The histogenesis of the gonad in rat embryos

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INTRODUCTION

With respect to the origins of the cells which contribute to gonad formation, the corticomedullary antagonism theory advanced by Witschi (1951) is one of the most popular. He proposed that indifferent gonads of vertebrate embryos contain both cortex and medulla, and that the ovary is formed by the prevalence of the cortex over the medulla, the reverse being the case for the testis, thus emphasising the differences in major constituent cells between the ovary and the testis. On the other hand, many authors have considered that the constituent cells of the ovary and testis have the same origin. Some have maintained that they originate from the coelomic mesothelium (Whitehead, 1904; Torry, 1945) or the mesenchymal cells (Fischel, 1930; Gropp & Ohno, 1966; Jirasek, 1971) or both (Gruenwald, 1942; Pinkerton, McKay, Adams & Hertig, 1961; Pelliniemi, 1976). In addition, several authors (Kolliker, 1898; Byskov & Linterun-Moore, 1973; Merchant, 1975; Upadhyay, Luciani & Zamboni, 1979; Zamboni, Bezard & Mauleon, 1979) have suggested the involvement of the mesonephric tubules. Thus, the elements involved in gonadogenesis still remain to be elucidated.

The present investigation has been conducted to elucidate the origins of the cells involved and the process of gonad formation. This has been done by histological examination of serial sections of the gonads and ultrastructural observations in rat embryos whose sexes had been identified by chromosomal analysis.

MATERIAL AND METHODS

Wistar strain rat embryos were used in this investigation. The day on which spermatozoa were detected in a vaginal smear was denoted as Day 0 of gestation. The gonads were removed on Days 12 –14 of gestation and subjected to experiment. The materials were dissected out quickly and fixed by immersion using a 2·5 % glutaraldehyde–4 % paraformaldehyde fixative in 0·1 M cacodylate buffer, pH 7·4, for 2 hours at 4 °C. This was followed by washing with the same buffer solution and postfixation with 1 % osmium tetroxide for 1 hour. After dehydration with ethanol series, each specimen was embedded in Epon 812.

Gonads from eight rat embryos obtained from four mother rats were examined. Two to four thousand sections forming an almost complete series, each section 0·5 μm thick, were prepared from each specimen and the 23000 sections were examined by light microscopy. Ultrathin sections corresponding to various appropriate light microscopical sections were prepared using an LKB Ultrotome. The ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963) and

examined with a Hitachi H-700 electron microscope. For the observation of chromosomes, parts of the embryos were cultured with Ham's F-10 culture solution and treated with a hypotonic solution (Hsu, 1952).

## RESULTS

Gonads at day 12·5 *of gestation*

With the light microscope, primordial germ cells were found immediately below the coelomic epithelium in the region of the gonadal ridge and also along the dorsal mesentery (Fig. 4). Mitotic figures were noted in large numbers in the coelomic epithelium, showing a tendency toward stratification of the epithelium (Fig. 1). Intrusion of the coelomic epithelium cells into the subepithelial regions was more prominent around the primordial germ cells. Regionally these structures formed the primary sex cords. One of the mesonephric tubules which branched from the cranial portion of the mesonephric duct was highly tortuous and divided further into six to eight branches (Figs. 1–3). Granules, darkly stained with toluidine blue, were observed in the cells of the mesonephric tubules (Fig. 2). At the distal portion of each of the branches of the mesonephric tubule, some were directly contiguous with, some in contact with, and others in close proximity to (but not in contact with) the coelomic epithelium (Figs. 1, 3). At the site of transition from mesonephric tubule to coelomic epithelium, cells constituting the mesonephric tubules and cells lining the coelomic epithelium were easily distinguished by virtue of their location and differences in their affinity for toluidine blue (Figs. 1, 3). Mesenchymal cells and blood vessels were noted between the mesonephric tubules.

With the electron microscope, a basal lamina was not found in any of the regions, including the locations where primordial germ cells were in contact with coelomic epithelium (Fig. 5). Cell membranes between primordial germ cells and coelomic epithelial cells were regionally very close to each other at intervals of about 20 nm. Typical cell junctions between germ cells and cells of the coelomic epithelium were not observed. Each primordial germ cell had a large, circular nucleus containing one or two clear nucleoli. The cytoplasm contained a well developed Golgi complex, spherical mitochondria, granular endoplasmic reticulum, granules demarcated by membranes, polysomes and coated vesicles (Fig. 6). Cell junctions were found between coelomic epithelial cells and subepithelial cells, and rupture of the basal lamina of the coelomic epithelium was noted. Each of these subepithelial cells had an oval nucleus containing one or two nucleoli. The cytoplasm contained a large number of circular or oval mitochondria with laminated cristae, granular endoplasmic

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Fig. 1. Fetal gonad at Day 12·5 of gestation. A tendency toward stratification of the coelomic epithelium (*CE*) is observed. Granules (arrowheads), darkly stained with toluidine blue, are observed in the cells of the mesonephric tubules. Mesonephric tubules are highly tortuous. Original mesenchymal cells and blood vessels (*BV*) can be noted between the mesonephric tubules. × 300.

Fig. 2. Fetal gonad at Day 12·5 of gestation. Arrowhead and *LG*, lysosome-like granules. × 700.

