

THE NORMAL FINE STRUCTURE OF OPOSSUM TESTICULAR INTERSTITIAL CELLS

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ABSTRACT

The interstitial tissue of the opossum testis includes interstitial or Leydig cells, macrophages, and small cells which morphologically resemble mesenchymal cells. The latter are thought to give rise to mature interstitial cells. The most prominent feature of the interstitial cell cytoplasm is an exceedingly abundant agranular endoplasmic reticulum. This reticulum is generally in the form of a meshwork of interconnected tubules about 300 to 450 A in diameter, but occasionally it assumes the form of flattened, fenestrated cisternae resembling those of pancreatic acinar cells, except for the lack of ribonucleoprotein particles on the surface of the membranes. The interstitial cells vary considerably in their cytoplasmic density. The majority are quite light, but some appear extremely dense, and in addition usually have a more irregular cell surface, with numerous small pseudopodia. These differences may well reflect variations in physiological state. Cytoplasmic structures previously interpreted as "crystalloids" consist of long bundles of minute parallel tubules, each about 180 A in diameter, which seem to be local differentiations of the endoplasmic reticulum. The mitochondria are rod-shaped, and contain a moderately complex internal membrane structure, and also occasional large inclusions that are spherical and homogeneous. The prominent juxtannuclear Golgi complex contains closely packed flattened sacs and small vesicles. The results of the present study, coupled with biochemical evidence from other laboratories, make it seem highly probable that the agranular endoplasmic reticulum is involved in the synthesis of the steroid hormones produced by the interstitial cell. This finding therefore constitutes one of the first functions of the agranular reticulum for which there is good morphological and biochemical evidence.

Testicular interstitial cells, or Leydig cells, are believed to be the major source of the steroid hormones produced by the vertebrate testis. When examined with the electron microscope, their cytoplasm is found to contain an unusually abundant agranular endoplasmic reticulum¹ (16,

9). The present study describes the fine structure of this reticulum in the interstitial cells of the opossum, and compares it with that of the corresponding component of other endocrine and exocrine secretory cells. It is suggested that the remarkable development of cytoplasmic mem-

¹ In this paper, the term "endoplasmic reticulum" will refer broadly to any of the non-Golgi membrane systems in the cytoplasm, whether they be cisternae, tubules, or vesicles. If the membranes bear numerous particles on their outer surface, they are called

granular endoplasmic reticulum, and if there are no adhering particles or very few, they are termed *agranular* endoplasmic reticulum. Although the Golgi elements are often continuous with the endoplasmic reticulum and may constitute part of a

branes in these cells is related to the biosynthesis of steroid hormones. This conclusion is supported by recent biochemical evidence from other laboratories which indicates that the principal enzymes involved in steroidogenesis are located in the microsome fraction. Of the several functions which have been proposed for the agranular reticulum, this appears to be one of the first for which there is complementary morphological and biochemical evidence.

MATERIALS AND METHODS

Opossums, *Didelphis marsupialis virginiana* Kerr, collected in north central North Carolina, were obtained from the Carolina Biological Supply Company. The animals were normal, except for one (Fig. 7) which was treated with human chorionic gonadotropin. Since the treatment had no noticeable effect on the structures illustrated, this micrograph is included with the normals. Bits of testis were fixed in chromate-dichromate-buffered 1 per cent osmium tetroxide (11) adjusted to about pH 7.6, and containing 5 per cent sucrose. The tissues were kept at 4°C. during fixation and subsequent steps until dehydration was completed. After 2 to 3 hours

general system of cytoplasmic membranes, it leads to unnecessary confusion to apply the term "agranular reticulum" to the membranes of the Golgi complex (33, 31, 32), especially in view of the structural complexity sometimes attained by the agranular endoplasmic reticulum.

The current nomenclature is clearly inadequate, since the terms "granular" and "rough" give the unfortunate implication that the membranes which constitute the endoplasmic reticulum are themselves granular or rough. It would be more accurate to refer to the granular reticulum as "endoplasmic reticulum with attached ribonucleoprotein particles," or some similar expression. But this is unwieldy, and we have thus chosen to use conventional terms, despite their shortcomings. Future developments may permit a more precise terminology based both on functional considerations and on more complete information as to the structural interrelationships of the various membrane systems of the cytoplasm.

fixation the tissues were washed briefly in water to remove excess dichromate, and were then postfixed in two 5-minute changes of 10 per cent neutral formalin (12). After ethanol dehydration, the tissues were infiltrated and embedded in a mixture of methyl and *n*-butyl methacrylates (1:9), catalyzed with 2 per cent Luperco CDB, and prepolymerized. Polymerization was completed at 60°C. Thin sections were cut on a Porter-Blum microtome, and were examined with an RCA EMU-3 electron microscope, or with a Siemens Elmiskop I. In some cases, sections were stained with lead hydroxide (44). Figs. 9 and 11 are from material that was embedded in Epon (23).

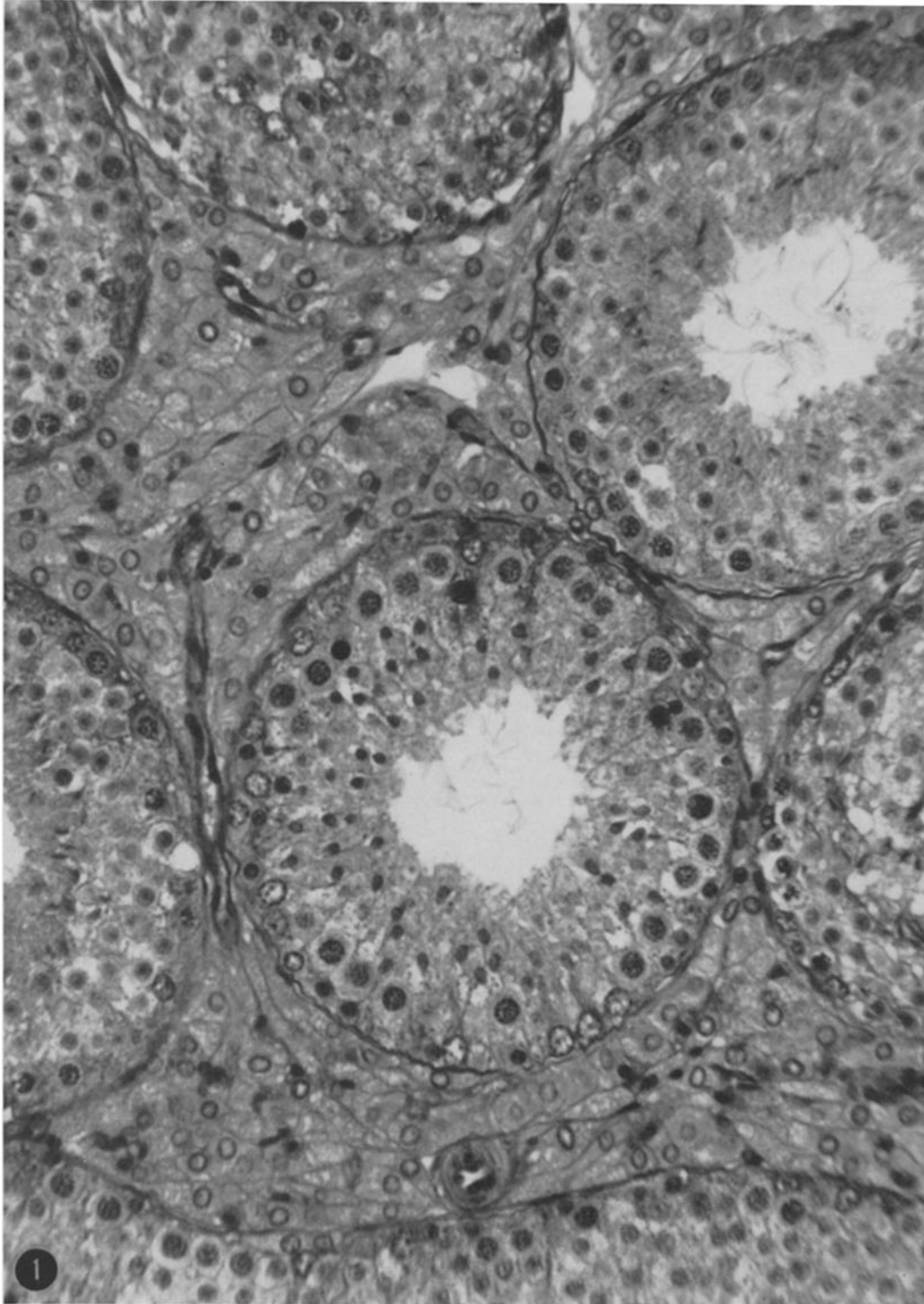
Tissues for examination with the light microscope were fixed in Stieve's fixative or in 10 per cent phosphate-buffered formalin. After the latter fixation, some material was subjected to the Perls test for ferric iron (20).

