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THE FINE STRUCTURE OF OOGONIA AND OOCYTES IN HUMAN OVARIES

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SUMMARY

Twenty-eight foetal and post-natal human ovaries (6 weeks *post conception* to 11 years *post partum*) have been examined with the electron microscope. Stages in the normal differentiation of germ cells (oogonia; oocytes at the pre-leptotene, leptotene, zygotene, pachytene, and diplotene stages of meiotic prophase) were identified by comparison with (*a*) corresponding cells in histological and squash preparations, and (*b*) similar cells in the rat ovary. Atretic cells (oogonia in mitosis; oocytes at pachytene and diplotene) were also examined.

The nuclei of oogonia contain an evenly dispersed fibrillar matrix which becomes organized into irregular strands in oocytes at pre-leptotene. At leptotene these strands become the sheaths surrounding unpaired, electron-dense axial threads or 'cores', which become associated in pairs during the transitory zygotene stage, and tend to be polarized within the nucleus. Single and paired threads are replaced by 'tripartite ribbons' (synaptinemal complexes) at pachytene; longitudinal subdivision of the lateral components of the ribbon is observed in some nuclei and may represent a later stage. At diplotene, essentially unpaired cores are observed as at leptotene, although they are thicker and more intimately related to the surrounding fibrillar sheath. The latter is also more highly organized than at earlier stages. This chromosomal structure is retained in oocytes in promordial follicles in post-natal ovaries. The dictyate stage observed in rat ovaries is not found in man.

The organization of the cytoplasm does not change markedly as oogenesis advances, although organelles become more numerous, and the internal structure more complex, as the cells enlarge towards the diplotene stage.

Oogonia degenerating during mitosis differ from normal cells in that the chromosomes become fused to form an irregular mass. Abnormal membrane-bound areas are also observed in the cytoplasm. Atretic oocytes at pachytene coalesce to form 'pools' of cytoplasm containing several nuclei in various stages of pyknosis. Atretic cells at diplotene may contain grossly swollen cytoplasmic organelles and clumped, homogeneous chromosomes. The 'atretic divisions' appear to be phagocytosed by somatic cells, but the means of elimination of degenerating meiocytes was not determined.

INTRODUCTION

The majority of authors who have studied the fine structure of germ cells in the mammalian ovary have confined their attention to the cytoplasm of oocytes in post-natal specimens. Much emphasis has been placed on the changing form of the plasma membrane of the oocyte and its relationship to the zona pellucida and surrounding follicular cells during the development of the Graafian follicle (e.g. Yamada, Muta, Motomura & Koga, 1957; Sotelo & Porter, 1959; Trujillo-Cenóz & Sotelo, 1959; Anderson & Beams, 1960; Tardini, Vitali-Mazza & Mansani, 1960, 1961; Odor, 1960, 1965; Franchi, 1960; Wartenburg & Stegner, 1960; Blanchette, 1961; Adams & Hertig, 1964; Hope, 1965). Except for observations on the nuclear envelope and the nucleolus, little reference has been made by these authors to nuclear structure.

Reports on the fine structure of meiotic chromosomes relate mainly to primary spermatocytes (cat, pigeon and man: Fawcett, 1956; Nebel & Coulon, 1962; mouse: Nebel, 1959; Nebel & Hackett, 1961; Woollam & Ford, 1964; rat: Sotelo & Trujillo-Cenóz, 1958; invertebrates: Moses, 1956*a*, *b*; Moses & Coleman, 1964; see also Sotelo & Wettstein, 1965). These authors have shown that meiotic chromosomes possess distinct electron-dense cores which are most highly organized at pachytene, when tripartite ribbons (also called 'synaptinemal complexes') are found. These are composed of two parallel electron-dense threads with a third finer strand in between.

The only comprehensive study of the meiotic chromosomes in female mammals is that of Franchi & Mandl (1962) for the rat. In order to identify the various stages of germ-cell differentiation with the electron microscope, these authors made use of the observation (Beaumont & Mandl, 1962) that most of the germ cells in the ovaries at a given age are at the same stage of development. They found that cells at leptotene contain fine, unpaired chromosomal threads which become associated in pairs during zygotene. Oocytes at pachytene contain tripartite ribbons (see above). Unpaired threads are again seen in cells at diplotene, while the nuclei of oocytes at the subsequent dictyate ('resting') stage seem to be devoid of organized chromatin material.

In Baker's (1963) extensive cytological and quantitative study of oogonia and oocytes in human ovaries, a number of differences were noted from the pattern of development shown in the rat. The close synchronization of stages found in the latter is not observed in man. Oogonia, which enter the prophase of meiosis at variable times from the 2nd month *post conception* (*p.c.*), coexist with oocytes at varying stages of differentiation. By the time of birth, however, the majority of germ cells are oocytes at the diplotene stage, although some (generally abnormal) oogonia and earlier meiocytes may still be present. A definitive dictyate stage cannot be detected in human oocytes, and some differences occur in the appearance of other cellular stages (see Baker, 1963).

The present study was therefore undertaken to compare the fine structure of oogonia and oocytes in foetal, neonatal and early post-natal human ovaries with corresponding cells in the rat.

MATERIALS AND METHODS

Specimens

The material used in the present study formed part of a large series of human ovaries which were collected during the course of a cytological and quantitative study (Baker, 1963). Eight foetal (6 weeks to 7 months *p.c.*) and 20 prematurely born and neonatal specimens were obtained from the Birmingham Regional Hospitals. The foetuses were derived from cases of spontaneous abortion or surgical delivery (hysterectomy or hysterotomy). Post-natal specimens were obtained from routine autopsies, the interval between death and fixation of the tissues varying between 4 and 30 h. The standard of preservation of ovarian tissues was carefully checked during the initial examination of each specimen with the electron microscope, and those which showed autolytic changes were discarded. In no case was the cause of death believed to have

induced pathological changes in the structure of the ovary. The age of the foetus was estimated (*a*) from the date of the mother's last menstrual period, where known, and (*b*) from measurements of crown–rump length using the methods and data of Streeter (1920, 1951).

Procedures

The ovaries, which had been stored at 4 °C since removal, were freed of adhering blood and cut into small pieces (about 1–2 mm^3). They were fixed either in chilled 1 % osmium tetroxide in veronal-acetate buffer (see Franchi & Mandl, 1964), or in 5 % glutaraldehyde in cacodylate buffer followed by treatment with the osmium fixative. The fixed tissues were washed in the appropriate buffer, dehydrated in a graded ethanol series, treated with 0·5 % phosphotungstic acid in absolute ethanol, and finally immersed in dry acetone. The material was subsequently embedded in Vestopal W (Ryter & Kellenberger, 1958). Thin sections (silver or gold) were cut on Porter–Blum MT-1 and MT-2 microtomes and were examined with Siemens Elmiskop I or Zeiss EM 9 electron microscopes. Some sections were stained on the grids with alcoholic uranyl acetate before examination.

Thick (0·5–1·5 μ) sections of the plastic-embedded tissue were stained with aqueous toluidine blue and examined with phase-contrast illumination. By means of suitable landmarks (blood vessels, edges of sections, flaws, etc.), individual cells could be studied in adjacent sections using both electron and phase microscopes. They could also be compared with cells in ovaries prepared by histological and squash techniques (Baker, 1963), and hence their developmental stage identified with confidence.

RESULTS

Normal germ cells

Oogonia. Oogonia at interphase are generally round or ovoid in form. The nucleus is regular in outline and contains a matrix of randomly dispersed fibrillar and granular material (Fig. 1). In contrast, the nucleoplasm of neighbouring somatic cells is partly condensed beneath the nuclear envelope, which consequently has a darker outline. Oogonia possess prominent nucleoli which take the form of coarse, irregular networks of granular material, similar to that in other mammals (Yamada *et al.* 1957; Franchi & Mandl, 1962).

Oogonia which are undergoing mitosis contain dense irregular masses of chromosomal material, closely surrounded by chains of vesicles or double membranes (Fig. 2).

The cytoplasm of both resting and dividing oogonia contains large ovoid mitochondria, usually with parallel cristae. Somewhat larger mitochondria containing circular profiles are also present (Fig. 1); these are presumed to be sections through villiform cristae, and have also been observed in germ cells in the rhesus monkey (Baker & Franchi, unpublished). The endoplasmic reticulum is poorly developed and usually represented by numbers of small vesicles, but occasionally a few flattened cisternae, bearing ribosomes, are seen in the region of the Golgi apparatus (Fig. 1).

The latter is a juxtanuclear structure sometimes seen in association with centrioles, and is slightly more prominent than that in the rat (Franchi & Mandl, 1962).