Fig. 3. Fetal gonad at Day 12·5 of gestation. The mesonephric tubule (*MNT*) which is ramifying from the cranial portion of the mesonephric duct is highly tortuous and further divides into six to eight branches. The distal portion of the mesonephric branch indicated by the arrowhead is directly contiguous with the coelomic epithelium. The coelomic epithelial cells are easily distinguished from the cells of the mesonephric tubules by differences in affinity for toluidine blue. × 900.
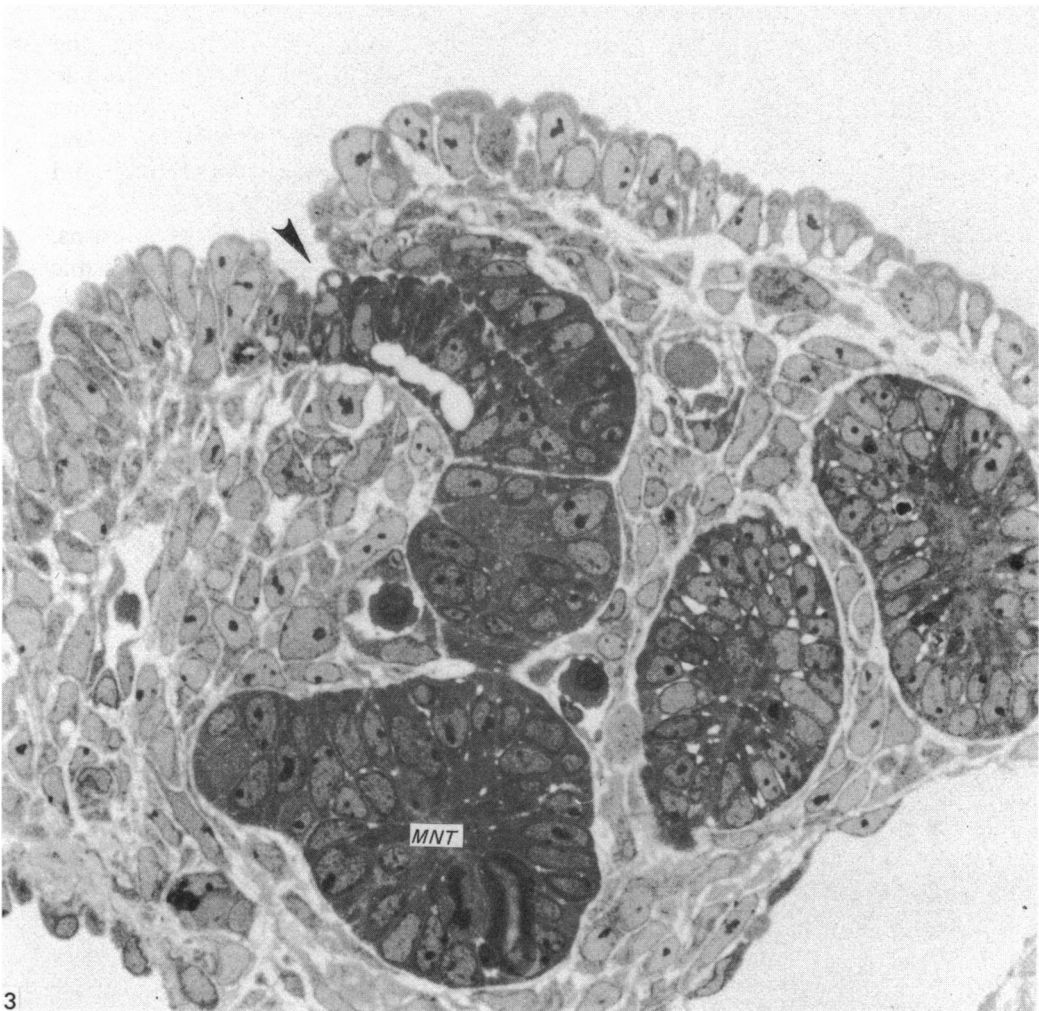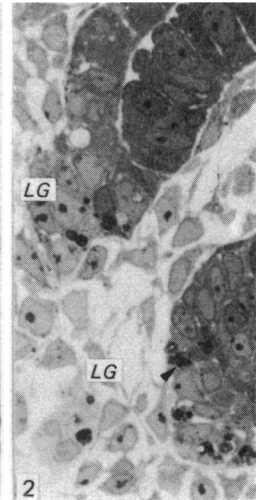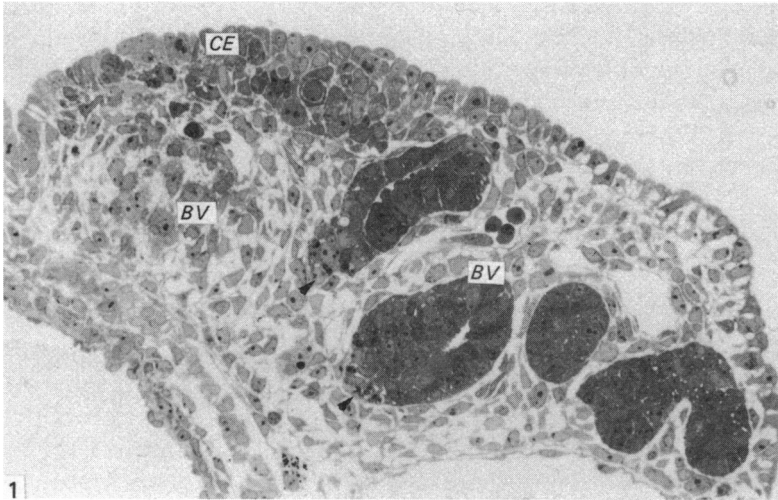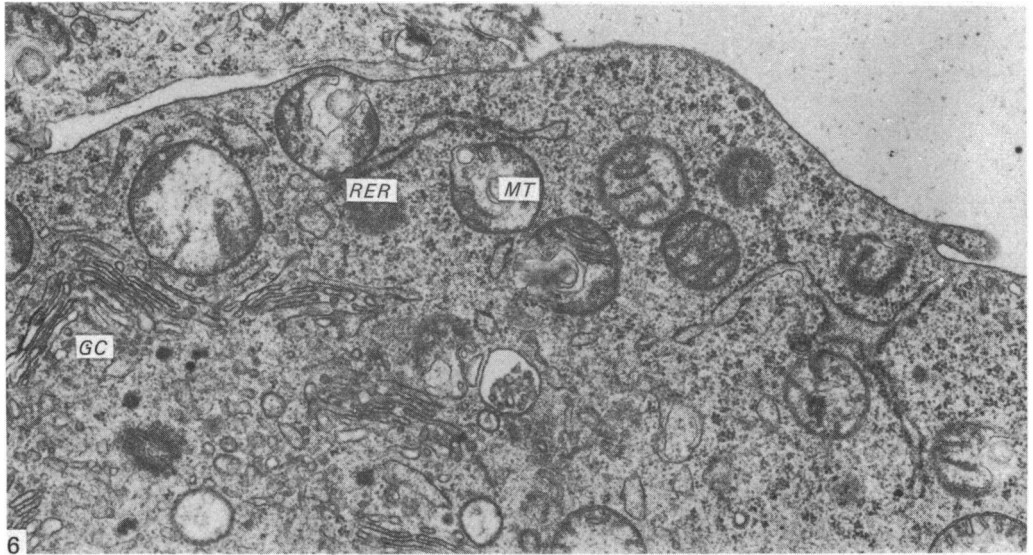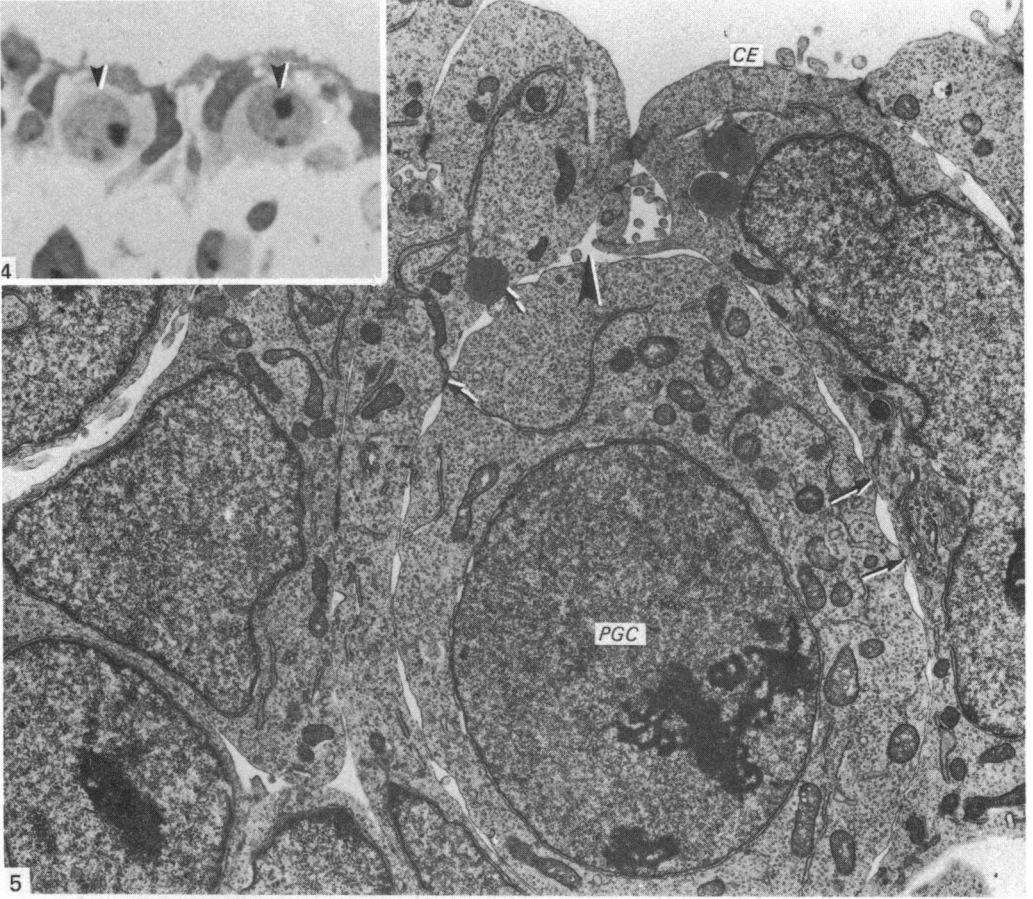
.eticulum and coated vesicles. Cell processes were also observed. Each of the cells composing the mesonephric tubules and duct had dark cytoplasm and a nucleus shaped like a slightly irregular circle. The cytoplasm contained mitochondria, granular endoplasmic reticulum and many ribosomes. Lipid droplets were often noted in these cells.