OBSERVATIONS

The opossum testis has an exceptionally abundant interstitial tissue (Fig. 1), composed primarily of large, polyhedral interstitial cells, which are closely apposed to one another and seem to fill the entire interstitial space. These epithelioid cells usually contain one nucleus, but are occasionally binucleate. Their cytoplasm is intensely acidophilic. The detailed morphology of these cells, as far as can be seen with the light microscope, has been described by Duesberg (13). He found the mitochondria to be numerous and rod-like, and they seemed to be more difficult to preserve for light microscopy than those of most other cell types. With appropriate impregnation techniques a Golgi apparatus was revealed, usually localized in one area near the nucleus, but often showing perinuclear extensions. A cytocentrum usually containing two centrioles seemed to be closely associated with the Golgi apparatus. In contrast to the interstitial cells of many other species, lipid droplets were uncommon and lipofuscin pigment seemed to be completely absent. Two kinds of crystalloids were described by Duesberg, one short and thick, with blunt ends, and the other thin, rod-like, and of varying length, pointed at

FIGURE 1

This photomicrograph of opossum testis shows the abundance of the interstitial tissue in this animal. This tissue, lying between the seminiferous tubules, is made up primarily of large interstitial or Leydig cells, which are believed to be the source of male steroid hormones. $\times 350$.



both ends. The former tended to occur singly, while the latter were less common and tended to be multiple. Nothing corresponding to the endoplasmic reticulum was visualized with the light microscope.

When thin sections of opossum testis are examined with the electron microscope at relatively low magnification (Fig. 2), the interstitial cells are seen to vary considerably in their density, some appearing light and others quite dark (Figs. 2 and 8). The basic similarity of the fine structure of all the cells, and the occurrence of cells of intermediate density, suggest that their diverse appearance reflects different phases of physiological activity of the same cell type, rather than the existence of distinct cell types. The following description of the cytology of the interstitial cells will be based mainly upon micrographs of the more numerous lighter cells.

The interstitial cells are not in intimate contact over most of their surface, as are typical epithelial cells. Instead they are rather loosely aggregated, with angular interstices between adjacent cells. Although generally polygonal in shape, the cells are far less regular in outline than they seem to be in histological sections. Their free surfaces often show numerous small pseudopodia. Such evidence of surface activity is particularly conspicuous on the darker cells (Fig. 8). The nucleus of the interstitial cells is large and regular in outline and has a compact nucleolus, which is not as prominent as in the interstitial cells of other species. In other respects the nuclear fine structure is in no way unusual, and deserves no detailed description.

The most striking cytological feature of the interstitial cells is the extraordinary abundance of their cytoplasmic membranes (Fig. 3). These

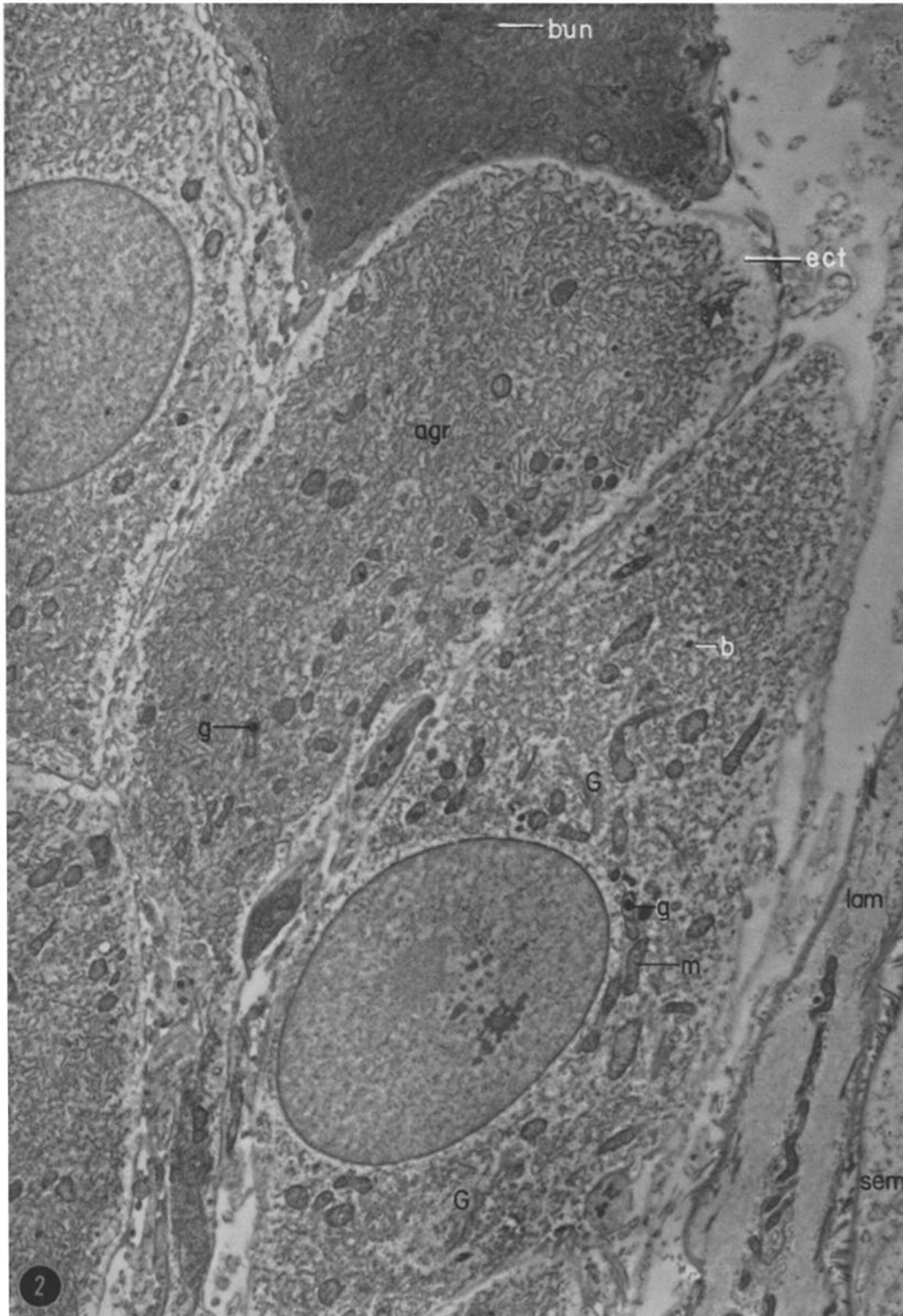
are usually in the form of a close-meshed agranular endoplasmic reticulum, which occupies the greater part of the cytoplasm, except for the Golgi region and a narrow ectoplasmic zone around the periphery of the cell (Fig. 2). The reticulum may be uniformly distributed or may be concentrated in some areas and sparse in others, giving the cell a mottled appearance when viewed at low magnification. Under the most favorable conditions of preservation, the reticulum consists of freely branching and anastomosing smooth-surfaced tubules, 300 to 450 Å in diameter, with an amorphous content of somewhat greater density than the surrounding cytoplasmic matrix. Occasionally, cells are observed in which most of the reticulum is in the form of flattened, fenestrated cisternae (Fig. 4) resembling those found in acinar cells of the pancreas, except for the lack of ribonucleoprotein particles on their surface. The occurrence of forms intermediate between the usual labyrinthine system of tubules and the parallel arrays of cisternae suggests that the latter arise from the former by rearrangement and coalescence, and that the two forms of the reticulum are freely interconvertible, depending upon local environmental conditions or functional requirements.

