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Changes in Testicular Leydig Cells and in Plasma Testosterone Levels Among Seasonally Breeding Rock Hyrax

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Seasonal changes in plasma testosterone levels and in various testicular parameters were studied in 41 adult male rock hyrax (*Procavia habessinica*). Among animals collected during the annual breeding season, testis weights and plasma testosterone levels were approximately five times greater than among animals collected outside the breeding season. Light microscopic measurements showed that increase in testis weight was largely due to enlargement of the seminiferous tubules. Histological sampling techniques indicated no significant change in Leydig cell numbers. Leydig cell size increased during the breeding season, and average Leydig cell volume showed significantly positive correlation with plasma testosterone level. With the electron microscope, Leydig cell hypertrophy was seen to involve changes in quantity and structure of several cytoplasmic constituents. Lipid droplets disappeared and smooth endoplasmic reticulum (SER) spread dramatically as the cells increased in size. In contrast to the sparse and heterogeneous assemblage of irregularly tubular and cisternal SER seen in nonbreeding animals, the extensive masses of SER in breeding animals appeared as relatively straight, unbranched tubules of uniform diameter. Peculiar membranous structures, possibly derived from the SER, were abundant in the periphery of Leydig cells from animals with high plasma testosterone levels. These findings suggest that there is a definite relationship between plasma testosterone levels and Leydig cell fine structure in seasonally breeding hyrax.

INTRODUCTION

In recent years, information regarding the location of enzymes in cell fractions from the testis has permitted the assignment of various biochemical steps in testosterone synthesis to cell organelles such as the smooth endoplasmic reticulum (SER) and mitochondria (see Christensen and Gillim, 1969). In spite of this significant progress, much remains to be learned about testosterone synthesis and secretion within the context of intracellular organization. While the role of the SER in steroid metabolism is well established and the prominence of this organelle in testicular interstitial cells has long been documented, functional justification for the diverse forms of SER seen in these cells is still missing. The significance of other organelles, such as the Golgi complex, is either unknown or highly speculative. In contrast to endocrine cells which produce protein hormones, structural evidence of secretory activity is not apparent in Leydig cells (see Fawcett *et al.*, 1969).

As a model system for studying relationships between Leydig cell structure and function, seasonally breeding mammals have much to offer. The light microscopic literature contains numerous reports of dramatic seasonal changes in Leydig cells from a variety of wild mammals. In view of the opportunity for functional correlations that a study of these changes might reasonably provide, it is surprising that so

little attention has been directed toward the fine structure of Leydig cells in seasonally breeding mammals. So far, only Belt and Cavazos (1971) appear to have begun work on this problem.

Another promising approach to the correlation of structure and function in testicular interstitial tissue is electron microscopic study of Leydig cells known to be secreting measured amounts of testosterone. However, most existing studies of the fine structure of Leydig cells have been performed without knowledge of the cells' activity in testosterone production. Only a few studies have included experimental manipulations that may have influenced the secretion of hormone by Leydig cells (Nishimura and Kondo, 1964; Hatakeyama, 1965; Schwarz and Merker, 1965; Murakami and Tonutti, 1966; deKretser, 1967; Merkow *et al.*, 1968a,b; Aoki, 1970). With the exception of deKretser's (1967) study, there is no direct evidence that the experimental manipulations, usually human chorionic gonadotropin (HCG) administration, have affected steroid secretion. deKretser (1967), who measured plasma testosterone levels in three oligospermic men before and after gonadotropin therapy, found certain changes in Leydig cell structure which accompanied a rise in plasma testosterone.

To the extent that plasma testosterone reflects Leydig cell function (Lipsett *et al.*, 1966), this relatively accessible parameter offers a valuable basis for correlative studies of Leydig cell fine structure. Recently, we have had the opportunity to perform such a correlative study on an African mammal, *Procavia habessinica*, which exhibits discrete periods of male reproductive activity accompanied by striking changes in testicular structure and in circulating testosterone levels.

MATERIALS AND METHODS

Between April, 1970 and November, 1971, 41 adult male hyrax (*Procavia habessinica*) were selectively shot at monthly intervals on lava outcroppings in the Rift Valley of central Kenya. Less than 3 min after an animal was shot, blood from the carotid arteries was collected in plastic bottles and immediately put on ice. Within 24 h, the blood samples were centrifuged and the plasma collected and stored at −20°C. Within 2 weeks of collection, frozen plasma samples were sent by air express to Searle Scientific Services, High Wycombe, England, where testosterone assays were performed using a competitive protein binding technique following purification by paper chromatography (Mayes and Nugent, 1968). The precision of the testosterone assay was approximately 10%.

Immediately after drawing the blood sample, one testis was fixed for electron microscopy by gentle injection of a formaldehyde–gluteraldehyde–trinitrocresol (FGC) fixative (Ito and Kanovsky, 1968) into the testicular vasculature through a blocked section of the abdominal aorta. Fixative was prevented from reaching the second testis by clamping its spermatic artery with a hemostat. The animal was then weighed to the nearest 0.1 kg and its unfixed testis was removed, dissected free of epididymis, and weighed to the nearest 0.1 g. Cross-sectional slices of this testis were submerged in freshly mixed Bouin's fixative and later processed for light microscopy according to a standardized schedule of paraffin embedding. In addition to Bouin's-fixed samples, testicular slices from some animals were fixed in 5% buffered formaldehyde and later sectioned on a freezing microtome without further preparation.

Five to 10 min after injection of FGC into one testis, an equatorial slice from this organ was minced, immersed in fresh FGC, and stored on ice for 1–6 h. Tissue blocks were postfixed overnight in 1% OsO_4, dehydrated rapidly in alcohols, passed through propylene oxide, and embedded in Epon 812 according to Luft (1961). Thin sections of this material were stained with uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965) and were examined in a Zeiss 9A electron microscope. Thick sections stained with toluidine blue were prepared for light microscopic study.

Quantitative histological study was performed on 0.5 μm Epon sections and 8 μm paraffin sections of testis from each animal, and on 20 μm frozen sections from four breeding and four nonbreeding animals. The majority of measurements of seminiferous tubule diameter and Leydig cell diameter were taken from paraffin sections (at least 40 measurements of each parameter per animal) while at least 20 measurements of each parameter per animal were taken from Epon sections. In spite of the fact that fewer measurements were available from Epon-embedded testis, these were used in constructing Table 1 since relatively little

TABLE 1

COMPARISON OF BODY WEIGHT, VARIOUS TESTICULAR PARAMETERS, AND PLASMA TESTOSTERONE LEVELS IN NONBREEDING AND BREEDING ROCK HYRAX

	Nonbreeding[a] (N = 20)	Breeding[a] (N = 21)	P[b]
Body weight (kg)	2.7 ± 0.1	2.7 ± 0.1	>0.9
Testis weight (g)	6.0 ± 0.3	35.9 ± 2.4	<0.001
Seminiferous tubule diameter (μm)	111 ± 5	222 ± 6	<0.001
Total volume of Leydig cells per testis (ml)	0.14 ± 0.02	0.36 ± 0.03	<0.001
Average volume of a single Leydig cell (ml)	$(1.61 \pm 0.13) \times 10^{-9}$	$(2.98 \pm 0.19) \times 10^{-9}$	<0.001
Total number of Leydig cells per testis	$(93 \pm 10) \times 10^{6}$	$(127 \pm 16) \times 10^{6}$	>0.05
Plasma testosterone level (μg/100 ml)	0.53 ± 0.10	2.71 ± 0.40	<0.001

[a] Values expressed as mean ± standard error of the mean.