Gonads at Day 13·0 *of gestation*

Large numbers of cell divisions were observed in the coelomic epithelium, mesenchymal cells and mesonephric tubules. In the distal portion of each mesonephric tubule, many clear cells which were lightly stained with toluidine blue formed cell cords (Figs. 7–9). These cell cords had caused the enlargement of the gonads and occupied their main part. Basal lamina, contiguous with that of the mesonephric tubule, was observed around the clear cell cords which were connected with the mesonephric tubules, but disappeared in the vicinity of the primordial germ cells. Mesenchymal cells and blood vessels were noted between the cell cords.

Gonads with XY chromosomes at Day 13·5 *of gestation*

In the subepithelial region of the coelom, there were well developed cell cords composed of clear cells, in which sporadic primordial germ cells were observed. These cords occupied the greater part of the gonads and observation of serial sections revealed that there were six to eight of them. Each cell cord was contiguous with each of the above mentioned mesonephric tubules branching from the mesonephric duct in the cranial portion of the gonads. These cell cords corresponded to the secondary sex cords, that is, the seminiferous tubules (Fig. 10). Stratification of the coelomic epithelium was observed, however, and the cells appeared to be more stretched out than in the early stages. In the mesenchyme between the cell cords, there were mesenchymal cells darkly stained with toluidine blue, each of which contained an oval nucleus, clear nucleoli, and narrow, long cytoplasmic processes (Fig. 10). Granules darkly stained with toluidine blue were observed in the cells lying at the site of transition from dark mesonephric tubules to clear cell cords (Figs. 10, 13, 14). The mesonephric tubules at this stage were highly tortuous, but an anastomosis between them was not noted and formation of the so-called 'rete' testis was not observed.

The ultrastructure of the primordial germ cells was almost the same as at Day 12·5 of gestation. Cell junctions were noted in small numbers between germ cells and clear cells of the cell cords (primordial Sertoli cells). In each primordial Sertoli cell, the nucleoplasm was slightly coarse with one or two nucleoli. The cell sometimes had a crescent-like shape. Rod shaped mitochondria, granular endoplasmic reticulum and lipid droplets were observed in the cytoplasm (Fig. 11). On the other hand,

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Fig. 4. Fetal gonad at Day 12·5 of gestation. Primordial germ cells (arrowheads) are observed immediately below the coelomic epithelium in the region of the gonadal ridge. Each primordial germ cell is a large, round cell with a large, circular nucleus in its centre. × 800.

Fig. 5. Fetal gonad at Day 12·5 of gestation. The basal lamina is not seen in any of the regions (arrowhead) where primordial germ cells (*PGC*) are in contact with the coelomic epithelium (*CE*). Cell membranes are close to each other in places (arrows). × 7500.

