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Light and electron microscopy of developing germ cells and follicle cells in the ovary of the golden hamster: twenty-four hours before birth to eight days *post partum**

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INTRODUCTION

Development of the mammalian ovary in late foetal and early postnatal stages, though well documented by light microscopy, has received limited attention at an ultrastructural level (see review by Hadek, 1965). The information available is confined to the following species: rat: Sotelo (1959), Odor (1960), Franchi & Mandl (1962); mouse: Chiquoine (1960), Parsons (1962), human and monkey: Baker & Franchi (1966).

Prepubertal follicles of the golden hamster, *Mesocricetus auratus*, have been described at 14 and 15 d *post partum* by Odor (1965) and at 8 and 26 d *post partum* by Weakley (1966), but descriptions of earlier stages have not as yet been published.

The hamster develops *in utero* faster than any other placental mammal (Graves, 1945), having a gestation period averaging 14 d 21 h *post coitum* (Bond, 1945), or 15 d 17 h from estimated time of ovulation (Knigge & Leathem, 1950). Its ovaries at birth, therefore, might be expected to be less advanced in development than those of rodents (or other species) with longer gestation periods.

The present paper describes the development of germ cells and follicle cells in the hamster ovary from approximately 24 h before birth to 8 d *post partum*, and compares these findings with those reported for the species mentioned above.

Since clear-out criteria for atresia have not been established for peri-natal germ cells at the ultrastructural level, an attempt is made to pin-point phenomena which are clearly associated with this degenerative process. Other phenomena which may possibly be atretic but which also occur in apparently healthy germ cells are pointed out and discussed.

MATERIALS AND METHODS

Litters of hamsters were removed from the mothers at approximately 24 h before birth, at birth, and at 2, 4, 6 and 8 d *post partum*. The females from each litter were killed by decapitation and ice-cold 4 % glutaraldehyde fixative in 0·1 M cacodylate buffer, pH 7·4, was introduced into the abdominal cavity so that fixation would commence without delay. The ovaries were then removed to fresh glutaraldehyde fixative for 4 h, post-fixed in 1 % osmium tetroxide (Millonig, 1962) for 1 h, dehydrated, and embedded in araldite. 1 and 3 μm sections of the tissues were stained with a 1:1 solution of 1 % methylene blue and 2·5 % borax for purposes of orientation and

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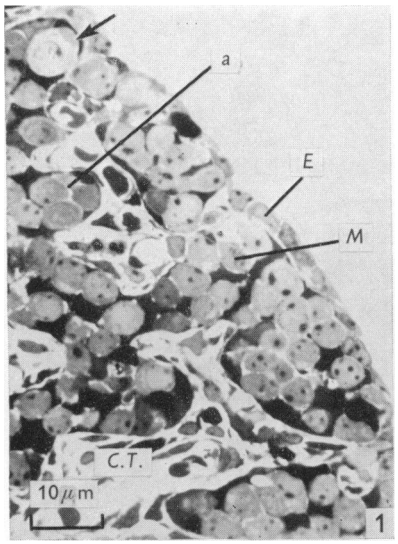
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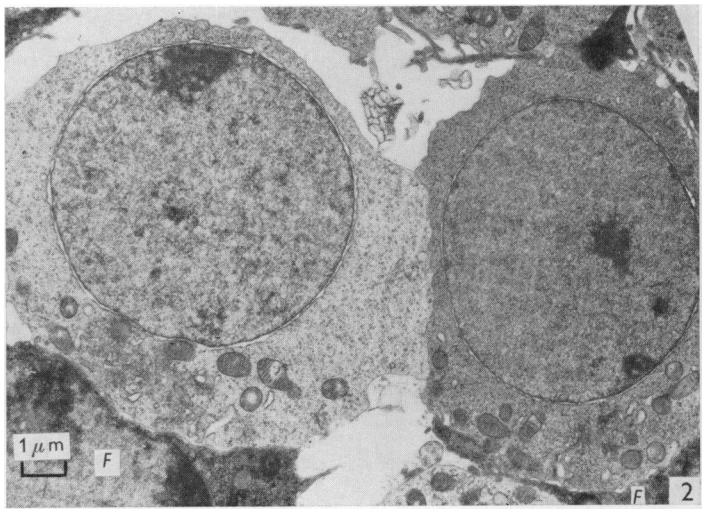
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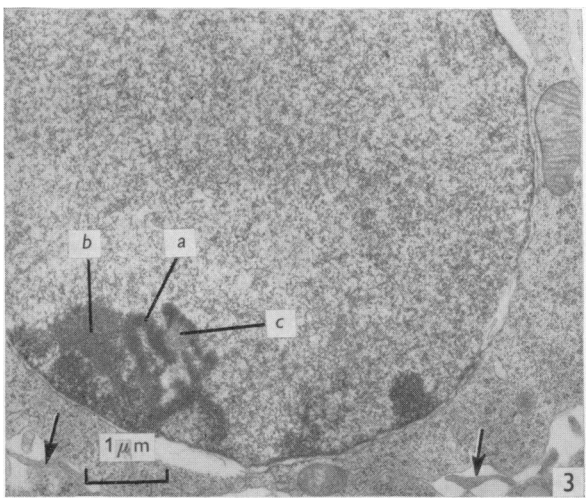
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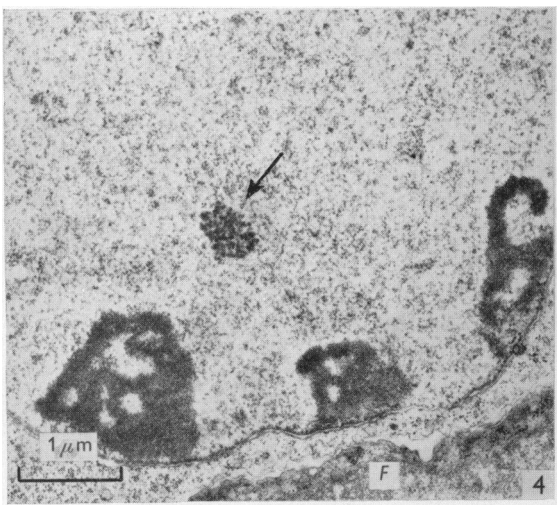
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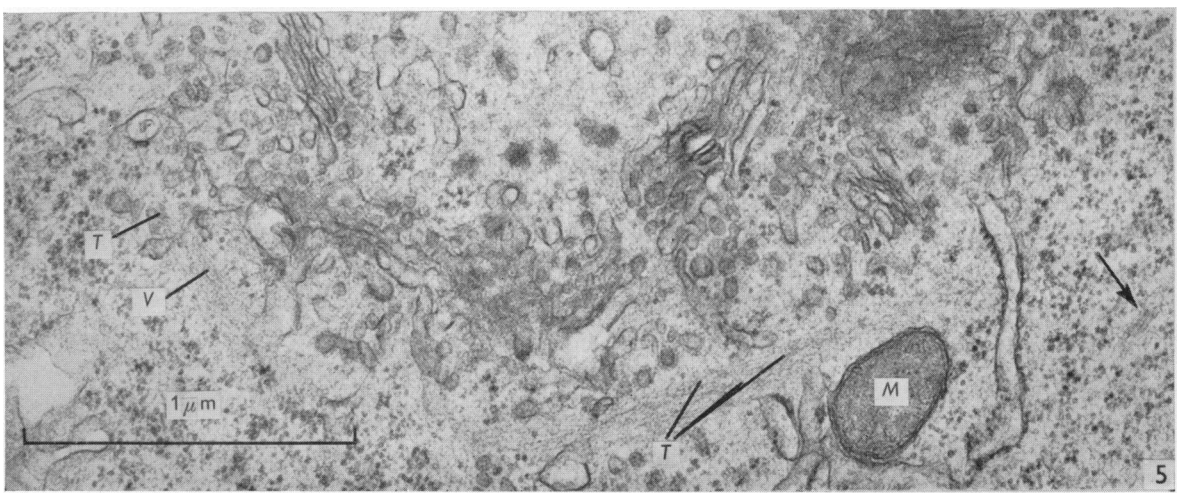
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study by light microscopy. Adjacent ultrathin sections of the same material were stained with uranyl acetate followed by lead citrate (Reynolds, 1963), and viewed with a Metropolitan Vickers EM 6 electron microscope.

RESULTS

Light microscopy

The ovary is covered by a single layer of epithelial cells which vary in morphology from flat to cuboidal. The cuboidal cells are convex on their outer aspect, giving a scalloped appearancd to the surface of the ovary.

Clusters of germ cells are present throughout the ovary and are separated from each other by cords of connective tissue through which course many blood vessels. In the foetal and newborn ovary the cords of connective tissue are wide and loosely packed; by 2 d *post partum* they have become dense narrow septa, two to three cells wide.

The number of germ cells per cluster is highly variable, ranging from two to over seventy. The clusters are larger toward the centre of the ovary, and developing follicle cells are scattered randomly among the germ cells. The follicle cells are flattened and distorted by tight packing between the germ cells. Toward the periphery the clusters are smaller, and are partially or completely delineated by a single layer of developing follicle cells (Fig. 1). Germ cells at the outer poles of these peripheral clusters may be completely surrounded by a single layer of flattened follicle cells. Both nucleus and cytoplasm of the majority of follicle cells stains intensely with methylene blue, greatly facilitating identification. As development proceeds, the larger central clusters break down into smaller clusters, and the ratio of follicle cells to germ cells increases. By 8 d *post partum* the clusters contain roughly 2 to 18 germ cells. Many

Fig. 1. Hamster ovary 24 h before birth. Follicle cells stain intensely, with methylene blue, outlining germ-cell clusters. The germ cells exhibit variable affinity for the stain; the larger ones toward surface of ovary are paler. Large pale germ cell at arrow has become completely surrounded by follicle cells. Nuclei of germ cells at (*a*) are darker than cytoplasm. Wide cords of connective tissue containing blood vessels separate the germ cell clusters. *M*, Mitotic germ cell. *E*, surface layer of epithelium; *CT*, connective tissue.

Fig. 2. Variations in electron density of oogonia in hamster ovary 24 h before birth. The outer nuclear membrane of both cells is reflected outward into the cytoplasm at many points. Mitochondria are concentrated around the Golgi region. The matrix of several mitochondria is 'patchy'. *F*, Follicle cells.

