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Comparison of Cytoplasmic Lamellae and Membranous Elements in the Oocytes of Five Mammalian Species*

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Summary. Certain cytoplasmic components of the ovarian oocytes of hamster, rat, mouse, guinea pig and cat are described and compared. Special attention is given to non-membranous cytoplasmic lamellae, concentric arrangements of the endoplasmic reticulum, and the morphology of various membrane-bound bodies. The possible significance of some of these entities is discussed.

Introduction

The presence of non-membranous cytoplasmic lamellae in the oocyte of the golden hamster has been demonstrated by fixation with 4% glutaraldehyde (WEAKLEY, 1966). The lamellae are not preserved by fixatives such as osmium tetroxide, formaldehyde and other short-chain aldehydes, and have not been reported in other mammalian species. The lamellae are digested by pepsin, but are partially resistant to extraction of the oocytes in 30% ethanol followed by distilled water (WEAKLEY, 1967b). The extraction procedures reveal an underlying skeletal lattice composed of what appear to be cubic units approximately 400 Å on a side.

Since published ultrastructural studies of mammalian oocytes have been carried out largely on material fixed in osmium tetroxide, it was decided to fix ovarian tissue from several mammalian species in glutaraldehyde to determine if the cytoplasmic lamellae are present, and to compare other aspects of oocyte morphology using this fixative. No attempt was made to do an exhaustive study on all stages of oocyte development in these species, and only salient points will be mentioned.

Materials and Methods

The species studied for comparison with the hamster were mouse, rat, guinea pig and cat. Ovaries were fixed initially by immersion in ice cold 4% glutaraldehyde, post-osmicated in Millonig's fixative (MILLONIG, 1962), and embedded in araldite. One μ sections of the araldite blocks were stained with methylene blue for purposes of orientation and study with the light microscope. Ultrathin sections for electron microscopy were stained with uranyl acetate followed by lead citrate (REYNOLDS, 1963).

* Since preparation of this manuscript, my attention has been called to a paper by A. C. ENDERS and S. J. SCHLAFKE (in Ciba Foundation Symposium on Preimplantation Stages of Pregnancy, 1965, pp. 29—54, Ed. G. E. W. WOLSTENHOLME and M. O'CONNOR, J. & A. Churchill, Ltd., London) in which "fibrous elements" are reported to occur in cells of four-and five-day blastocysts in four species of small rodent. These appear morphologically identical to the non-membranous lamellae in the oocytes described above, indicating that these structures persist in the trophoblastic cells during cleavage.

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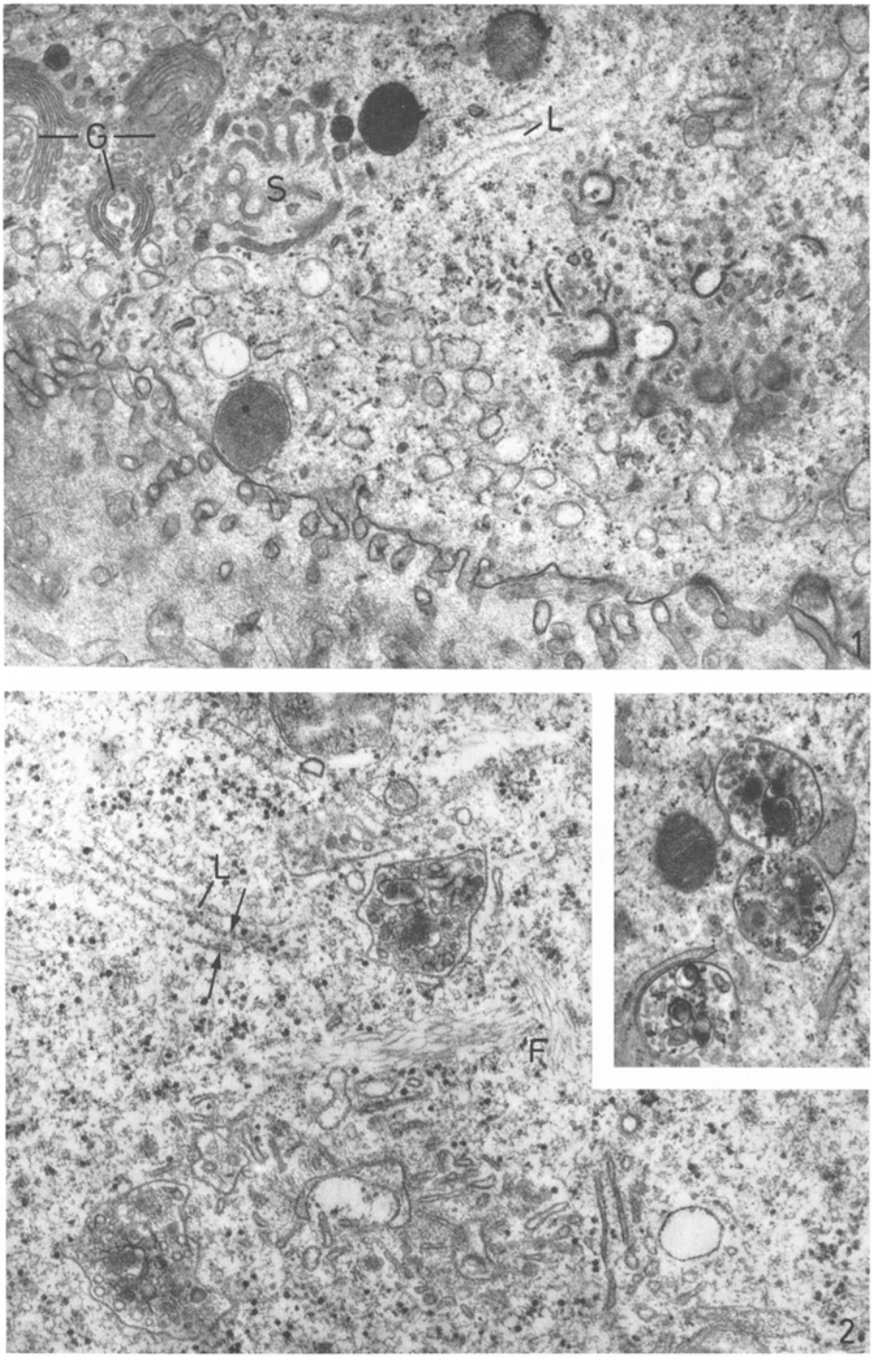