Oocytes at pre-leptotene and leptotene. Some of the germ cells seen in association with oogonia are characterized by the condensation of the nuclear matrix into irregular strands (Fig. 3). A nucleolus is present, although this appears to be smaller, denser and more vacuolated than in oogonia. The organization of the cytoplasm is similar to that already described but in general the cell and its nucleus often appear more irregularly shaped. Cells with these features are believed to be at the pre-leptotene stage of meiotic prophase.

The nuclei of oocytes at the leptotene stage contain numerous fine, electron-dense threads which are on average 450 Å thick (Figs. 4, 5). They tend to be oriented in one direction, as are the chromosomes in histological preparations. Each thread is enclosed by a sheath of fibrillar material similar to that present at pre-leptotene. The outer limits of the sheath are diffuse since the fibrils merge with those in the surrounding nuclear matrix. One or more small nucleolar bodies are observed, as well as an occasional larger nucleolus, which is more homogeneous than that present at earlier stages.

In general, cytoplasmic organization is similar to that described for oogonia. Prominent pores are evident in some regions of the nuclear envelope, however, and in others there are small areas over which the two membranes are more than usually separated (Figs. 5, 6; see also below). Mitochondria are often closely associated with the nuclear envelope; this association is also encountered, though to a lesser extent in cells at zygotene and pachytene.

Zygotene. Oocytes at the zygotene stage are difficult to identify in both the light and electron microscopes. In thick sections of plastic-embedded material, cells containing a marked nuclear 'bouquet' of chromosomes are believed to be at this stage. The 450 Å threads seen in adjacent thin sections also tend to be polarized (Figs. 4, 6). There are signs of pairing between some threads, particularly near the nuclear envelope (Fig. 6), where the threads are often thickened at the point of contact with the inner membrane. Many short segments of unpaired threads, and occasionally of tripartite ribbons, are also seen in the nucleus. The latter perhaps indicate a more advanced stage of synapsis at some points along the length of the chromosomes (see p. 219). The threads are surrounded by a sheath of fibrillar material as at leptotene.

The cytoplasm is also similar to that in cells at earlier stages.

Pachytene. The nuclei of many oocytes seen in thick sections possess thick chromosomes which mostly lack the polarized configuration seen at earlier stages. These cells correspond to those at the pachytene stage visible in histological preparations. In thin sections the nuclei contain many segments of tripartite ribbons, although occasional unpaired threads are seen. Variations in the appearance of the ribbons are thought to be due to their helical twisting and undulation in and out of the plane of the section (Fig. 9). The overall width of the ribbon is about 2000 Å and that of each lateral arm 450 Å (range 390–500 Å). The fine central strand is 200–220 Å wide. In some nuclei the ribbons are further complicated by the longitudinal subdivision of the lateral arms (Figs. 8, 10), although not necessarily all the profiles within one nucleus show

this feature; it is particularly evident near the nuclear envelope where (as at earlier stages) the terminal portions of the threads are expanded. The dimensions of the modified ribbon differ from those given above and are more variable. Its width may be as much as 2800 Å and the width of the bifid lateral arms, 670–890 Å. Each component of the lateral arm is 230–300 Å wide, but at the nuclear envelope it may measure as much as that of the undivided arm (about 450 Å). The thickness of the central strand of the ribbon is unaltered.

The outer limits of the sheaths of fibrillar material surrounding the ribbons are more clearly demarcated from the nuclear matrix than at earlier stages of meiosis (Figs. 9, 10). Tightly coiled fibrils, 100–200 Å in diameter, are present in the sheath, together with granules of similar size; these may represent fibrils in cross-section. Near the tripartite axis, fibrils appear to be continuous with the substance of the lateral arms. A more detailed examination is required to determine the precise structural relationship between the chromosomal axis and its sheath.

Oocytes at pachytene are larger cells than oogonia or the earlier meiocytes and their cytoplasm is more complex. Spherical and ovoid mitochondria are plentiful and their parallel cristae well developed. While most mitochondria are scattered randomly in the cytoplasm, those which remain close to the nucleus are more flattened, with their long axes lying parallel to the nuclear envelope (Fig. 8). Vesicles of the endoplasmic reticulum are abundant and vary considerably in size; few ribosomes are seen in association with them. The Golgi apparatus is prominent at one pole of the nucleus and contains groups of distended cisternae and numerous smaller vesicles (Fig. 10). Some of the latter correspond in size to those found elsewhere in the cytoplasm. Occasional organelles which resemble multivesicular bodies (Sotelo & Porter, 1959) are also observed.

Diplotene. Oocytes at diplotene are larger than those at earlier stages of meiotic prophase and may be partially or wholly surrounded by flattened follicular cells. The nucleus contains unpaired, electron-dense threads, the continuity of which is interrupted at irregular intervals, presumably due to undulation in and out of the plane of section (Fig. 11). Each thread (which may be homologous to a lateral arm of the ribbon present at pachytene) is 700–1400 Å thick, although its outlines are not always sharply demarcated from the surrounding material. In favourable sections, however, two or more longitudinal strands some 100–200 Å thick are visible over short distances. The whole thread is closely invested by a sheath similar in composition to that observed at pachytene (Fig. 12). Its organization is, however, more complex; in view of our current interest in comparing nuclear structure in the oocytes of a number of mammals, a more detailed report on the fine structure of these chromosomes will be published elsewhere.

The nucleoli are large and are uniformly granular but may contain clear, vacuole-like areas. Smaller nucleoli of similar form are also observed, usually in close association with the chromosomal sheaths. The nuclear envelope bears well-developed pores but at some points the membranes are more widely separated to form 'pockets' which often contain vesicular profiles (Fig. 11).

The cytoplasm of oocytes at diplotene is similar to that at pachytene except that the

association of mitochondria with the nuclear membrane is much less marked. In the larger primordial follicles, mitochondria tend to congregate in one half of the oocyte, are less regular in outline, and their cristae may project radially into the lumen of the organelle. The Golgi apparatus contains fewer distended cisternae; instead, short tubular profiles are present. The endoplasmic reticulum is represented by small vesicles, the majority of which occur in the vicinity of mitochondria. Occasionally, irregular spaces bounded by a single membrane are present; these contain small vesicles similar to those in the nuclear pockets (see above) and may be continuous with them.

Germ cells undergoing degeneration

An analysis of atretic changes during human oogenesis has revealed that germ cells may degenerate at any one of 3 distinct stages of differentiation (Baker, 1963). These 3 cell types were identified during the course of the present study, although they were less frequent than would have been expected from the histological preparations.

Degenerating oogonia undergoing mitosis (*'atretic divisions'*). Atretic cells at metaphase contain irregular, clumped masses of chromatin, apparently formed by the fusion of individual chromosomes (Fig. 14). The nuclear membrane is absent, but many vesicles, some of which are enlarged, occur within and around the chromatin mass. The cytoplasm contains both normal and abnormal mitochondria; the latter are slightly swollen and have disrupted cristae. The vesicles of the endoplasmic reticulum are also somewhat swollen, and areas of cytoplasm containing vesicles and mitochondria are surrounded by an irregular double membrane (Fig. 14). Similar inclusions are typical of 'atretic divisions' in foetal rat ovaries (Franchi & Mandl, 1962). As in the rat, degenerating oogonia are frequently seen wholly or partly engulfed in the cytoplasm of neighbouring somatic cells, indicating that they are eliminated by phagocytosis (Fig. 13).

Degenerating meiocytes (*'Z' cells*). While some oocytes appear to undergo disruptive changes 'individually' (i.e. within intact plasma membranes), others degenerate simultaneously, forming 'pools' in which several nuclei exist without intervening plasma membranes (Fig. 15). Some of the nuclei in a pool appear relatively normal while others are grossly changed. In some of the latter the chromosomal sheaths are contracted and more electron-dense than in normal cells, though the nucleoplasm in others is uniformly dense. Stacks of 'annulate lamellae' may occur on parts of the nuclear envelope (Fig. 16). The cytoplasm of the pool contains both normal and abnormal organelles which sometimes appear to have invaded the disrupted nuclei.

The pools of degenerating cells are found in the ovaries of specimens aged 5 months *p.c.* to full term, a period during which some 60% of the population of germ cells is lost (Baker, 1963). They are visible in thick sections as pyknotic masses. The means whereby they are finally eliminated has yet to be determined.