[b] P is the probability that the population means of the two samples are identical.

shrinkage occurred during Epon embedding as compared to paraffin embedding. Shrinkage due to paraffin or Epon embedding was assessed in eight testes from which frozen sections were also obtained. Shrinkage, expressed as a decrease in linear dimensions relative to the frozen material, varied from testis to testis. It approached 30% in some paraffin-embedded specimens but remained less than 5% in Epon-embedded material.

Estimates of the total volume of Leydig cells in the testis were made by two applications of Chalkley's (1943) sampling technique. Using a ten-point grid in a 10× ocular, paraffin sections of testis were examined with a 10× objective lens at 40 sites randomly selected by predetermined movements of the microscope stage. By counting the dots distributed over interstitial tissue, the proportion of testis occupied by interstitial tissue was estimated. Using the same ocular grid, interstitial areas of the same sections were examined with the 40× objective lens at a number of randomly selected sites sufficient to accumulate a total of 400 points divided between Leydig cells and other interstitial components. In this way, the proportion of interstitial tissue occupied by Leydig cells was estimated. It should be recognized that potentially unequal shrinkage of various testicular components during paraffin embedding would adversely affect the accuracy of this technique. In paraffin sections, it is difficult to assess changes in linear dimensions of extracellular components of the testis. Epon sections would be more favorable since the intimate and apparently natural relationship of various tissue components in this medium renders unequal shrinkage unlikely. However, the inherently small area of Epon sections causes them to be unsuitable for application of Chalkley's (1943) technique at the level of the light microscope.

OBSERVATIONS

Populations of *Procavia habessinica* in central Kenya exhibit a discrete period of sexual activity once each year. The duration of this activity is approximately 3 months with maximum breeding usually occurring within a 6-week period. Although constant from year to year in a given population, the breeding season varies among geographically isolated populations within the Central Rift Province of Kenya. Striking differences are seen between various reproductive parameters of adult males collected during the breeding season and those of adult males collected during the remaining 9 months of the year (see Table 1). The testes of an average breeding male are almost six times heavier than those of its nonbreeding counterpart. Hypertrophy of the testes largely involves an expansion of the seminiferous tubules with simultaneous stimulation of spermatogenic activity (Figs. 1 and 2). Associated with testicular hypertrophy is a significant elevation of plasma testosterone levels. The relationship between plasma testosterone

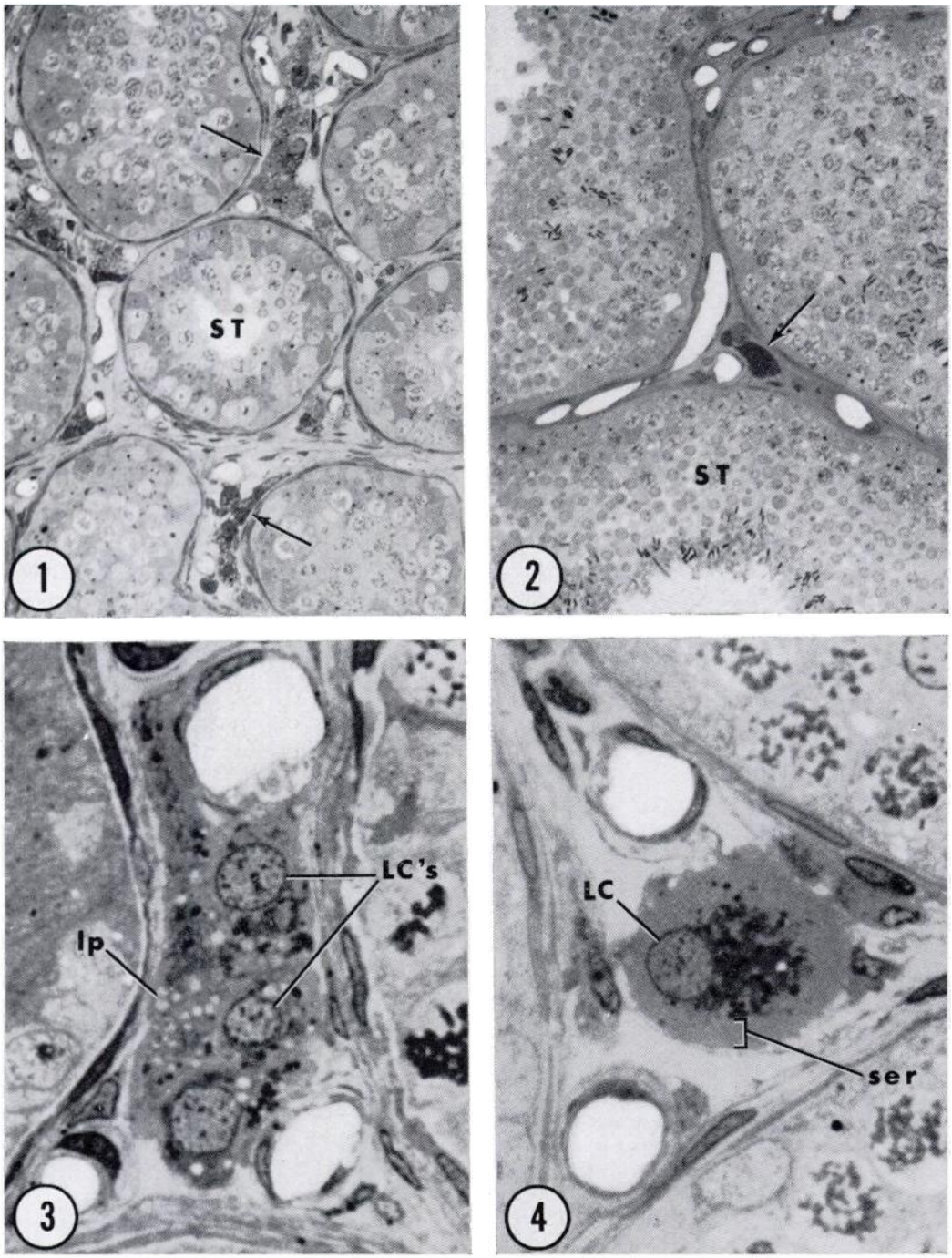


FIG. 1. During nine months of the year when no reproductive activity occurs, *Procavia* testes are relatively small, and show little or no spermatogenesis. A section from the testis of a nonbreeding animal is seen here. Note the relatively small diameter of the seminiferous tubule (ST) and the scarcity of spermatids in the germinal epithelium. Clusters of Leydig cells (at arrows) can be seen in the interstitial tissue, ½ μm Epon section stained with toluidine blue, PC 112, 6.3 g testis, 0.42 μg/100 ml plasma testosterone. ×200.