Fig. 6. Fetal gonad at Day 12·5 of gestation. Each primordial germ cell has a large amount of cytoplasm containing well developed Golgi complexes (*GC*), spherical mitochondria (*MT*), granular endoplasmic reticulum (*RER*), granules demarcated by membranes, polysomes, and coated vesicles. × 18700.
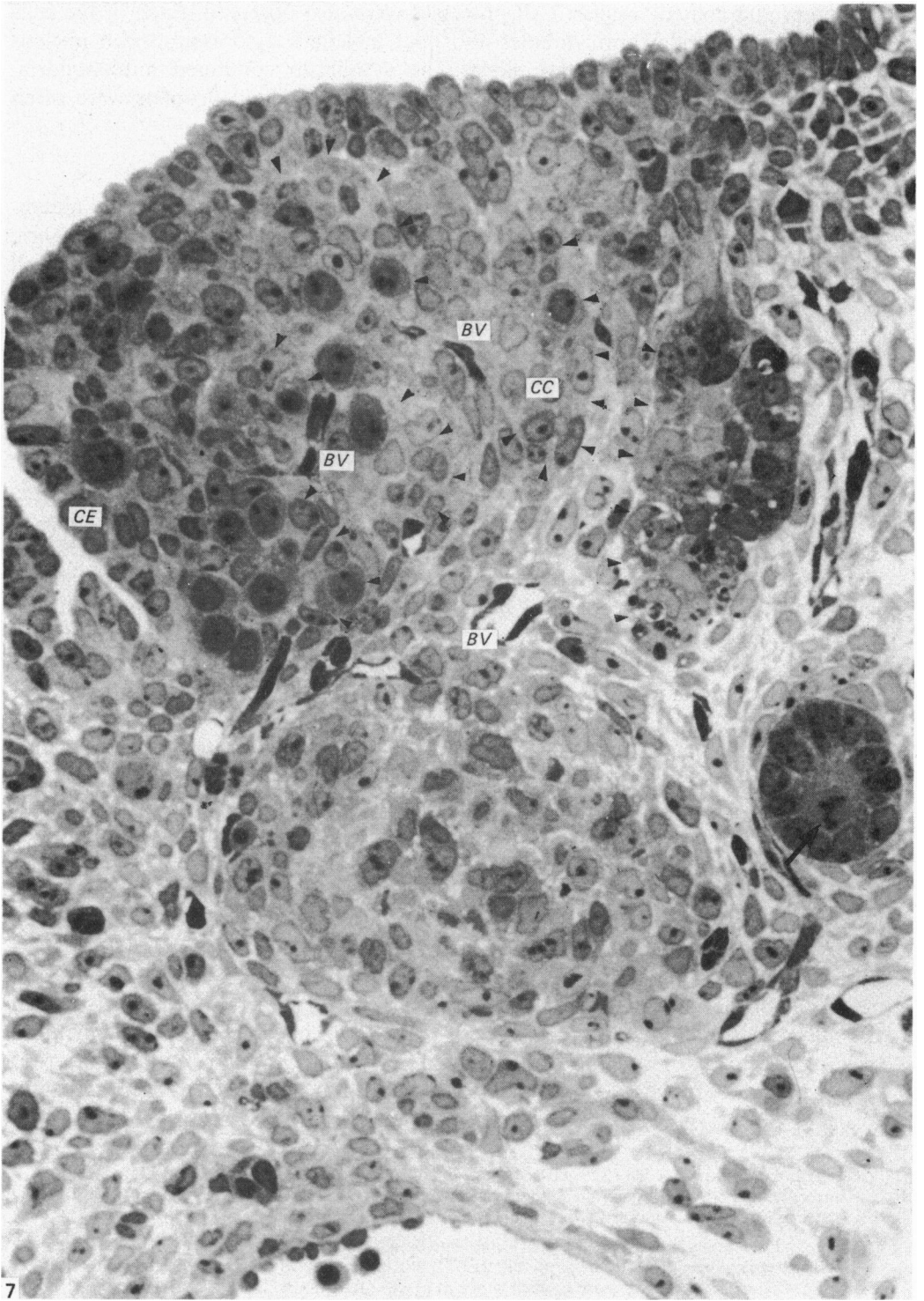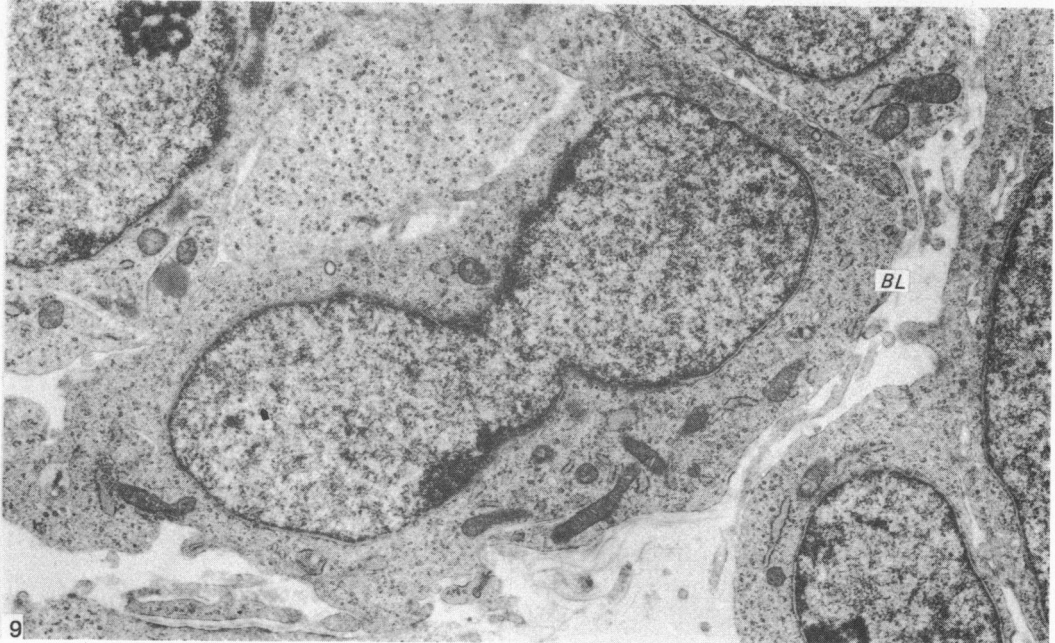
Fig. 7. Fetal gonad at Day 13·0 of gestation. In the distal portion of each mesonephric tubule, many clear cells which are lightly stained with toluidine blue are observed to have formed cord-like structures (arrowheads). These clear cell cords (*CC*) have caused the rapid enlargement of and occupy the main part of the gonads. Originally mesenchymal cells and blood vessels (*BV*) are noted between the cell cords. Cell division is seen in the mesonephric tubule (arrow). *CE*, coelomic epithelium. × 800.
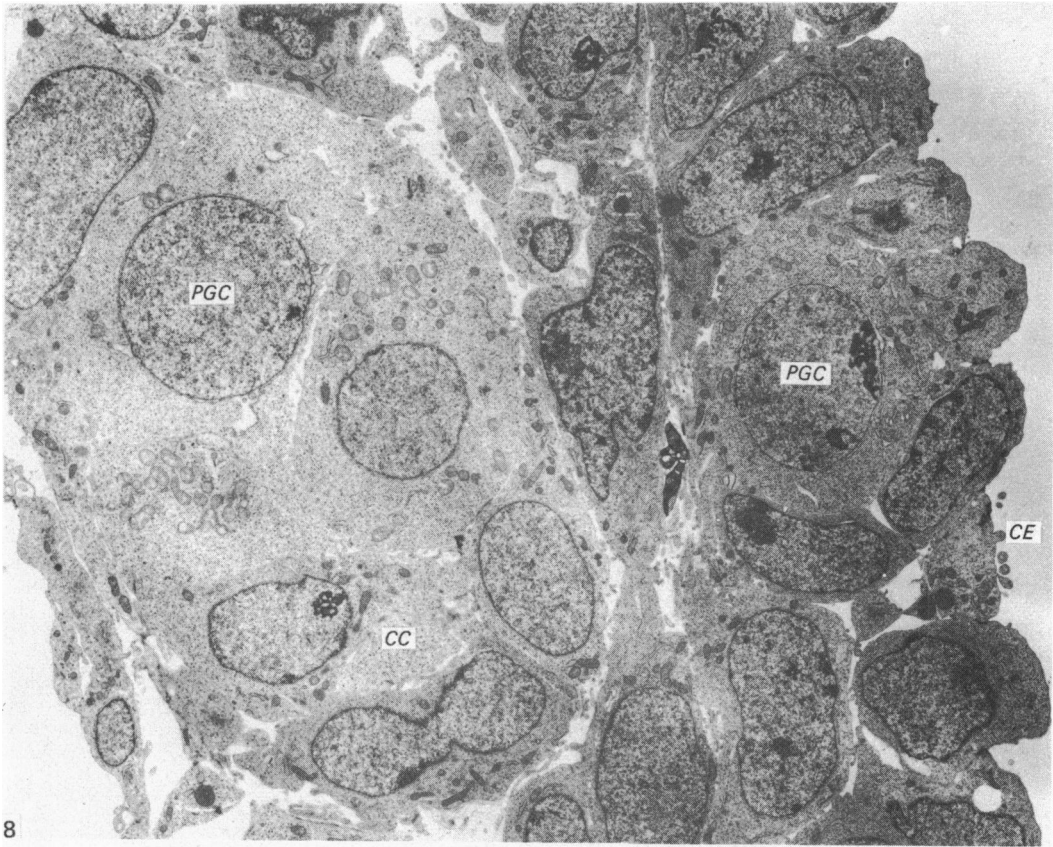
Fig. 8. Fetal gonad at Day 13·0 of gestation. From examination of electron microscope serial sections, this clear cell cord (*CC*), in a lower portion of the gonad, was found to be contiguous with a mesonephric tubule and was not in contact with the coelomic epithelium (*CE*). A primordial germ cell (*PGC*) can be seen in the cell cord. × 3000.

