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ELECTRON MICROSCOPIC STUDIES ON FETAL AND EARLY POSTNATAL TESTICULAR INTERSTITIAL CELLS OF MICE

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INTRODUCTION

Androgen secreted by the testis in rodents is known to play important roles in the differentiation of the reproductive and central nervous system during prenatal and early post natal periods. Knowledge of the morphological events occurring inside the testicular interstitial cells during this period is necessary to understand the functional states of the testis.

The present study was designed to investigate electron microscopically the prenatal and early postnatal changes inside the mouse testicular interstitial cells and to associate the changes with the physiological events which have been well documented.

MATERIAL AND METHODS

In this study dd-N strain mice bred in this laboratory were used. The mature female mice were put with the male over night and the next morning the vaginal plugs were examined. The day the plugs were found was designated as "day 0" of gestation. In this system of designation birth usually occurred until night on day 19 of gestation. Male fetuses were removed from their mothers under ether anesthesia at days from 13 to 19 of gestation. This was performed between 10 and 11 a.m. Postnatal mice were sacrificed at 3, 5, 7 and 10 days of age. In addition, the testes immediately and about 12 hours after birth were also studied. Testes from mice 3 to 7 months of age were served as controls. The testes from fetal and postnatal mice were immersed in 1% phosphate buffered OsO_4 with or without cutting into small pieces and fixed at 0°C for 1 to 2 hours according to their size. After fixation the tissues were dehydrated in ascending series of ethanol and embedded in Epon 812 with the method recommended by Coulter (1967). These embedded tissues were cut at a thickness of about 1 μ on a Porter-Blum MT-1 type ultramicrotome and stained with 1% methylen blue in 1% borax for a perspective view by light microscopy. Ultrathin sections were obtained on the

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same microtome and stained doubly with uranyl acetate and lead citrate. The stained ultrathin sections were examined with a Hitachi HU-11 A type electron microscope.

OBSERVATIONS

As the ultrastructure of the testicular interstitial cells of adult mice has been described in detail by Christensen and Fawcett (1966), here only two electron micrographs of the mature testicular interstitial cells of mice are shown as a basis for comparison with those of the cells from mice during development (Fig. 1, 2).

The surface of the testis from the 13-day-old fetus is covered by one layer of cuboidal cells surrounded by a basement membrane. Subjacent to the membrane lies a zone of several cell layers which may differntiate to the future tunica albuginea. Further, between zones consisting of typical mesenchymal cells, are seen a few immature seminiferous tubules with one or two cell layers lining a narrow canaliculus (Fig. 3). Between the tubules a wide interstinum exists. This results in a small ratio of the seminiferous tubules to the interstinum in this stage of development. Most cells occurring in the interstinum are easily identified as fibroblasts by their fine structural characteristics such as large flattened cisternal profiles of rough endoplasmic reticulum filled with lamellar cristae, small sized Golgi complex and abundant free polyribosomes. Among these fibroblasts appear sporadically cells with larger cytoplasm as compared with that of the fibroblasts. The cytoplasm contains a well developed tubular endoplasmic reticulum with ribosomes partially on its surface, relatively well developed Golgi complex and round to oval mitochondria with a few cristae. Large amounts of polyribosomes are observed to be scattered in the rest of cytoplasm. These cells have also dense bodies and lipid droplets, but are few in number (Fig. 4).

Cells with distinct smooth endoplasmic reticulum first occur among the fibroblasts in the interstinum of the testis of the 14-day-old fetus (Fig. 5). These cells are round to oval in shape and are provided with enlarged cytoplasm due mainly to the development of smooth endoplasmic reticulum. In these cells rough endoplasmic reticulum is present in small amount, but configurations indicating association of the reticulum with the smooth one are frequently observed. The mitochondria are rather pleomorphic and variable in size. In general, they are larger than those of the fibroblasts and have more numerous tubular cristae and fairly dense matrix. Images of adhesion of the mitochondria to each other are occasionally seen.