FIG. 2. During the breeding season, *Procavia* testes increase in size and show intense

level and various testicular parameters is shown in Table 2.

The number of Leydig cells was similar in testes of breeding and nonbreeding males and showed no relationship to plasma testosterone level. It is interesting that, among the Leydig cell parameters studied, only cell size showed significant correlation with plasma testosterone. Increased cell size was due to cytoplasmic hypertrophy; the nuclear dimensions showed no change between breeding and nonbreeding males. At the light microscopic level, it was evident that increased size of Leydig cells in breeding males was accompanied by distinct changes in the cytoplasm (Figs. 3 and 4). With the electron microscope, these changes were clearly seen to involve disappearance of lipid droplets and proliferation of membranous organelles, especially smooth endoplasmic reticulum (SER) (Figs. 5 and 6). Associated with the increase in SER was a distinct compartmentation of the cytoplasm. In Leydig cells from breeding males, the few remaining lipid droplets, along with most mitochondria and dense bodies, tended to reside in a discrete mass of central cytoplasm (Figs. 4 and 8). The peripheral cytoplasm was largely occupied by broad expanses of SER (Figs. 4 and 11). The relatively sparse cytoplasm of Leydig cells from nonbreeding animals showed little evidence of compartmentation, a mixture of membranous organelles, lipid droplets, and dense bodies occurring uniformly throughout the cytoplasm (Figs. 3 and 7).

TABLE 2

SAMPLE CORRELATION COEFFICIENTS (r) BETWEEN PLASMA TESTOSTERONE AND VARIOUS TESTICULAR PARAMETERS IN ROCK HYRAX (N = 41)

	r	P[a]
Testis weight	+0.78	<0.001
Average volume of a single Leydig cell	+0.64	<0.001
Total volume of Leydig cells per testis	+0.28	>0.04
Total number of Leydig cells per testis	−0.07	>0.3

[a] P is the probability that the population correlation coefficient is equal to zero.

Smooth endoplasmic reticulum in breeding hyrax occurred as unbranched tubules of uniform diameter (Fig. 11). Packing of these tubules was normally quite close, so that little space intervened among them. In nonbreeding animals, SER rarely appeared in masses sufficiently large to permit comparison, but when it did, it was an irregular network of fenestrated cisternae and tubules of irregular diameter (Fig. 9). Fenestrated cisternae of the SER occasionally formed concentric layers around lipid droplets in nonbreeding hydrax (Fig. 10). Although a few small lipid droplets were sometimes found in breeding

spermatogenesis. A section from the testis of a breeding animal is shown here. Note the increased diameter of the seminiferous tubule (ST) and the abundance of spermatids in the germinal epithelium. Leydig cells (at arrow) are widely scattered in the interstitial tissue, ½ μm Epon section stained with toluidine blue, PC 101, 40.0 g testis, 5.80 μg/100 ml plasma testosterone. ×200.

FIG. 3. Leydig cells (LC's) of nonbreeding animals tend to occur in clusters. Their cytoplasm characteristically contains numerous lipid droplets (lp) which are sufficiently large to be easily seen with the light microscope, ½ μm Epon section stained with toluidine blue, PC 110, 5.7 g testis, 0.85 μg/100 ml plasma testosterone. ×1000.

FIG. 4. A typical Leydig cell (LC) of a breeding animal frequently occurs alone. The cytoplasm rarely shows lipid droplets which are appreciable with the light microscope. These cells characteristically show a homogeneous band of peripheral cytoplasm that is known from electron micrographs to consist largely of smooth endoplasmic reticulum (ser). The center of the cell contains granules of varying density which represent an accumulation of mitochondria, lysosomes, and lipofuscin pigment granules, ½ μm Epon section stained with toluidine blue, PC 97, 41.6 g testis, 5.50 μg/100 ml plasma testosterone. ×1000.