Fig. 3. Oogonium from new born hamster. The nucleolus has three components: a heavy electron-dense reticulum (*a*); an extensive electron-dense granular component (*b*); and a small, round, less electron-dense component (*c*). The nuclear membrane is breaking down, presumably in preparation for mitotic division. Cytoplasmic processes from associated follicle cells are seen at arrows.

Fig. 4. Oogonium from foetal hamster. Three peripheral nucleoli, sectioned through the reticular component, are present. A cluster of electron-dense granules ranging in diameter from 400 to 950 Å appears in the nucleoplasm (arrow). *F*, Associated follicle cell.

Fig. 5. Golgi zone in oogonium from foetal hamster. The Golgi apparatus consists of several stacks of lamellae and associated vesicles. About the periphery of the Golgi zone are masses of fibrous material which appears to contain small tubules (*T*) or vesicles (*V*). Microtubules are scattered in the cytoplasm (arrows). A tubule of rough endoplasmic reticulum and other tubules of uncertain type are seen at right, associated with a mitochondrion (*M*) in which cristae are not seen.

germ cells by that time have become separated from the clusters and are enclosed in unilaminar follicles, a few of which have cuboidal granulosa cells. One follicle was observed at 8 d which had two layers of cuboidal granulosa cells.

In the foetal ovary a few follicle cells are seen which do not take an intense stain with methylene blue. At subsequent stages more of these 'pale' follicle cells are observed, although the majority still stain intensely. The germ cells, although staining much less intensely than the dark follicle cells, exhibit a range of affinity for methylene blue which correlates with germ-cell size: the smaller cells stain more deeply than the larger ones. In the foetal ovary the germ cells vary in diameter from 7 to 16 μm and have a fairly deep colour range. The nuclei of the small dark germ cells stain with about the same intensity as does the cytoplasm. As the germ cells increase in size, the cytoplasm starts to lose methylene-blue affinity before the nucleus does. By 4 d *post partum*, however, a few larger germ cells have lighter nuclei than cytoplasm, and this trend continues as the germ cells grow. Germ cells with similar staining characteristics tend to occur together in groups, suggesting a certain synchrony of development between neighbouring cells. Peripheral germ cells tend to be larger and paler than those toward the centre of the ovary. By 8d *post partum* germ-cell diameters range from 9 μm to 30 μm, and the larger germ cells are almost colourless.

The germ cells have large round-to-oval nuclei which are central or slightly eccentric at the earlier stages. The eccentricity increases with development, the larger germ cells accumulating cytoplasm at one pole of the cell. Within this area of cytoplasm a few granules measuring about 1 μm in diameter are visible. One to four large peripheral nucleoli are present in the germ-cell nuclei before birth. Number and size decreases after birth, one or two being present at 8 d *post partum*. Before birth a nucleolus may be so large (up to 4 μm in diameter) as to form a 'cap' at one pole of the nucleus. After birth they become distinctly reticular in appearance and seldom exceed 2 μm in diameter. By 8 d *post partum* many of them have become elongate and lie parallel with the nuclear membrane.

Up to eighteen germ cells per section are seen to be in mitosis in the foetal ovary. These increase dramatically in number, up to forty mitoses per section being observed in newborn material. The maximum has dropped to fourteen per section by 2 d *post partum*. Only the smaller dark cells are involved in mitotic activity, which becomes rare by 6 d *post partum* and is not seen at all by 8 d. Cells at the same stage of mitosis tend to occur together in groups, again suggesting synchrony of development between neighbouring germ cells.

Except for nucleoli, most non-mitotic germ cell nuclei in the foetal ovary appear devoid of structure. In the newborn, however, a few germ cells have in their nuclei one or two elongate condensations of material which stain less intensely than do mitotic chromosomes, and are more variable in size and shape. They always occur in nuclei in which the nuclear membrane is intact, and are similar to the meiotic condensations described in 1–3 μm sections of plastic-embedded material by Moses (1956, 1958). By 2 d *post partum* these meiotic condensations are seen in most germ cells except for the smaller, darker ones. They are often associated with the nucleoli, around which they may form a crescent. By 6 d *post partum* the larger germ cells which have become separated into unilaminar follicles seldom show meiotic phenomena and appear to have reached the dictyate phase. At 8 d, all germ cells appear

to be either in meiotic prophase, have reached the dictyate stage, or have become atretic.

Atretic phenomena are present at all stages but reach a maximum at 6 and 8 d *post partum*. In the foetal ovary a few germ-cell nuclei appear atretic, with clumped chromatin in a very pale ground substance. Deeply staining, rounded masses are sometimes seen in the cytoplasm of germ cells, between germ cells and follicle cells, and within the cytoplasm of the follicle cells. They average 4·8 μm in diameter and appear to be atretic material originating in the oocyte and subsequently phagocytosed by the follicle cells. After extrusion from the germ cells, the masses become denser, more deeply staining, and appear to be broken down within the cytoplasm of the follicle cells, where they are seen as small fragments. In the newborn, in addition, large cytoplasmic vacuoles measuring up to 2 μm in diameter are seen in the germ cells. At 6 and 8 d *post partum*, up to thirty germ cells per section appear atretic: nuclear chromatin is condensed and the cytoplasm appears clumped and curdled. Other germ cells, though not unequivocally atretic, have suspiciously dark nuclei, which may represent the beginning of atresia.

Germ cells *Electron microscopy* (24 *h before birth*)

Nucleus. The background nucleoplasm has electron density approximately similar to that of the background cytoplasm in most cases, although it is slightly darker in a few cells. Neighbouring germ cells are usually of about equal electron density, but a few show a considerable difference, as in Fig. 2.* The degree of electron density appears to correlate with the degree of affinity for methylene blue at the optical level. A heavy scattering of granular and filamentous material is quite evenly distributed throughout the nucleus. About half the nuclei have condensations of chromatin material along the nuclear margin, often oriented at right angles to the nuclear membrane, as in Fig. 6. Nuclear pores occur at irregular intervals and are closed by diaphragms. The outer layer of the double nuclear membrane is often reflected outward into the cytoplasm (Fig. 2).

The nucleoli are massive. Study of a large number of nucleoli at all stages suggest that each nucleolus is composed of three regularly occurring components (Fig. 3): (1) a heavy, electron-dense reticular network; (2) a large mass of electron-dense granular material; (3) a smaller, round, less electron-dense ball of more finely granular material.

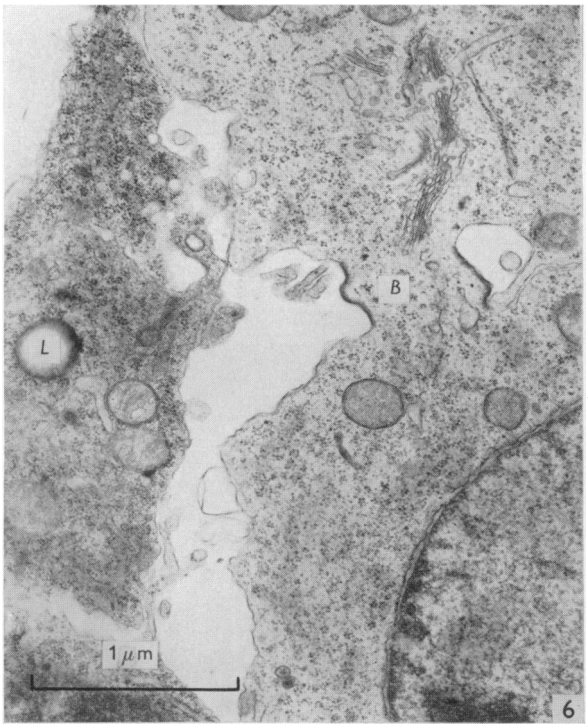
The nucleoli differ from one stage to another mainly with respect to size and number rather than with respect to nucleolar components.

The 'caps' of deeply staining material seen in some nuclei by light microscopy are seen by electron microscopy to be extra-large nucleoli, sectioned through the granular component.

Clusters of electron-dense granules measuring from 400 to 950 Å in diameter are sometimes observed in a nucleus (Fig. 4), at all stages studied.

Most of the mitotic cells observed are in early prophase, the nuclear membrane still intact and the chromosomes incompletely condensed, giving a 'speckled' appearance to the nucleus. No meiotic chromosome cores were seen.