The agranular reticulum in the interstitial cells seems to be exceptionally labile, for unusual care must be taken in specimen preparation to retain the tubular or cisternal form of this organelle. The routine procedures for fixation and embedding, which successfully preserve the endoplasmic reticulum in many other cell types, usually result in fragmentation of the interstitial cell reticulum into a mass of empty-appearing vesicles (Fig. 9). In methacrylate-embedded material it was found

FIGURE 2

A low-power electron micrograph showing several interstitial cells, one of which (above) contains cytoplasm of considerably greater density than the others. The most striking feature of the interstitial cells is the abundant agranular endoplasmic reticulum (*agr*), which fills their cytoplasm and is in the form of a network of interconnected tubules. At the periphery of the cells is an ectoplasmic zone (*ect*), which is relatively free of endoplasmic reticulum. The cytoplasm also contains mitochondria (*m*), in which large, homogeneous granules (*g*) sometimes occur (see Fig. 10). Various extensions of the Golgi zone (*G*) are seen around the nucleus. Small, dark bodies (*b*) of unknown nature are also found in the cytoplasm. The denser cell contains an oblique section through a bundle (*bun*) of minute tubules, but it is difficult to make out detail because of the great density of the cytoplasm.

The edge of a seminiferous tubule (*sem*) is seen at lower right, and is flanked by one of the cells (*lam*) which contribute to the lamina propria of the tubule. $\times 6,200$.



that more consistently satisfactory preservation was achieved with chromate-dichromate-buffered fixative, followed by formalin postfixation, than with fixatives buffered with acetate-veronal or *s*-collidine (4). An Epon embedding technique (23) was tried several times, using each of the three fixatives specified above, but was generally unsuccessful in maintaining the form of the reticulum, although the over-all quality of preservation was otherwise very good. In the two micrographs of Epon-embedded material, Fig. 11 shows swelling of the reticulum, and Fig. 9 shows more extensive swelling into empty-appearing vesicles. The latter occurred most commonly. It is difficult to understand how tubules that have been fixed can swell and break down into vesicles, but this would appear to happen, since the techniques were the same except for the embedding medium.

Although particles are absent on the membrane surfaces of the agranular reticulum, particles presumed to be ribonucleoprotein do occur in small numbers in the cytoplasm between the tubules or cisternae (Fig. 4).

The mitochondria are generally rod-shaped (Figs. 2 and 5), but branching and cup-shaped forms (10) (Fig. 11) are not infrequent. Their diameter averages about half a micron, and the length of their profiles as seen in section is usually 2 or 3 microns, but in exceptional cases may extend to more than 5 microns. The internal membrane structure is relatively simple, consisting of irregularly oriented short cristae and slender tubules. The small granules commonly seen in the matrix of mitochondria are not present, but larger spherical inclusions are not uncommon (Figs. 2 and 10). These are similar in appearance to the dense globules found by Nilsson (27) in mitochondria of the uterine epithelium of estrous mice. They have the osmiophilia and homogeneous character of lipid, but their actual chemical nature is unknown.

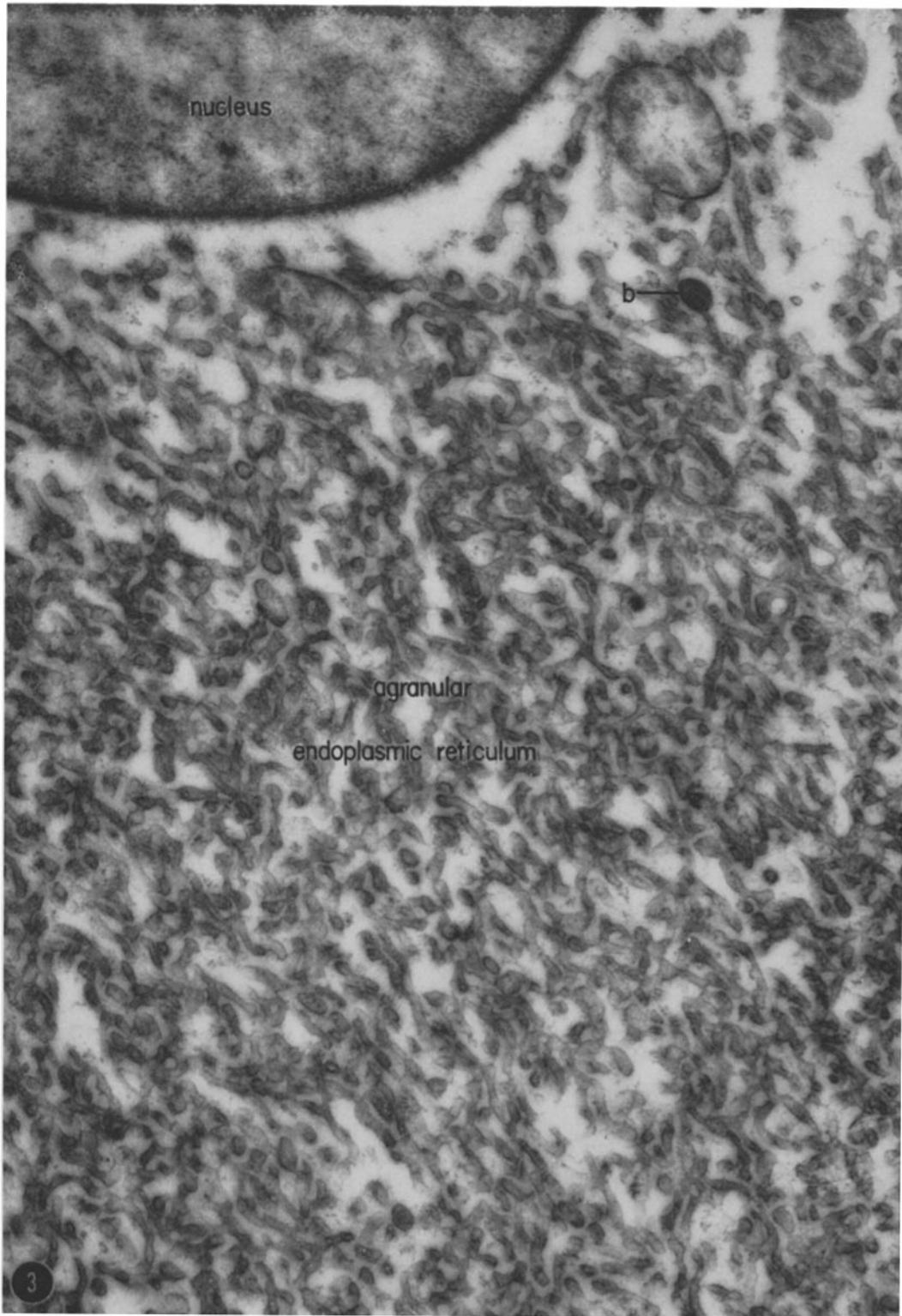
Adjacent to the nucleus is a prominent centrosomal region (Fig. 5), which is relatively free of agranular reticulum, but contains a pair of

centrioles and a variable number of Golgi elements that correspond to the dictyosomes of light microscopy. Each of the latter consists of from three to five closely spaced, flattened sacs surrounded by numerous small vesicles about 400 Å in diameter. Similar vesicles are present in smaller number throughout the cell center. Although the bulk of the Golgi complex is located in this region, isolated elements may be found elsewhere, usually in a juxtannuclear position (Fig. 2). This finding is consistent with Duesberg's (13) observation that the Golgi apparatus occasionally extends around the nucleus.