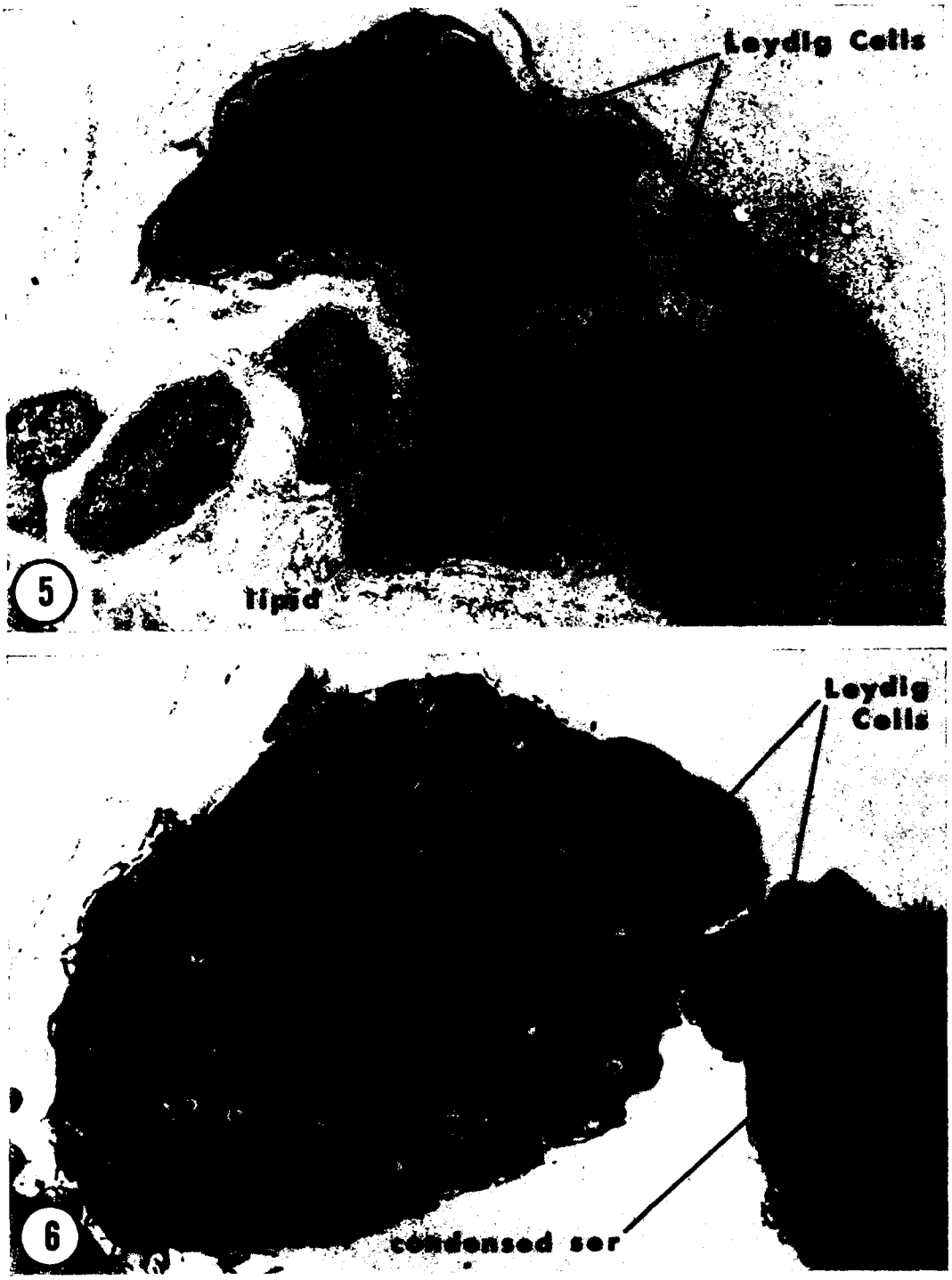


FIG. 5. The abundance of large lipid droplets inside Leydig cells of non-breeding hyrax is readily apparent with the electron microscope. These lipid droplets are most numerous near the plasmalemma, PC 9, 5.3 g testis, 0.17 μg/100 ml plasma testosterone. $\times$3500.

FIG. 6. Lipid droplets have not been observed near the plasmalemma of Leydig cells during the breeding season. A few, relatively small lipid droplets occur in the central cytoplasm

animals, they were not observed in special relationship with the SER (Fig. 8).

Leydig cells from breeding animals were further distinguished by unique membranous structures frequently seen near the plasmalemma (Figs. 6, 12, and 13). These structures appeared as irregular spheroidal masses of smooth membrane in the form of closely wound tubules and flattened cisternae. The tubules were of finer caliber than those of the ordinary SER (Figs. 12 and 13). We have conveniently referred to these membranous bodies as "condensed SER," although there is no proof that they are derived from the SER. Condensed SER occurred in an ectoplasmic zone between the plasmalemma and underlying masses of ordinary SER (Figs. 12 and 13). Myelin figures were another feature peculiar to Leydig cells of breeding hyrax. Some myelin figures appeared in the central cytoplasm (Fig. 8), but most were found in the ectoplasmic zone (Fig. 14).

Among the common features shared by Leydig cells of breeding and nonbreeding hyrax were lipofuscin pigment granules and presumed lysosomes (Figs. 7 and 8). Mitochondria were similar in both groups, with mixed tubular and lamellar cristae usually seen. Although rough endoplasmic reticulum was relatively sparse in both groups, it tended to localize in the central cytoplasm of Leydig cells from breeding males (Fig. 8).

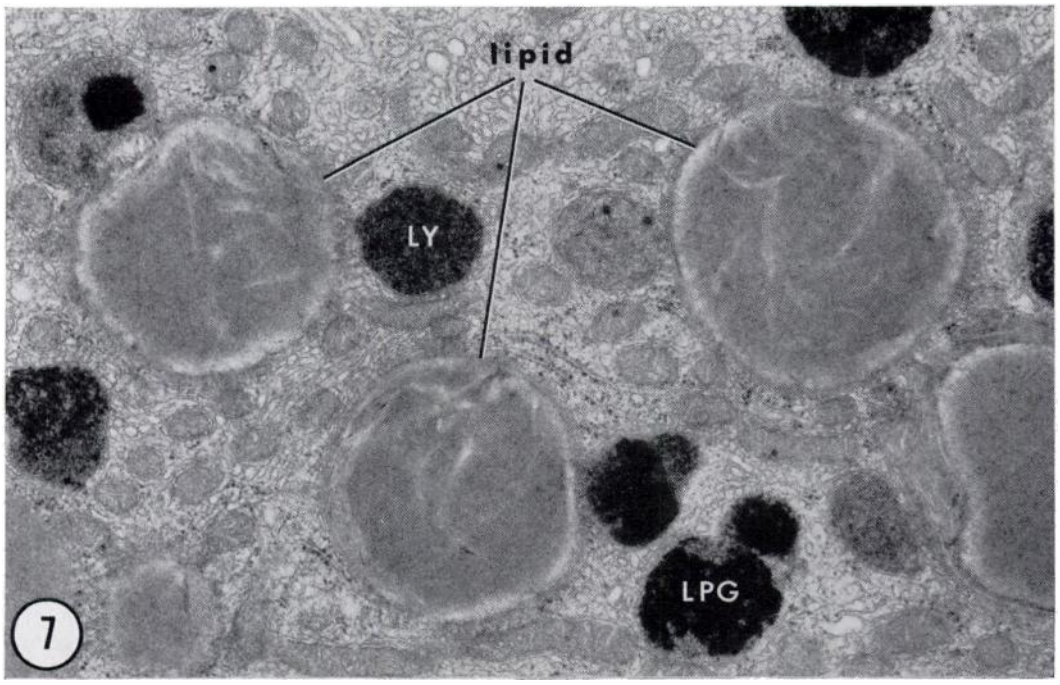
DISCUSSION

Most studies of Leydig cell fine structure have utilized material from such continuously breeding species as laboratory rodents and humans. Unless experimental manipulation of Leydig cells is involved, such studies offer little opportunity for correlation of structure and function. A number of workers have approached this problem by comparing control populations of Leydig cells with others stimulated by administered gonadotropins, usually human chorionic gonadotropin (HCG). Even though most studies of the effects of gonadotropin on Leydig cells have not assessed testosterone production, it is reasonable to assume that the gonadotropin acted to stimulate cell function. Hence, the numerous changes in Leydig cell structure which have been recorded after gonadotropin administration may be changes associated with increased synthesis and secretion of testosterone.

Several reports indicate that Leydig cells respond to hormone stimulation by increasing in size. Murakami and Tonutti (1966) found that chorionic gonadotropin administered to rats resulted in pronounced hypertrophy of Leydig cells. Enlarged Leydig cells in mice were produced experimentally by stimulation of pituitary gonadotropin secretion (Ichihara, 1967a,b). Leydig cell hypertrophy followed administration of HCG to immature guinea pigs (Merkow *et al.*, 1968a,b). Aoki (1970) reported cytoplasmic hypertrophy in Leydig cells of immature mice after HCG administration.