* In the figures all material for electron microscopy was stained with uranyl acetate and lead citrate.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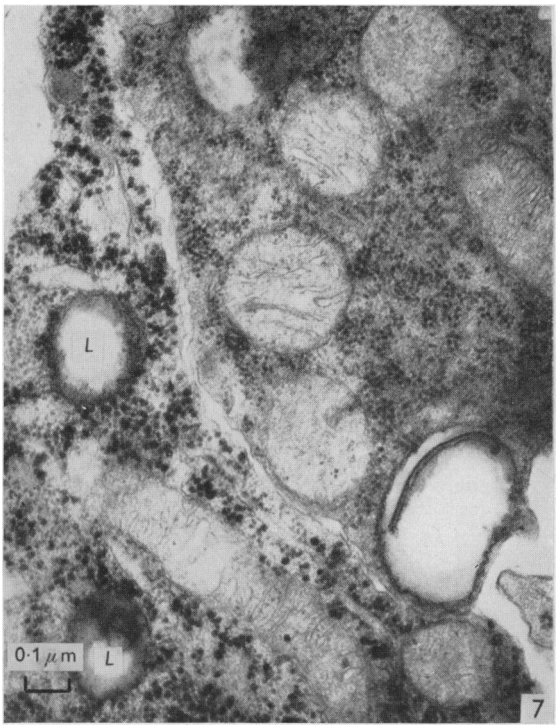
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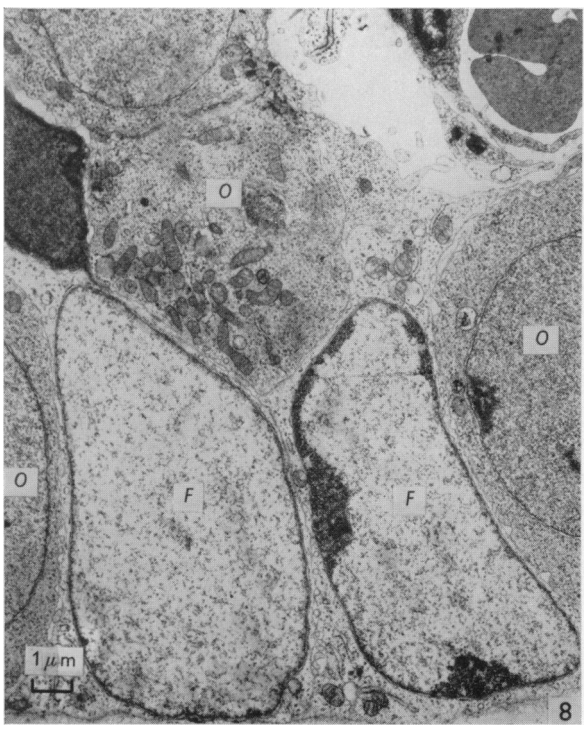
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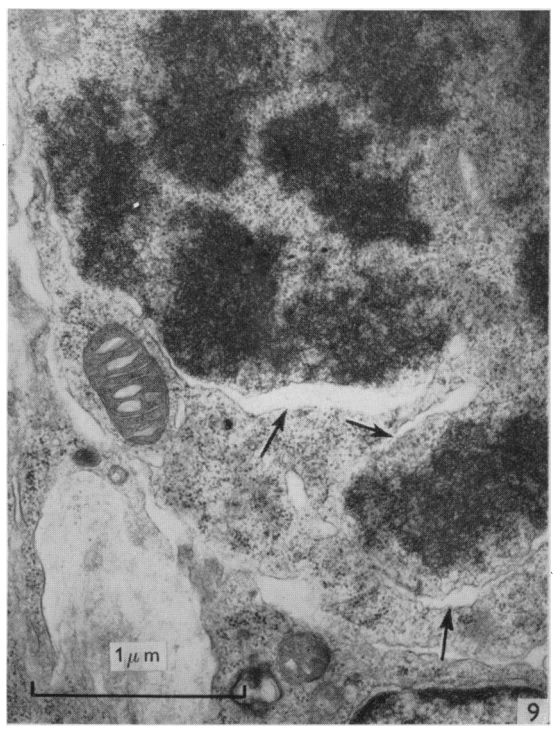
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Cytoplasm. Although the cytoplasmic ground substance of most of the germ cells is relatively pale, a few cells exhibit considerable electron density, as seen in Fig. 2. Free ribosomes are abundantly scattered throughout the cytoplasm and may occur singly, in clusters or in chains. A few single tubules of rough endoplasmic reticulum are randomly scattered in the cytoplasm (Fig. 5). More numerous are vesicles and tubules of smooth endoplasmic reticulum. A small number of microtubules has been observed in some germ cells (Fig. 5).

The Golgi apparatus is mostly lamellar in form and is confined to one area, although it may be quite massive and include five or more stacks of lamellae. In some cells it is associated with elongate granular-filamentous masses containing fine tubules (Fig. 5). Similar masses are also seen (at this and at succeeding stages) closely associated with the nuclear membrane (Fig. 11) and with mitochondria (Figs. 10, 18).

Mitochondria are round to elongate, and their matrix, which is moderately electron dense, may have lighter 'patches' containing vesicles (Fig. 2). Cristae usually run at right angles to the long axis of the mitochondrion (Fig. 3), but may be few in number or absent (Figs. 5, 6). The mitochondria may be scattered at random in the cytoplasm, but are most frequently located at one pole of the cell, in association with the Golgi apparatus (Fig. 2). Single mitochondria are often in close association with the nuclear membrane.

The periphery of the germ cells is free of microvilli but often has a scalloped appearance. Formation of pinocytotic vesicles is common. In germ-cell clusters the plasma membranes of two adjacent cells may run closely parallel for a distance of a micron or more, or the two membranes may both be scalloped and therefore separated by a varying distance.

Cytoplasmic bridges are often seen at this and at succeeding stages between germ cells (Fig. 6).

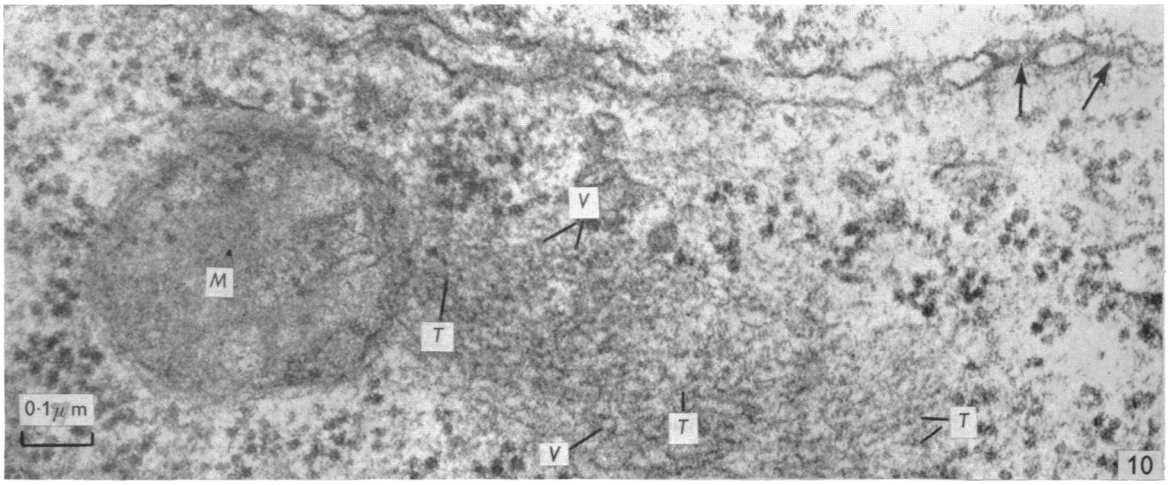
Atretic phenomena. Since the degenerative phenomena observed at each stage, appear to differ only in frequency of occurrence, they are described together in a later section.

Fig. 6. Cytoplasmic bridge (*B*) between germ cells. The lateral limits of the bridge are electron-dense. Golgi lamellae extend down toward the bridge and vesicles of smooth endoplasmic reticulum appear within the bridge. Cristae are not evident within the mitochondria, and the mitochondrial boundaries are indistinct in places. Note chromatin condensations occurring beneath and at right angles to nuclear membrane. The follicle cell associated with the germ cells has typical electron-dense cytoplasm and contains a lipoid droplet (*L*).

Fig. 7. Cytoplasm of two adjacent follicle cells. Clusters of ribosomes averaging 180 Å in diameter are present in both cells. Cell at left also contains larger, more electron-dense particles averaging 300 Å in diameter. Mitochondrial membranes appear indistinct.

Fig. 8. Two pale follicle cells (*F*) associated with three oogonia (O). The nuclei of the follicle cells are smooth in contour and almost devoid of chromatin except along nuclear membrane. Two peripheral nucleoli are present in follicle cell at right.

Fig. 9. Mitotic oogonium from newborn hamster. Remnants of the nuclear membrane are associated with the chromosomes (arrows). Mitochondrial cristae have enlarged to form electron-lucent vacuoles.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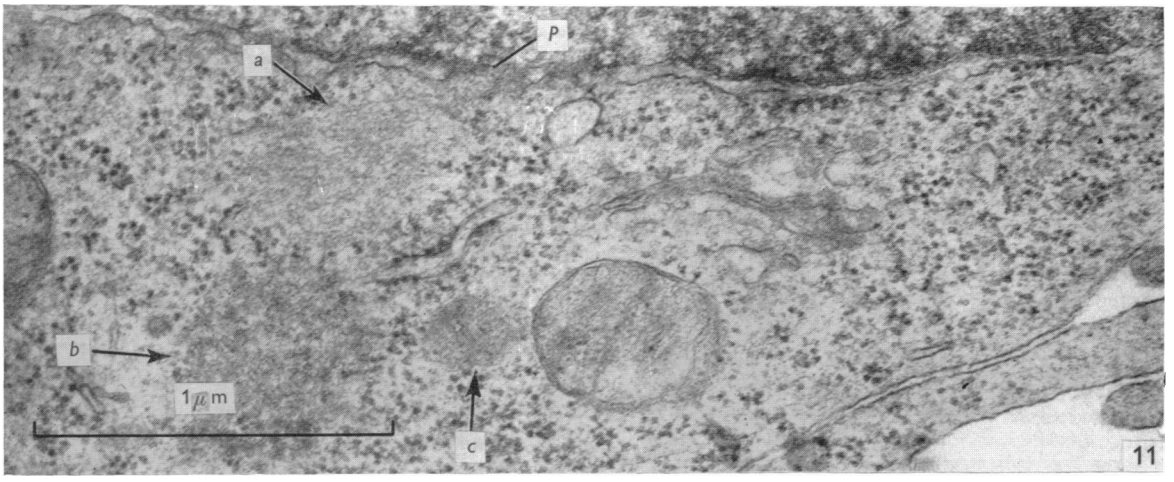
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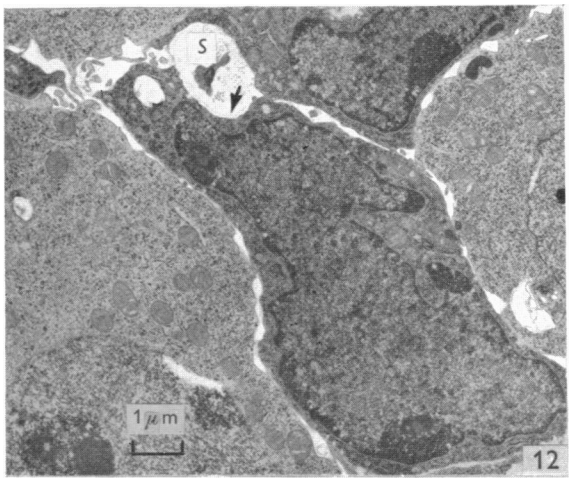
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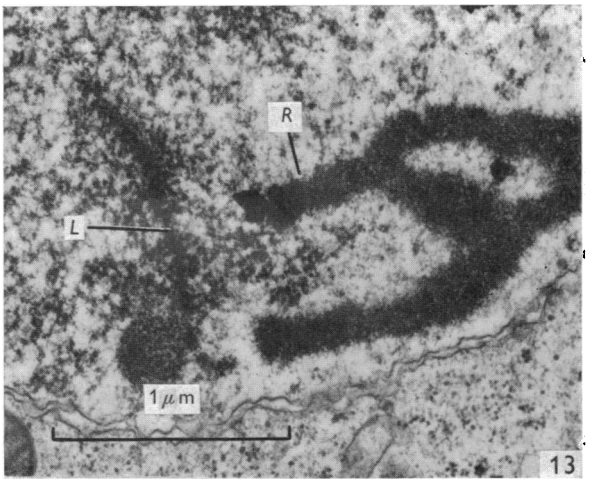
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Follicle cells

The follicle cells usually lie along one side of a germ cell and send out thin cytoplasmic processes along the germ-cell margin. A few germ cells are almost completely surrounded by two or more follicle cells; others may be associated with follicle cell cytoplasm for only a small porton of their circumference. The follicle cell cytoplasm may be so attentuated that a portion of its nucleus (often containing a nucleolus) comes within 1000 Å of the plasma membrane of the oocyte.