Fig. 9. Fetal gonad at Day 13·0 of gestation. The basal lamina (*BL*) is seen partially to surround the cord. × 8800.
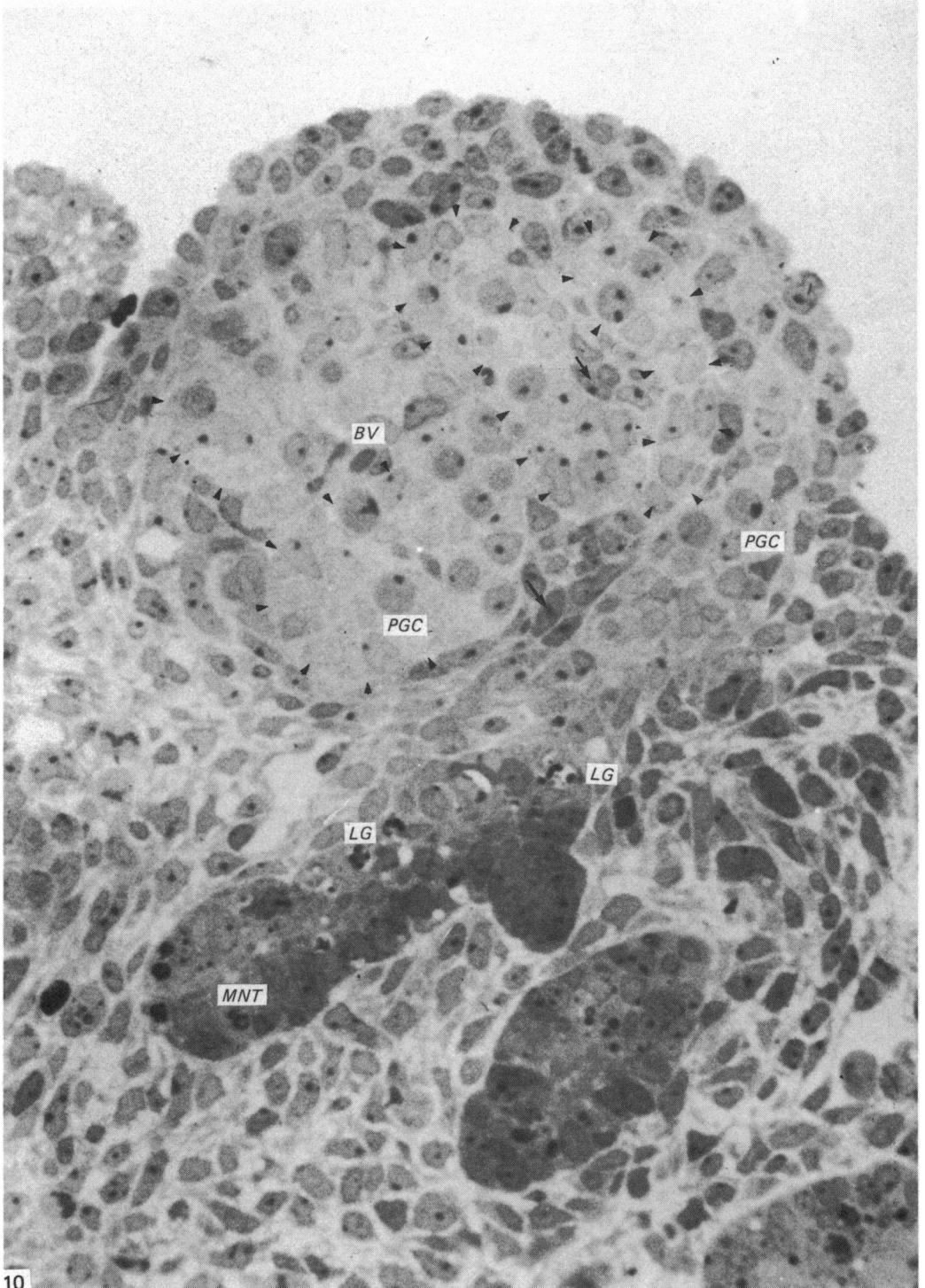
Fig. 10. Fetal gonad of a male at Day 13·5 of gestation. In the subepithelial region of the coelom, two or three strata of coelomic epithelium are seen, below which well developed cell cords (arrowheads) composed of clear cells and containing primordial germ cells (*PGC*) are present, occupying the greater part of the gonad. Mesenchymal cells (arrows) are observed between cell cords. *BV*, blood vessel; *LG*, lysosome-like granules; *MNT*, mesonephric tubule. × 800.