Figs. 1 and 2 (for legends see p. 111)

Extraction procedures as described previously (WEAKLEY, 1967b) were carried out on oocytes of those species in which the cytoplasmic lamellae were demonstrated.

The hamster tissues used for purposes of comparison had been collected over the past three years and were fixed by various methods, principally glutaraldehyde used alone or followed by osmium tetroxide, osmium tetroxide alone, or formaldehyde followed by osmium tetroxide.

Results

Cytoplasmic Non-Membranous Lamellae

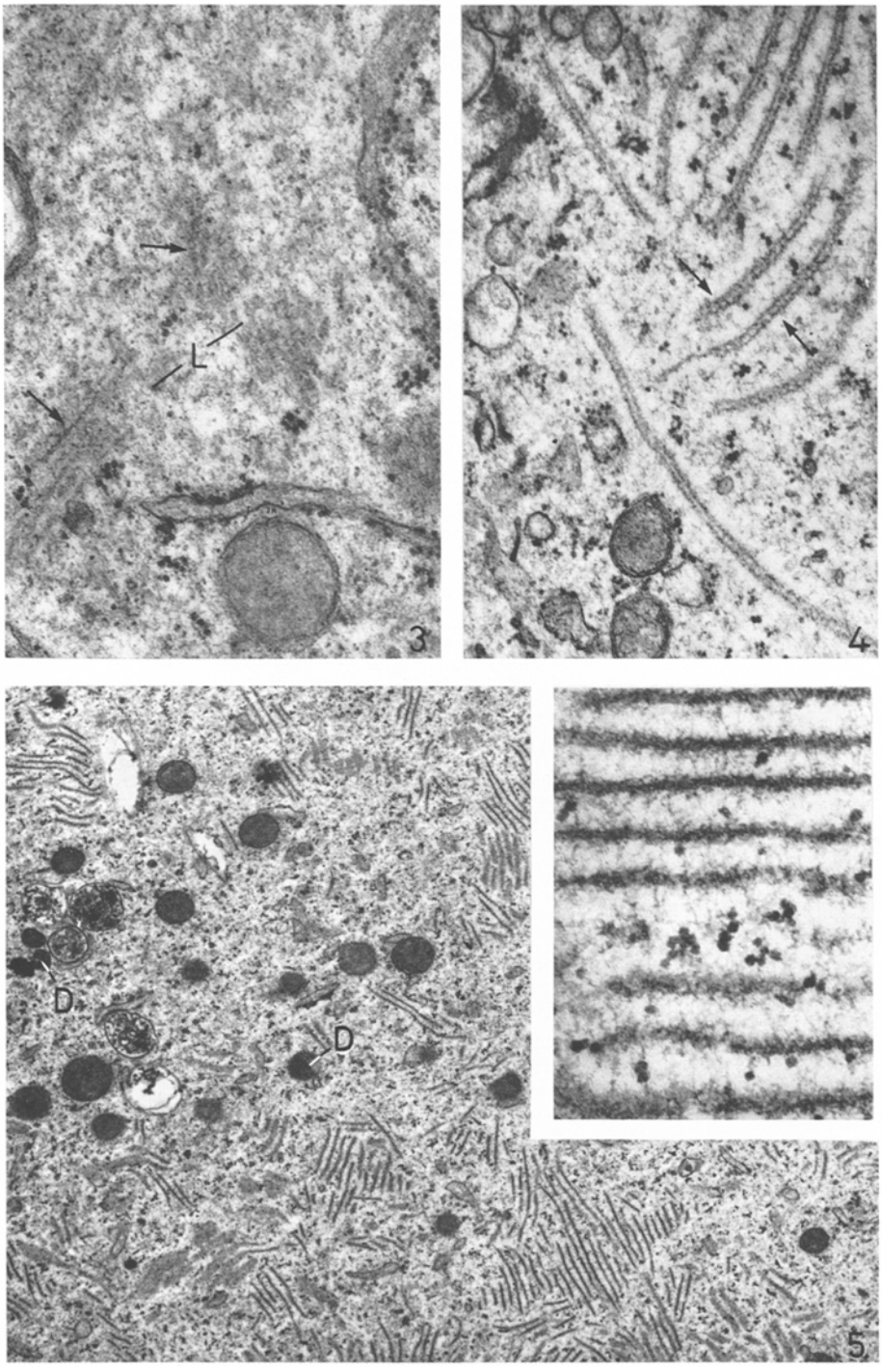
In none of the species studied are cytoplasmic non-membranous lamellae present in primary follicles surrounded by a single layer of flattened follicle cells. In the hamster they are invariably present as single lamellae by the time the single layer of follicle cells has attained the cuboidal granulosa form. In rat and mouse at this stage, however, only an occasional filamentous structure can be observed in the cytoplasm, and it is not until a second layer of granulosa cells has been added to the follicle in the rat and a third layer in the mouse, that the lamellae are unequivocally present. In the cat and guinea pig, lamellae are not evident at any stage studied.