The structures in the cytoplasm previously described as "crystalloids" (13) are found not to have a true crystalline organization. In electron micrographs of low magnification they have the appearance of arrays of slender filaments oriented more or less parallel to one another. Upon examination at higher magnification (Fig. 6), it becomes evident that they are actually bundles of minute straight tubules about 180 Å in diameter and of indefinite length. The hollow nature of these subunits is clearly seen in cross-sections (Fig. 7), where as a rule each has a dense annular profile and an interior of low density. In some cases, however, the cross-section is shaped like a figure eight (*d*, Fig. 7). Such doublets are strongly reminiscent of the nine peripheral fibers found in the interior of cilia. In longitudinal sections, adjacent parallel tubules are often connected laterally by delicate bridges (Figs. 6 and 7). There are also occasional clear examples of continuity between these minute tubules and neighboring elements of the agranular reticulum (Fig. 6). The existence of such communications suggests that the bundles of straight tubules represent an unusual local differentiation of the endoplasmic reticulum. The bundles of minute tubules vary considerably in the number of their constituent tubules, in the closeness of their packing, and in their over-all length. Not all interstitial cells contain them. In those that do, they may form a single large bundle or multiple smaller ones. These two configurations

FIGURE 3

A view at higher power of interstitial cell cytoplasm, showing the elaborate meshwork of interconnecting tubules which constitutes the agranular endoplasmic reticulum. There is biochemical evidence indicating that this reticulum is involved in the biosynthesis of the steroid hormones secreted by these cells. At the upper right is one of the small bodies (*b*) commonly seen in the cytoplasm. $\times 33,000$.



were probably what gave Duesberg (13) the impression that there were two different kinds of "crystalloids." The bundles are often quite loosely organized, with their tubules occurring in groups of varying size interspersed with larger elements of the endoplasmic reticulum (Fig. 7). In these loose aggregations, seen in cross-section, the tubules appear to be randomly arranged. In the more compact bundles, however, they occasionally exhibit square packing (Fig. 9), instead of the hexagonal pattern one would expect from close ordering of cylindrical structures. In such instances, the small bridges between adjacent tubules are quite prominent, and it is possible that they may play a role in maintaining this unusual pattern.

Small spherical or ovoid bodies about 0.2 micron in diameter are frequently encountered in the cytoplasm (Figs. 2, 3, and 5). They are usually limited by a single membrane, and contain vesicles, tubules, or lamellae embedded in a dense matrix. The significance of these bodies and their possible relationship to the "microbodies" (39, 2) and "multivesicular bodies" (43) of other authors is not clear.

In addition to the Leydig cells, the interstitium of the adult testis contains macrophages and relatively undifferentiated persisting mesenchymal cells which are believed to be capable of developing into typical Leydig cells. In electron micrographs of opossum testis, these primitive cells (Fig. 12) have a large nucleus and relatively scant cytoplasm containing a few mitochondria, a small Golgi complex, and some free ribonucleoprotein particles, but little or no endoplasmic reticulum. They thus appear less differentiated than typical fibroblasts. Occasional cells of this kind do show somewhat more cytoplasm and areas of agranular reticulum. These are tentatively

interpreted as early intermediate stages in the differentiation of the primitive cells into Leydig cells.

Macrophages occur commonly among the interstitial cells (9), and may be confused with them when examined with the light microscope. They are easily distinguished in electron micrographs, however, by the different character of their endoplasmic reticulum, by their numerous large granular inclusions, and by the presence of ferritin in their cytoplasmic matrix. A more detailed description of their fine structure is not pertinent here. Their phagocytic nature was established by examining with the light microscope testis tissue from animals injected with trypan blue. Furthermore, the Perls test for ferric iron, which gives a blue reaction with ferritin, was positive in the macrophages and not in the interstitial cells. Although Duesberg (13) described secretory granules occurring in some opossum Leydig cells, we were unable to corroborate this observation, and it seems probable that he mistook some cytoplasmic granules of macrophages for secretory products in the endocrine cells.

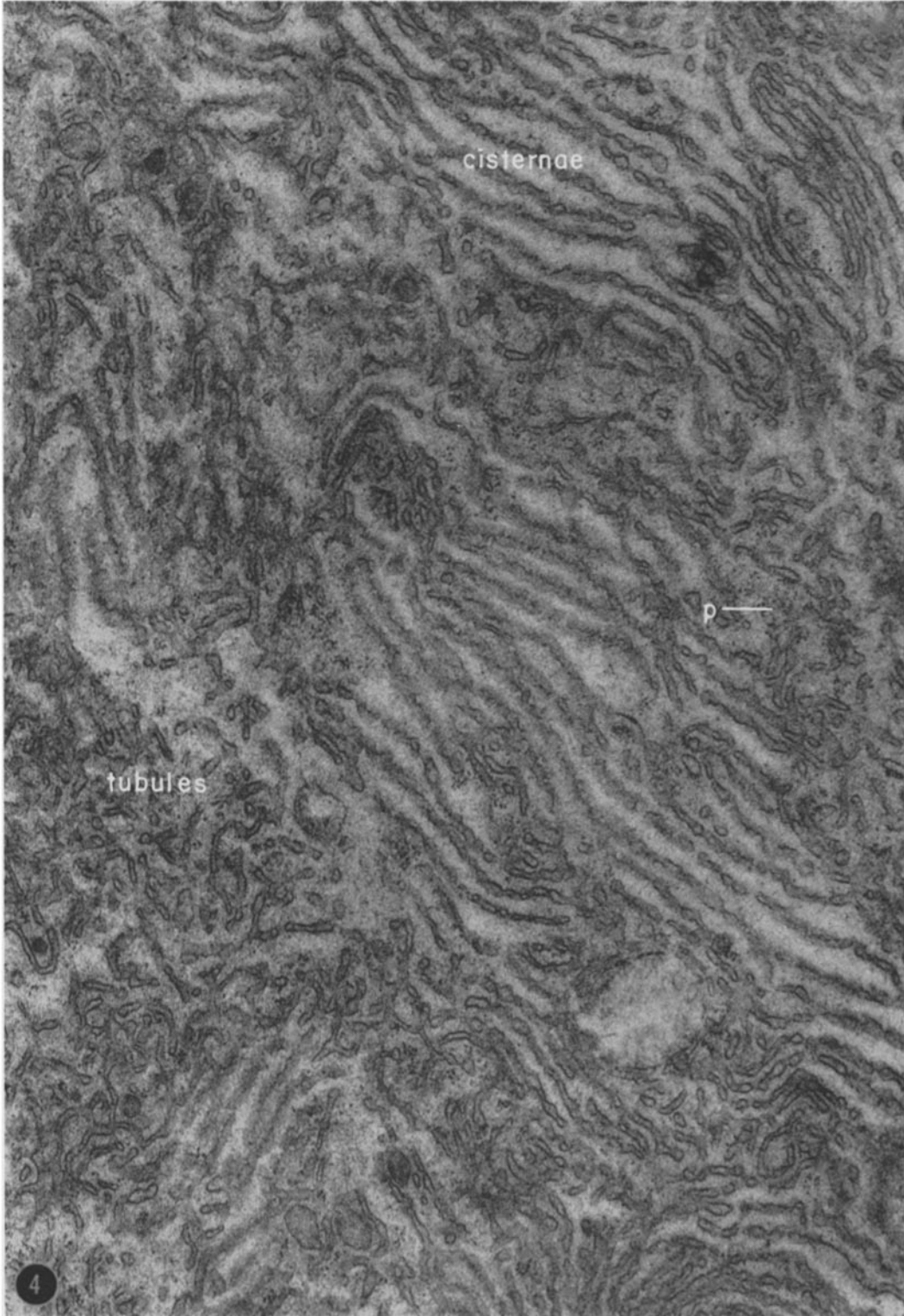
DISCUSSION

Cytologists assigned to the basophilic substance of the cytoplasm an important role in the secretory activity of cells long before modern cytochemical and biochemical methods established its chemical nature. When it became technically feasible to bring the higher resolution of the electron microscope to bear upon this constituent of cells, it was found that membrane-limited tubules or parallel cisternae of what we now know as endoplasmic reticulum were usually concentrated at the same sites that exhibited the most marked basophilia and the greatest specific ultraviolet absorption. Al-