From fetal day 14 onward, the cells with a large amount of smooth endoplasmic reticulum by which they can now be identified as testicular interstitial cells enlarge progressively and attain a relative plateau of their

development by 16 to 17 days of fetal life. This enlargement of the cells is mainly due to an increase of smooth endoplasmic reticulum. This reticulum comes to occupy most parts of the cytoplasm. The rough endoplasmic reticulum appears in a form of parallel arrays of several flattened cisternae here and there in the cytoplasm. The mitochondria also become numerous. Although the shape and size of the mitochondria are also variable, they, in general, are round with many tubular cristae and of large diameters. The number of cristae and density of matrix of these mitochondria also increase with development. The Golgi complex is rather well developed and usually located at the perinuclear region, consisting of fine vesicles and lamellae. In the vicinity of the Golgi region dense bodies tend to increase toward the end of fetal life. Lipid droplets are small in number and are distributed sporadically or in a small group in the cytoplasm. Glycogen is frequently seen to be aggregated in small parts of the cytoplasm. This material is still observed in such a form in the early postnatal days, but in the adult is not seen in a congregated form. Mitotic figures of the interstitial cells are often encountered at this period of development.

As described above, the testicular interstitial cells enlarge with progression of fetal life, and their size at fetal day 19 becomes comparable with that of the adult (Fig. 2, 6). However, the appearance of the cytoplasm of the fetal interstitial cells at this stage is rather different from that of adult ones. The cytoplasm of the adult interstitial cells shows a more elaborated appearance than that of the fetal ones (Fig. 2). The close association of both types of endoplasmic reticulum with mitochondria seen in the adult interstitial cells is one of the examples. Membranous whorls, double-walled tubules, "small granules" among the mitochondria and a large number of lipid droplets cannot be seen in the fetal interstitial cells.

The interstitial cells immediately after birth appear almost the same as that of cells from the 19-day-old fetuses. But 12 hours after birth the interstitial cells begin to exhibit a tendency of decrease of the cytoplasm (Fig. 7). In contrast, the number of lipid droplets tends to increase. These tendencies become clearer with age and five days after birth the cells reach a certain regressive stage (Fig. 8). Such reduction of the cytoplasm is mainly due to a decrease of the smooth endoplasmic reticulum and mitochondria. The rough endoplasmic reticulum also decreases and is dispersed in a form of small flattened cisternae without forming parallel arrays. The Golgi complex is reduced in size. This cytological state is seen to remain 10 days after birth without any significant changes (Fig. 9).

DISCUSSION

The present study demonstrated that in mice distinct testicular interstitial

cells evidenced by a presence of smooth endoplasmic reticulum occur on day 14 of fetal life, developing rapidly to a plateau at a certain active level during the rest of fetal life, and that the enhanced interstitial cells thereafter regress gradually without drastic changes between before and after birth to be in a quescent stage until five days after birth.

Very recently Russo and Rosas (1971), using the same species, have reported the developmental changes of testicular interstitial cells. They divided the stages of differentiation and development of Leydig cells in the fetal life into the following three Phases: Phase 1 (day 12 to day 15; the day the vaginal pluge were found was designated as "day 1" of gestation), was marked by the initial recognition of immature Leydig cells. Phase 2 (day 16 to day 19) was characterized by a development of smooth endoplasmic reticulum and an increase of mitochondria in these cells. Phase 3 (day 19 to birth) was a period when lipid and glycogen contents in the Leydig cells reached a maximum level. According to them, close to the time of birth, the smooth endoplasmic reticulum decreased and became vesicular. Furthermore, Russo (1971) described that the cytoplasm of Leydig cells from birth to 10 days of age contained a poorly developed smooth endoplasmic reticulum, clusters of glycogen particles and numerous lipid droplets. These observations generally agree with the present data.

Concerning the onset of steroidgenesis in the testicular tissues, considerable work has been reported.