Most of the follicle cells exhibit a highly electron-dense background nucleoplasm and cytoplasm (Fig. 2), which corresponds to the intense affinity of these cells for methylene blue stain in 1–3 μm sections.

The nucleus of the follicle cells is oval to elongate and highly irregular in contour. Chromatin is distributed unevenly and randomly throughout the nucleoplasm, with an almost continuous condensation beneath the nuclear membrane. One or two nucleoli are also located beneath the nuclear membrane. They appear either as uniformly electron-dense masses, or may contain a small area of less electron density.

The cytoplasm of the follicle cells usually has a higher content of free ribosomes than does that of the germ cells. Ribosomes measuring apprixomately 180 Å in diameter occur in clusters throughout the cytoplasm except within the Golgi area. A few cells have been encountered which also contain larger, more electron-dense particles averaging about 300 Å in diameter. Figure 7 shows the two types of particle in neighbouring cells.

Long strands of rough endoplasmic reticulum are often closely associated with mitochondria and contain an amorphous material. A few small vesicles and short tubules of smooth endoplasmic reticulum are present.

The Golgi apparatus is composed of elongate stacks of cisternae associated with a few small vesicles. Cilia are occasionally observed in the Golgi region, originating from the distal centriole.

Mitochondria are numerous, round to moderately elongate, with cristae running at right angles to the long axis. The matrix of the mitochondria may have the same density as the background cytoplasm, or may be paler.

Lipoid droplets commonly occur in the follicle cell cytoplasm (Figs. 6, 7). Some of them are homogeneous in appearance; others have lost their central areas,

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Fig. 10. Portion of oocyte from newborn hamster. Tubular structures are visible in the diaphragm of the nuclear pores (arrows). A mitochondrion (*M*) is associated with a mass of filamentous material which appears to contain tubules (*T*) and vesicles (*V*).

Fig. 11. Three masses of filamentous-tubular material (arrows) in cytoplasm of germ cell from newborn hamster. Mass (*a*) is closely associated with the nuclear membrane at the site of a nuclear pore (P). Mass (*b*) is free in the cytoplasm; mass (*c*) is near a mitochondrion.

Fig. 12. Two dark follicle cells from ovary of newborn hamster. The nuclear contour is highly convoluted. The two cells enclose a space (*S*) containing collagen fibres and are separated from it by basement membrane (arrow). The follicle cell cytoplasm is heavily laden with free RNP particles.

Fig. 13. Leptotene chromosome core (*L*) lies within an electron-dense mass of chromosome material and is associated at one end with a round ball of granular substance. The reticular component of the nucleolus (*R*) partly surrounds some of the chromosome material.

presumably by extraction in the processing fluids. Pinocytotic vesicles are commonly observed along the margins of the cell.

The follicle cells are not separated either from the germ cells or from themselves by basement membrane. However, when one margin of the cell abuts on a tissue space containing collagen, this margin is separated from the space by a basement membrane (Fig. 12).

A few follicle cells were encountered which lack the usual electron density (Fig. 8). Too few were seen to make a meaningful comparison of their morphology with that of the dark follicle cells, but superficially the nuclei appear to have a smoother contour and to contain less chromatin; the cytoplasm appears to have fewer ribosomes. Lipoid droplets were not seen within the cytoplasm.

Electron microscopy (newborn)

Germ cells

Nucleus. The nucleoli are more compact and appear less prominent than before birth. In favourable sections the diaphragms of the nuclear pores are seen to be penetrated by tubular structures (Fig. 10).

Many germ cells show a wide separation of the nuclear membranes at one or more places along the nuclear circumference, leaving an electron-lucent gap between nucleus and cytoplasm (Fig. 3). At these gaps one or both of the nuclear membranes may have disintegrated. It is possible that this represents the beginning of nuclear membrane dissolution prior to mitotic division. In mitotic cells, widely separated remains of the double nuclear membrane persist near the prophase chromosomes (Fig. 9).

No meiotic chromosome cores are seen. However, in a few nuclei elongate accumulations of tightly packed electron-dense granules, probably corresponding to the condensations seen by light microscopy, are present.

Cytoplasm. Typical rough endoplasmic reticulum is not encountered at this or later stages, although an occasional vesicle may have one or two ribosomes attached to its cytoplasmic surface. The Golgi apparatus is still mostly lamellar in form. The cristae of some mitochondria, especially in mitotic cells, are enlarged and contain an electron transparent substance (Fig. 9). The enlargement may be sufficiently great to form large central cavities within the mitochondrion. Both 'normal' and enlarged cristae may occur within the same mitochondrion.

At times the mitochondria are associated with filamentous-tubular material resembling that seen near the Golgi apparatus in Fig. 5. Figure 10 shows such a mass apparently attached at one end to a mitochondrion. Figure 11 shows three such masses in the cytoplasm, one of which is associated with the nuclear membrane at the site of a nuclear pore.

Follicle cells

The follicle cells have not changed significantly since 24 h before birth. Some of the nuclei are deeply indented by cytoplasmic tunnels (Fig. 12). Cisternae of the Golgi apparatus often lie close to and parallel with the cell membrane, thus being in close proximity to the plasma membrane of the germ cells.

Germ cells *Electron microscopy* (2 *d post partum*)

Nucleus. Although all three components of the nucleoli are seen at this stage, most prominent is the heavy, rounded, compact reticular mass. Many nucleoli are associated with elongate masses of granular chromosome material. The masses also occur without apparent association with nucleoli, and are often denser toward the centre, along the long axis. Many are clearly associated with a leptotene chromosome filament (Fig. 13), which usually lies at right angles to the nuclear membrane, approaching it or actually contacting it. Small beads roughly 250 Å centre to centre and measuring approximately 110 Å in diameter are attached to the outer aspects of the filament (Fig. 14). At times a filament is seen to branch. The filaments appear to twist, and margins are ill defined; thus accurate measurement of width is extremely difficult. Estimates have ranged from 280 to 700 Å. At high magnification the filaments are seen to be double in nature, composed of two closely approximated strands measuring roughly 140 Å in width (Fig. 14).

In two instances filaments were observed lying parallel to one another about 1200 Å apart, and one typical tripartite chromosome core was seen. The inner aspects of the lateral filaments of the tripartite core were beaded. The outer aspects were too obscured by electron-dense chromosome material to ascertain whether or not beading is present.

Cytoplasm. Smooth endoplasmic reticulum at this stage falls into two categories:

(1) Round-to-oval vesicles ranging in diameter from 500 to 1200 Å, and short cisternae of approximately the same diameter. The profiles often contain membranous material and may have a moderately electron-dense amorphous substance associated with the inner aspect of the bounding membrane (Fig. 16).

(2) Small vesicles *c.* 300 Å in diameter and short, slender tubules.

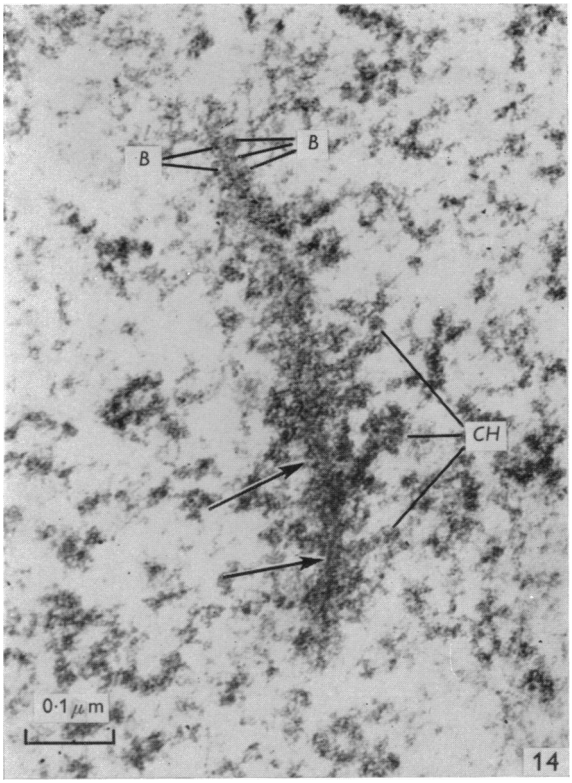
The Golgi apparatus has an increased number of vesicles associated with its lamellae. The mitochondria have not changed appreciably.

Follicle cells

The follicle cells at 2 d *post partum* are essentially the same as at the newborn stage.