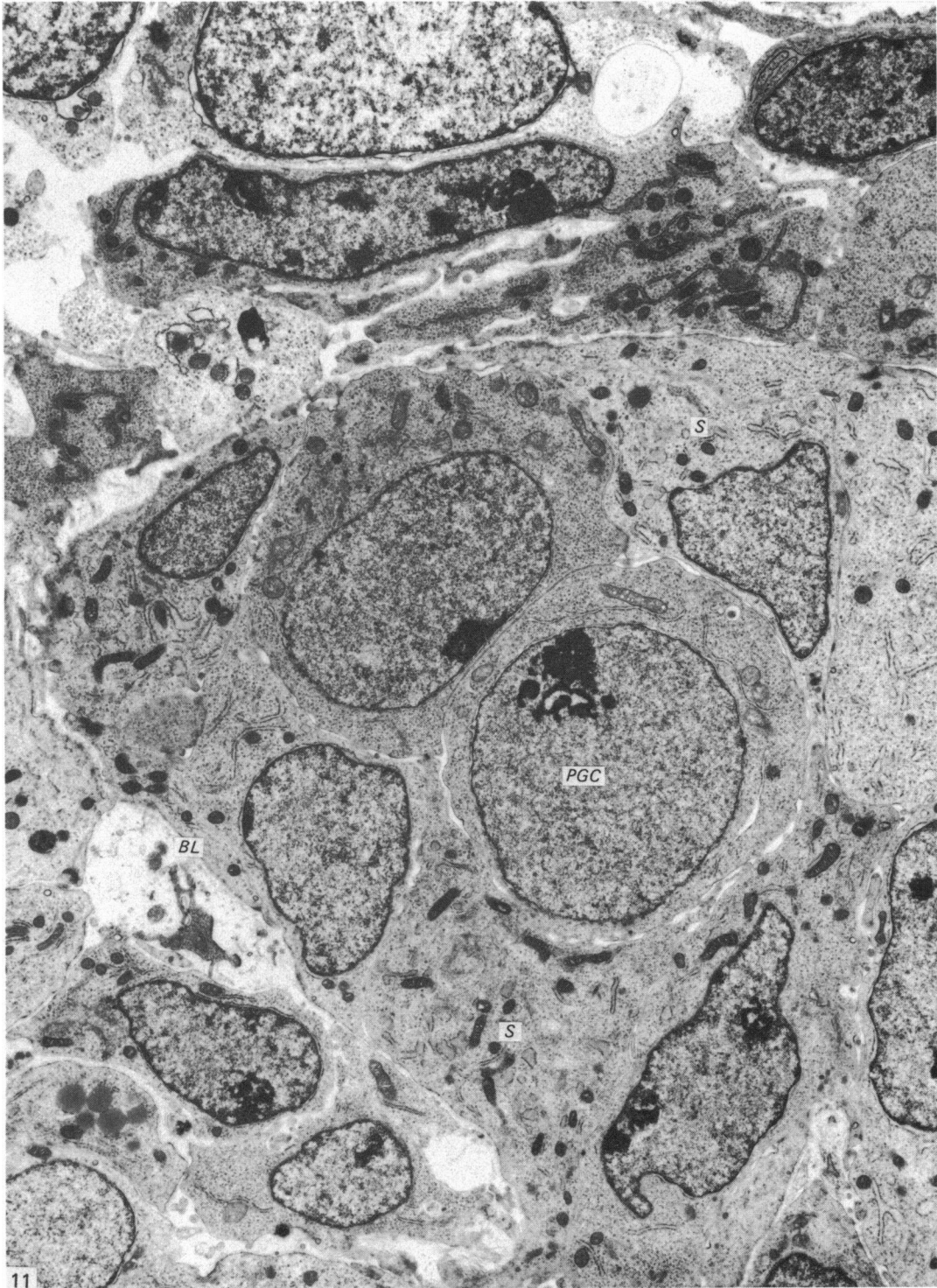
Fig. 11. Fetal gonad of a male at Day 13·5 of gestation. In the primordial Sertoli cell (*S*), the nucleoplasm is slightly coarse and one or two nucleoli are contained in the nucleus. The cytoplasm appears crescent-like because of cell junctions. *BL*, basal lamina; *PGC*, primordial germ cell. × 5600.
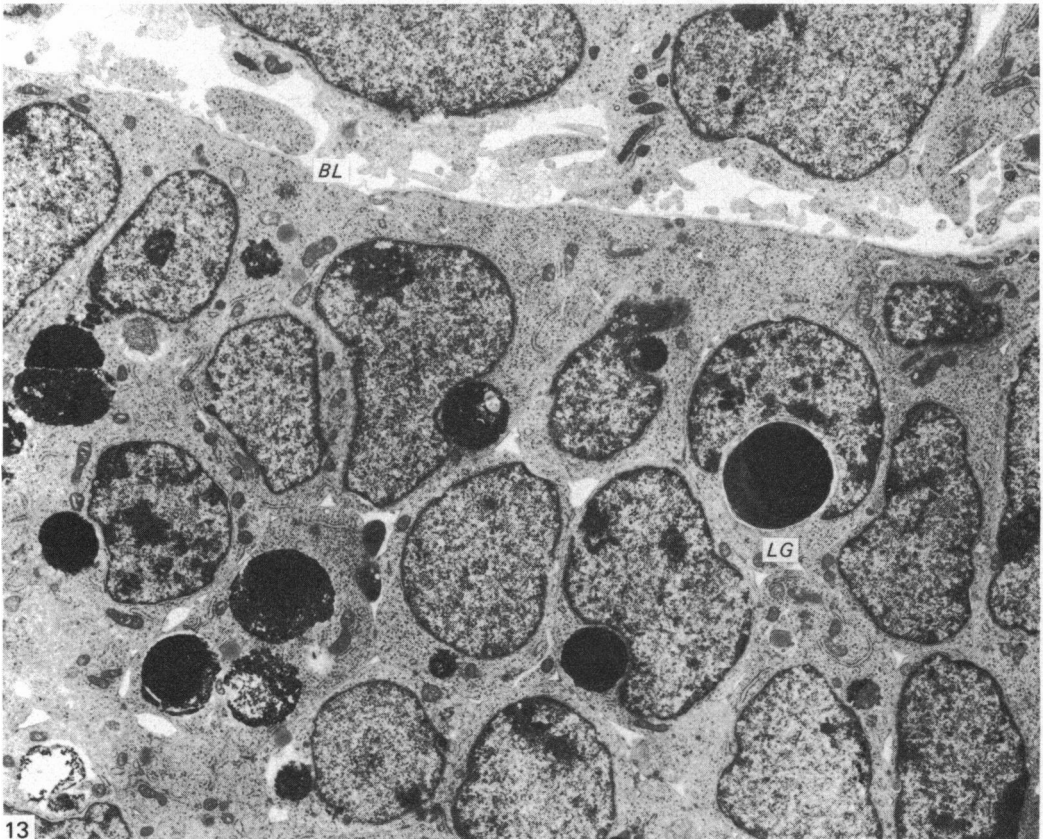
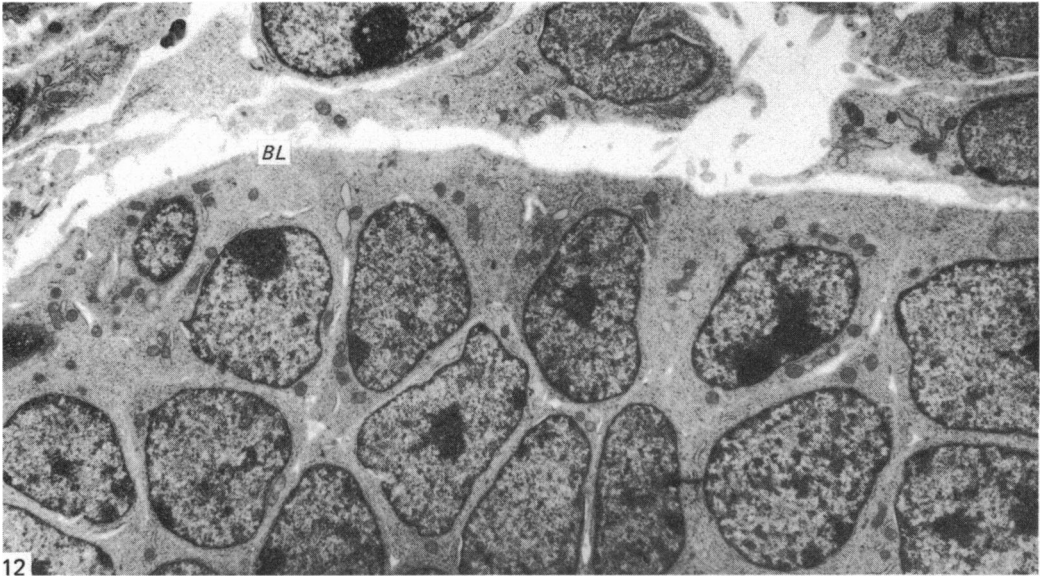Fig. 12. Fetal gonad of a male at Day 13·5 of gestation. The wall of each mesonephric tubule located on the dorsal side is composed of dark cells. *BL*, basal lamina. × 4600.