Baillie and Griffiths (1964 b) demonstrated histochemically 3 β-hydroxysteroid dehydrogenase activity in the testis of fetal mice from 11 days of gestation. Noumura *et al.* (1966), employing an *in vitro* system, showed that capacity of the rat fetal testis to convert progesterone to androgen was detected from 13 days of fetal life. The time of onset of the steroidgenesis in these two studies is in good agreement with the time when the testis can be distinguished from the ovary in the respective species. Electron microscopical information in this study and in the literature (Russo and Rosas, 1971; Narbaitz and Adler, 1967), however, shows that typical smooth endoplasmic reticulum that is well known to play an important role in the steroid synthesis, has not detected in any cells in the fetal testis at such an early stage of fetal life. Accordingly, it would seem to be considered that a small amount of androgen may be produced in certain cells without complete differentiation of the interstitial cells, since the enzymes to convert progesterone to androgens are said to be present also in the cytoplasmic matrix (Fawcett *et al.*, 1969). On the other hand, many other morphologists reported that the steroidgenesis occurred more lately, *i.e.*, just before or during the differentiation of the male reproductive tracts (Black and Christensen, 1969; Pelliniemi and Niemi, 1969; Narbaitz and adler, 1967). The occurrence of the characteristic interstitial cells determined by electron microscopy agrees in time with the duct dif-

ferentiation. This agreement is of interest, because the duct differentiation is subject to the effect of androgens secreted by the testis.

The hypertrophy of the mouse interstitial cells in late fetal days was well evidenced by many histochemical and biochemical studies which showed an enhancement of the steroid synthesis during the period (Baillie and Griffiths, 1964 b; Hitzeman, 1962; Lipsett and tullner, 1965). Furthermore, several electron microscopical studies also confirm well the event (Russo and Rosas, 1971; Pelliniemi and Niemi, 1969; Black and Christensen, 1969).

The testicular interstitial cells of mice immediately after birth did not differ so much in their fine structure from those of mice just prior to birth, though both types of endoplasmic reticulum in the former cells tended to slightly decrease and conversely lipid droplets to increase in number. This tendency was more evident in the cells from mice about 12 hours old. Black and Christensen (1969), using guinea pig testes, documented similar results. But the observed fine structural difference in appearance was not so drastic as expected from the light microscopical observations of Roosen-Runge and Anderson (1959), showing that the rat interstitial cells were remarkably degranulated at the first postnatal day. Although what the degranulation described by them means is unclear, it may be explained by a possibility that the increase of lipid droplets is considered as the degranulation by light microscopy.

The postnatal regressive changes of the mouse interstitial cells suggest that the activity of steroid biosynthesis may decline gradually in the postnatal days in mice, remaining at a low functional level in the second week of life. This view was well supported by several lines of evidence which follow.

Resko *et al.* (1968) estimated the androgen concentrations in plasma and testes of developing rats by gas chromatography and reported that high concentrations of testosterone were found in both materials from newborn rats, and that there was a decline in the concentrations which lasted up to about the age of 30 days. Steinberger and Ficher (1968) investigated the capacity of developing rat testes to convert progesterone to testosterone and showed that testes from newborn rats were capable of converting up to 59% of progesterone to testosterone and that then the capacity declined and on day 20 was not detected almost completely. Lipsett and Tullner (1965) found a similar tendency in the rabbit testes. Hitzeman (1962) reported that the activity levels of the oxydative enzyme systems and 3 β-hydroxysteroid dehydrogenase fell during the early postnatal days in the mouse and that mitotic figures of the Leydig cells decreased continuously in the early postnatal days, being static at the age of 15 days. My morphological observations confirm well these findings in the literature.

It has been well documented that the masculine differentiation of the hypothalamus is effected by androgen secreted by the testis during the early

postnatal period in the rat and mouse. This presupposes that the interstitial cells in this critical period may be provided with the capacity of biosynthesizing this hormone. However the mouse interstitial cells start to regress shortly after birth, and some retainment of the active cytological images seen in the late fetal days may be suggestive of the activity of this tissue needed for the neural differentiation.

SUMMARY

Mouse testes from fetal day 13 to postnatal day 10 were observed. Interstitial cells were initially recognized from fetal day 14. These cells thereafter enlarged progressively and reached a peak during the late fetal days.

Birth did not induce any significant cytological changes in the interstitial cells, but signs of regression appeared in them shortly after birth. The regression became pronounced on day 5. Such cells showed reduction in cytoplasmic size due to a decrease of the cell organelles, and a prominent aggregation of lipid droplets. The interstitial cells on day 10 still retained such hypofunctional morphology.