Fig. 1 shows a stack of four single lamellae (L), cut in transverse and oblique section, in an oocyte from a bilaminar follicle in the hamster. Fig. 2 shows a similar stack (L) cut transversely in an oocyte at the same stage in the rat. Also present are two stacks of fine filaments (F) running at right angles to one another. These may possibly be developing lamellae. The fine filaments within a stack run roughly parallel to one another but may branch or anastomose. They occasionally exhibit a beaded appearance. The fine filaments are seldom present at later stages, although the lamellae invariably are. Fig. 3 shows lamellae (L) in a mouse oocyte, 3-layer follicle. Far fewer lamellae are present in the mouse than in the rat and hamster, and they stand out less clearly from the background cytoplasm.

By the time the hamster oocyte is surrounded by three layers of follicle cells, its lamellae have doubled in width. Each lamella in transverse section now appears as two parallel lines connected together by cross pieces at periods of approximately 400 Å (Fig. 4, arrows). In oocytes of the rat and mouse, the lamellae do not become double structures, as illustrated by Figs. 5 and 6, which show the lamellae in oocytes from graafian follicles.

Fig. 1. Peripheral cytoplasm of hamster oocyte in 2-layer follicle. A stack of 4 single lamellae (*L*) cut in transverse and oblique section is seen toward upper center. A cluster of horseshoe-shaped membranes surrounded by vesicles and short cisternae is seen at right. Peripheral to this are larger vesicles of smooth endoplasmic reticulum. At upper left, Golgi bodies (*G*) are closely associated with a lattice of smooth membranes (*S*) containing electron-dense material. Two dense bodies are seen to right of lattice and another is located between the Golgi bodies. A cortical granule lies just beneath plasma membrane. × 25.600. All material has been fixed with 4% glutaraldehyde followed by osmium tetroxide unless otherwise stated

Fig. 2. Cytoplasm of rat oocyte in 2-layer follicle. A stack of cytoplasmic lamellae (*L*) is seen at upper left. The periodic beaded nature of the lamellae is evident at arrows. At *F*, two stacks of fine filaments run at right angles to one another. Below these is a horseshoe-shaped body enclosed by smooth membrane and surrounded by vesicles and smooth cisternae. Two large membrane-bound bodies containing vesicular and granular material are present. × 48.000. Similar membrane-bound bodies enclosing what appear to be ribosomes are seen in inset. (× 25.600)



Figs. 3—5 (for legends see p. 113)

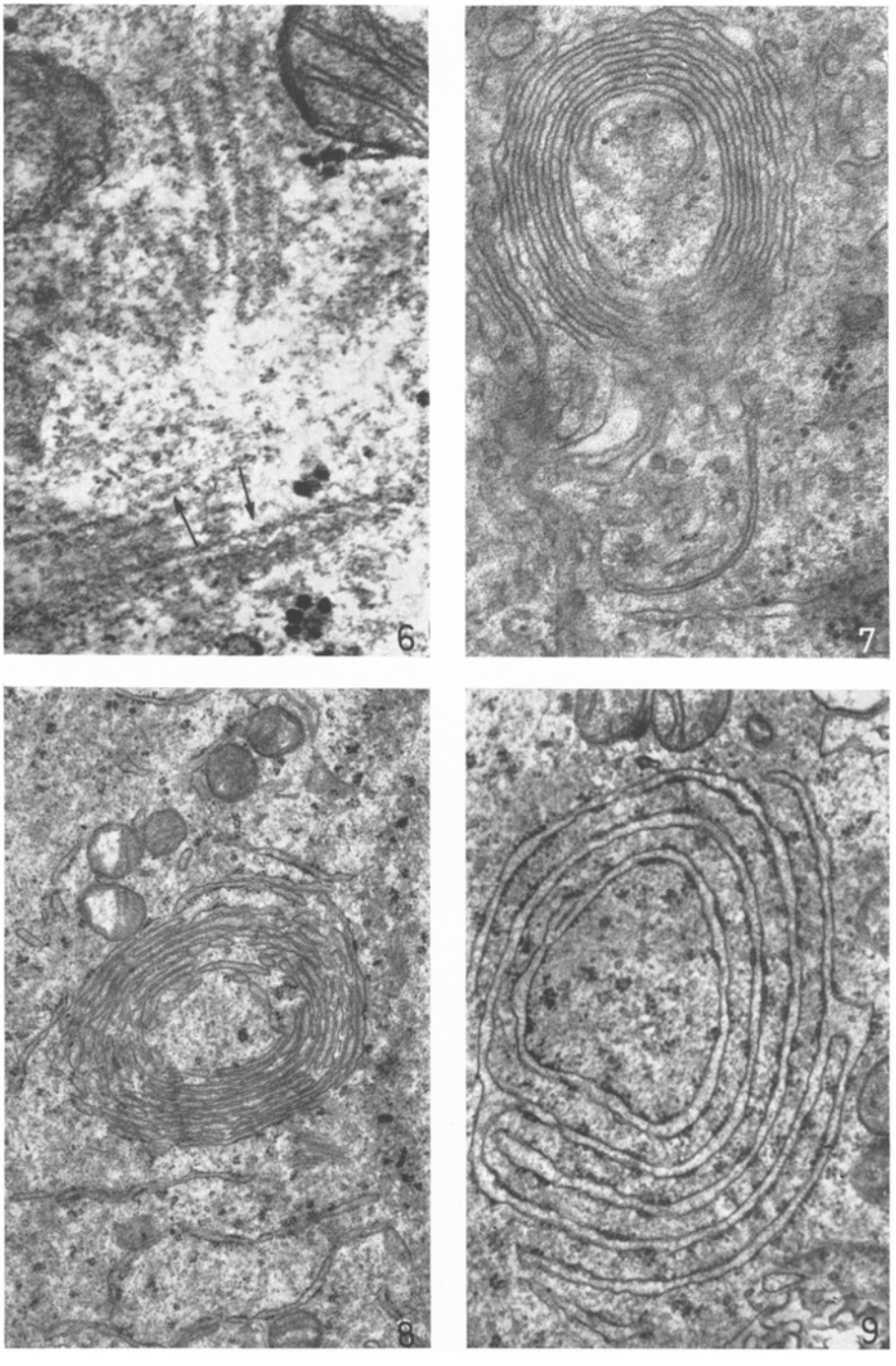
In the hamster, the lamellae when single are long and straight, and have been followed in the plane of section for as much as 7 μ. After they become double they frequently exhibit a circular or horse-shoe arrangement around clusters of mitochondria, large ribosomes and vesicles of smooth endoplasmic reticulum. Neither rat nor mouse lamellae develop this orientation. They remain rather short, single lamellae, usually in stacks of five to ten sheets running parallel to one another in straight or only slightly curved lines. They usually do not extend for more than one or two μ in the plane of section. The mouse lamellae, particularly in earlier oocytes, are associated with amorphous material which makes elucidation of their structure particularly difficult. They stand out slightly more clearly in the graafian follicle; otherwise their morphology does not appear to change with maturity. The lamellae within a stack are closer together than in either rat or hamster oocytes. In favourable sections their periodic beaded nature may be seen at any stage (Fig. 6, arrows). In the rat, the form of the lamellae is more clearly delineated in the younger oocytes, and a beaded structure can be distinguished (Fig. 2, arrows). In the multilaminar follicle the structure has been obscured by the presence of electron-dense amorphous material. Fine filaments, which appear to be extensions of this amorphous material, interconnect the parallel lamellae (Fig. 5, inset). Fig. 13 shows the lamellae in tangential section. Here, also, the underlying structure is obscured by amorphous material.