Germ cells *Electron microscopy* (4 *d post partum*)

Nucleus. The ground substance of the nucleus is usually paler than that of the cytoplasm, but large condensations of granular chromosome material are more common and more electron dense than at the 2 d stage. The reticular component of the nucleoli is not as distinct, tending to be 'fuzzy' and less clearly demarkated from the surrounding nucleoplasm. Leptotene chromosome filaments are still seen, but more common are the tripartite chromosome cores typical of synaptic chromosomes (Fig. 15). Electron-dense granular chromosome material surrounds the outer aspects of the tripartite cores. There is some evidence of beading along the inner aspect of the lateral filaments. Fine threads connect the lateral filaments with the central filament. The tripartite cores may be located anywhere within the nucleus, but are most frequently seen near and at right angles to the nuclear membrane.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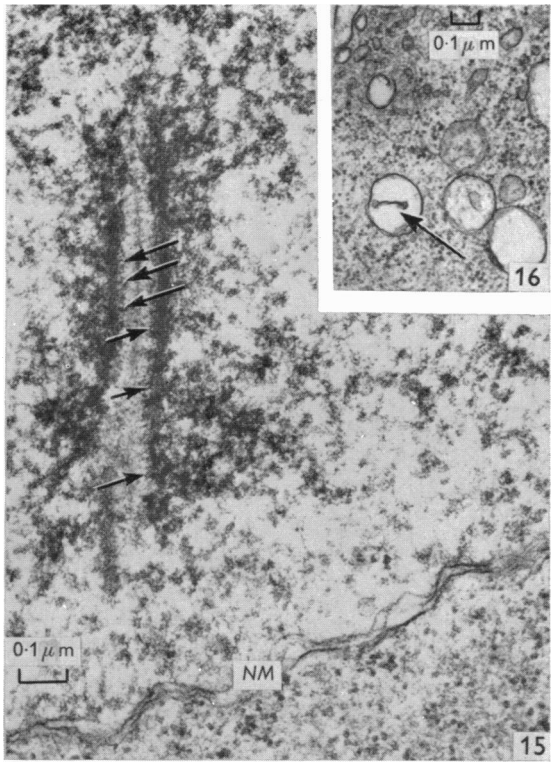
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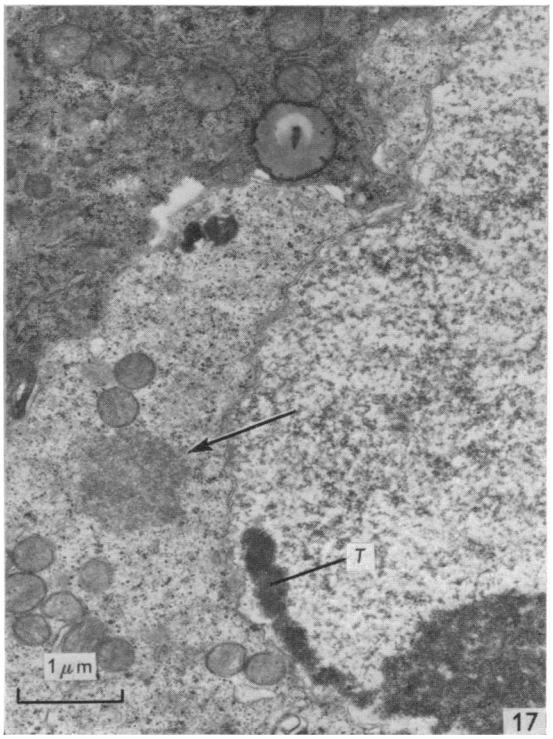
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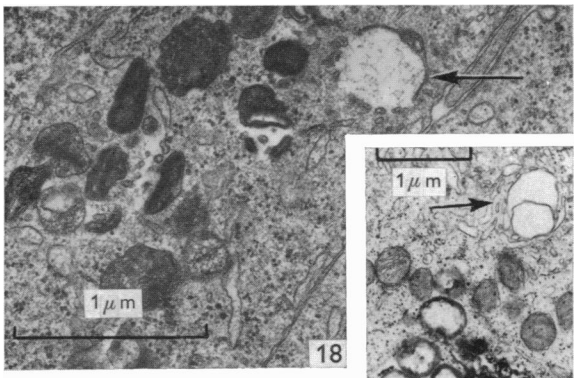
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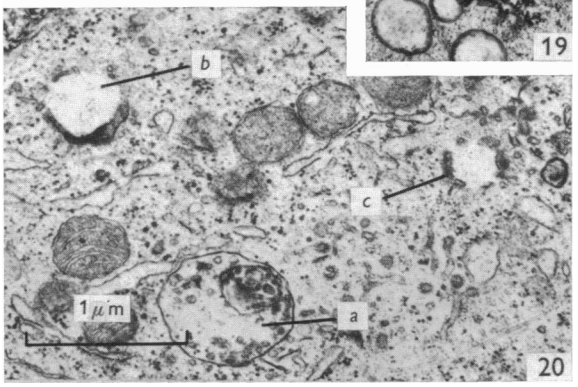
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Cytoplasm. A distinctive feature of the larger vesicles of smooth endoplasmic reticulum is the presence within many of them of electron-dense rods with bulbous ends (Fig. 16). A few rods of this sort were seen at the 2-d stage and rarely in the newborn. The vesicles are seen scattered throughout the cytoplasm, but tend to concentrate around the Golgi region.

Mitochondria have increased in number and occur in clusters of four or five as well as singly. The cristae tend to be irregular, and may be enlarged.

Follicle cells

The follicle cells are essentially similar to those at the 2-d stage.

Electron microscopy (6 *d post partum*)

Germ cells in polyovular follicles

Nucleus. The contours of the nuclei tend to be scalloped. The background nucleoplasm is in general considerably lighter than the background cytoplasm. All three nucleolar components are seen, but the heavy, compact reticulum is now less frequently encountered than are the dense granular masses. These masses often contain light, tunnel-like areas which are positioned at right angles to the nuclear membrane. A 'tail' of reticular material is sometimes seen to originate from the nucleolus proper and lie beneath the nuclear membrane (Fig. 17).

Leptotene chromosome filaments are no longer observed, but many nuclei contain tripartite complexes.

Cytoplasm. Figures 18 and 20 show large rings of smooth membrane which appear to be breaking down into small vesicles and short tubules. Otherwise there is no change in the endoplasmic reticulum.

Fig. 14. Leptotene chromosome core is seen at high magnification to be 2-stranded (arrows). Beads (*B*) are attached to the outer aspects of the core. The core is surrounded by 'branches' of granular chromosome material (*CH*).

Fig. 15. Tripartite chromosome core in nucleus of oocyte from 4-d-old hamster. The core is surrounded by electron-dense chromosome material. Fine threads connect the two lateral filaments of the core with the central filament. Beading occurs along the inner aspect of the lateral filaments (arrows). The core is positioned at right angles to nuclear membrane (*NM*).

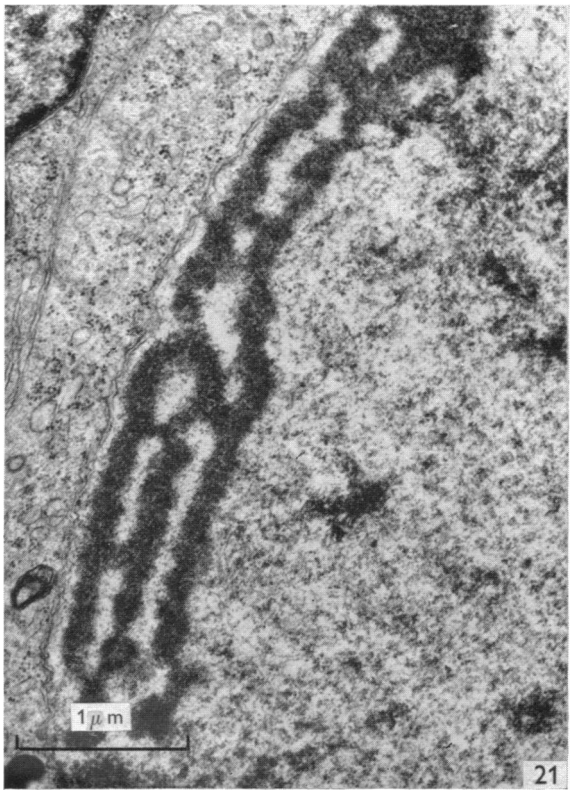
Fig. 16. Area near Golgi zone of oocyte from 4-d-old hamster. Many vesicles of the larger category of smooth endoplasmic reticulum are present and contain membranous and granular material. An electron-dense rod with bulbous ends is present in one of them (arrow).

Fig. 17. Portions of oocyte and follicle cell from 6-d-old hamster. A 'tail' (*T*) from nucleolus lies parallel to nuclear membrane. A granular-fibrillar-tubular mass of material lies in the cytoplasm near a mitochondrion (arrow). A cytoplasmic process from the follicle cell indents the oocyte cytoplasm and approaches the nuclear membrane.

Fig. 18. Electron-dense inclusions in cytoplasm of oocyte from 6-d-old hamster. Some of the inclusions contain vesicular or granular material; others contain concentric membranes. A ring of smooth endoplasmic reticulum is breaking down into small vesicles (arrow).

Fig. 19. Meiotic oocyte, 6-d-old hamster: area of cytoplasm containing bodies with electron-dense periphery and paler homogeneous interior. Note also swollen Golgi apparatus (arrow).

Fig. 20. Inclusion (*a*) with electron-lucent interior containing vesicular, membranous and amorphous substance. At upper left (*b*) a ring of smooth endoplasmic reticulum is breaking down into vesicles and short tubules. At upper right (*c*) vesicles and short tubules disposed in a circle around a clear area may represent a later step in the same process.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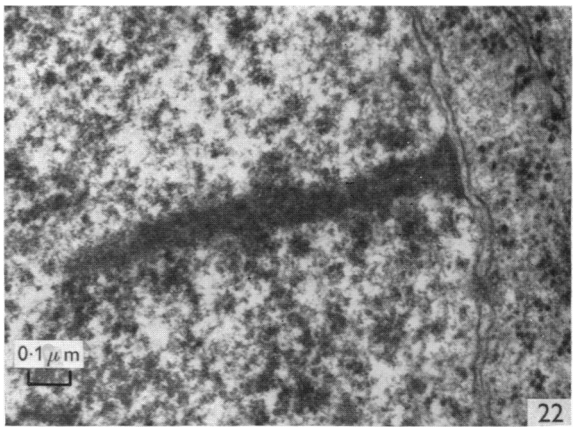
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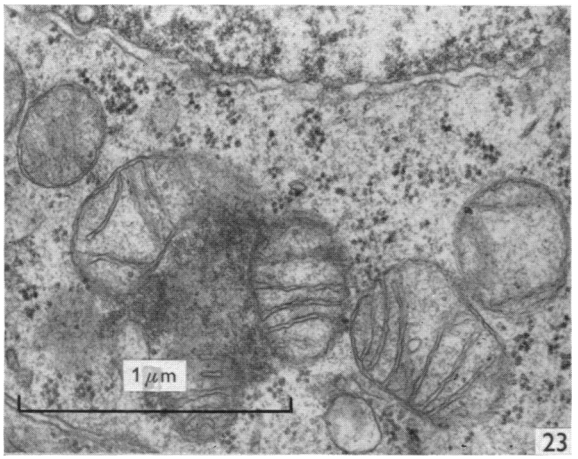
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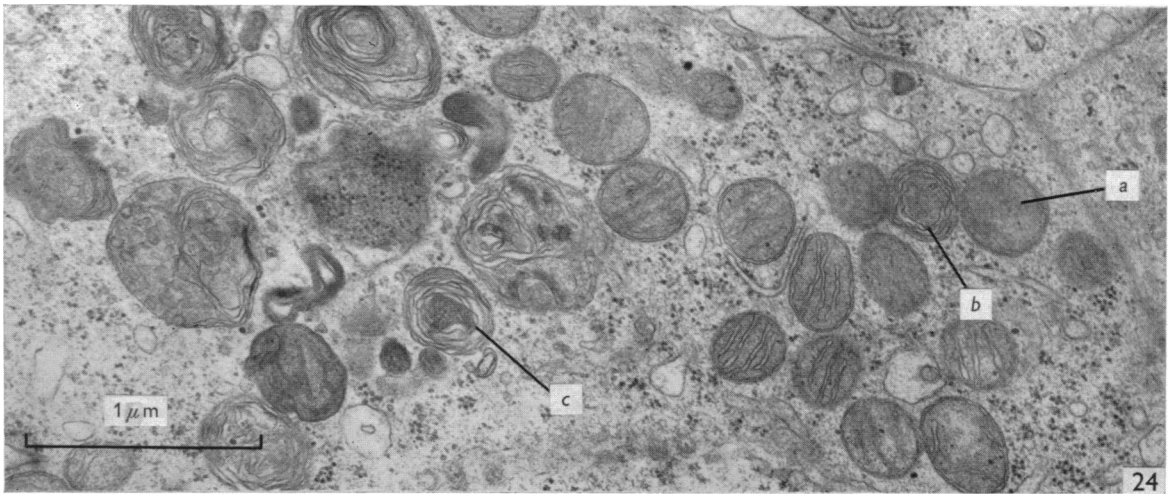
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Golgi material is massive, and may occupy the entire space between nuclear membrane and plasma membrane. More vesicles are present among the lamellae than at earlier stages. The lamellae may be narrow and occur in tight stacks, or may be swollen and contain electron-transparent material (Fig. 19).