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EXPLANATIONS OF FIGURES

FIG. 1. Survey electron micrograph of the testis of an adult mouse. In the upper portion of the Fig. a small part of a seminiferous tubule (T) is seen. The tuble is surrounded by a continuous sheath consisting of "peritubular cells (P)" (Ross, 1967). The interstinum which occupies the lower large portion of the Fig. contains well developed interstitial cells (I), portions of fibroblasts (F) and macrophages (MP), and two vessels (V). ×2,875

FIG. 2. Higher power electron micrograph of portions of the interstitial cells from an adult mouse. The picture shows the usual fine structure of the interstitial cells with smooth (sER) and rough (rER) endoplasmic reticuli, mitochondria (M), Golgi complex (G), dense bodies (D), "small granules (g)" (Christensen and Fawcett, 1966), lipid droplets (L) and membranous whorls (W). ×10,600

FIG. 3. Portion of a central region of the testis from a 13-day-old fetus. The interstinum in this region is characterized by a dense population of cells of mesenchymal origin. In the left upper corner of the Fig. a part of the seminiferous tubule (T) with a degenerative cell is seen. To the right lies a part of the densely populated interstinum. Capillary (C). ×2,250

FIG. 4. Portion of a cytoplasm-rich cell found in the interstinum from the testis of a 13-day-old fetus. This cell is provided with well developed rough endoplasmic reticulum (rER) in a form of small flattened cisternae, oval or round mitochondria (M) of relatively large size and Golgi complex (G). Portions of typical fibroblasts (F) are seen around the cell. ×10,600

FIG. 5. Portion of an interstitial cell (I) from the testis of a 14-day-old fetus. This cell has features which characterize the steroid producing cells; the presence of distinct smooth endoplasmic reticulum (sER) and large mitochondria (M) with tubular cristae. This cell also contains Golgi complex (G), rough endoplasmic reticulum (rER) and lipid droplets (L). Portions of surrounding fibroblasts (F) are seen. ×10,600

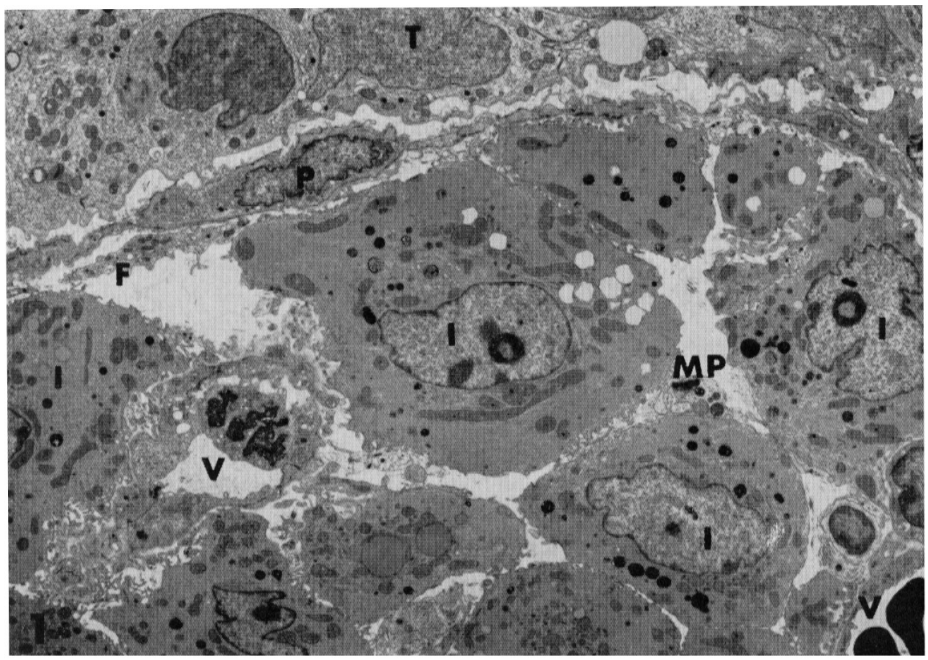
FIG. 6. Portion of the interstinum of the testis from a 19-day-old fetus. At the center of the Fig. a large interstitial cell (I) is seen with an abundance of smooth endodlasmic reticulum (sER), a number of round mitochondria (M), a few profiles of rough endoplasmic reticulum (rER) in parallel arrays, and Golgi complex. A small number of lipid droplets (L) and dense bodies (D) are also observed. A few fibroblasts (F), portions of the other interstitial cells and a capillary (C) are present in the surroundings. ×4,200