FIGURE 4

The agranular endoplasmic reticulum may occasionally take the form of flattened, fenestrated cisternae resembling those found in acinar cells of the pancreas, except for the lack of ribonucleoprotein particles on the surface of the membranes. At lower left the reticulum is still in the tubular configuration. Micrographs showing intermediate stages indicate that the reticulum may pass from one form to the other.

A few small particles (*p*), presumed to be ribonucleoprotein, are often seen free in the cytoplasm between the tubules or cisternae, and are particularly evident in this micrograph. These particles are not attached to the membranes, and their primary function is thought to be that of supplying the cell's protein needs. There is biochemical evidence that the particles do not have a direct part in the synthesis of the steroid hormones. $\times 33,000$.



though initially it was thought that the membranes themselves or their content might be responsible for the basophilic staining and specific absorption observed in these areas with the light microscope, it was subsequently concluded that the affinity for basic dyes resides in a small particulate component of the cytoplasm closely associated with the surface of the membranes (28). The granules were later isolated and identified as ribonucleoprotein (30). They have since been the subject of intensive biochemical investigation to elucidate their role in protein synthesis. Studies of the so called granular endoplasmic reticulum have been pursued by both morphologists and biochemists to the neglect of other forms of the reticulum which lack associated ribonucleoprotein particles, but which may nonetheless have equally important functions in the economy of cells.

Attention was early directed to the occurrence in spermatids and Sertoli cells of an endoplasmic reticulum which did not have attached particles (8). Palade (29) subsequently described a number of cell types wherein agranular membranous elements constituted the only or the predominant form of the endoplasmic reticulum. These included leucocytes, mast cells, adipose cells, parietal cells of the gastric mucosa, cells of the seminal epithelium, and cells of the adrenal cortex. These agranular membranes were often seen to be continuous with elements of the granular endoplasmic reticulum, and it was evident that these were simply two forms of the same basic organelle. An extensive development of agranular membranes in striated muscle has been described by Porter and Palade (36) and others, and designated the "sarcoplasmic reticulum." Tight networks of interconnected tubules similar to those described here in interstitial cells of the testis have been reported in certain cells of the meibomian gland

(31) and of the olfactory epithelium (34), in fetal adrenocortical cells (38), in the pigment epithelium of the eye (37), and in cells of the mouse corpus luteum (45). The widespread occurrence of agranular reticulum in cell types of very different function attests to its importance and suggests that it will be found to have a diversity of functions.

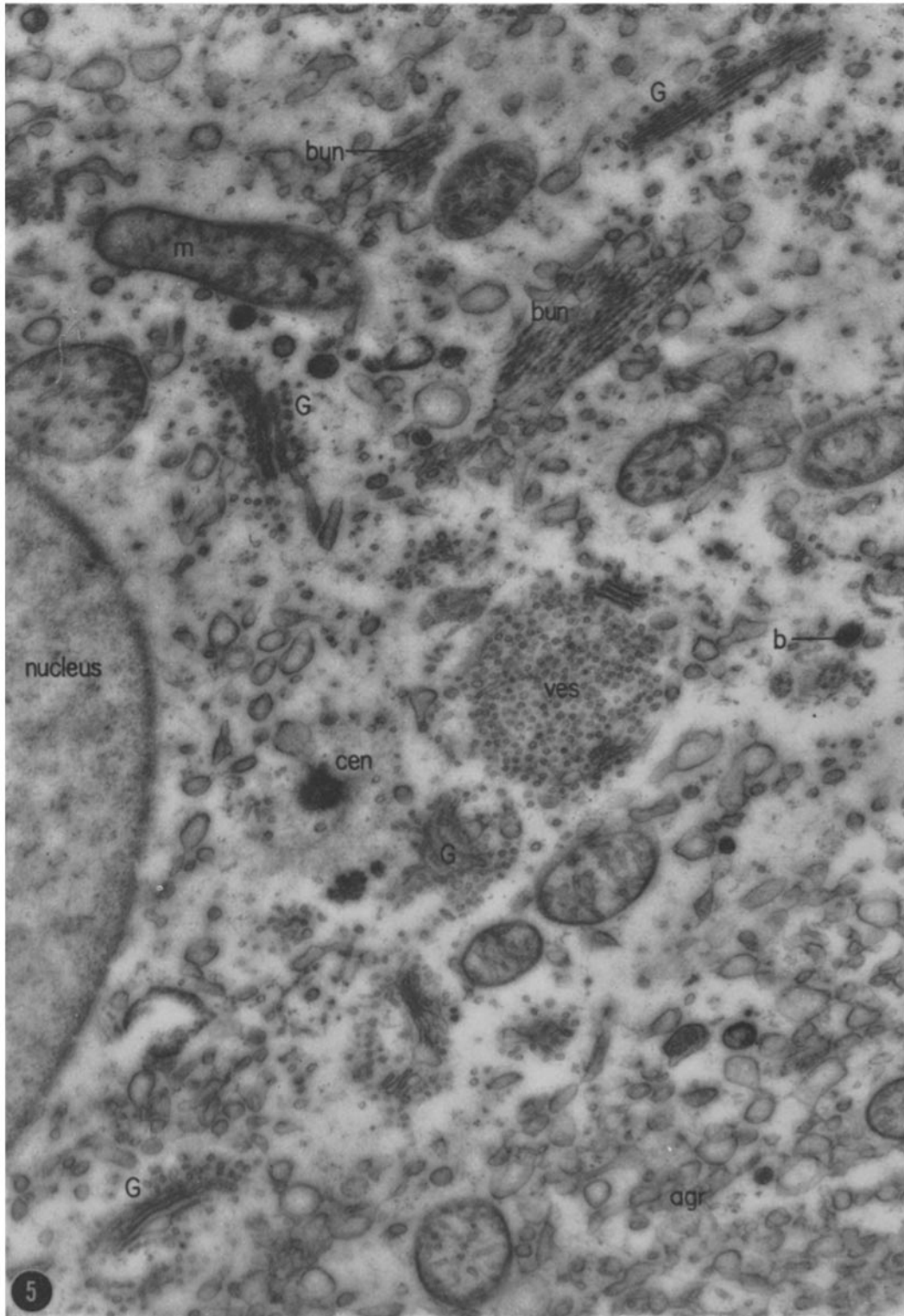
Correlated fine structural and biochemical studies of the kind which established the function of the granular reticulum have not yet been carried out on the agranular reticulum. To date, the functions that have been tentatively attributed to this organelle have been based upon indirect or circumstantial morphological evidence. Fawcett (14), observing masses of agranular reticulum in the livers of animals refeeding after a period of fasting, speculated that this might represent a stage in the regeneration of the granular endoplasmic reticulum. Porter and Bruni (35) have found abundant agranular reticulum in liver cells after experimental inhibition of glycogen synthesis. Interpreting this as a possible compensatory hypertrophy, they speculate that this form of the reticulum may be involved in the glycogen metabolism of the liver cell.