The number of mitochondria has increased dramatically. They may occur singly or in large or small clusters. A few photographs suggest that intermitochondrial substance is beginning to accumulate within a few clusters. In a few mitochondria the cristae are arranged parallel to the mitochondrial membrane. Though the majority of mitochondria still have regular cristae, they are often sparsely distributed, which may account for the apparent absence of cristae in some.

In all of the germ cells, cytoplasmic inclusions are seen. They are highly variable in morphology and number, and are most frequently located around the Golgi zone, intermingled with mitochondria and smooth endoplasmic reticulum. The following types are seen:

(1) (The most numerous type): electron-dense bodies of varying shape containing other material of higher electron density (Fig. 18). The darker contents often appear membranous, and may form concentric lamellar systems about the periphery of the body.

(2) Large membrane-bound bodies with a pale background, containing vesicular, membranous or amorphous substances (Fig. 20).

(3) (Rarely encountered): bodies slighty larger than mitochondria with an electron-dense periphery and a paler, homogeneous interior which resemble lipoid droplets (Fig. 19).

Germ cells in separate unilaminar follicles

Most of the germ cells in separate unilaminar follicles have apparently reached the dictyate stage of meiosis. No tripartite chromosome cores were observed. In a few, however, diplotene chromosome cores were seen: i.e. single ribbons of electron-dense material measuring roughly 900 Å in width.

Follicle cells

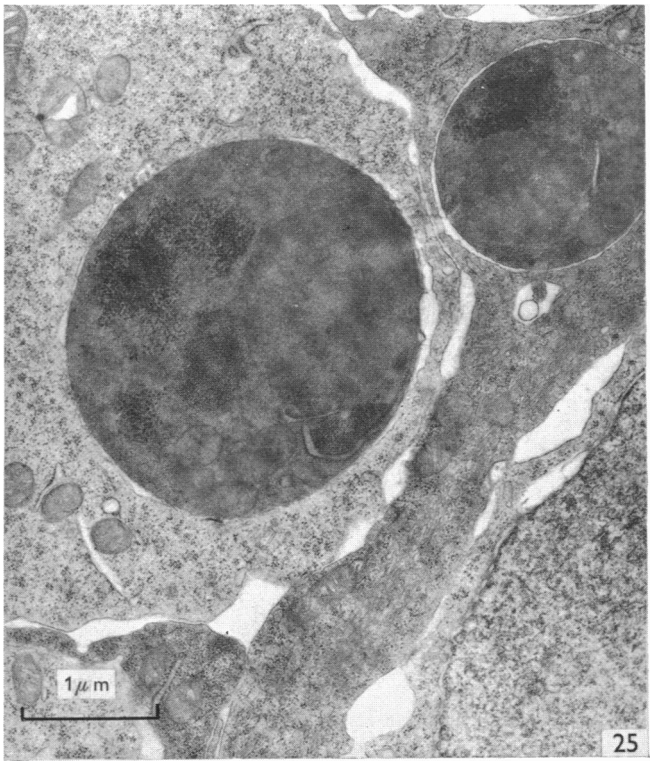
There are few changes in the follicle cells. The cytoplasmic lipoid droplets may have a granular, electron-dense outer border of variable width (average about 600 Å) (Fig. 17).

Fig. 21. Nucleolus which has elongated into a narrow reticulum stretched beneath nuclear membrane. Oocyte from 8-d-old hamster.

Fig. 22. Diplotene chromosome core in nucleus of oocyte from 8-d-old hamster. The core is widest at nuclear membrane where a distinct zone of attachment is seen.

Fig. 23. Mitochondria in oocyte from 8-d-old hamster. Intermitochondrial substance is beginning to accumulate between three of the mitochondria. The mitochondrial membranes appear interrupted so that the mitochondrial matrix seems continuous with the intermitochondrial substance. Cirstae are not seen in mitochondrion at upper left and are few in number in one at right.

Fig. 24. Mitochondria and bodies containing concentric lamellar membrane systems. Some mitochondria appear devoid of cristae (*a*); in others the cristae parallel the mitochondrial membrane (*b*); body at (*c*) cannot be identified unequivocally as either a mitochondrion or as a lamellar body and appears to be transitional between the two. It contains a central area of granular material.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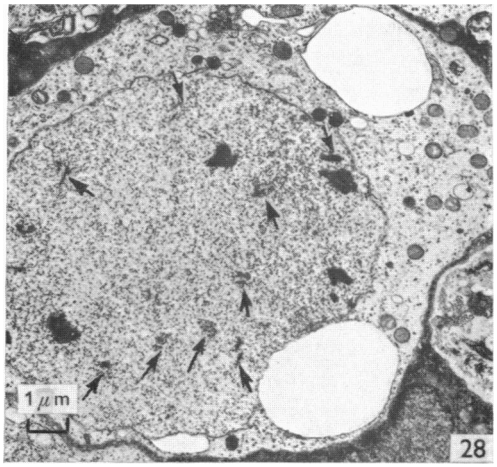
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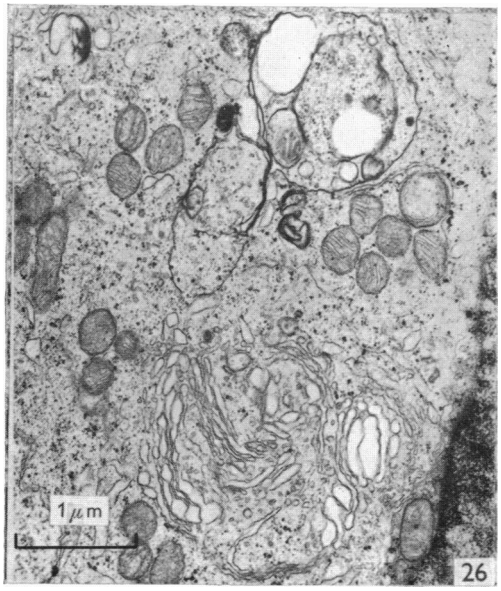
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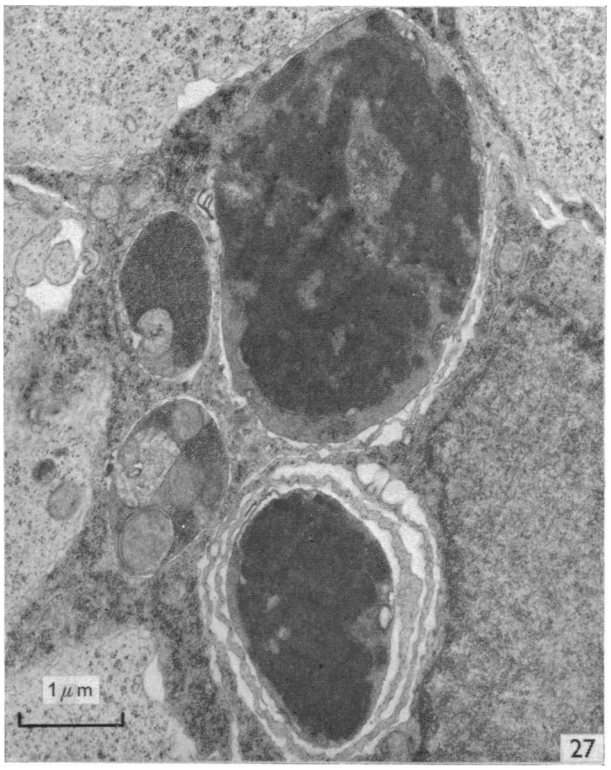
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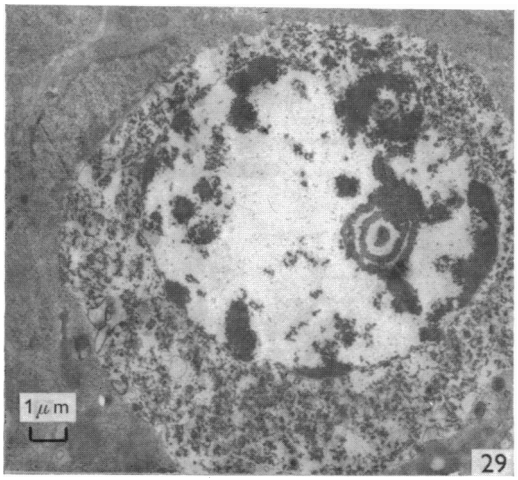
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Slender processes from the follicle cells are seen to indent the oocyte cytoplasm deeply (Fig. 17), and may appear to contact the nuclear membrane.