Fig. 13. Fetal gonad of a male at day 13·5 of gestation. The cells at the site of transition from dark to clear cells contain 1–3 μm granules (*LG*) of homogenous composition which are demarcated by a membrane. *BL*, basal lamina. × 5200.
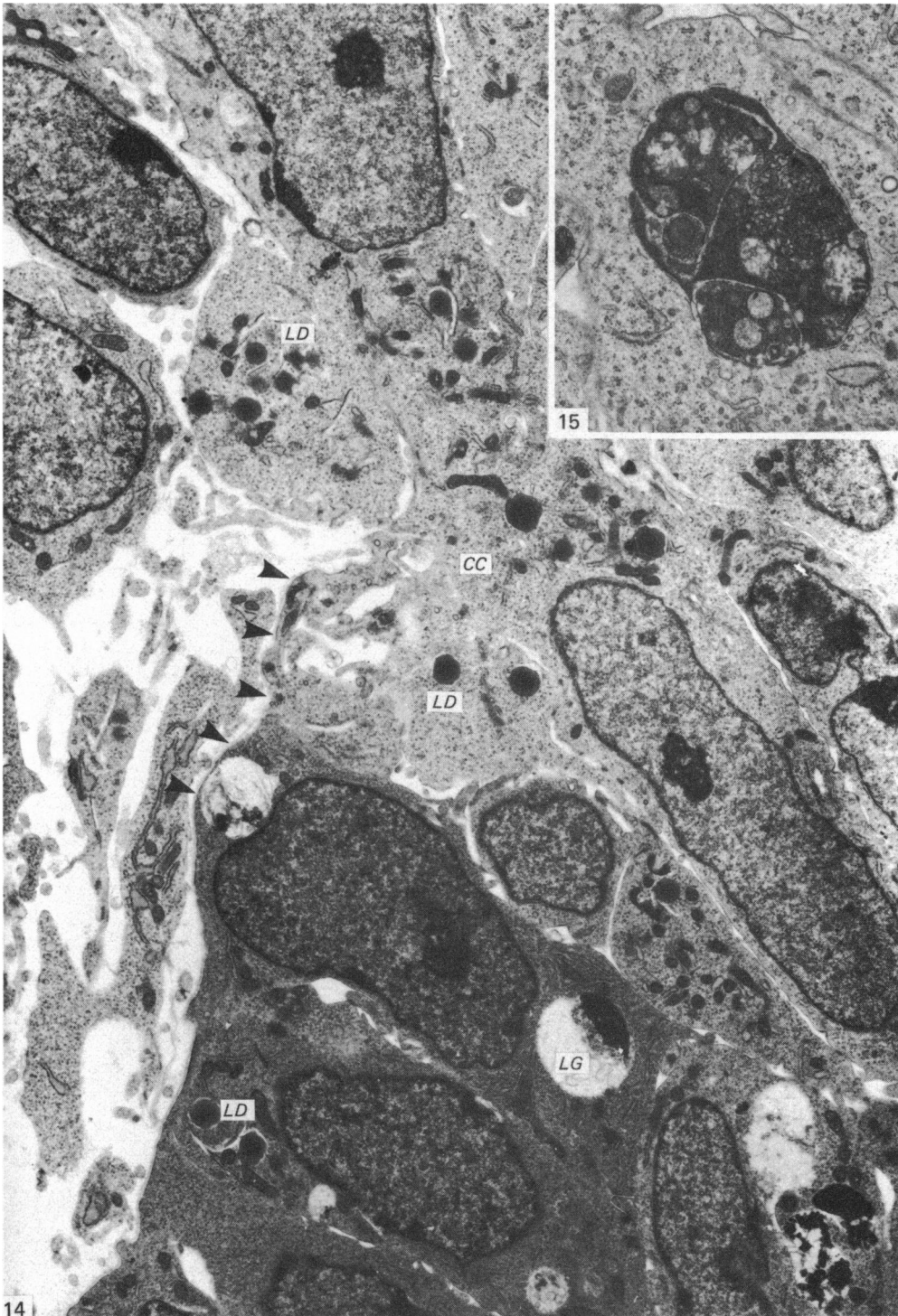
Fig. 14. Fetal gonad of a male at Day 13·5 of gestation. Cells at the site of transition from dark to clear cells. Each cell cord (*CC*) is contiguously surrounded by the basal lamina (arrowheads) of the mesonephric tubule. A large number of lipid droplets (*LD*) and large granules (*LG*) are seen in the clear cells composing the cell cords. × 4000.