The abundance and complexity of the agranular reticulum in opossum Leydig cells suggests that this organelle may play an important role in the endocrine secretory activity of the interstitial tissue, but morphological observations alone obviously cannot establish the agranular reticulum as the site of synthesis of steroid hormones. However, independent biochemical investigations have already provided evidence which strongly supports this thesis. The current concept of steroid hormone biosynthesis (Fig. 13) includes cholesterol as a precursor of testicular steroids. There is evidence that this cholesterol is made in the testis, and not

FIGURE 5

This micrograph shows a juxtannuclear Golgi area, and within it one of the centrioles (*cen*). Scattered through the area are several Golgi elements (*G*), consisting of stacks of from three to five flattened sacs, surrounded by small vesicles about 400 Å in diameter. A cluster of these vesicles (*ves*) can be seen near the center of the field. The agranular endoplasmic reticulum (*agr*), seen in the surrounding cytoplasm, is sparse within the Golgi area.

The internal structure of the mitochondria (*m*) is not very highly developed, and consists of tubules and short cristae. Several small, dense bodies (*b*) are present. In the upper part of the field are oblique sections through two small bundles of minute tubules (*bun*). $\times 25,000$.



derived from the plasma (25). Bucher and McGarrahan (6) have shown that cholesterol can be synthesized from labeled acetate by the combined microsomal and soluble fractions of rat liver homogenates, the rate-limiting reaction occurring in the microsomal fraction (7). Lynn and Brown (24) have found that the enzymes which convert progesterone into androstenedione are located in the microsomal fraction of guinea pig and rat testis homogenates. The testis is known to produce some estrogens, and Baggett *et al.* (1) have demonstrated that estrogenic steroids can be synthesized from testosterone by homogenates of stallion testis and of human placenta. In the latter tissue, Ryan (40) found that the androgen to estrogen conversion occurs in the microsomal fraction. The usual microsomal fraction is known to consist predominantly of vesicular fragments of the endoplasmic reticulum, bearing on their outer surface ribonucleoprotein particles whose number depends on the character of the reticulum in the tissue homogenized. Although ribonucleoprotein granules are necessary for protein synthesis, it is unlikely that such particles are appreciably involved in the steroid syntheses cited above, since in each case the enzymes concerned are stable to ribonuclease digestion at concentrations well above those which inhibit protein synthesis by homogenates of liver or pancreas. It has further been shown that in the case of cholesterol synthesis by rat liver homogenates (6), the early microsomal subfractions, containing a high percentage of membrane vesicles, are more active than later microsomal subfractions, consisting mostly of ribonucleoprotein particles. It thus appears that

most of the insoluble enzymes involved in the synthesis of testicular steroids are associated with the membranous component of the microsomal fraction.

It is not possible from the biochemical data on cell fractions to distinguish clearly between a situation in which one is dealing with an agranular endoplasmic reticulum, and one where there is a granular reticulum in which the enzymes are associated only with the membranes. The latter seems to be a possibility in the case of cholesterol synthesis by the liver, for a considerable increase in granular reticulum has been found in hepatic cells stimulated to very high levels of cholesterol production (22, 21). In the interstitial cells of the testis, however, electron micrographs leave no doubt that the reticulum is agranular in opossum, man (16), albino rat (9), guinea pig (15), and the fish *Lebistes* (17). The major contribution of testicular interstitial cells to the microsomal fraction would therefore consist of small vesicles resulting from the fragmentation of the agranular endoplasmic reticulum. It seems justifiable to conclude from the available biochemical evidence and from the observations on the fine structure of the interstitial cells that the membranes of the agranular reticulum in this cell type are involved in the biosynthesis of steroid hormones.

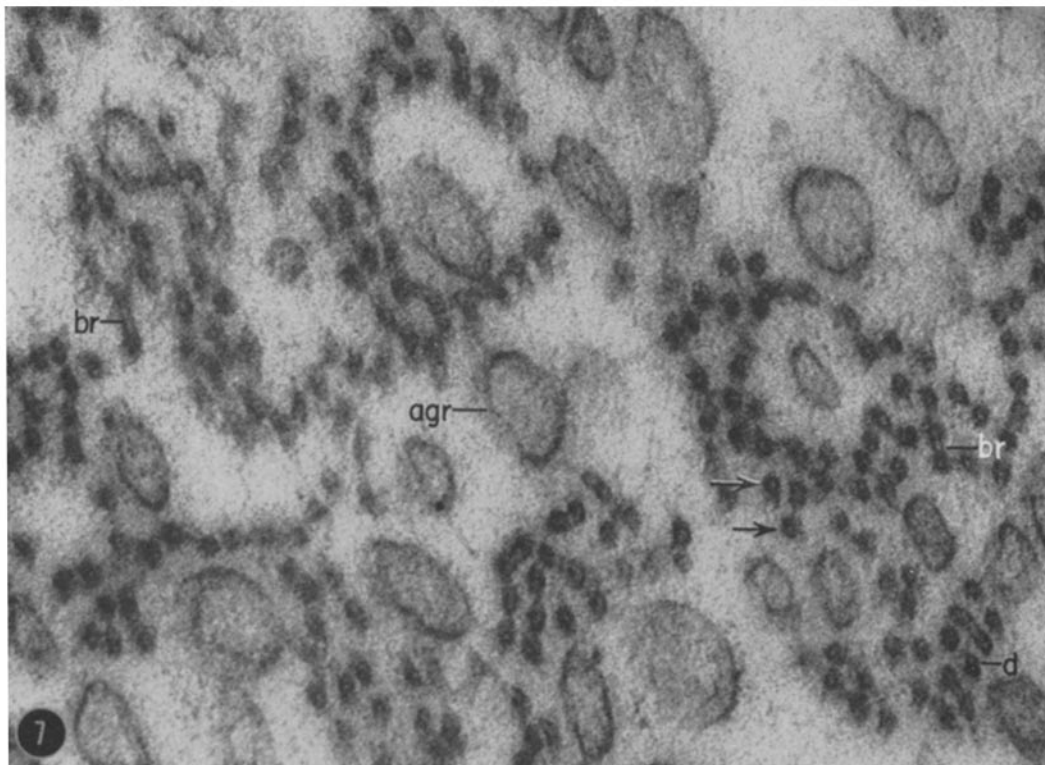
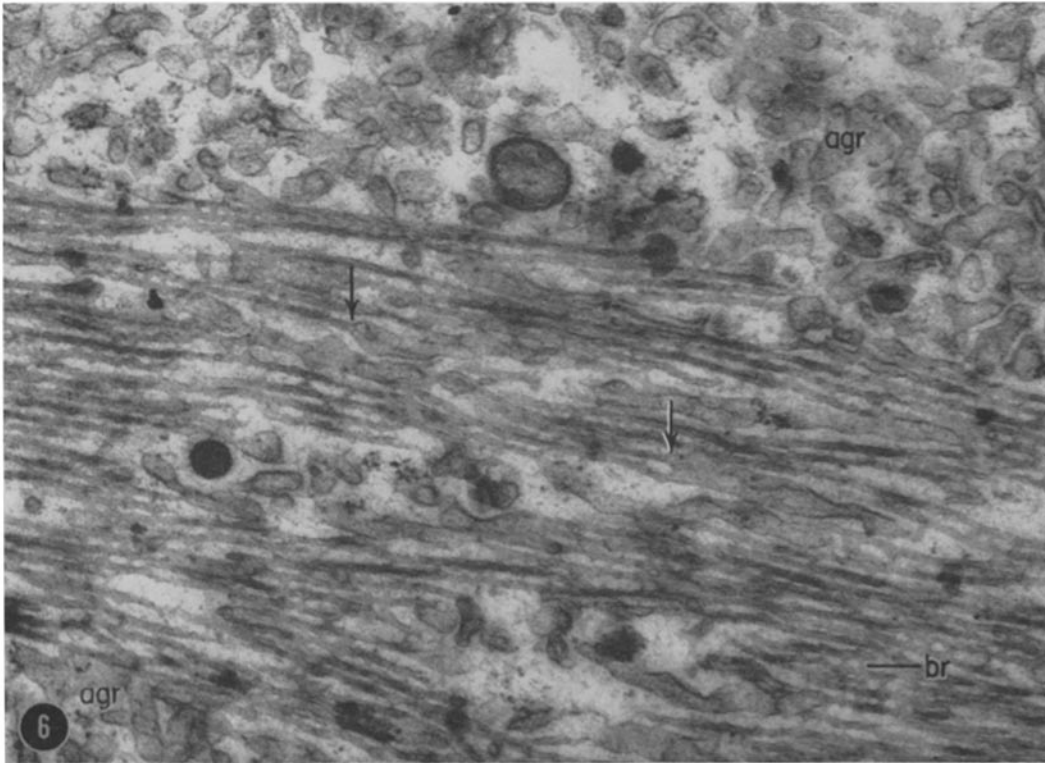
Dark cells have been described in many organs in stained preparations studied with the light microscope, and it has long been a subject of debate whether these differences in staining were real or artifactual. This controversy remains unresolved. The dense cells observed in electron micrographs of interstitial tissue do not seem to be