Accurate measurement of transverse sections of the lamellae in rat and mouse was not possible because of the absence of sharp outlines and the depth of focus inherent in electron micrographs. In the hamster, measurements on routinely processed material could be checked against extracted tissue in which the lamellar substructure was clearly visible (WEAKLEY, 1967b). Unfortunately, the lamellae in the rat and mouse did not survive the extractions, so no additional information was available from this source. Estimates derived from measurements taken at several different magnifications indicate that when the lamellae first appear they average roughly 150 Å in width in rat and hamster, and rather less than this in the mouse. By the graafian follicle stage, lamellar width in the mouse appears not to have increased at all, but that in the rat has risen roughly to 240 Å, due apparently to accretion of amorphous material onto the underlying structure. In the hamster, however, the lamellae have doubled in width, apparently through the linking together of two single lamellae by cross pieces between adjacent periodic beads. The lamellae now measure up to 400 Å in width.

Fig. 3. Cytoplasm of mouse oocyte in 3-layer follicle. Two stacks of lamellae (*L*) run at roughly right angles to one another. Beading is seen at arrows. Two strands of rough endoplasmic reticulum are present. × 51.200

Fig. 4. Cytoplasm of hamster oocyte in 3-layer follicle showing transverse sections of lamellae. The lamellae have doubled in width and each appears as two parallel lines connected by cross pieces at intervals of approximately 400 Å (arrows). × 36.800

Fig. 5. Cytoplasm of rat oocyte in Graafian follicle. Stacks of lamellae are shown in transverse, oblique and tangential sections. The structure of the lamellae is obscured by amorphous material, continuations of which appear to interconnect adjacent lamellae (inset). Several dense bodies (*D*) and larger bodies containing membranous and granular material are present. × 12.800 (Inset × 36.000)



Figs. 6—9 (for legends see p. 115)

Lamellae are associated with large ribosomes (300—400 Å in diameter) in rat and hamster only. Associated ribosomes in the mouse average 180 Å.

Concentric Endoplasmic Reticulum

Systems of concentrically arranged endoplasmic reticulum were observed in oocytes of the hamster, mouse and cat. In all three species the systems surround areas of cytoplasm which are void of organelles except for a few tiny vesicles and clusters of free ribosomes. In the hamster, a concentric system was encountered only once, although a great deal of material has been studied. The system appeared in the oocyte of a single-layer follicle from an 8-day-old hamster (Fig. 7). Its membranes were free from ribosomes. In the mouse, concentric arrays were quite common in oocytes in 3-layer follicles. In one case two arrays were observed in a single section of an oocyte. In the mouse the concentric systems are free from attached ribosomes except for the inner and outer cisternae (Fig. 8). Bridges are occasionally seen to connect adjacent cisternae. Strands of rough endoplasmic reticulum may lie nearby in the cytoplasm, possibly originating from the concentric arrays. Mitochondria are closely associated with the periphery of the concentric systems. In the cat, all the concentric cisternae are studded with ribosomes, and conspicuous bridging occurs between cisternae (Fig. 9). As in the mouse, mitochondria are closely associated with the periphery of the system. No examples of concentric endoplasmic reticulum were observed in the rat, but this may be due to the limited number of specimens studied at each stage. ADAMS and HERTIG (1964) have already reported the concentric specializations of rough endoplasmic reticulum which appear in small primary oocytes in the guinea pig.