The testes of breeding hyrax contained Leydig cells that were approximately twice as large as in nonbreeding animals. This finding agrees with Wislocki's (1949) report of Leydig cell hypertrophy during the period of greatest sexual activity in white-tailed deer. The correlation of Leydig cell volume and plasma testosterone levels in hyrax has a parallel in roe deer, where Short and Mann (1966) found that Leydig cell size was positively related to testicular testosterone levels. If one can assume that both plasma and testicular testosterone

among mitochondria and various dense bodies. The peripheral cytoplasm is occupied largely by smooth endoplasmic reticulum and by occasional bundles of narrow cisternae and fine-caliber tubules (condensed ser) which appear to be related to the smooth endoplasmic reticulum. See Fig. 12 for a detailed view of the structure indicated here, PC 13, 55.5 g testis, 2.70 μg/100 ml plasma testosterone. ×3500.



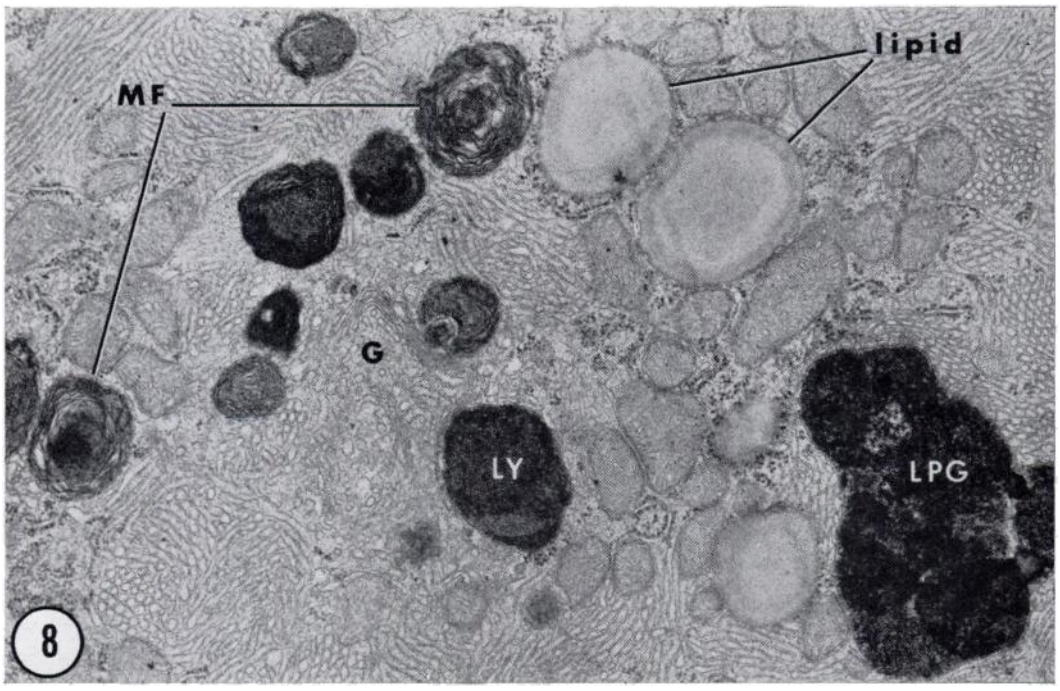


FIG. 7. The large lipid droplets in Leydig cells outside the breeding season are variable in appearance and may sometimes appear less homogeneous than their smaller counterparts during the breeding season. Smooth and rough endoplasmic reticulum, mitochondria, lysosomes (LY) and lipofuscin pigment granules (LPG) occur throughout the cytoplasm of Leydig cells from nonbreeding animals, PC 112, 6.3 g testis, 0.42 μg/100 ml plasma testosterone. ×27,000.

FIG. 8. In Leydig cells from breeding animals, only the central cytoplasm, as shown

levels are a function of testosterone secretion by Leydig cells, it appears that Leydig cell size may be an index of secretory activity.

In the face of considerable evidence that Leydig cell size may be proportional to functional activity, there is one report which demonstrates the importance of monitoring not only size but also numbers of cells. Schoen (1964) found that HCG stimulation in rats caused Leydig cell hypertrophy and increased androgen biosynthesis, but with such an increase in Leydig cell numbers that androgenic activity per cell was diminished. Significant hyperplasia of Leydig cells apparently did not occur in breeding hyrax, suggesting that androgenic activity per cell may actually increase as cells enlarge in this species.

It is worth mentioning here that the number of Leydig cells in the pubertal rat testis does not correlate with accessory sex organ growth, which is presumably governed by circulating testosterone levels (Clegg, 1966). Among other possible explanations for this finding, Clegg (1966) considered that it may be due to changes in the amount of androgen produced per cell during puberty. Our finding that Leydig cell numbers in hyrax are unrelated to plasma testosterone levels agrees with Clegg's (1966) study of pubertal rats, and we favor the same explanation.

There are other parallelisms between seasonally breeding hyrax and pubertal mammals. For example, the loss of lipid droplets in Leydig cells of breeding hyrax also occurs in pubertal rats (Niemi and Ikonen, 1963). Clegg (1966) found that the pubertal Leydig cells of rats contain lipid only until the phase of maximum accessory sex organ growth. He stated, "It is tempting to suggest that the depletion (of lipid) which occurs at this time is due to a sudden outpouring of androgenic hormone." Earlier, Lynch and Scott (1951) had demonstrated that lipid was retained in Leydig cells of rats treated with androgen. They attributed this finding to removal of Leydig cell stimulus, presumably by inhibition of pituitary gonadotropin secretion. Evidence has subsequently accumulated in support of lipid depletion in Leydig cells stimulated by gonadotropin. Merkow *et al.* (1968a,b) found decreased numbers of lipid droplets in Leydig cells of immature and adult guinea pigs after HCG administration. Dramatic depletion of lipid droplets also occurred in Leydig cells of immature mice following HCG administration (Aoki, 1970).

These findings, as well as results from our study of hyrax, agree with Deane's (1958) view that lipid droplets "represent stores of potential precursor materials that may be converted into steroid hormones when the proper stimulus occurs." Deane (1958) goes on to say that lipid droplets in steroid-producing cells contain detectable quantities of cholesterol by the Schultz test. It hardly needs emphasizing that cholesterol is an important intermediate in testosterone synthesis (see Hall, 1970).

It should not be assumed that all available evidence tends to associate depletion of lipid droplets with stimulation of Leydig cell function. For example, Merkow *et al.*

here, contains appreciable rough endoplasmic reticulum, mitochondria, lysosomes (LY), lipofuscin pigment granules (LPG), and lipid droplets. Myelin figures (MF) are commonly found in the central cytoplasm, but they also occur near the cell surface (see Fig. 14). These myelin figures have not been observed in Leydig cells from nonbreeding animals. Even in the central cytoplasm, smooth endoplasmic reticulum is a prominent organelle, but it tends to be more abundant in the peripheral areas where most other organelles are not found. Golgi complexes (G) are poorly developed and rarely seen in Leydig cells of *Procavia* regardless of reproductive activity. When they do occur, they tend to be restricted to central areas of the cell, PC 13, 55.5 g testis, 2.70 μg/100 ml plasma testosterone. ×27,000.

