in the cytoplasm of some spermatogonial cells.

In control experiments, no striking differences were observed using denatured LH-Fe or FSH-Fe, unlabeled gonadotropins added to the tracer, ferritin alone, or rat serum albumin labeled with ferritin. From 30 min to 3 hr after the injection, the electron-dense molecules were seen in the lumina of small vessels of the intertubular spaces; from 3 to 12 hr, a steady accumulation of particles was seen around the peritubular cells and attached to the basal lamina of the seminiferous tubule wall (Figs. 1 and 2). From 3 to 12 hr, few dense particles were occasionally observed in scattered vacuoles in the cytoplasm of Sertoli and immature Leydig cells.

Discussion. In order to assess the results of studies on the distribution of intravenously injected labeled gonadotropins, it has been assumed (3) that these substances are intact when they diffuse out from the vessels and reach their specific sites of localization; therefore, only the labeled hormone and not a labeled metabolic product of that hormone or the label itself, is being observed. This assumption is supported by data which indicate that bonds between proteins and labels are stable (12, 13, 14), and also by the observation that in contrast to the selective localization of the labeled hormones, the labels alone, labeled nonhormonal proteins, or labeled denatured hormones are slowly incorporated by some cells in sparing amounts and fail to show any specific localization. Furthermore, the rapid rates of intracellular accumulation of labeled gonadotropins makes it improbable that significant amounts of them were degraded into fragments responsible for the localization observed. Thus, it is reasonable to assume that the distribution of the materials seen in tissues reflects that of the injected hormones.

Light microscopic studies (3) failed to show differences between mature and immature Leydig cells; furthermore, ferritin-labeled LH revealed by the Prussian blue reaction seemed to be randomly deposited in the round or oval morphologic types of interstitial cells. Recently, De Kretser *et al.* (15), using radioautography at the optical level, demonstrated in 20-day-old rats a similar localization of ^{125}I-labeled human LH. The electron microscopic observations presented here indicate that only the so-called immature Leydig cell incorporates LH-Fe. These results, together with the evidence that native LH may act on the maturation of the Leydig cells (16), suggest that the localization reported here may be mainly related to the trophic action of this hormone. Taking into account the ovine origin of the LH and the partial denaturation which follows labeling, the possibility that some phagocytic activity of the interstitial cells could obscure the specificity of the incorporation reported for labeled LH has already been considered (3). However, in the present study it was observed that those cells responsible for that incorporation lacked the general features of macrophages. On the other hand, it has been reported that India ink particles injected as an indicator of phagocytic activity were taken up by those cells resembling macrophages, whereas Leydig cells had little or no phagocytic capacity (17). Contrary to the negative finding of our previous work (3), the subcellular localization of LH-Fe in cytoplasmic structures of the Sertoli cell could be representative of another site of specific accumulation, or may be due to the contamination of this hormone with FSH, particularly if the high dose used is considered. Nevertheless, it should be remembered that small doses of LH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are required for the maintenance of the seminiferous tubule function and that this hormonal influence might be exerted directly on the tubules (18). The observation that dense particles in cytoplasmic structures of peritubular cells appeared when LH-Fe was injected, is suggestive of another site of selective cell accumulation.

The results obtained with FSH-Fe were in accordance with those found at the optical level (3). Shortly after the injection, the Sertoli cell cytoplasm appeared filled with single-membrane-bound structures containing dense particles. The significance of the erratic finding of particles in the spermatogonial cells remains to be clarified, particularly because they were absent in primary spermatocytes and other cells of the germinal epithelium. The presence of a few particles in immature Leydig cells when FSH-Fe was injected might also be attributed to the slight contamination of this hormone with LH.