Other Special Configurations of Cytoplasmic Membranes

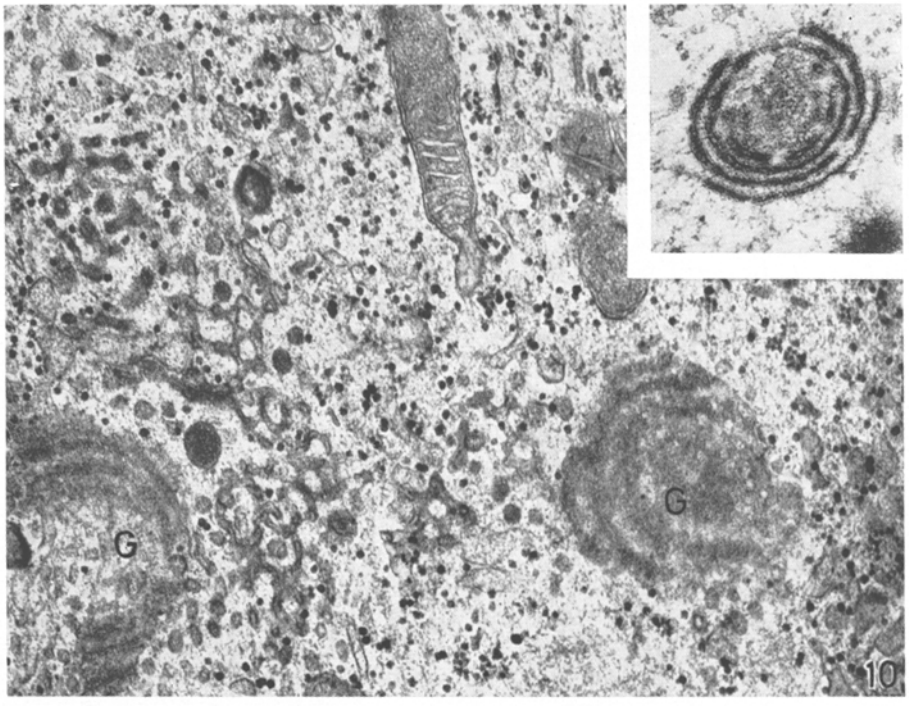
Smooth membranous elements in the rat and hamster frequently exhibit a configuration in which one or more narrow cisternae with expanded ends takes the shape of a horseshoe, with smaller cisternae or vesicles radiating away from

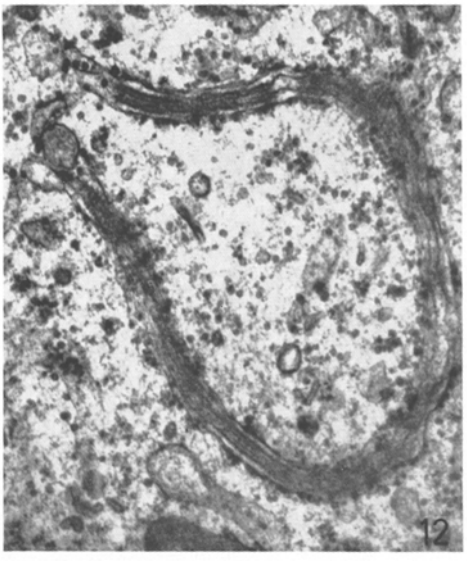
Fig. 6. Cytoplasm of mouse oocyte in Graafian follicle. The beaded nature of the mature single lamellae is clearly visible (arrows). × 108.000

Fig. 7. Cytoplasm of small primary oocyte from 8-day-old hamster. A concentric array of smooth endoplasmic reticulum encircles an area of cytoplasm containing free ribosomes and tiny vesicles. × 54.400

Fig. 8. Cytoplasm of mouse oocyte in 3-layer follicle. A concentric system of endoplasmic reticulum surrounds an area of cytoplasm containing clusters of free ribosomes and a few tiny vesicles. The inner and outer membranes of the system are associated with a few ribosomes; the membranes in the main body of the system are smooth. Several strands of rough endoplasmic reticulum lie close by in the cytoplasm. Mitochondria are closely associated with the system. × 16.000

Fig. 9. Concentric system of rough endoplasmic reticulum in the cytoplasm of a small primary oocyte in the cat. The concentric cisternae are interconnected by bridges. The system encloses an area of cytoplasm containing tiny vesicles and clusters of free ribosomes. Mitochondria are seen near periphery of the system. × 36.000





Figs. 10—12 (for legends see p. 117)

the outer aspect of the horseshoe (Figs. 1, 2 and 13). In the rat, such horseshoe-shaped cisternae may encircle what appear to be masses of ribosomes (Fig. 13), and are sometimes associated with multivesicular bodies. Groups of multivesicular bodies associated with vesicles and short cisternae of smooth membrane are common at all stages in the mouse, as reported by YAMADA *et al.* (1957), but clusters of horseshoe-shaped cisternae have not been observed.

In the hamster, lattices of smooth membranes filled with electron-dense material are common (Figs. 1, 10). They are usually associated with modified Golgi bodies (WEAKLEY, 1966), examples of which are seen in Fig. 10, and also with electron-dense bodies of varying size. Similar lattices and modified Golgi bodies were not encountered in the other species. The morphology of the modified Golgi bodies tends to vary somewhat with the method of fixation employed. When glutaraldehyde is used alone, the contents of the cisternae comprising the bodies assumes a beaded appearance and the cisternal membranes are seen as negative images (Fig. 10, inset).

Rough endoplasmic reticulum is not abundant in the oocytes studied, although in the mouse it is present in greater amounts than in the other species. In the mouse, ribosomes are sparsely and irregularly scattered along the cisternae, which are often seen in pairs with the ribosomes confined to the outer aspect of each pair (Fig. 11). A similar arrangement is very occasionally encountered in the hamster (Fig. 12), but here the two cisternae are separated by a structured electron-dense material. In the guinea pig and hamster, narrow cisternae of endoplasmic reticulum may be encountered running roughly parallel to one another with a number of expanded cisternae lying between them (Fig. 18). Ribosomes occur only sporadically along the narrow strands and not at all on the expanded cisternae.