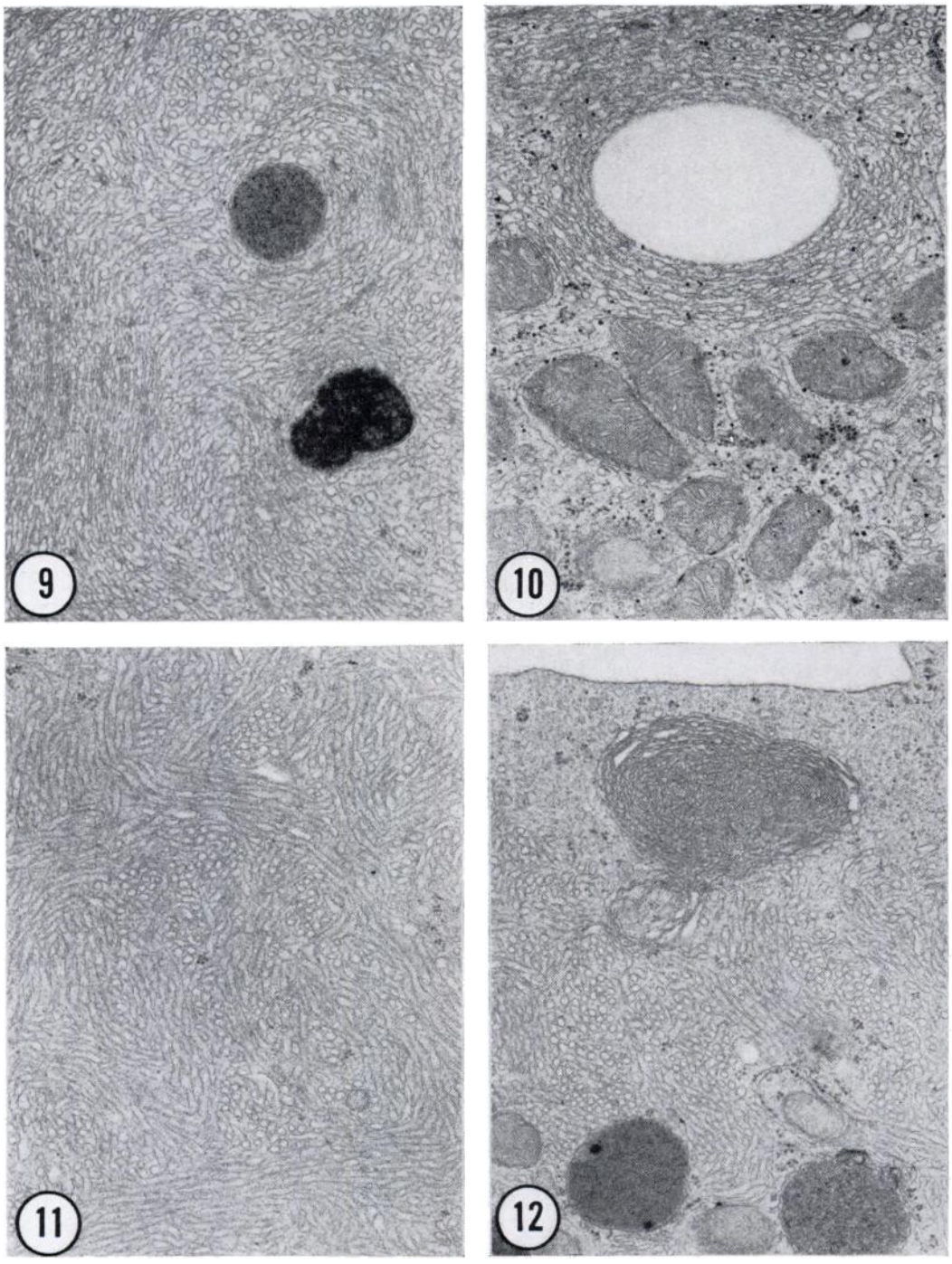


FIG. 9. Large areas of smooth endoplasmic reticulum rarely occur in Leydig cells from nonbreeding animals. When such areas are found, the organelle presents a varied appearance. As seen here, it may consist of a mixture of fenestrated cisternae and irregular tubules of varying diameter, PC 67, 3.6 g testis, 0.16 μg/100 ml plasma testosterone. ×27,000.

FIG. 10. Smaller lipid droplets in Leydig cells from nonbreeding animals are sometimes surrounded by concentric arrays of fenestrated cisternae of the smooth endoplasmic reticulum.

(1969) found more lipid droplets in Leydig cells of immature Mongolian gerbils after HCG administration. Furthermore, maximum Leydig cell lipid content coincides with sexual activity in such seasonally breeding mammals as white-tail deer (Wislocki, 1949) and European moles (Lofts, 1960). These cases serve to emphasize the difficulties involved in comparing species whose biosynthetic pathways for androgen production have not been determined.

Early electron microscopic observations of Leydig cells from certain species revealed such an abundance of SER that the investigators were encouraged to believe this organelle important in testosterone production (Christensen and Fawcett, 1961). In subsequent years, accumulating biochemical evidence has verified the major role of SER in steroid hormone synthesis (see review by Christensen and Gillim, 1969). Therefore, it comes as no surprise to see that SER has increased markedly in Leydig cells from hyrax whose testosterone levels are elevated. Increased amounts of SER have also been reported in experimentally stimulated Leydig cells from mice (Ichihara, 1967b, Aoki, 1970), guinea pigs (Merkow *et al.*, 1968a,b), and humans (deKretser, 1967). Interspecific comparisons suggest that the quantity of SER seen in Leydig cells may be related to endogenous cholesterol synthesis (Christensen, 1965). Hence, it is possible that the disappearance of lipid droplets and proliferation of SER in breeding hyrax both reflect increased demands on intracellular sources of cholesterol for use in testosterone synthesis.

Proliferation of SER in hyrax Leydig cells was associated with a pronounced tendency for compartmentation of the cytoplasm. Expanses of SER occupied peripheral areas, while rough endoplasmic reticulum, mitochondria, and dense bodies were largely confined to a central region of the cell. Christensen and Fawcett (1966) first commented on cytoplasmic segregation in Leydig cells of mice, and Ichihara (1970) later described the phenomenon during postnatal development, where SER increased in amount and tended to be located further from the nucleus with advancing age. This again emphasizes the parallelism between seasonal changes in hyrax and pubertal changes in other mammals which breed continuously.