Atretic oocytes at the diplotene stage. Few of the oocytes at diplotene examined in ovaries aged 4 months *p.c.* to full term were undergoing degeneration. It may be that the high incidence of atresia recorded in histological preparations was related to the use of relatively harsh fixatives. On the other hand, the small pieces of tissue used

for electron microscopy may not have provided fully representative samples. Our observations on oocytes degenerating at the diplotene stage are therefore preliminary and need to be confirmed and extended.

In the cells examined, the chromosomes are condensed into homogeneous strands and are generally more osmiophilic, but the nucleolus remains relatively unchanged. The nuclear envelope may be incomplete and annulate lamellae sometimes occur on its cytoplasmic surface. Cytoplasmic organelles are grossly swollen and fill the entire volume between the nucleus and the plasma membrane (Fig. 17). Osmiophilic material is deposited in some mitochondria. Presumably, an examination of further specimens will reveal more advanced stages in degeneration and help to determine the ultimate fate of these cells.

DISCUSSION

The results of the present study show that the fine structure of the nucleus in oogonia and oocytes in human ovaries is similar in most respects to that of corresponding cells in the rat (Franchi & Mandl, 1962). Electron-dense chromosomal threads or 'cores' of the same basic form are observed in both species, and they show similar variations in structure through the stages of differentiation of the oocyte. Some important differences in both nucleus and cytoplasm have, however, become apparent.

The earlier cytological and quantitative study of foetal human ovaries (Baker, 1963) showed that a cellular stage exists in which fine thread-like associations of chromatin appear in the nucleoplasm. This stage was identified as pre-leptotene. Our observations with the electron microscope would seem to confirm this: cells closely resembling resting oogonia possess a nucleus in which the usually dispersed fibrillar matrix is organized into irregular strands. These resemble the sheaths surrounding the chromosomal cores in oocytes at leptotene. Similar cells have been observed in the ovary of the foetal rhesus monkey (Baker & Franchi, in preparation), but have not been identified in the rat (Franchi & Mandl, 1962).

Oocytes at leptotene in human ovaries bear a close similarity to those in the rat. Identification of the zygotene stage in the human is more difficult: in the rat, definition is aided by the fact that oocytes pass through the successive stages of meiotic prophase in a synchronized fashion (Beaumont & Mandl, 1962). With the electron microscope, pairing between the electron-dense threads is clearly evident (Franchi & Mandl, 1962). Cells believed to be at a comparable stage of prophase in human ovaries possess chromosomes which are frequently polarized and in which the fine cores seen at leptotene are associated in pairs along at least part of their length. The nuclei also contain segments of unpaired threads and, occasionally, of tripartite ribbons. Our interpretation of these observations is that pairing of homologous chromosomes starts at one point and proceeds along their length. Thus the coexistence of single, paired and tripartite threads within one cell becomes readily understood. The initiation of pairing of homologues at specific regions appears to be of common occurrence during gametogenesis in both plant and animal species, and it is probable that pairing proceeds along chromosomes at different rates (see Suomalainen, 1952).

Recent observations (A. McDermott, personal communication) indicate that this phenomenon also occurs in human spermatocytes. While these authors' studies refer to male germ cells, the present observations suggest that the zygotene stage proceeds in a similar manner in the female. This might also explain why it seems transitory and difficult to identify in histological preparations of human ovaries (Baker, 1963). It is noteworthy that, while it is generally accepted that the tripartite complex represents the pachytene stage, a distinctive electron-microscopical appearance for the zygotene stage has hitherto been described only for the rat oocyte (Franchi & Mandl, 1962).

The tripartite ribbon, which is present in the nucleus of many oocytes at pachytene in young specimens, probably represents the fully synapsed condition of the homologous chromosomes, and hence the core of the bivalent. A second and possibly subsequent stage has been observed in cells whose dimensions approach those of oocytes at early diplotene. In these cells, the lateral elements of the ribbon are seen to be split longitudinally, although it is not possible from an examination of thin sections to ascertain whether the splitting has taken place along the entire length of the threads. This complex ribbon has also been observed in spermatocytes in the pigeon (Nebel & Coulon, 1962), several mammals (Woollam & Ford, 1964) and invertebrates (Moses, 1960). Moses considers that the double-strandedness of the lateral elements may only become manifest late in meiotic prophase. From the classical descriptions of meiosis (see White, 1952; Sinnott, Dunn & Dobzhansky, 1958), and as suggested by previous authors, it would seem that the stage at which double-stranded lateral arms are observed signifies the formation of chromatids.

The present study also shows that the nucleus in the oocyte at diplotene differs in a number of important respects from that in corresponding cells in the rat (Franchi & Mandl, 1962). In the latter, unpaired chromosomal threads similar in thickness to those present at leptotene reappear at diplotene. At the dictyate stage which rapidly ensues, the nucleus assumes an interphase-like appearance; no semblance of threads remains. In histological preparations the nuclei lose their affinity for stain and the chromatin is irregularly arranged (Beaumont & Mandl, 1962). Human oocytes, on the other hand, retain well-defined basiphilic chromosomes which bear faint lateral projections (Baker, 1963); squash preparations show that they remain in a configuration typical of diplotene, even after the oocyte becomes enclosed in a primordial follicle (Ohno, Klinger & Atkin, 1962; Manotaya & Potter, 1963; Baker, 1963). Our studies on the fine structure of these chromosomes have shown that they possess an unpaired, electron-dense core which corresponds more nearly in size to the bifid lateral arms of the tripartite ribbons of cells at pachytene, than to the chromosomal core at leptotene. The fine fibrils and granules in the surrounding chromosomal sheath frequently seem to be arranged symmetrically and it is possible to define both simple and more complex loop-like projections arising from the region of the core. These observations have led us to conclude (Baker & Franchi, 1966*a*, *b*) that human oocytes in primordial follicles contain lampbrush chromosomes which are in some ways similar to those described in newt oocytes (Callan & Lloyd, 1960*a*, *b*). There are also strong indications, both from this study and from other work with the electron microscope (Nebel & Coulon, 1962), that a lampbrush-type structure may be present at the pachytene stage.

The above findings emphasize a further difference between meiotic chromosomes in human and rat oocytes. In human oocytes an organized fibrillar sheath surrounds the chromosomal cores at all stages of meiotic prophase and, indeed, seems to provide the first indication of the appearance of chromosomes in early oocytes (pre-leptotene: see above). In the rat it is rare or absent, except at the points of contact of tripartite ribbons with the nuclear envelope at pachytene (Franchi & Mandl, 1962). It is clear that the meiotic chromosomes possess similar sheaths in male germ cells in a wide variety of species, including man (Fawcett, 1956). Recent studies indicate that oocytes in several species also possess them (chick: Greenfield, 1966; mouse: Tsuda, 1965; guinea-pig: J. M. Ioannou, personal communication; monkey: Baker & Franchi, in preparation). It would appear, therefore, that the structural organization of meiotic chromosomes in the female rat differs not only from that in the human oocyte but also from that in the other vertebrates which have been studied.

The cytoplasm in human oogonia and oocytes does not appear to undergo profound structural changes as prophase advances. The endoplasmic reticulum consists mainly of apparently isolated vesicles as in several other mammals (compare the guinea-pig; Adams & Hertig, 1964). Changes in the internal structure of mitochondria (pp. 215, 218) are difficult to interpret on the basis of a morphological study. A notable feature, however, is their association with the nuclear envelope, particularly in oocytes at leptotene, zygotene and pachytene. The relationship, if any, of this phenomenon to the presence of 'pockets' formed by the separation of the nuclear membranes remains to be established. The latter are most highly developed at diplotene, when the space often contains small vesicular structures and occasionally more complex organelles which bear some resemblance to miniature mitochondria. Moreover, they appear to be prominent when mitochondria increase in numbers. The possibility that they are a fixation-induced artifact cannot be completely ruled out, although nuclear pockets do not occur in neighbouring somatic cells. They are, however, developed in the oocytes of rhesus monkeys (Baker & Franchi, in preparation). A more detailed study of these regions of the nuclear envelope will form the basis of another report.

Despite our care in selecting specimens it is still possible that technical artifacts may have influenced our diagnosis of degenerating cells. Nevertheless, 3 'waves' of degeneration which affect germ cells in the rat (Beaumont & Mandl, 1962; Franchi & Mandl, 1962) affect the same stages in human ovaries, and the pattern of degeneration in each case is almost identical to that found in the rhesus monkey, where the ovaries were placed in fixative within 5 min of removal. It is thus unlikely that the gross changes visible in germ cells could have been caused by autolysis.