FIG. 7. Group of interstitial cells (I) from the testis of a mouse 12 hours after birth. The cells exhibit tendencies of an increase of lipid droplets (L) in number and a small reduction of the cytoplasm. ×4,200

FIG. 8. Portion of the testis of a 5-day-old mouse. Near the center of the Fig. a group of interstitial cells (I) are illustrated. These cells show small cytoplasm with a small amount of the cell organelles and conspicuous aggregation of lipid droplets (L). An area of glycogen droplets (GL) is seen in the cytoplasm of an interstitial cell. To the left of the Fig. a small portion of a seminiferous tubule (T) and its cell sheath are seen. Fibroblasts (F) and a mrcrophage (MP) are dispersed around the interstitial cell group. ×4,200

FIG. 9. Portion of the testis of a 10-day-old mouse. On the right of the Fig. a group of

interstitial cells (I) which are provided with small cytoplasm is seen. In the left portion of the Fig. a part of a seminiferous tubule (T) surrounded by immature "peritubular cells" is present. Between the interstitial cell group and the tubule there are a capillary (C) and portion of fibroblasts (F). ×4,200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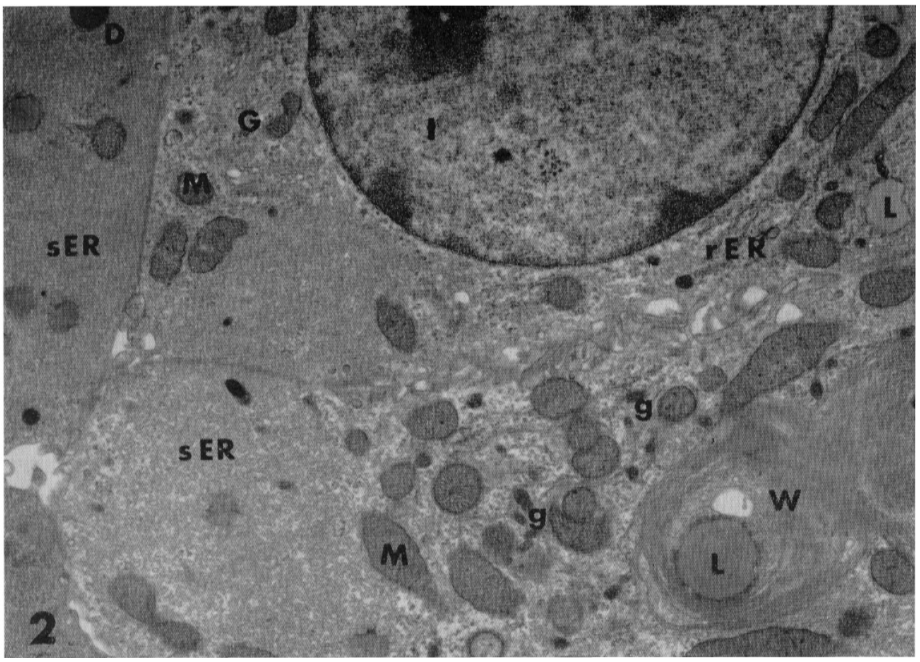
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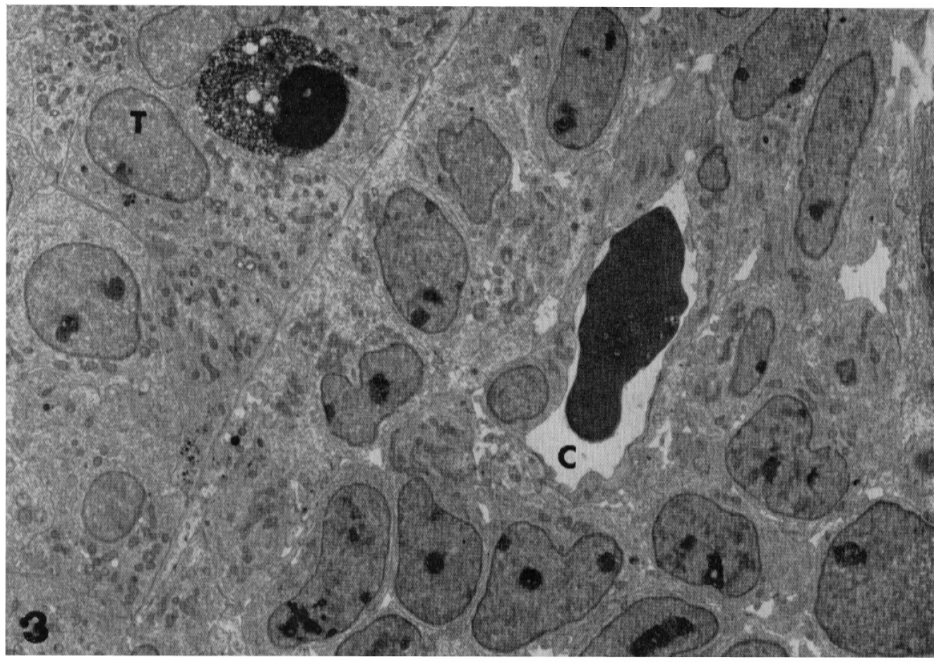
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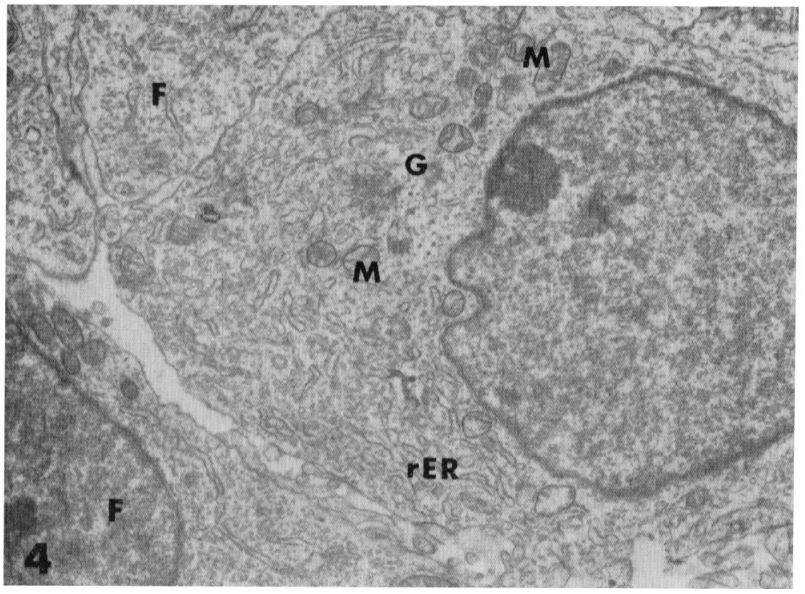