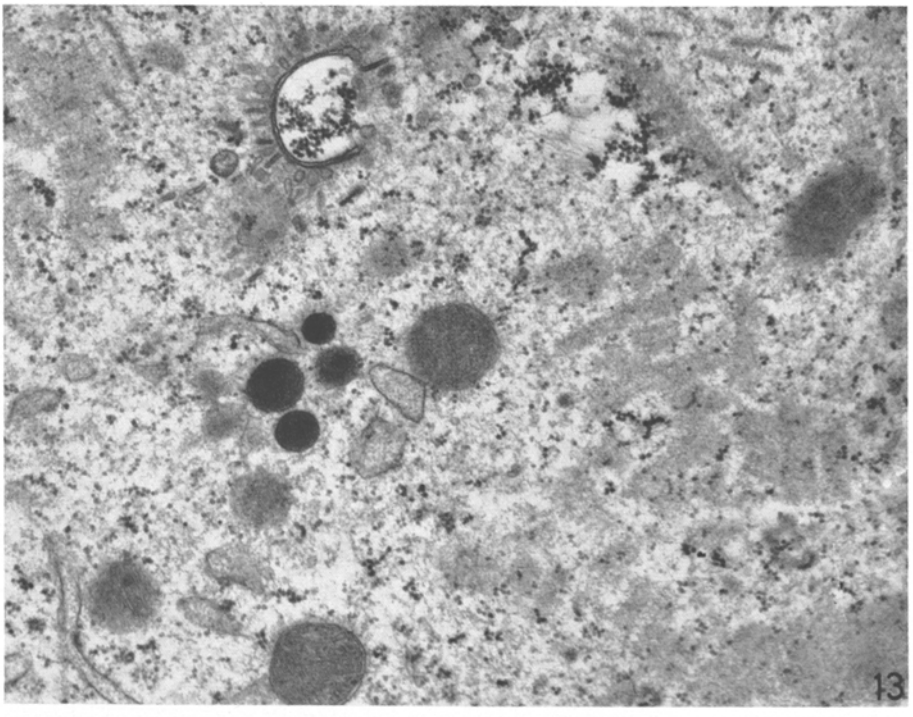
Cytoplasmic Bodies

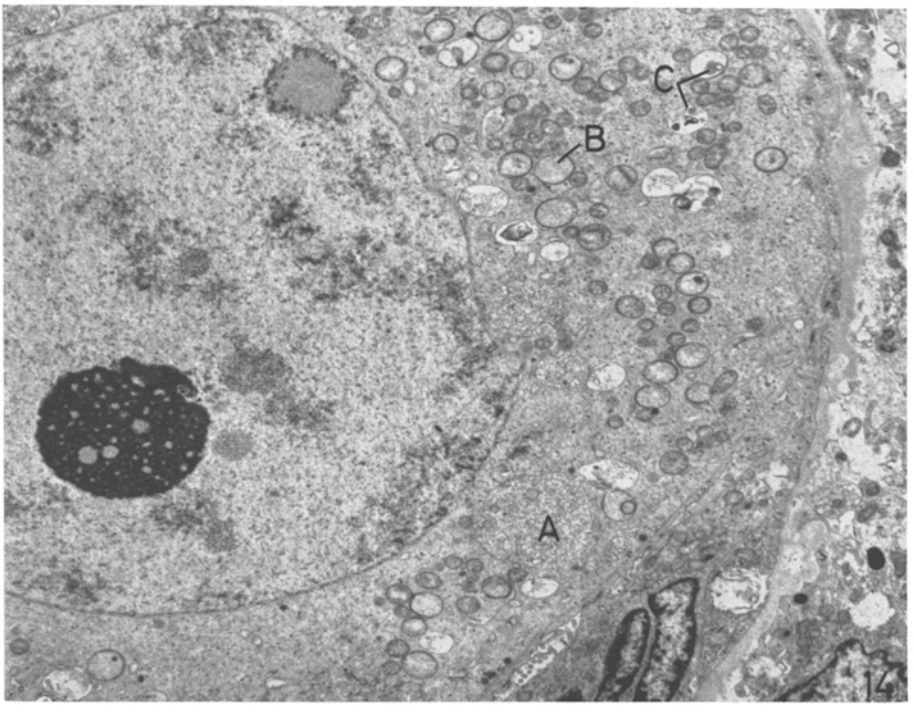
In the rat, round or irregularly-shaped membrane-bound bodies measuring roughly 1 μ across are a prominent feature at all stages (Fig. 2). They most frequently contain an accumulation of small vesicles and two or three larger vesicles with granular contents. They may also contain highly electron-dense particles resembling ribosomes (Fig. 2, inset). Smaller round electron-dense bodies are also common in the rat (Fig. 13), and are present in guinea pig (Fig. 18) and hamster (Fig. 1).