The morphologic events described, especially those concerning the predominant localization of FSH-Fe and secondarily of LH-Fe in the Sertoli cell cytoplasm, the lack of ostensible accumulation in the germinal cells, and data from other authors related to the morphologic changes induced by FSH on the Sertoli cells (19, 20), suggest a possible role of the Sertoli cell as an intermediate site of gonadotropin storage for the ultimate action on spermatogenesis. Concerning this intratubular distribution, it should be mentioned that several authors have suggested the existence of barrier mechanisms in the testis, which would prevent the entry of substances into the seminiferous tubules. Kormano (21) and Kormano and Penttilä (22) have reported that the basement membrane of those tubules does not allow the passage of L-3, 4-dihydroxyphenylalanine, 5-hydroxytryptamine and 5-hydroxytryptophan. A barrier to protein passage has been proposed by Johnson and Setchell (23) because of the low protein content of the rete testis fluid of the ram, as compared to that of the serum. This latter point, however, would need further clarification since Pande *et al.*, using a different method for fluid collection, have shown that fluid from rat (24) and monkey and goat (25) testes contain more protein than the serum. There is histological evidence (26) which indicates that the intratubular diffusion rate of injected labeled serum proteins is much slower for globulins than for albumin, the former being accumulated in the seminiferous tubule wall The present electron microscopic observations suggest that the basal lamina of the seminiferous tubules could act as an obstacle, limiting the access of significant amounts of macromolecules such as ferritin, denatured labeled hormones, or ferritin-labeled homologous serum albumin; these control preparations showed poor, or no localization inside the tubules,

FIG. 1. Area of peritubular tissue of rat testis after the injection of homologous serum albumin labeled with ferritin. Accumulation of dense molecules can be seen in the basal lamina (bm) of the seminiferous tubule wall and around the peritubular cells (p). cf: collagen fibrils; m: Sertoli cell mitochondrion; × 35,000.

FIG. 2. Higher magnification of the zone enclosed in Fig. 1. Arrows show the ferritin particles; × 65,000.

FIG. 3. Portion of an immature Leydig cell cytoplasm (ILC) of a rat testis 3 hr after the injection of ferritin-labeled LH. Ferritin particles appear condensed with varied density in dense bodies (db); Agr: agranular endoplasmic reticulum; gr: granular endoplasmic reticulum; N: nucleus; m: mitochondrion; v: vesicles; × 30,000.

FIG. 4. Same as preceding at higher magnification; dense bodies containing ferritin particles are seen; × 45,000.

FIG. 5. Portion of a Sertoli cell cytoplasm (SC) of a rat 3 hr after the injection of ferritin-labeled FSH; dense particles are seen within dense bodies (db). Key references as in Fig. 3; × 32,000.

FIG. 6. Basal portion of the Sertoli cell 30 min after the injection of ferritin-labeled FSH. Dense particles appear inside vacuoles of different size (arrows), and adjacent germinal cell cytoplasm (GC) is also seen; × 90,000.

but high accumulation in their basal lamina. In contrast to that, no such accumulation was observed after the injection of FSH-Fe; this fact, together with the early intratubular appearance of the labeled hormone, indicate a rapid passage through the basal lamina which does not offer a barrier to its diffusion. The observation that FSH-Fe reaches the cytoplasmic structures of the Sertoli cell despite barrier mechanisms that could operate for other macromolecules, increases the functional significance of its intratubular distribution.

The process of cellular incorporation of LH-Fe and FSH-Fe, seems apparently to be similar to that described in other cells, as far as the uptake of macromolecules is concerned (27). It is then suggested that the specificity of the incorporation of labeled gonadotropins, as compared with the control preparations, resides in the accelerated and greater uptake of these hormones by the target cells.

Summary. Adult male rats were intravenously injected with a single dose of FSH-Fe or LH-Fe; ferritin alone, unlabeled-hormones plus ferritin, denatured labeled hormones, and homologous albumin similarly labeled were used as controls. Electron microscopic studies of testis from rats injected with FSH-Fe demonstrated a predominant accumulation of the ferritin particles in vesicles and dense bodies of the Sertoli cell cytoplasm. After the injection of LH-Fe, the ferritin molecules appeared predominantly in the same organelles of immature Leydig cells, in peritubular structures, and in lesser amount in vesicles of Sertoli cells. The injection of control preparations resulted in fewer ferritin particles detected in the pertibular tissue and occasionally in Leydig or Sertoli cell cytoplasms.

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