Electron microscopy (8 *d post partum*)

Germ cells in polyovular follicles

Nucleus. Many of the nucleoli at this stage have elongated into a heavy, narrow reticulum stretched linearly beneath the nuclear membrane (Fig. 21).

Bands of chromosome material are not of as frequent occurrence as at the 4- and 6-d stages. Many of the nuclei contain tripartite chromosome cores, which do not differ appreciably from those seen at 4 and 6 d. A few nuclei show the single cores of diplotene chromosomes (Fig. 22). Other nuclei appear to be free of meiotic phenomena.

Cytoplasm. The Golgi apparatus again is massive and many of the lamellae are greatly swollen.

Mitochondria occur in about the same numbers and have the same morphology as at 6 d. A few clusters are seen within which intermitochondrial substance is beginning to form (Fig. 23).

In some germ cells the mitochondria are associated with cytoplasmic bodies containing concentric lamellar membrane systems. Transitional forms suggest that the mitochondria may be changing into lamellar bodies (Fig. 24). A variety of other cytoplasmic inclusions is also present, as at 6 d.

Follicle cells

The major difference in the follicle cells at this stage is an increase in the number of lipoid droplets in the cytoplasm. Again, the droplets may have an electron-dense granular peripheral band.

Cytoplasmic extensions of the follicle cells are again seen to penetrate deeply into the oocyte cytoplasm.

Atretic phenomena at the ultrastructural level.

Several phenomena were seen by electron microscopy at all stages studied, both in oogonia and in meiotic germ cells, which were assumed to be atretic in nature:

Fig. 25. Atretic phenomena in a germ cell from newborn hamster. A large electron-dense granular body is present in the peripheral cytoplasm. Three mitochondria, a strand of rough endoplasmic reticulum, particles resembling ribosomes, and masses of larger particles are seen within the body. A similar but smaller body is present in the cytoplasm of the neighbouring follicle cell.

Fig. 26. Cytoplasm of atretic oocyte from 6-d-old hamster. Areas of cytoplasm containing ribosomes, smooth endoplasmic reticulum and a mitochondrion are sequestered by smooth membranes.

Fig. 27. A badly shrunken germ cell, devoid of cytoplasmic organelles, has been engulfed by a follicle cell. Bodies containing mitochondria and ribosome-like granules are also present within the follicle cell cytoplasm and may represent the missing organelles of the germ cell.

Fig. 28. Atresia in a meiotic oocyte from a 6-d-old hamster. Large vacuoles have appeared in the cytoplasm, which otherwise appears normal. Sections through meiotic chromosome cores are seen at arrows.

Fig. 29. Atretic germ cell from 8-d-old hamster. Nuclear membrane has broken down and the nucleoplasm has dispersed. A few chromatin clumps remain, together with the skeleton of the nucleolus. The cytoplasm is completely disorganized and lacks organelles.

(1) Nucleus appears normal, nuclear membrane is intact, and meiotic chromosome cores may be present. The cytoplasm also appears normal except for small areas resembling autophagic vacuoles where organelles are sequestered within single or double membranes (Fig. 26).

(2) Same as no. 1 except that the nucleus is in mitotic division, the chromosomes appearing to have coalesced.

(3) Large, round, electron-dense bodies containing a variety of material are present in the cytoplasm. Besides granulation of various sorts, cytoplasmic components such as mitochondria and endoplasmic reticulum are often discernible within them (Fig. 25). Neighbouring follicle cells are seen to contain similar bodies. At a later stage in this sort of degeneration the germ cell, severely shrunken and devoid of cytoplasmic organelles, may be seen within a follicle cell, together with vacuoles which contain what are presumably some of its missing organelles (Fig. 27).

(4) Nucleus still usually appears normal and may contain meiotic cores, but large vacuoles are present in the cytoplasm (Fig. 28). The cytoplasm may otherwise appear essentially normal, or may be in process of obvious disintegration. Amorphous regions of vesicular and membranous material sometimes accompany the vacuolation.

(5) The chromatin within the nucleus is clumped and highly electron-dense. The nuclear membrane may be intact, or may have partially broken down. The nucleoplasm is patchily granular and portions of it may be sequestered within membranes. The cytoplasm is vacuolated and disorganized, the mitochondria highly abnormal.

(6) The nuclear membrane has partially or completely broken down. The nucleoplasm has dispersed, leaving large empty spaces between chromatin masses. The latter appear homogeneous and structureless rather than granular. The skeleton of the nucleolus may remain as concentric electron-dense circles attached at one side to a rounded mass (Fig. 29). The cytoplasm is completely disorganized—a mass of granular and membranous material without discernible organelles.

Nos. 5 and 6 appear to be successive stages of the same degenerative process.

DISCUSSION

Mitotic and meiotic phenomena

As expected from the short period of gestation, the ovary of the hamster at birth is not well developed. A few germ cells appear to have reached pre-leptotene, but the great majority are still oogonia, and mitotic activity is at its peak. By 6 d *post partum* a few germ cells have reached the dictyate phase. Study of ovarian tissue from 8 d to sexual maturity will be necessary to ascertain when meiosis ceases entirely. Since polyovular follicles have been reported in the ovaries of 16-, 26- and 36-d-old hamsters (Odor, 1965) and in the adult (Kent, 1962), it is conceivable that meiosis continues to occur in the adult of this species.

Despite the immaturity of its ovaries at birth, the hamster is sexually mature at approximately 1 month. This extremely rapid development compares with 40–70 d in the rat, 40–50 d in the mouse and 4–7 months in the rabbit.

The timing of meiotic prophase in the hamster resembles that of the rabbit. Teplitz & Ohno (1963) report that the rabbit is the only mammalian species yet studied in which oogenesis occurs entirely after birth. Leptotene is not identified

until 1 d *post partum*, and follicle development begins only after 14 d (Peters, Levy & Crone, 1965). In the rat, leptotene commences 3–4 d before birth (Franchi & Mandl, 1962), 5 d before birth in the mouse (Borum, 1961) and 7 months before birth in the human (Baker & Franchi, 1966).

In general, the meiotic complexes of the hamster resemble those of the rat (Franchi & Mandl, 1962) and the primate (Baker & Franchi, 1966). The hamster complexes have an investing sheath of electron-dense material associated with the lateral filaments as do those of the primate. Those of the rat do not have this sheath. The complexes of other mammalian species have not been described at the ultrastructural level.

Changes in Germ-cell organelles

Nucleoli

Changes in the nucleolus from pre-meiotic to dictyate stages involve mainly a spatial rearrangement of nucleolar parts and a relative increase or decrease in size of the three components. The prominence of the electron-dense granular component decreases as meiosis proceeds. The reticular component undergoes both morphological and positional changes, the most marked occurring at 8 d *post partum*. The prominence of the less dense granular component appears to increase with germ-cell development. Multiple small masses of this latter material are common in oocytes in primary follicles of the adult (Weakley, 1966), so that its functional importance appears to be greatest during the dictyate phase when meiosis is suspended.

Nuclear contour

The nucleus of the oogonium is rounded and has a relatively smooth contour. As development proceeds, the contour becomes more and more scalloped. In growing oocytes in the adult hamster ovary this is carried even further, with nuclear lobes penetrating far into the cytoplasm (Weakley, 1966). This probably reflects increased interaction between nucleus and cytoplasm as growth proceeds. Such convolution is common in metabolically active cells (Moses, 1964). Yamada, Motomura & Koga (1957) have also reported progressive folding and indentation of the nuclear envelope in mouse oocytes.

Endoplasmic reticulum and Golgi apparatus

A few elongate cisternae of endoplasmic reticulum bearing regularly spaced ribosomes are typically present in the oogonia 24 h before birth. At birth, however, these disappear from the germ cells and only an occasional vesicle or tubule is seen thereafter to have a few irregularly spaced ribosomes attached to its cytoplasmic surface. Protein synthesis in the developing germ cells would appear, then, to be largely confined to the free ribosomes.

Scattered vesicles of smooth endoplasmic reticulum are present in oogonia, but it is not until meiotic prophase has been established at 2 d *post partum* that they fall into the two clearly defined categories seen in the adult (Weakley, 1966). Both categories may be closely associated with the Golgi apparatus, as well as randomly distributed in the cytoplasm, so that origin from Golgi membranes cannot be ruled out. Electron-dense rods with bulbous ends are seen free in the cytoplasm in increasing

numbers as oocyte development proceds. Since they are first observed within the large vesicles of category 1, it is assumed that they are liberated by breakdown of these vesicles.

The Golgi apparatus changes mainly with respect to size. In the oogonium it is mostly lamellar with few vesicles and occupies a relatively small area. By 6 d *post partum* it has become massive, the ratio of vesicles to lamellae has increased, and the lamellae may be greatly swollen. It is assumed that this increase in size of the apparatus occurs preparatory to its breakdown into smaller segments, which occurs when the follicle cells surrounding separate oocytes become cuboidal in form. Swollen Golgi lamellae are of such universal occurrence in the oocytes at 8 d *post partum* that it is thought to represent a functional process rather than degenerative vacuolation. Such swelling is seldom seen after the apparatus has dispersed into smaller segments. It is inferred, therefore, that whatever products are elaborated or collected within the swollen lamellae are for use during that critical period when the polyovular follicles are breaking down into monovular follicles and the dictyate stage of meiosis is reached. Franchi & Mandl (1962) have reported that distended Golgi vesicles are also characteristic of diplotene in the rat.

Mitochondria

At all stages mitochondria are seen in which a number of narrow cristae cross the mitochondrion at right angle to its long axis. In addition to these mitochondria, other more unusual types are observed. In the embryo, the mitochondrial matrix may contain 'patchy' areas of less electron density, sometimes associated with tiny vesicles. At all stages one or more cristae within a mitochondrion may be enlarged to form a small chamber. This phenomenon is most striking in mitotic cells, but is also seen in meiotic germ cells. Such chamber formation has been reported in oocytes of the mouse (Yamada *et al.*, 1957) and rat (Franchi & Mandl, 1962), and in spermatocytes of the rat (André, 1962), but timing appears to differ with species.