Fig. 10. Two modified Golgi bodies (*G*) partially obscured by fluffy granular material. Between the two systems is an extensive latticework of smooth membranes filled with a moderately electron-dense material. Small dense bodies are associated with the lattice. × 38.400. Inset shows Golgi body fixed with glutaraldehyde alone. Contents of cisternae have a beaded appearance and bounding membranes appear as negative images. × 48.000

Fig. 11. Typical configuration of endoplasmic reticulum in mouse oocyte. The cisternae occur in pairs with a few ribosomes attached to the outer aspects of the pair. × 43.200

Fig. 12. Paired cisternae of rough endoplasmic reticulum in the hamster oocyte. Ribosomes occur only on outer aspects of pair. Between the cisternae is a moderately electron-dense material displaying longitudinal lines. Fixation: Formaldehyde followed by osmium tetroxide. × 40.000





Figs. 13 and 14 (for legends see p. 119)

In the cat, a striking feature of the cytoplasm in the smaller oocytes is the large number of membrane-bound bodies of several types. The most frequent and consistently occurring type (Figs. 14a and 15) contains fragments of membrane associated with an amorphous substance, and occasionally a small inclusion which may be lipid. The bounding membrane of these bodies is frequently discontinuous. Other membrane-bound bodies (Fig. 14b) contain a pale, homogeneous substance with no apparent structure, or may enclose an assortment of granular or membranous material (Fig. 14c). In follicles with antra, the membrane-bound bodies are larger and contain highly electron-dense masses of material in an apparently structureless ground (Fig. 16). At this time also, large lipoid bodies are present, often displayed in a ring around the periphery of the oocyte (Fig. 17).

In the guinea pig, membrane-bound areas with a pale homogeneous content and an irregular outline are present in oocytes from multilaminar follicles (Fig. 18). These have been described by ADAMS and HERTIG (1964) as "expanded endoplasmic reticulum".

Cytoplasmic bodies in the hamster oocyte, particularly large myelin figures and lipoid bodies, have been described in previous publications (WEAKLEY, 1966, 1967a).

Discussion

Cytoplasmic Non-Membranous Lamellae

These structures have so far proven to be present only in three closely related species of small rodent. In the larger guinea pig no such material is preserved, nor is it evident in the cat. The lamellae are most highly developed in the hamster, where the single lamellae appear to pair up into double structures as the oocyte grows. This pairing may account for their greater stability to the extraction procedures.

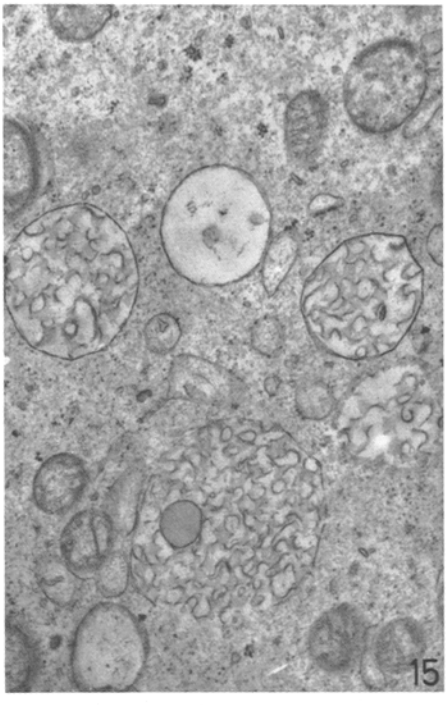
This marked difference in stability between closely related species raises the question as to whether similar structures in the guinea pig and cat are present *in vivo* but too delicate to be preserved even by glutaraldehyde fixation.

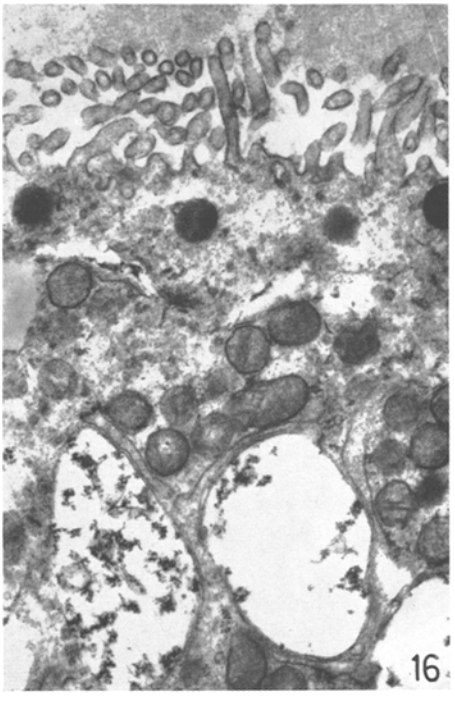
In the larger oocytes of both rat and hamster, large numbers of mitochondria are associated with the lamellae. However, only the highly developed lamellae in the hamster oocyte assume a concentric orientation around the mitochondria.

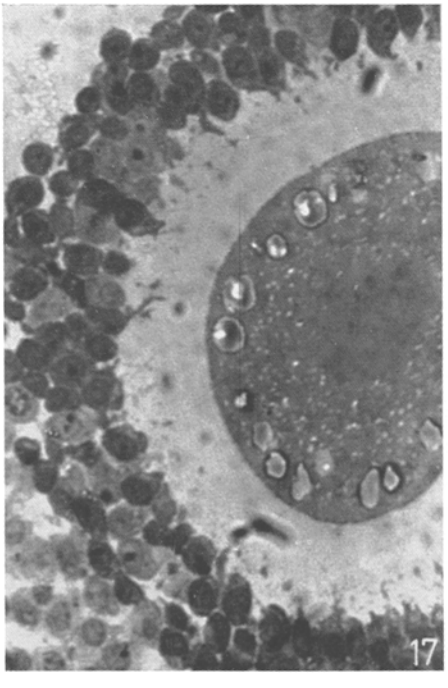
The lamellae are least well developed in the mouse, and they are not associated with large single ribosomes as they are in the rat and hamster. If the function