The form assumed by 'atretic divisions', affecting oogonia in mitosis, and their mode of elimination, is broadly similar in both rat and human ovaries. The 'pools' of degeneration, affecting mainly oocytes at pachytene, are of particular interest since (*a*) intervening plasma membranes appear to have broken down before marked changes are visible in the cytoplasm, and (*b*) they have not been reported for the rat. Despite the confluence of these cells, the presence of nuclei varying in appearance from essentially normal to grossly abnormal suggests that specific internal nuclear factors determine the rate of degeneration. As far as is known, this pattern of atresia is confined to

primate ovaries, and may be related to the arrangement of germ cells into cords (see van Wagenen & Simpson, 1965).

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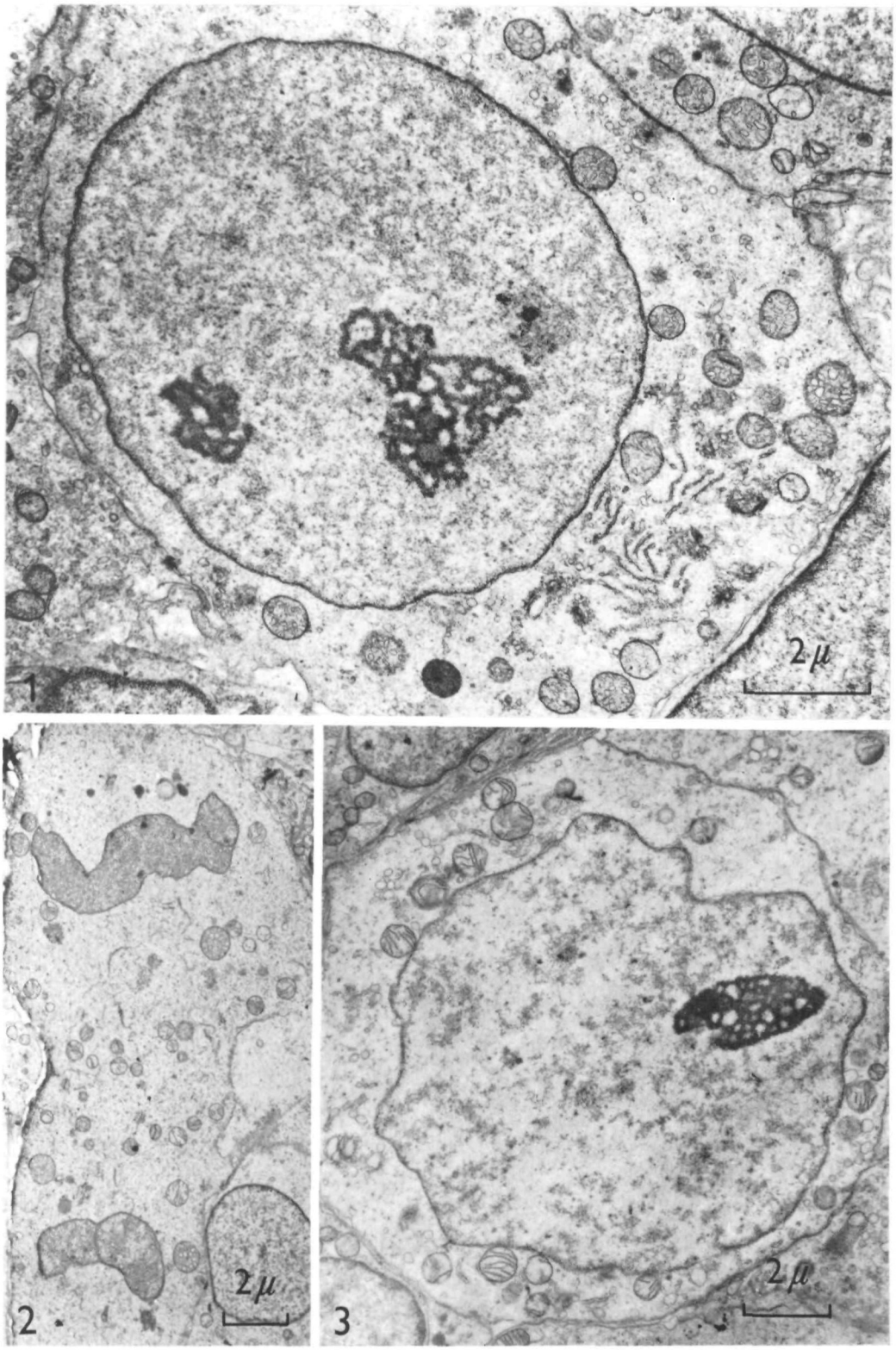
Fig. 1. Normal oogonium at interphase. The nucleus contains randomly dispersed fibrillar material and the nucleolus is in the form of an irregular reticulum. Many mitochondria contain villiform cristae.

Fig. 2. Oogonium at mitotic telophase. The nuclear envelopes have re-formed around the granular masses of chromatin. Most mitochondria contain parallel cristae. A cytoplasmic constriction is present at the equatorial region.

Fig. 3. Oocyte at the pre-leptotene stage of meiotic prophase. Indications of thread-like condensations of the fibrillar material can be seen in the nucleus. The nucleolus is more compact than in oogonia (see Fig. 1).



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(*Facing p.* 224)

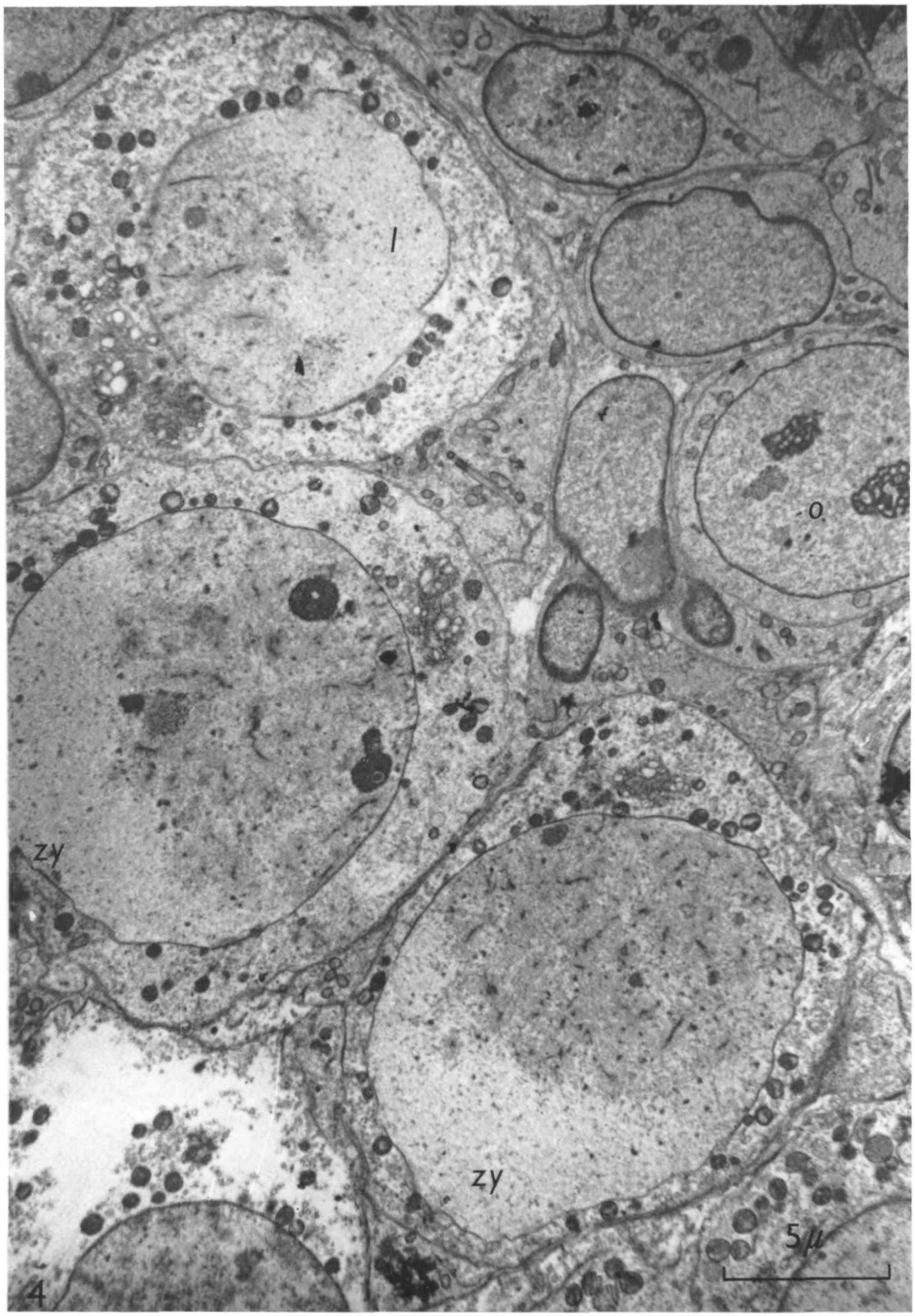
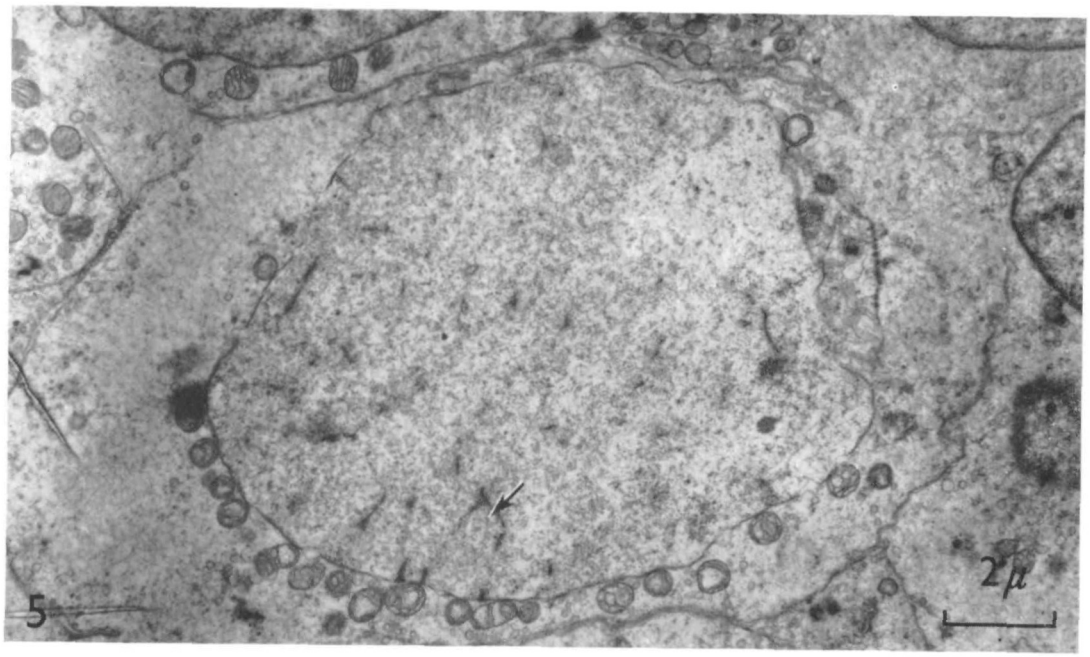