At 6 and 8 d *post partum*, mitochondria are observed in which the cristae parallel the outer membranes rather than crossing the mitochondrion. At 8 d such mitochondria are often associated with bodies containing complex membrane systems (Fig. 24). In some instances it is impossible to differentiate between the mitochondria and these bodies. A transition between the two forms seems possible. Odor (1960) has also reported an apparent transformation of mitochondria into myelin figures in the rat oocyte; André (1962) in the rat spermatocyte. Similar changes have been linked with yolk production in invertebrates and lower vertebrates (e.g. Favard & Carasso (1958); Balinsky & Devis (1963); Dalcq (1963), Ward (1962)).

Mitochondria do not commence to form clusters in the oocytes until 4 d *post partum*, and electron-dense material does not start to accumulate within the clusters until 6 d *post partum*. This 'intermitochondrial substance' was reported in oocytes of the adult hamster by Odor (1965), but has not been described in other mammalian oocytes. The interior of the mitochondria may appear continuous with the intermitochondrial substance, as in Fig. 23. This raises the possibility on morphological grounds that the mitochondria give rise to the intermitochondrial substance, as suggested by Odor (1965). However, indistinct outlines have been characteristic of some oocyte mitochondria at stages when intermitochondrial substance is not

present (e.g. Figs. 10, 11), and are also seen in follicle cell mitochondria (e.g. Fig. 7).

The components of the filamentous-granular-tubular material which is seen from birth to 8 d *post partum* in association with the nuclear membrane, mitochondria, Golgi apparatus and free in the cytoplasm (Figs. 5, 10, 11, 17) are similar to those comprising the intermitochondrial substance. The latter, however, appears more condensed and electron-opaque. Ornstein (1956), Miller (1962) and Balinsky & Devis (1963) have reported the presence in amphibian oocytes of similar material, possibly ribonucleoprotein in nature. This is first observed associated with nuclear pores, and later becomes surrounded by clusters of mitochondria. The mitochondria then proliferate rapidly to form the Balbiani body.

If this filamentous-granular-tubular material in the hamster oocyte is identical to the intermitochondrial substance, and if it takes origin from the nucleus, then its passage through the nuclear pores should be increasingly evident as the mitochondrial clusters increase in number. Such does not appear to be the case in the tissues studied. It seems more likely that the material serves as a messenger to trigger mitochondrial production of the intermitochondrial substance. The intermitochondrial substance, since it has not been reported in other mammalian eggs, may represent the remnants of a developmental process (e.g. yolk production) which has lost its final stages (see Balinsky & Devis, 1963). Alternatively, it may serve some purpose specifically tailored for the needs of the hamster oocyte, or may simply be a mitochondrial waste product.

Cytoplasmic inclusions

Concentric lamellar membrane systems, whether they are derived from mitochondria or not, appear to be standard equipment in oocytes from 6 d *post partum* on. Some are not particularly electron dense and contain granules interior to the peripheral concentric membranes, as in Fig. 24. Others appear at first as small (average 0·2 μm diameter) irregularly shaped electron-dense bodies with concentric membranes surrounding a homogeneous-appearing centre. At later stages of oocyte development (Weakley, 1966) the lamellar membrane systems are round, much larger (measuring up to 1·8 μm in diameter) and are filled throughout by the concentric lamellae. At this time they are often intimately associated with multivesicular bodies, which have not been observed during the perinatal period. The lamellar membrane systems continue to be present until the oocyte has reached its maximum size. That they appear suddenly at 6 d *post partum*, are almost universally present, and show a regular sort of development argues against their being degenerative phenomena. It is suggested that they may be centres for the generation of cellular membranes to be used in the rapidly expanding oocyte.

The other inclusions so prevalent at 6 and 8 d *post partum* cannot be definitely classed as atretic, although their presence in mammalian eggs has been considered degenerative by some authors (e.g. Odor, 1960; Franchi & Mandl, 1962). In the hamster ovary they appear in germ cells which seem normal in all other respects, and are of such universal occurrence at the 6- and 8-d stages that it is difficult to write them off as degenerative in nature. At 6 and 8 d atretic phenomena are the most common, but this is also the time when many germ cells are reaching the dictyate stage of meiosis when rapid growth and maturation will occur. It is conceivable that

the inclusions contain enzyme systems which operate only during this circumscribed period when many oocytes are about to pass into the dictyate phase.

Staining affinity of the oocytes and synchronization of development

Differences in staining affinity toward methylene blue after glutaraldehyde fixation are clearly seen to correlate with germ-cell size and stage of development. Oogonia routinely have a high affinity for methylene blue; with the onset of meiotic prophase this affinity lessens as the germ cell begins to increase in size. This suggests a dilution of the stained material. Germ cells occurring together spatially tend to have a similar affinity for methylene blue and to be in identical stages of meiotic prophase. This apparent synchronization may be made possible by the presence of the intercellular bridges which are seen to connect adjacent germ cells (see Fawcett, Ito & Slautterbach (1959)). The bridges may also be a means of disseminating follicle cell products to those germ cells in the polyovular follicles which have no direct contact with a follicle cell. Observation of several incomplete bridges suggests that they may be transient in nature. The bridges resemble morphologically those described between oocytes in the rat by Franchi & Mandl (1962).

Follicle cells

The follicle cells associated with polyovular clusters change very little morphologically from 24 h before birth through 8 d *post partum*. Lipoid droplets are present in the cytoplasm at all stages, but show an increase in number at 6 and 8 d *post partum*. Indentation of the cytoplasm of the germ cells and contact with the nuclear membrane by follicle cell processes is seen from 4 d *post partum* on, and may represent a triggering mechanism for certain developmental processes within the germ cell.

A few follicle cells are present at each stage which do not stain intensely with methylene blue, and do not exhibit high electron density. That these cells must differ from the dark cells in some important way chemically seems evident. Whatever material is present in the 'dark' cells apparently manifests itself through a reaction with the glutaraldehyde fixative, since follicle cells in material fixed in osmium tetroxide alone do not have this extreme electron density (Weakley, unpublished observations). Further cytochemical procedures are now being carried out to try and pinpoint the nature of the reacting substance. It is not clear whether the 'light' follicle cells are simply 'dark' cells which have lost their electron-dense component, or whether they are a separate type of cell entirely. The smooth nuclear contour, paucity of ribosomes and lack of lipoid droplets in the few cells which were encountered suggest that a fundamental difference in cellular equipment may exist.

The existence at all stages of a few cells which contain masses of 300 Å particles, raises the question of the existence of yet a third follicle cell type. The particles may represent a type of large ribosome, but the cells containing them are so seldom seen that cytochemical tests at the ultrastructural level do not appear feasible. Cells containing these particles have not been observed after the follicle cells assume the cuboidal form.

Atresia

Knigge & Leathem (1956), using light microscopy, describe two types of atresia in the hamster oocyte. Both types occur in oocytes larger than 30 μm, and are preceded by changes in the follicle cells. Neither type appears to resemble changes seen in perinatal oocytes by light microscopy.

More pertinent is the ultrastructural study of Franchi & Mandl (1962) of atresia during the perinatal period in the rat. They designate three types of atresia: 'atretic divisions' occurring in oogonia as other germ cells reach leptotene; 'Z' cells occurring in late pachytene; and atretic diplotene cells. The 'atretic divisions' appear identical to those seen in oogonia of the hamster 2 d after birth. The 'Z' cells are comparable to the more advanced stages of atresia observed in hamster oocytes (Fig. 29). Cells similar to the atretic diplotene cells of Franchi & Mandle were not seen in the hamster tissues studied, probably because of the low incidence of diplotene cells present by 8 d *post partum*.

Franchi & Mandl do not describe the type of organelle sequestration shown in Figs. 25 and 27 or the giant vacuoles shown in Fig. 28. Both of these phenomena occur in oocytes as well as in oogonia in the hamster. The cytoplasmic phenomena which Franchi & Mandl designate as characteristic of atretic divisions in the rat are also seen in cells which have reached meiotic prophase in the hamster. The differences in atretic phenomena in the rat and hamster seem to be relatively minor, however, and may reflect tissue sampling rather than species differences.

SUMMARY

One prenatal and four postnatal stages of ovarian development in the golden hamster have been studied by light and electron microscopy. Fixation with glutaraldehyde followed by osmium tetroxide was employed.

At 24 h before birth and at birth the ovary is poorly developed and germ-cell clusters are widely separated by loose, undifferentiated connective tissue. By 2 d *post partum* the connective tissue cells have become tightly packed together into narrow septa which separate the germ-cell clusters. The clusters break up into smaller polyovular follicles as development proceeds, and the ratio of follicle cells to germ cells increases.

In the hamster, unlike all mammalian species yet studied except the rabbit, meiosis appears to occur entirely after birth. Mitoses are seen as late as 6 d *post partum*, but not at 8 d *post partum*. Early leptotene appears to be commencing in a few germ cells at time of birth, and is well established by 2 d *post partum*. A few synaptic chromosomes are present at 2 d; tripartite cores are predominant at 4, 6 and 8 d. A few diplotene cores are present at 6 and 8 d. Meiotic chromosomes are evident until the individual oocyte is completely surrounded by follicle cells and has separated from the polyovular follicle. Nuclei in most of the germ cells in separate follicles appear to have reached the dictyate stage by 6 d *post partum*. Also at that time an occasional oocyte has become surrounded by two layers of follicle cells.

Descriptions of the components of the meiotic chromosomes of the hamster largely agree with those found in male and female germ cells of other species.

Developmental changes in the nuclear and cytoplasmic organelles of the germ cells are described and discussed.

Accumulations of filamentous-tubular material are observed in the cytoplasm of the germ cells both before and after birth. They are frequently associated with mitochondria and Golgi apparatus, and may be of nuclear origin.

Intercellular bridges are present between oocytes in polyovular follicles at all stages studied.

An attempt is made to pinpoint certain phenomena which are clearly atretic, and to point out other phenomena which cannot as yet be definitely designated as either degenerative or developmental in nature.

The follicle cells are nearly all highly electron dense 24 h before birth, but increasing numbers lose this electron density as development proceeds. A few follicle cells have been observed to contain 300 Å particles which may be a type of large ribosome. Lipoid droplets are present within the cytoplasm at all stages. These increase in number at the 6 and 8 d stages. Nucleoli are peripherally located, and due to mechanical deformation of the follicle cells between germ cells these nucleoli frequently closely approach the cell membranes of one or more oocytes. The Golgi apparatus is flattened and peripherally distribued so that it, too, lies close to the plasma membrane of the germ cells. At 6 and 8 d, cytoplasmic processes from the follicle cell are commonly seen to indent the oocyte so deeply that they contact the nuclear membrane.

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