FIGURE 6

A longitudinal section through one of the bundles of parallel minute tubules which occur in the cytoplasm of some interstitial cells. The tubules are interconnected along their length by frequent bridges (*br*). In certain places (arrows), the minute tubules appear to be continuous with elements of the agranular endoplasmic reticulum (*agr*), which extend in among them from the surrounding cytoplasm. The bundles may thus constitute a local differentiation of the agranular reticulum. $\times 38,000$.

FIGURE 7

Cross-section of groups of minute tubules occurring within a large bundle. Elements of the agranular reticulum (*agr*) are interspersed. The minute tubules, about 180 Å in average diameter, are clearly seen to be hollow (arrows), with a dense annular profile and an interior of low density. Included in the section are several bridges (*br*) between tubules. The tubules at times show a doublet structure (*d*) in cross-section. $\times 115,000$.



the result of artifact. There is no reason, however, to assume that cells that are dense in electron micrographs correspond to cells that are dark in stained histological sections. Therefore, in referring to the conspicuous variations in density of the interstitial cells in electron micrographs, we have intentionally avoided designating them as "dark cells" and "light cells," even though these terms accurately describe their appearance. The use of such terms would imply the existence of two distinct categories of cells, whereas, in fact, cells of all intermediate degrees of density can be found. The variations may reflect different phases in a cycle of physiological activity of a single cell type, rather than the existence of distinct cell types. The extraordinary density of some of the cells is due in part to exceptionally high concentrations of

cytoplasmic membranes, but some of the increased density also resides in the cytoplasmic matrix. It is possible that increased concentration of steroids dispersed throughout the cytoplasm of these cells might contribute to their greater osmiophilia. These dense cells also show evidence of greater activity of their surface, which may possibly be associated with release of their product. Similar variations in cytoplasmic density have been described in electron microscope studies of steroid-secreting cells of the adrenal cortex (18) and corpus luteum (19, 45).

Extensive biochemical research has made it appear that most of the known vertebrate steroid hormones may be synthesized by means of a common pathway, outlined in Fig. 13 (adrenal cortical steroids are derived predominantly from

FIGURE 8

The cytoplasm of three interstitial cells, showing the variation in cytoplasmic density. It will be noted that the increased density of the cells at left and below is due not only to a greater concentration of cytoplasmic membranes, but also to an increased density of the background cytoplasmic matrix. Several pseudopodia (*ps*) extend into the intercellular space, and from the density of their matrix are clearly derived from the denser cells, which characteristically show greater surface activity. The diverse appearance of the lighter and denser cells is thought to reflect different phases of physiological activity of the same cell type. \times about 20,000.

FIGURE 9

A cross-section of minute tubules in the closely packed pattern which is occasionally observed. It is possible that bridges (*br*) between the tubules aid in maintaining the square packing array.

Although the minute tubules seem well preserved in this micrograph, the agranular endoplasmic reticulum has fragmented and swollen into empty-appearing vesicles. Epon embedding technique (23). \times 37,000.

FIGURE 10

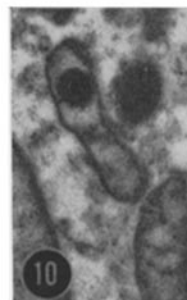
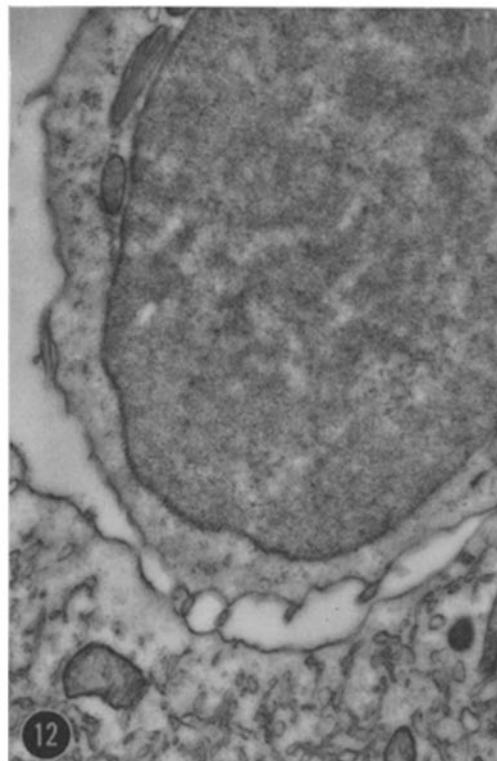
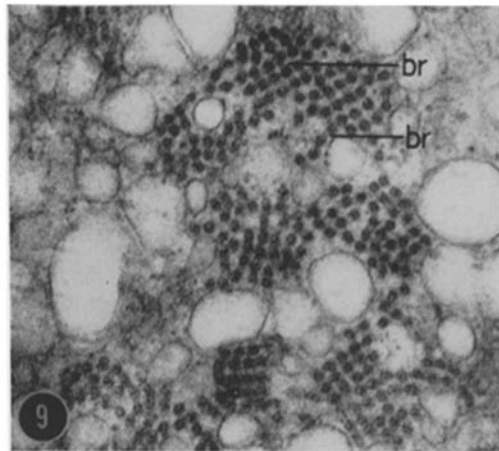
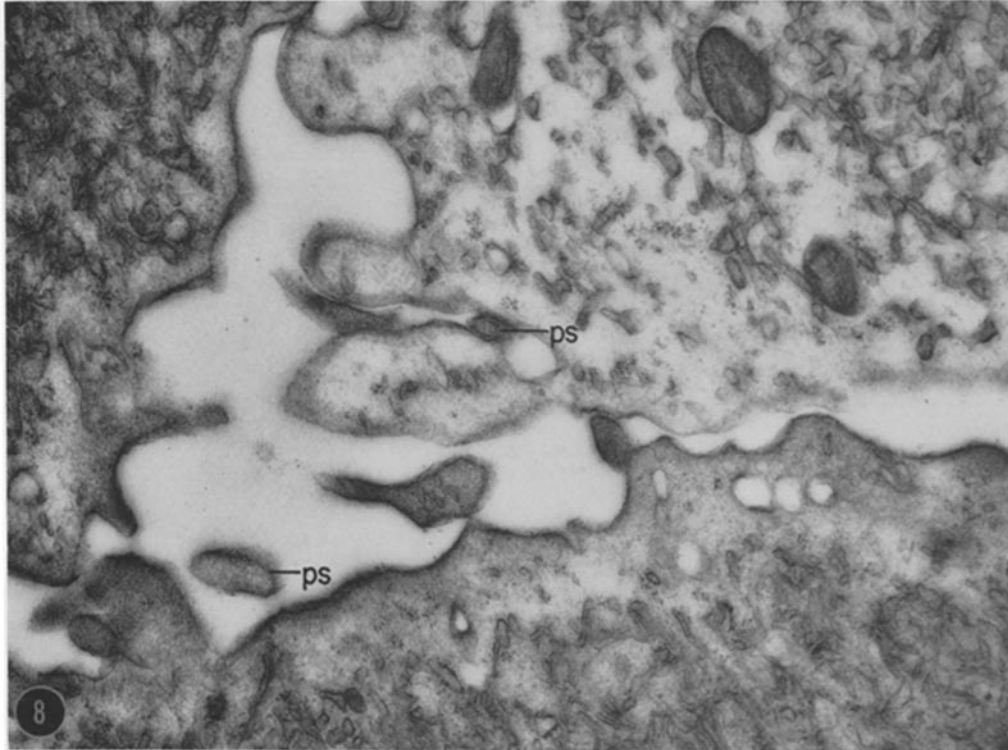
The mitochondria often contain large, spherical inclusions which show the osmiophilia and homogeneity of lipid, although their actual chemical nature is unknown. This micrograph is an enlargement of one of the mitochondria labeled *g* in Fig. 2. \times 18,000.