Fig. 4. Survey electron micrograph showing oocytes at leptotene (*l*) and zygotene (*zy*); compare the oogonium (*o*). These oocytes contain polarized chromosomes with fine electron-dense cores (see also Figs. 5, 6).



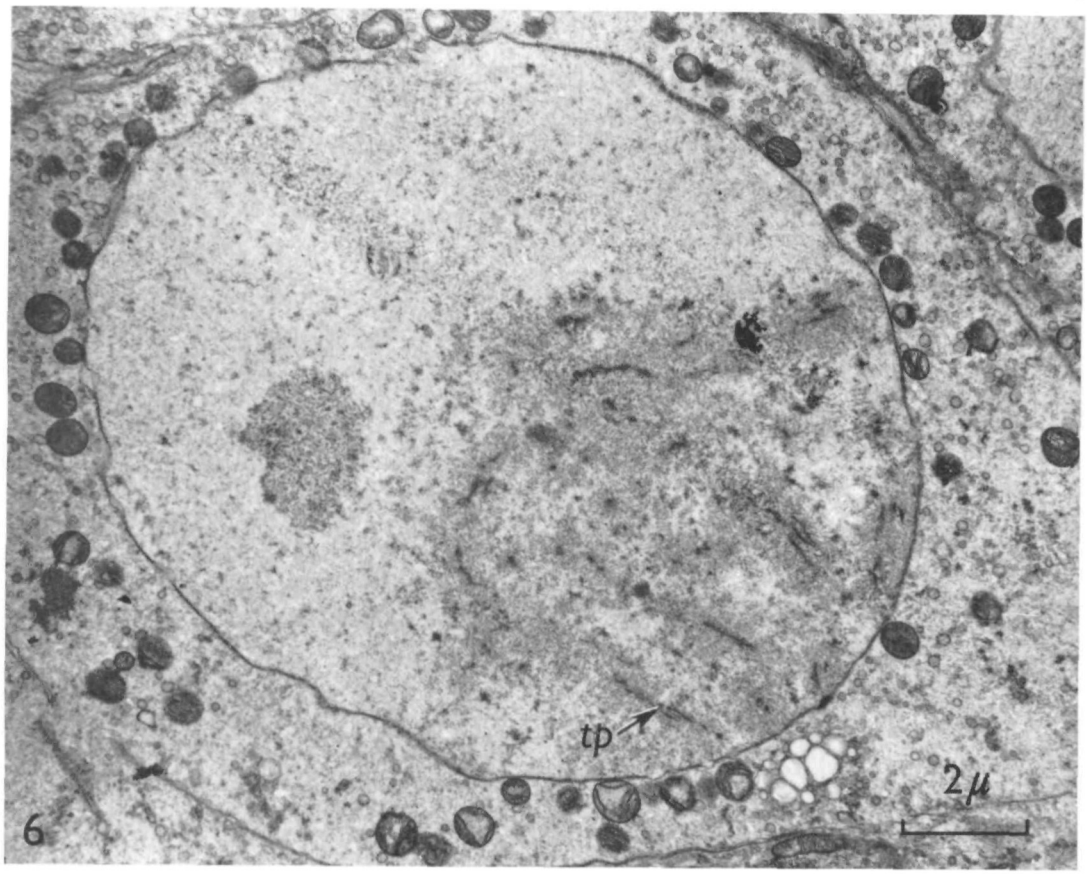
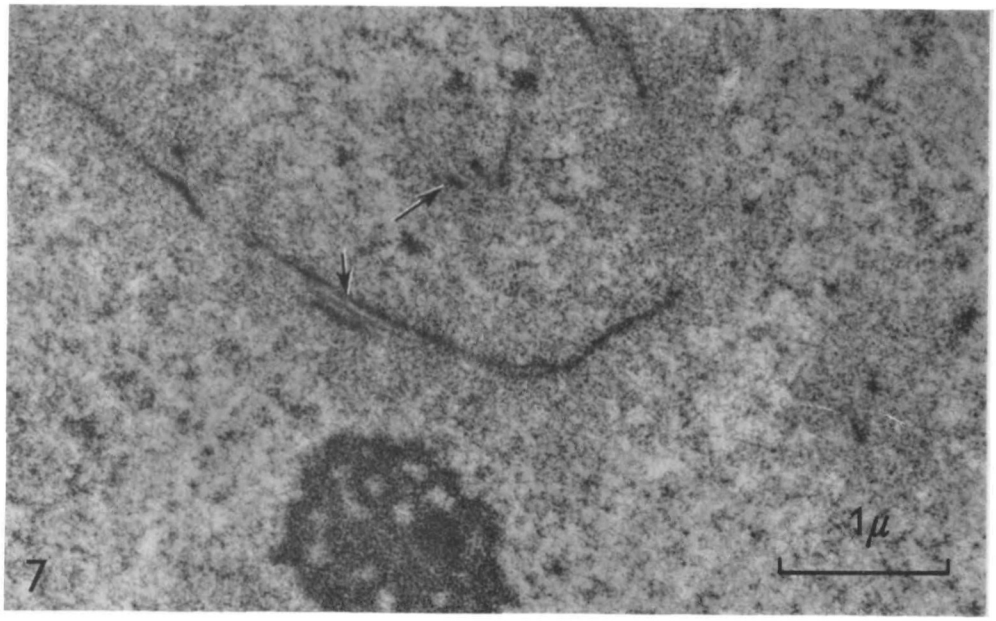


Fig. 5. Oocyte at leptotene, showing unpaired chromosomal threads (arrow) which are thickened at the inner nuclear membrane. Mitochondria are closely applied to the nuclear envelope.

Fig. 6. Oocyte believed to be at the zygotene stage. Varying degrees of pairing between the dense threads can be seen, including tripartite ribbons (*tp*; see p. 216). The chromosomal sheaths of fibrillar material are clearly shown.

Fig. 7. Oocyte at pachytene. Part of the nucleus showing typical tripartite ribbons cut both longitudinally and transversely (arrows); the fibrillar sheath is also evident. A small nucleolus is present in the nucleoplasm.