The significance of cytoplasmic compartmentation in Leydig cells thought to be actively involved in testosterone production is obscure. If species which exhibit segregation of SER and mitochondria do in fact have the cholesterol side chain cleavage system localized in mitochondria, then potential problems of substrate transport within the cell arise (Christensen and Fawcett, 1966). Under these circumstances, one must imagine the movement of substrate between enzymes situated in quite different parts of the cell. Recently, Flint and Armstrong (1971) have reported

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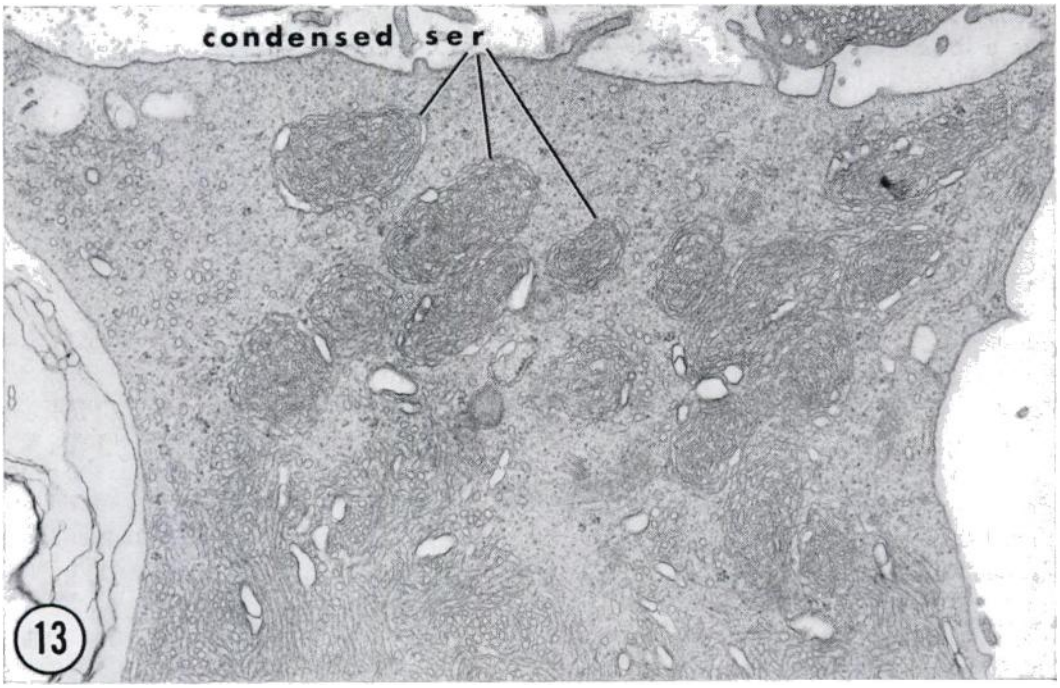
This association has not been observed in Leydig cells during the breeding season, PC 21, 8.1 g testis, 0.16 μg/100 ml plasma testosterone. ×27,000.

FIG. 11. The peripheral cytoplasm of Leydig cells from breeding animals is often occupied by masses of smooth endoplasmic reticulum. Here, the organelle consists of tightly packed tubules of uniform diameter, PC 13, 55.5 g testis, 2.70 μg/100 ml plasma testosterone. ×27,000.

FIG. 12. Interposed between the ordinary smooth endoplasmic reticulum and the plasmalemma of Leydig cells from breeding animals are occasional bundles of narrow cisternae and fine-caliber tubules. Such bundles of membranous profiles are presumed to be related to the smooth endoplasmic reticulum and have been conveniently termed "condensed ser." They are almost always found embedded in cytoplasmic ground substance which, apart from scattered granules resembling ribosomes, contains few organelles, PC 13. ×27,000.



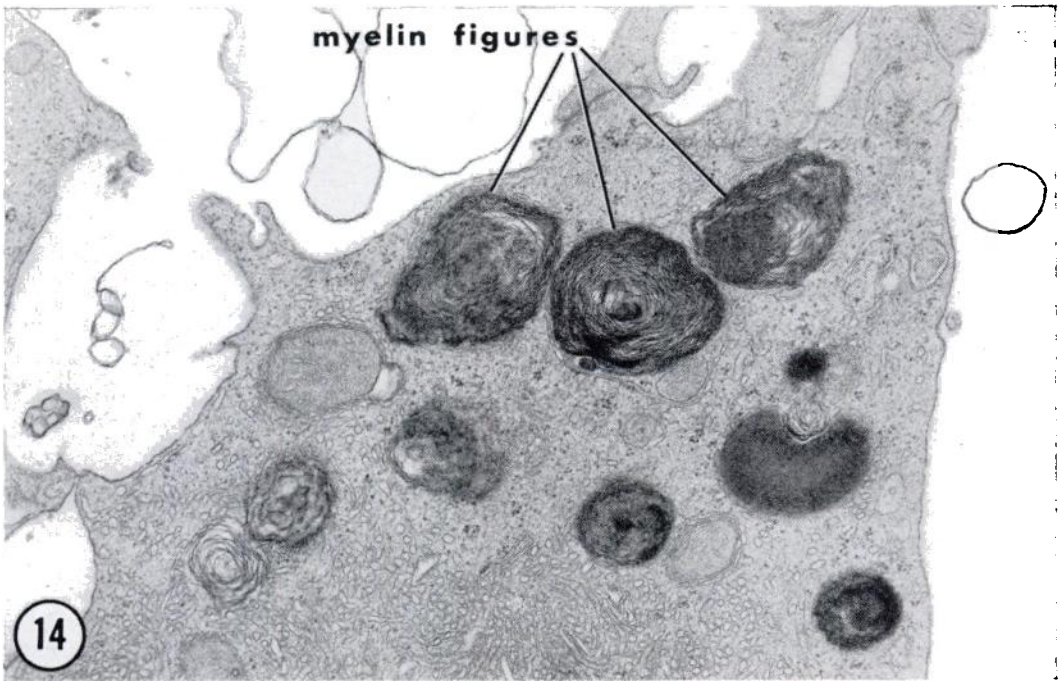


FIG. 13. During the breeding season, extensions of Leydig cell cytoplasm often contain numerous bundles of "condensed ser." These bundles occur between the cell surface and underlying masses of ordinary smooth endoplasmic reticulum, PC 55, 35.3 g testis, 1.51 μg/100 ml plasma testosterone. ×22,000.

FIG. 14. In breeding animals, myelin figures are also seen in extensions of Leydig cell cytoplasm. Like the bundles of "condensed ser," they are interposed between the smooth endoplasmic reticulum and the plasmalemma. They also occupy areas of cytoplasmic ground substance that contain few organelles other than presumed ribosomes, PC 55. ×22,000.

the existence of cholesterol side chain cleavage enzyme in microsomes isolated from the corpora lutea of cows and rats. If such a microsomal enzyme were present in Leydig cells, the spatial segregation of SER and mitochondria would be less troublesome.