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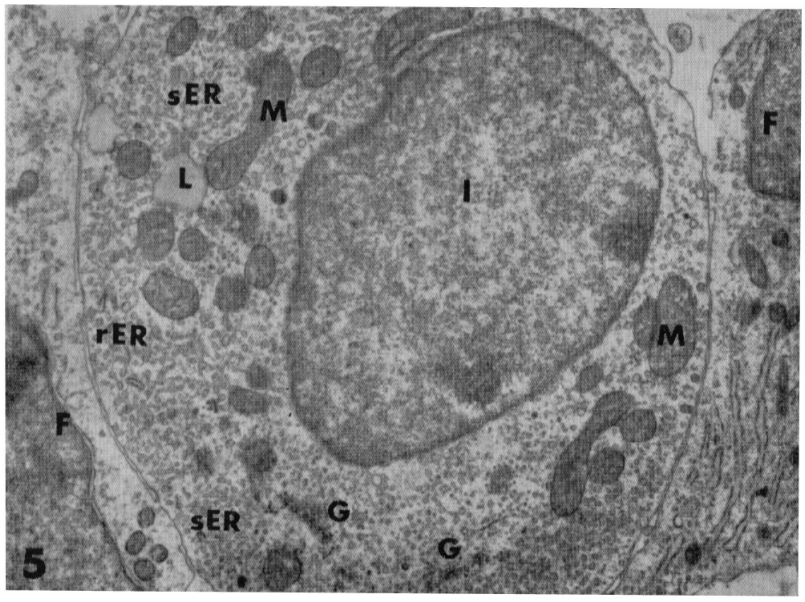
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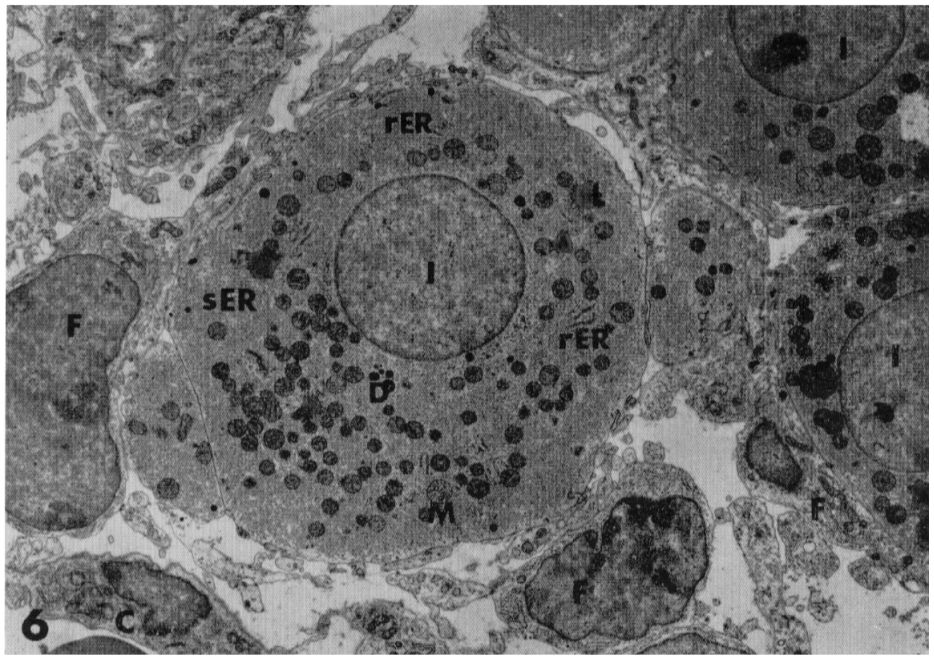
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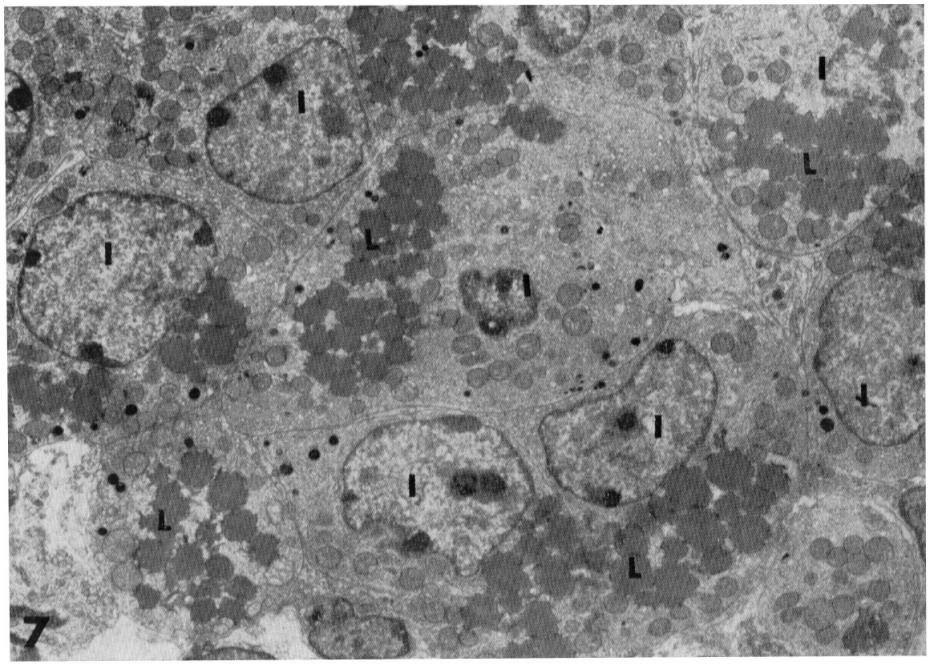
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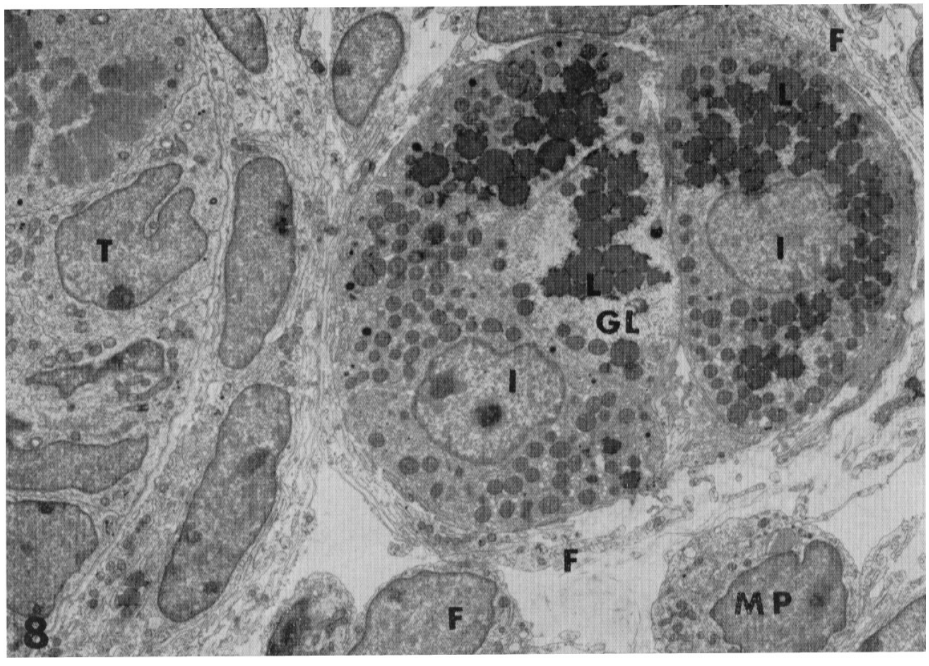
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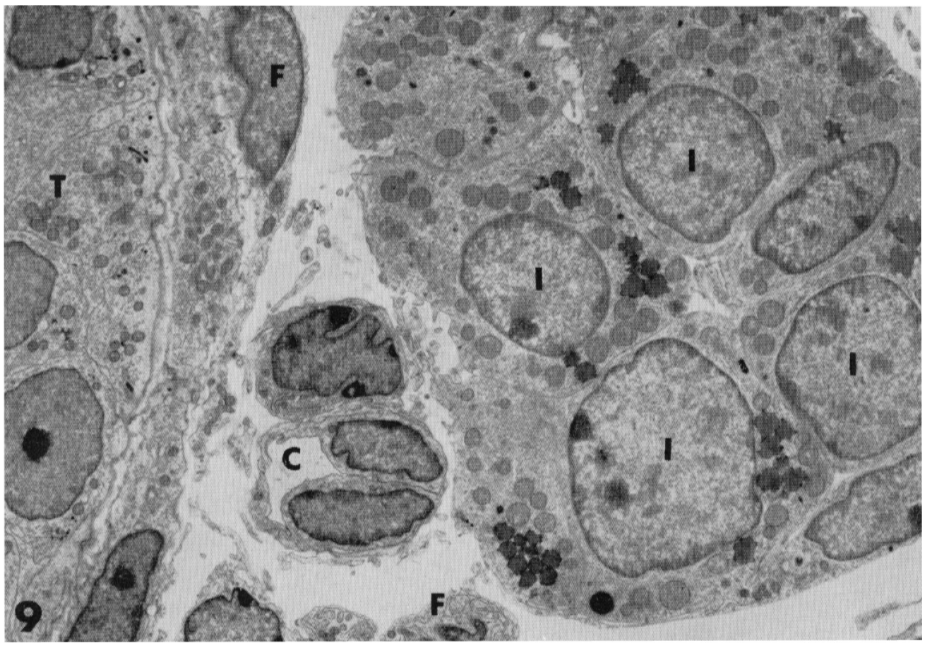
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