Fig. 8. Oocyte at pachytene. The mitochondria, which are closely associated with the nuclear envelope, are flattened. Portions of tripartite ribbons show longitudinal division of the lateral arms (arrows; see also Fig. 10).


7
1μ

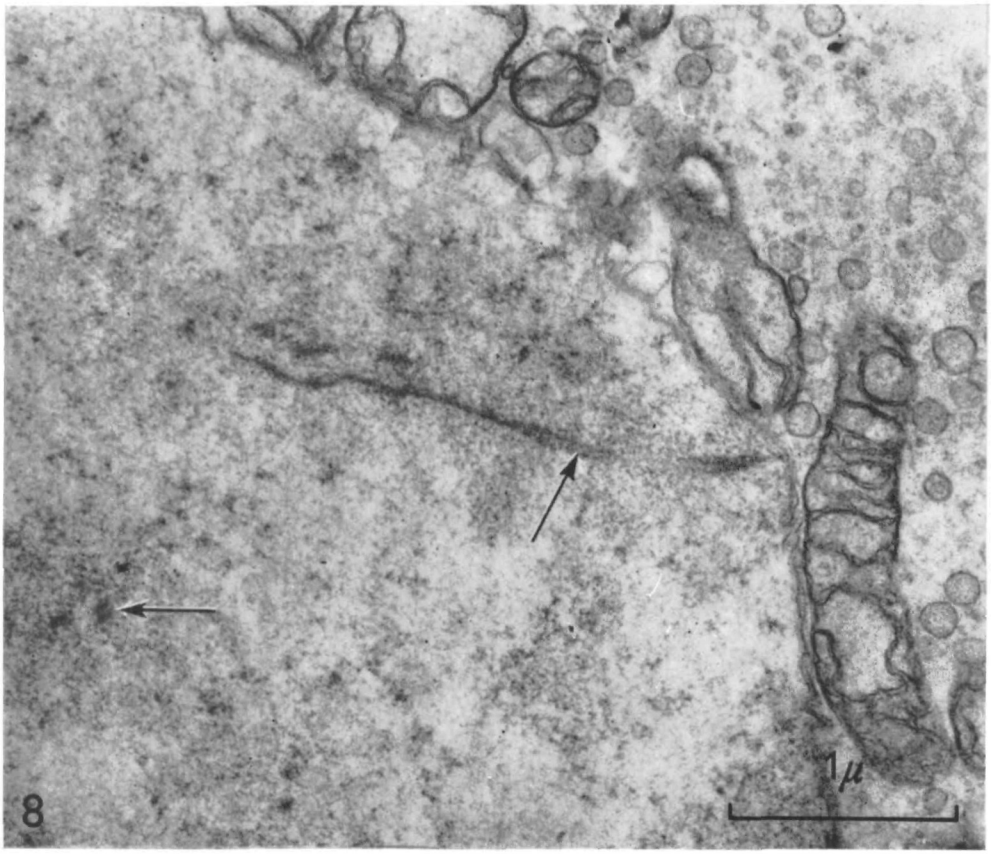
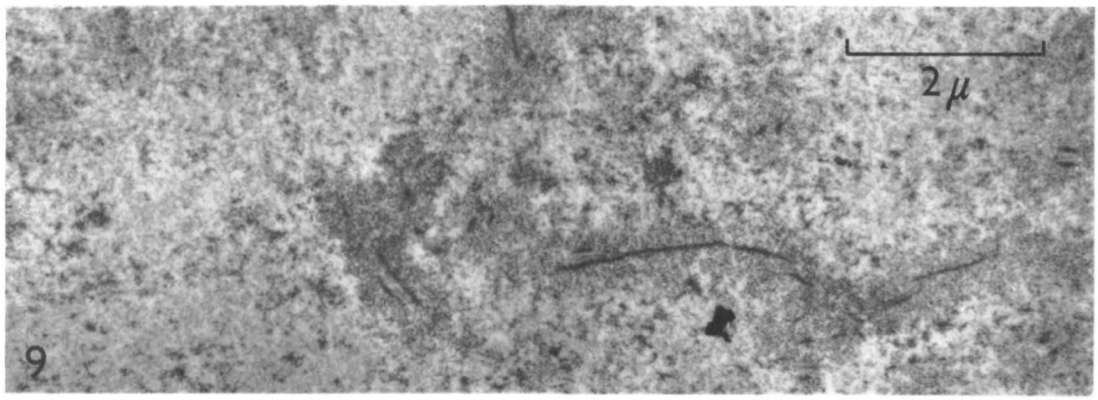

8
1μ

Fig. 9. Portion of the nucleus of an oocyte at pachytene, showing tripartite ribbons with their associated fibrillar sheaths. There are some indications of spiral twisting of the ribbon.

Fig. 10. Oocyte at pachytene showing the longitudinal division of the lateral arms of the chromosomal ribbon. This is particularly evident at the nuclear envelope, where the terminal portion of the complex is thickened. The fibrillar sheath appears to be more highly organized than that at earlier stages (Fig. 9). Distended Golgi cisternae can be seen in the adjacent area of cytoplasm.