Regarding the significance of certain patterns of organization in the SER, it is now generally accepted that combinations of tubular and cisternal profiles, as opposed to vesicular conformations, more accurately reflect the form of SER in living cells (Christensen and Gillim, 1969). So far, it has not been possible to attach functional significance to the distinction between tubular and cisternal SER. In fact, Christensen (1965) suggested that the two forms may be freely interconvertible in Leydig cells of the guinea pig. Both tubular and cisternal profiles of SER occurred in Leydig cells of hyrax, but they were seen together only in nonbreeding animals. In breeding hyrax, all SER tended to occur as uniform tubules. This observation suggests that tubular SER may be associated with increased testosterone production, at least in hyrax.

In some other species, there is suggestive evidence that certain arrangements of smooth membrane may be associated with elevated hormone synthesis. For example, experimental stimulation of Leydig cells resulted in more abundant membranous whorls in rats (Murakami and Tonutti 1966) and in mice (Ichihara, 1967b). Whorls of smooth membrane appear in Leydig cells of mice at puberty when testosterone production is thought to increase sharply (Ichihara, 1970). Concentric arrays of smooth membrane have also been found in Leydig cells of androgen-secreting tumors (Cervos-Navarro *et al.*, 1964).

Membranous whorls such as those first described by Carr and Carr (1962) were not seen in hyrax. However, bundles of fine-caliber tubules and narrow cisternae did occur in breeding hyrax. Although their significance is unknown, the association of these bundles of "condensed SER" with high plasma testosterone levels encourages the speculation that the two are related. Considering the paucity of secretory structures in steroid-producing cells (see Gillim *et al.*, 1969), it would be particularly gratifying if the "condensed SER" found under the plasmalemma of hypertrophied Leydig cells could be shown to play a role in testosterone secretion. It might be argued that this structure is unlikely to play a major role in testosterone secretion, since it has not been reported in other species. The most similar structures in the literature are Crabo's (1963) nests of fine-caliber tubules in Leydig cells of the rabbit, but these presumed derivatives of the ordinary SER differ from those in hyrax in that they neither occur in discrete bundles nor preferentially reside near the cell surface. Christensen (1965) reported very fine interconnecting tubules in Leydig cells of the guinea pig, but they seemed to be restricted to cells showing evidence of poor fixation. Christensen (1965) felt that these narrow tubules might occur as a result of chemical or functional heterogeneity in the smooth reticulum. He considered it unlikely that the fine tubules were derived from the Golgi complex.

In steroid-producing cells of other species in which "condensed SER" does not occur, a well-developed Golgi complex has been implicated in conjugation and secretion of hormone (Long and Jones, 1967). In hyrax Leydig cells, the Golgi complex is rarely found, and when seen, it is rudimentary (Fig. 8). Hence, the appearance of "condensed SER" in Leydig cells of breeding hyrax may be related to the paucity of Golgi complexes in these cells. It is conceivable that the function of the Golgi complex, whatever it may be in steroid-secreting cells, has been usurped by bundles of "condensed SER" in hyrax.

Myelin figures were another characteristic feature of Leydig cells from breeding hyrax. Like the bundles of "condensed

SER," many myelin figures occurred in the peripheral cytoplasm. Myelin figures have sometimes been attributed to poor fixation; however, in hyrax these structures were commonly seen in cells that otherwise appeared to be well fixed. Although there is no available evidence to suggest that some SER may be consumed in the process of testosterone synthesis and secretion, it is conceivable that a series of structural transformations occur. It is possible that bundles of "condensed SER" and myelin figures represent two stages in the transformation of SER to dense residual bodies in active Leydig cells of hyrax.

Lipofuscin pigment was prominent in the central cytoplasm of Leydig cells of breeding hyrax. In guinea pigs (Merkow *et al.*, 1968a) and in humans (deKretser, 1967), residual bodies, or lipofuscin pigment granules, have increased in response to hormonal stimulation of Leydig cells. Although the biochemistry of pigment in residual bodies of steroid-secreting cells is poorly understood (Christensen and Gillim, 1969), it is considered that they contain indigestible products from the oxidation of lipids within lysosomes (deKretser, 1967). Whether lysosomes or lipofuscin pigment deposits are involved directly in steroid synthesis and secretion remains unclear. However, it would be consistent with certain tenets of the lysosome theory if exhausted organelles of steroid-secreting cells, such as fragments of the SER, eventually became incorporated in dense residual bodies.

Due to the reported involvement of mitochondrial enzymes in cholesterol side chain cleavage in the rat testis (Toren *et al.*, 1964), one might expect this organelle to show some indication of increased functional activity in stimulated Leydig cells. However, mitochondrial changes resulting from experimental gonadotropic stimulation of Leydig cells seems to vary from species to species. In humans, treated with HCG, the number of mitochondria per Leydig cell was often increased and these mitochondria contained more cristae (deKretser, 1967). Aoki (1970) reported a striking increase in mitochondrial pleomorphism in Leydig cells after treatment of immature mice with HCG. In contrast, mitochondrial changes were not noted in immature guinea pigs after HCG administration (Merkow *et al.*, 1968a). An increase in numbers of mitochondria was suspected but not rigorously documented in Leydig cells of breeding hyrax. Otherwise, size and shape of the organelles, as well as frequency of cristae, were similar in breeding and nonbreeding animals.

SUMMARY

Male rock hyrax have enlarged testes and elevated plasma testosterone levels during their annual breeding season. Testicular hypertrophy in breeding animals does not involve hyperplasia of Leydig cells. However, the average Leydig cell volume, which doubles during the breeding season, shows significantly positive correlation with plasma testosterone level. Marked changes in the structure of enlarged Leydig cells include disappearance of lipid droplets and proliferation of SER. The inverse relationship between lipid droplets, which may store cholesterol, and SER, which may synthesize cholesterol, is consistent with the possibility that seasonal fluctuations in testosterone secretion are accompanied by changing intracellular requirements for steroid hormone precursors. The abundant SER of enlarged Leydig cells occurs as relatively straight, unbranched tubules of uniform diameter. In contrast, the relatively sparse SER in Leydig cells outside the breeding season appears as a heterogeneous assemblage of irregularly tubular and cisternal profiles. The seasonal variations in appearance of SER suggest that the structure of this organelle, as well as its abundance, may change according to functional demands. A peculiar membranous structure, which may be derived from the SER, frequently occurred in the periphery of Leydig cells

from animals with high plasma testosterone levels. The abundance of these structures and the scarcity of Golgi complexes raises the possibility that the function of the Golgi complex, which is well-developed in steroid-secreting cells of other species, may be assumed by the bundles of "condensed SER" in enlarged Leydig cells of hyrax.

Our study of hyrax has shown that changes in Leydig cell ultrastructure accompany seasonal fluctuations in plasma testosterone level. These findings confirm the usefulness of seasonal breeders in studies designed to correlate structure and function in steroid-secreting cells.

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