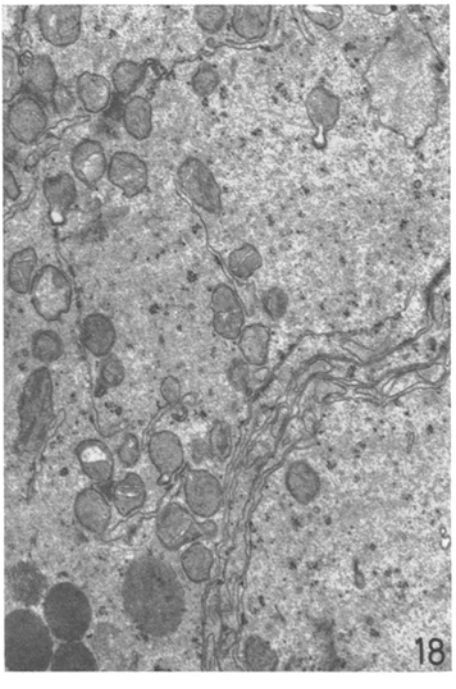
Fig. 13. Cytoplasm of rat oocyte in Graafian follicle showing tangential sections of cytoplasmic non-membranous lamellae. Four electron-dense bodies are seen to left of center. Above is a horseshoe-shaped cisterna of smooth endoplasmic reticulum with expanded ends. Its outer aspect is surrounded by vesicles and short cisternae of smooth membrane. It encloses electron-dense bodies resembling ribosomes. × 32.000

Fig. 14. Cat oocyte in follicle with single layer of flattened follicle cells. Nucleus contains a single large reticular nucleolus, several masses of fine granules, and scattered chromatin. The cytoplasm is filled with various types of membrane-bound bodies interspersed with mitochondria. The most frequently occurring type (*A*) contains fragments of membrane associated with amorphous material. Type *B* contains a pale homogeneous material. Type *C* contains a variety of membranous and granular materials. × 22.500









Figs. 15—18 (for legends see p. 121)

of the lamellae is concerned with protein synthesis, as their close association with large ribosomes and mitochondria in hamster and rat oocytes would suggest, the presence of more rough endoplasmic reticulum in the mouse oocyte might balance the poor lamellar development in this species. Also, it should be recalled in this connection that concentric systems of endoplasmic reticulum, associated with ribosomes at their inner and outer limits, are more common in the mouse than in the other species studied.

General Comparison of the Membranous Cytoplasmic Components of the Cytoplasm

The most striking differences in the oocytes of the five species studied concern variations in their systems of cytoplasmic membranes. Quite probably these variations, which occur in a rapidly expanding cytoplasm, are simply different methods of coping with the problem of how to manufacture enough membranous components to serve the growing oocyte. In rat and hamster the horseshoe-shaped smooth membranes and surrounding vesicles may function as "breeding areas" for smooth membranes. In the mouse the large accumulations of multivesicular bodies may serve a similar function, if the observations of PASTEELS and DE HARVEN (1963) are correct. Multivesicular bodies are seen in all the species studied, and some functional or developmental relationship with the horseshoe-shaped profiles of rat and hamster cannot be ruled out.

In the hamster, lattices of smooth membrane filled with electron-dense material are so consistently associated with modified Golgi bodies as to make their origin from the Golgi apparatus appear likely.

In mouse, cat and guinea pig the concentric arrays of endoplasmic reticulum could conceivably be sites of origin for cytoplasmic membranes. Alternatively, they may simply represent a convenient way of segregating metabolites for rapid assembly. Such concentric systems have been reported many times in the oocytes of invertebrates (e.g. REBHUN, 1956; AFZELIUS, 1957; SOTELO and TRUJILLO-CENOZ, 1957; PASTEELS and DE HARVEN, 1963; REVERBERI, 1966). They are sometimes associated with mitochondria and Golgi material, and it has been

Fig. 15. Higher magnification of bodies of type A in cat oocyte. The bounding membrane of body toward bottom is discontinuous. It contains a round structure resembling a lipid droplet. × 24.000

Fig. 16. Membrane-bound bodies in cat oocyte from Graafian follicle. The bodies contain clumps of electron-dense material. The bounding membranes appear discontinuous. Cortical granules are present at periphery of oocyte. × 27.200

Fig. 17. Cat oocyte in Graafian follicle. Homogeneous area in center represents the nucleus; cytoplasm is packed with large vesicles. Lipoid bodies ring oocyte periphery. Methylene blue staining. × 575

Fig. 18. Endoplasmic reticulum in guinea pig oocyte from multilayer follicle. Several narrow cisternae run roughly parallel to one another with expanded cisternae lying between them. Membrane-bound area containing pale homogeneous material is seen at upper right. Dense bodies are present at lower left. × 16.900

suggested that they represent the so-called "Balbiani's body" of the light microscopists. Since dense juxtanuclear accumulations of mitochondria (BALINSKI and DEVIS, 1963; ANTEUNIS *et al.*, 1966) and masses of Golgi material (ANDERSON and BEAMS, 1960; ZAMBONI and MASTROIANNI, 1966) have also been identified as "Balbiani's body" by electron microscopists, this question is still open. All three of these phenomena have been observed in the hamster oocyte at different stages of development, and are dealt with in a separate publication (WEAKLEY, 1967c).

Cytoplasmic Bodies

The salient feature of these bodies is their differing morphology, both between species and within the same oocyte. Very likely some of these entities are lysosomes or autophagic vacuoles. Others may contain enzymes of developmental importance. Some may represent reserve material equivalent to yolk. The round electron-dense bodies in rat, guinea pig and hamster are very similar morphologically to bodies identified as yolk platelets in invertebrates (e.g. see PASTEELS and DE HARVEN, 1963). The myelin figures common in the hamster oocyte and also observed in the rat oocyte may represent reserve material for synthesis of cytoplasmic membranes.

Morphological studies cannot determine the functions of these widely differing entities. Clarification must await further biochemical and cytochemical studies.

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