2 μ
9

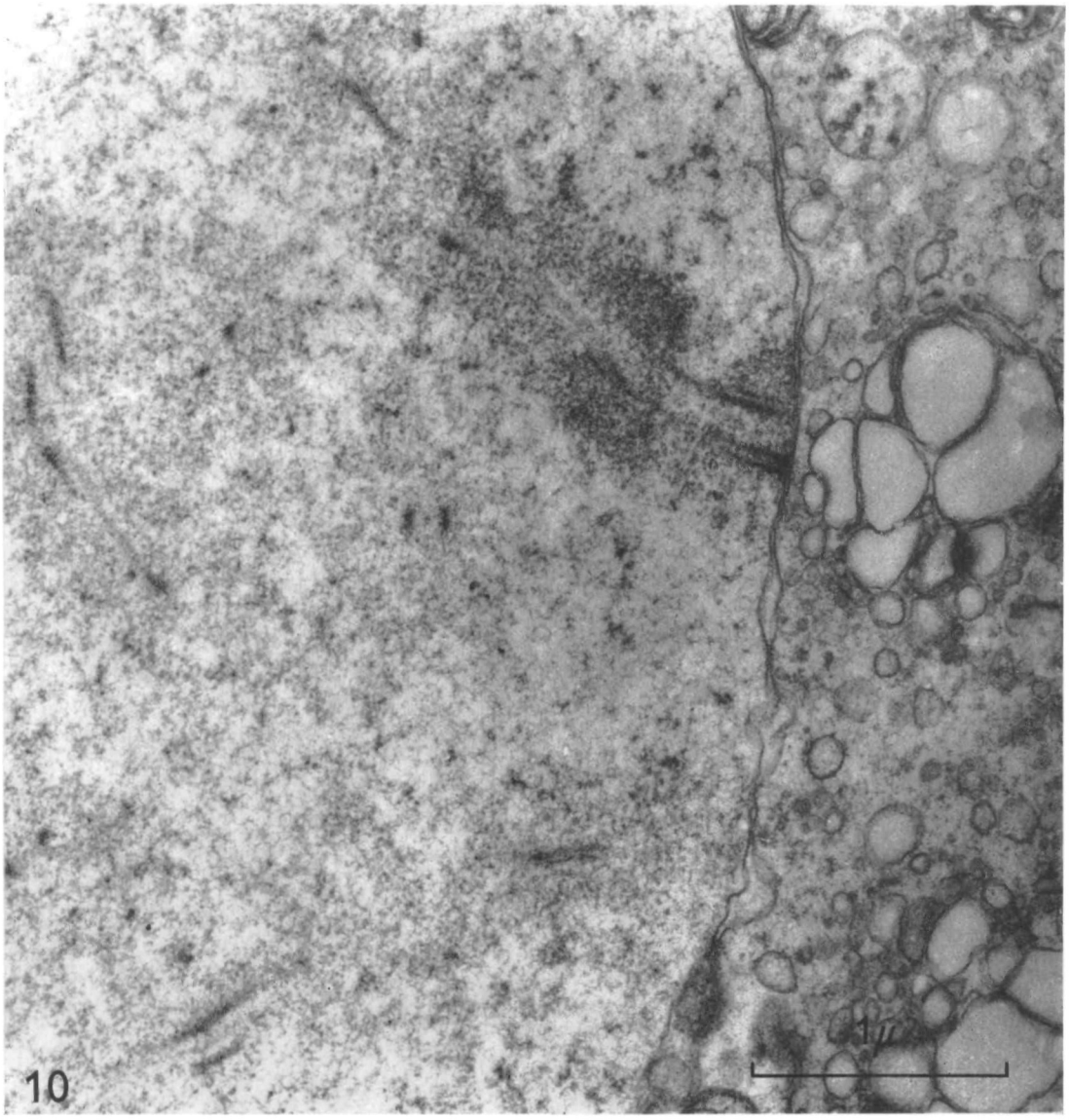
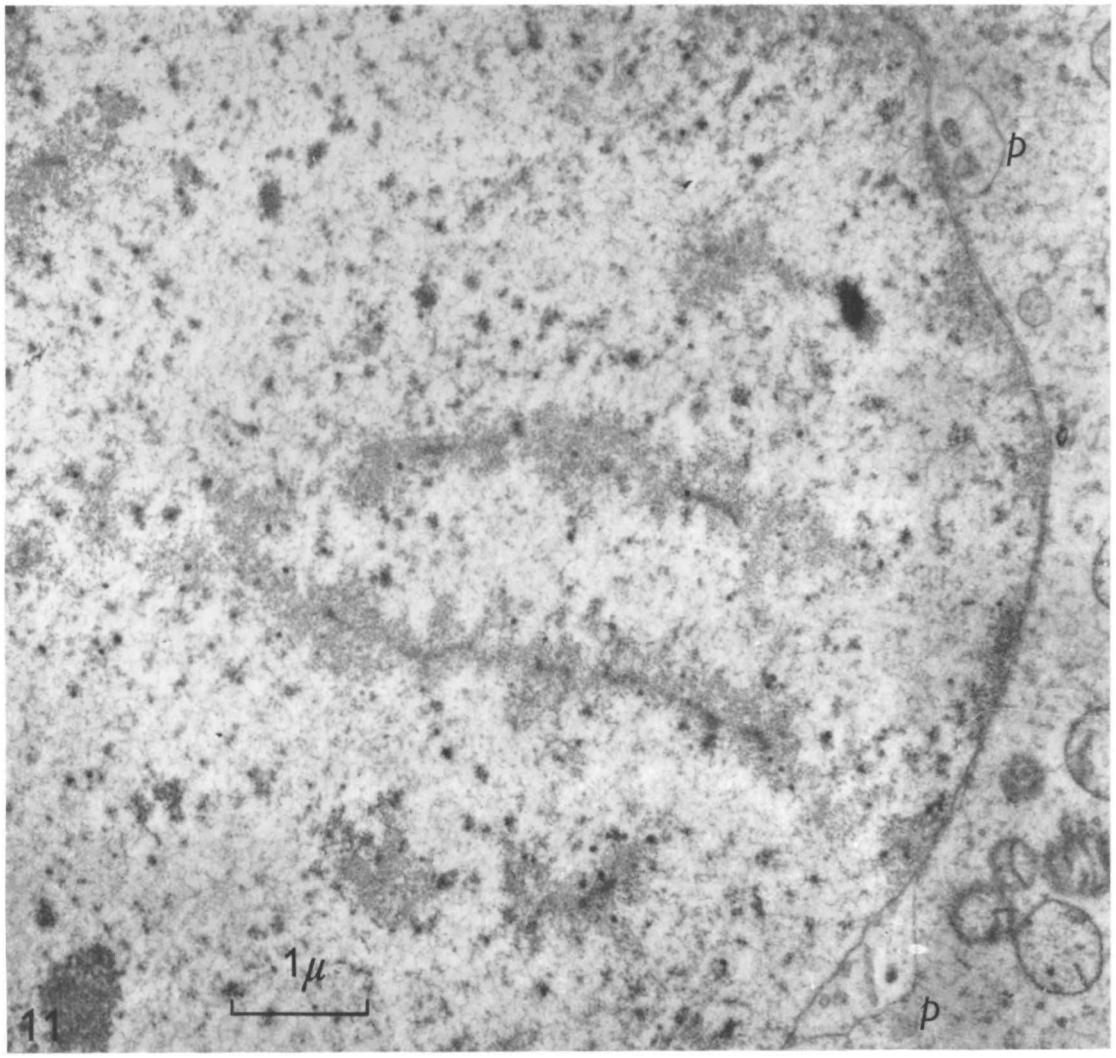

1 μ
10

Fig. 11. Oocyte at diplotene. Portions of 2 chromosomes with apparently unpaired cores (see p. 217), each of which is surrounded by a complex fibrillar sheath. The membranes in regions of the nuclear envelope separate to form 'pockets' (*p*) containing small membrane-bound profiles.

Fig. 12. Chromosome at the diplotene stage. Portions of the core can be seen (arrows); they are surrounded by a sheath of fibrillar and granular material.


p
1μ
11
p

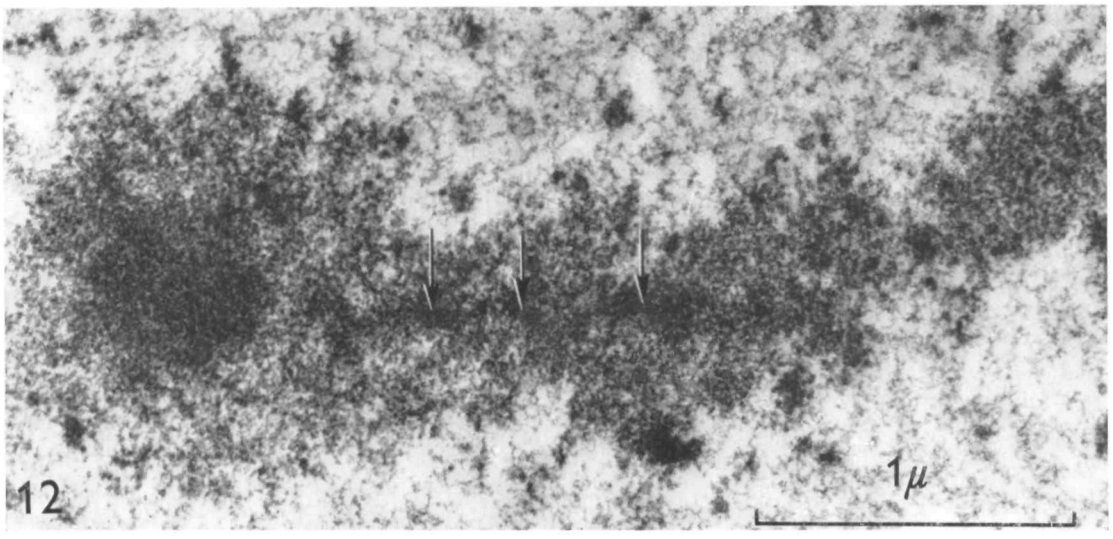

12
1μ

Fig. 13. Advanced stage of degeneration of an oogonium undergoing mitosis. The cell appears to have been engulfed by a somatic cell.

Fig. 14. Oogonium degenerating at metaphase (atretic division). The cell contains a fused mass of chromatin which lacks a nuclear envelope. Some organelles are surrounded by irregular membranes (arrow).

Fig. 15. Low-power micrograph showing a 'pool' of degenerating oocytes. Nuclei showing varying degrees of abnormality can be seen without intervening plasma membranes. A normal oogonium (not part of the pool) is shown at top left.