FIGURE 11

A section through two concentric cup-shaped mitochondria (10). The outer cup is sectioned horizontally, the inner one obliquely. Four other cup mitochondria were visible in the same section of this cell. Epon embedding technique (23). \times 14,000.

FIGURE 12

One of the relatively undifferentiated cells which occur commonly in the interstitium, and which in their fine structure resemble mesenchymal cells. They are believed to give rise to mature interstitial cells. The cytoplasm seen below is that of an adjacent interstitial cell. \times about 15,000.



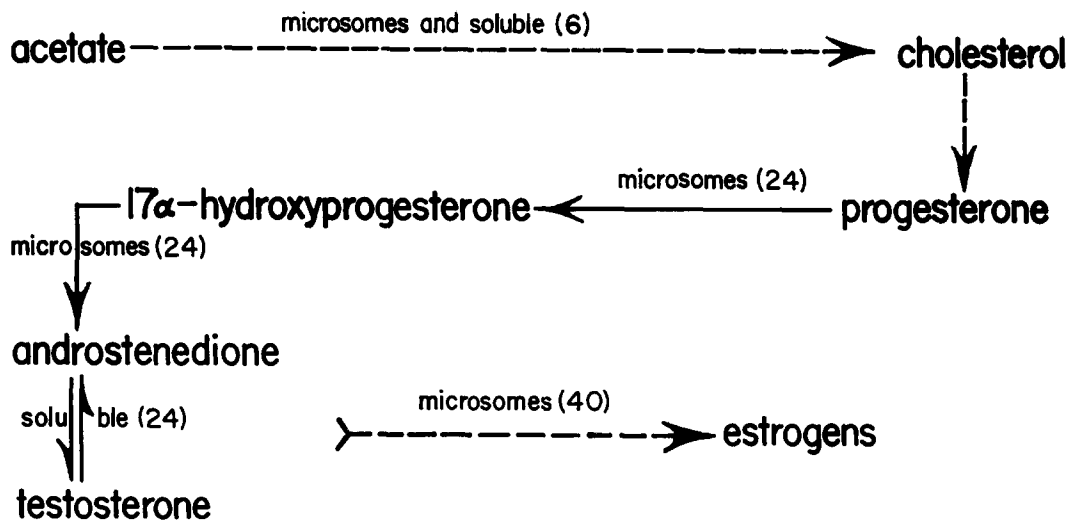


FIGURE 18

This simplified scheme of the biosynthesis of major steroid products of the testis indicates in what homogenate fractions the enzymes have been found. The contribution of testicular interstitial cells to the microsome fraction would consist predominantly of membrane vesicles resulting from the fragmentation of the abundant agranular endoplasmic reticulum. The cell contains only a small quantity of ribonucleoprotein particles, which, judging from biochemical evidence, probably play no appreciable role in the biosynthesis (see Discussion). Numbers indicate references in the bibliography, the data deriving as follows: Reference 6, rat liver; reference 24, guinea pig and rat testes; reference 40, human placenta.

progesterone and 17α -hydroxyprogesterone). It seems somewhat unlikely that the enzymes involved in this common pathway would be located in entirely different sites within the various cell types that secrete steroid hormones. If the agranular reticulum is involved in the biosynthesis of androgens in the interstitial cells, one might expect to find a similar development of this organelle in other steroid-secreting organs, such as the adrenal cortex, corpus luteum, or theca interna of the Graafian follicle. This is indeed the case in human fetal adrenal cortical cells (38), in lutein cells of the mouse corpus luteum (45), and in ovarian interstitial cells in mice (26). However, in the majority of electron microscope studies of the adrenal cortex, the endoplasmic reticulum has been reported to be poorly developed, and to consist of individual vesicles (46, 18). In the present study, when preservation was not optimal the reticulum of interstitial cells broke up into vesicles like those generally described in the cells of the adrenal cortex. The great difficulty encountered in preserving the continuity of the reticulum by routine methods of specimen prepara-

tion suggests to us that the cytoplasmic membranes of the interstitial cells may have properties quite different from those of other cell types where they are readily preserved by these same routine methods. It is conceivable that this unusual instability of the cytoplasmic membranes may apply also to other steroid-producing organs, and that by appropriate techniques of preservation it may prove possible to demonstrate a more or less continuous agranular reticulum in these cases.

The mitochondria in the adrenal cortex are found to be large and numerous, and to have an atypical internal structure consisting of tubules or vesicles instead of cristae (3). Investigators of the fine structure of the adrenal cortex have been inclined to implicate these unusual mitochondria in the synthesis of corticosteroids, rather than the cytoplasmic membranes. Some have suggested that the mitochondria are transformed into lipid droplets containing the hormone (18), but others (2) deny this and suggest instead that both mitochondria and lipid droplets arise from small, dense bodies that are morphologically similar to those described in the present study. Still others

believe that the secretory product accumulates within the vesicles or tubules of the mitochondria, and subsequently passes out into the vacuolar system of the cytoplasm (42). Although at least one of the late steps in corticosteroid biosynthesis is found in the microsome fraction (41), there is evidence that oxidation of the 11 position of the steroid nucleus, one of the last steps in the synthesis of some of the most important corticosteroids, is carried out by enzymes that occur in mitochondria of the bull adrenal cortex (5). Thus it may prove to be true that in the adrenal cortex both the endoplasmic reticulum and the mitochondria play important roles in the biosynthesis of steroid hormones. In testicular interstitial cells, on the other hand, the small number and relative structural simplicity of the mitochondria do not suggest that they have any significant role in the synthetic activity, other than as an energy source, and the biochemical data seem to bear out this impression.

In the cells of exocrine glands where there is an elaborate development of the granular endoplasmic reticulum in relation to protein secretion, the synthesis apparently takes place at the ribonu-

cleoprotein particle; there is no evidence that the membranes themselves participate. The function of the membrane-limited system of tubules and cisternae seems to be to convey the product to the Golgi region, where it is concentrated and segregated in vacuoles for temporary storage. In the interstitial cells described here, no secretory droplets or granules accumulate in the cytoplasm, and the Golgi complex, although well developed, shows no sign of receiving or concentrating the product. Although it is conceivable that the reticulum may be concerned with intracellular transport, the membranes probably have as their major function the abundant synthesis of the steroid product. Studies of the fine structure of these cells gave no clues as to the intracellular localization of the hormone or the mechanism of its release.

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