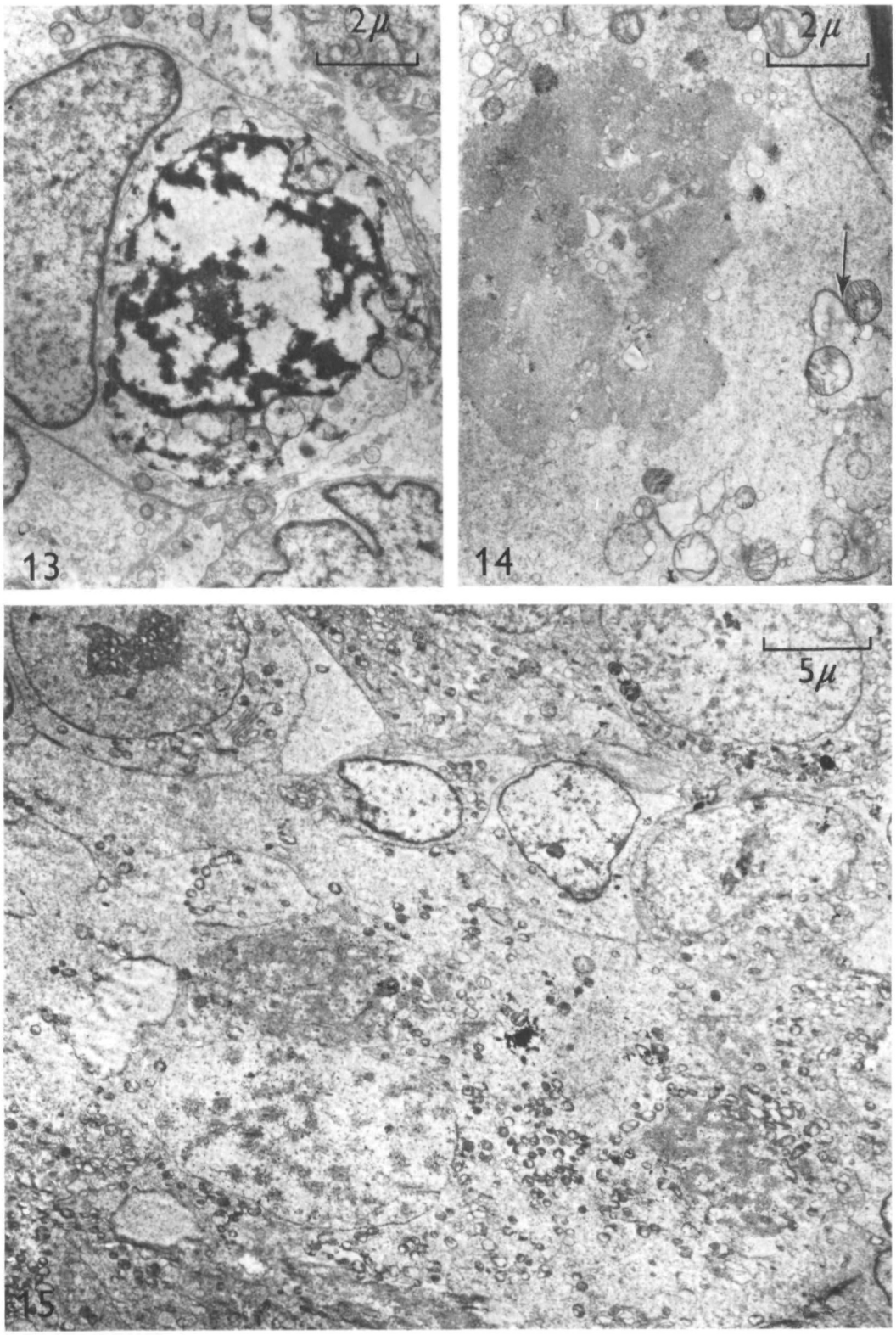
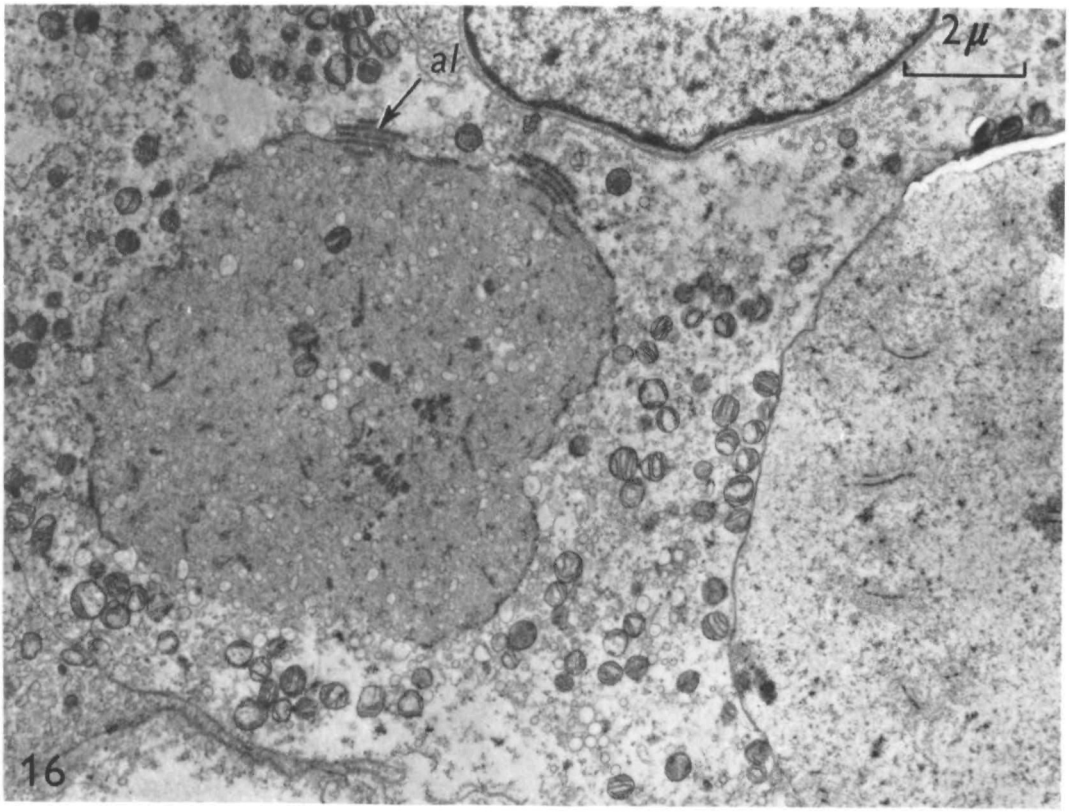
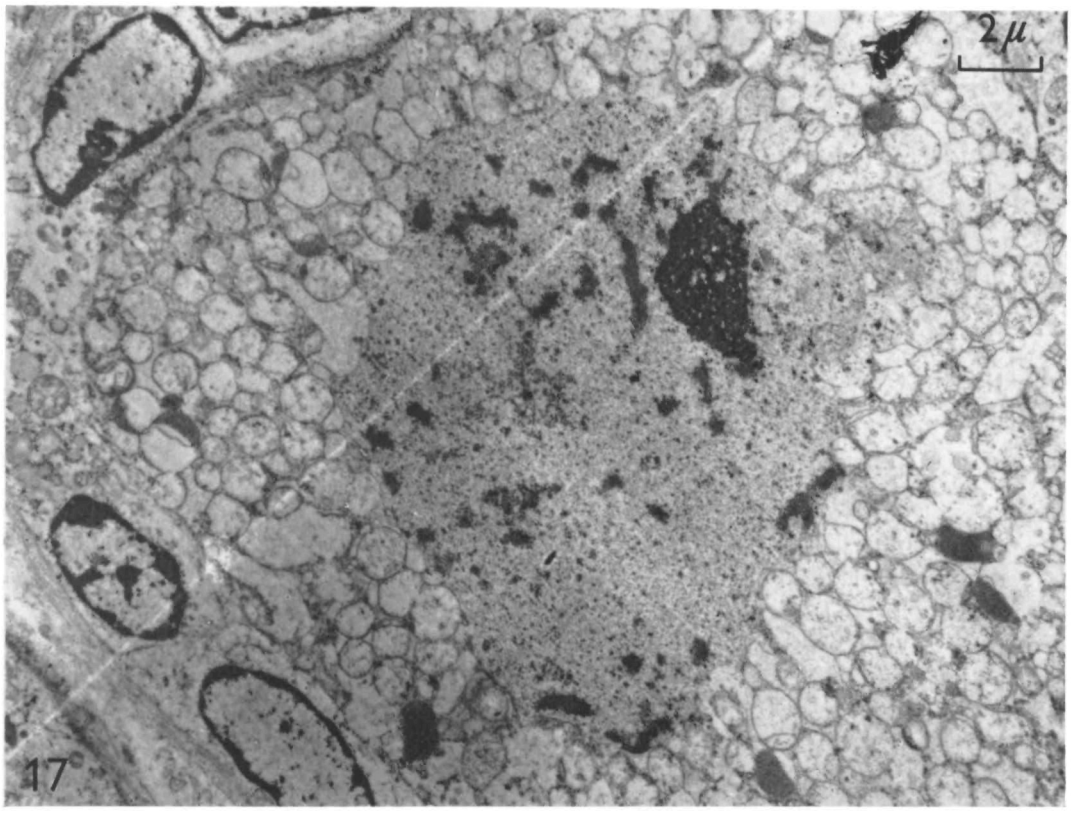

2μ
13
2μ
14
5μ
15

Fig. 16. Part of a pool containing abnormal and apparently normal oocyte nuclei. The former shows the development of annulate lamellae (*al*) and invasion by cytoplasmic organelles.

Fig. 17. Oocyte undergoing degeneration at the diplotene stage. The distorted nucleus contains highly condensed chromosomes and the cytoplasmic organelles are grossly swollen.


al
2μ
16


2μ
17