Fig. 15. Fetal gonad of a male at Day 13·5 of gestation. A secondary lysosome-like granule is noted in the cord cells. × 13 600.
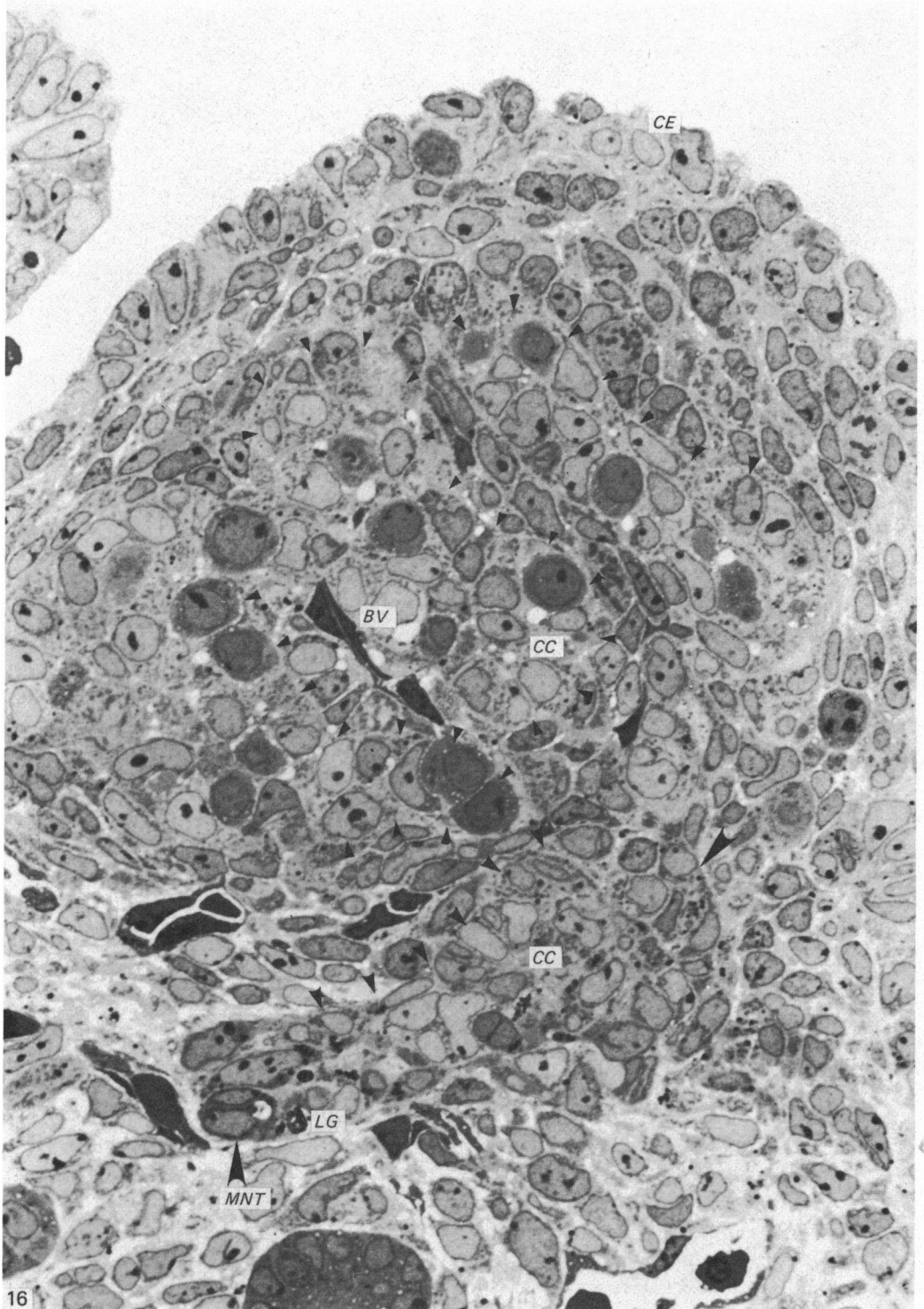
Fig. 16. Fetal gonad of a female at Day 13·5 of gestation. Cell cords (*CC*) containing primordial germ cells are present below the coelomic epithelium (arrowheads) and occupy the greater part of the gonad. Although stratification of the coelomic epithelium (*CE*) is seen, the coelomic epithelial layer appears to be stretched more than during the earlier stages. In serial sections, the number of these cell cords was six to eight, each of which was contiguous with the mesonephric tubules (*MNT*). In the cells at the site of transition from mesonephric tubules to cell cords, many granules (*LG*), darkly stained with toluidine blue, are observed. *BV*, blood vessel. × 800.
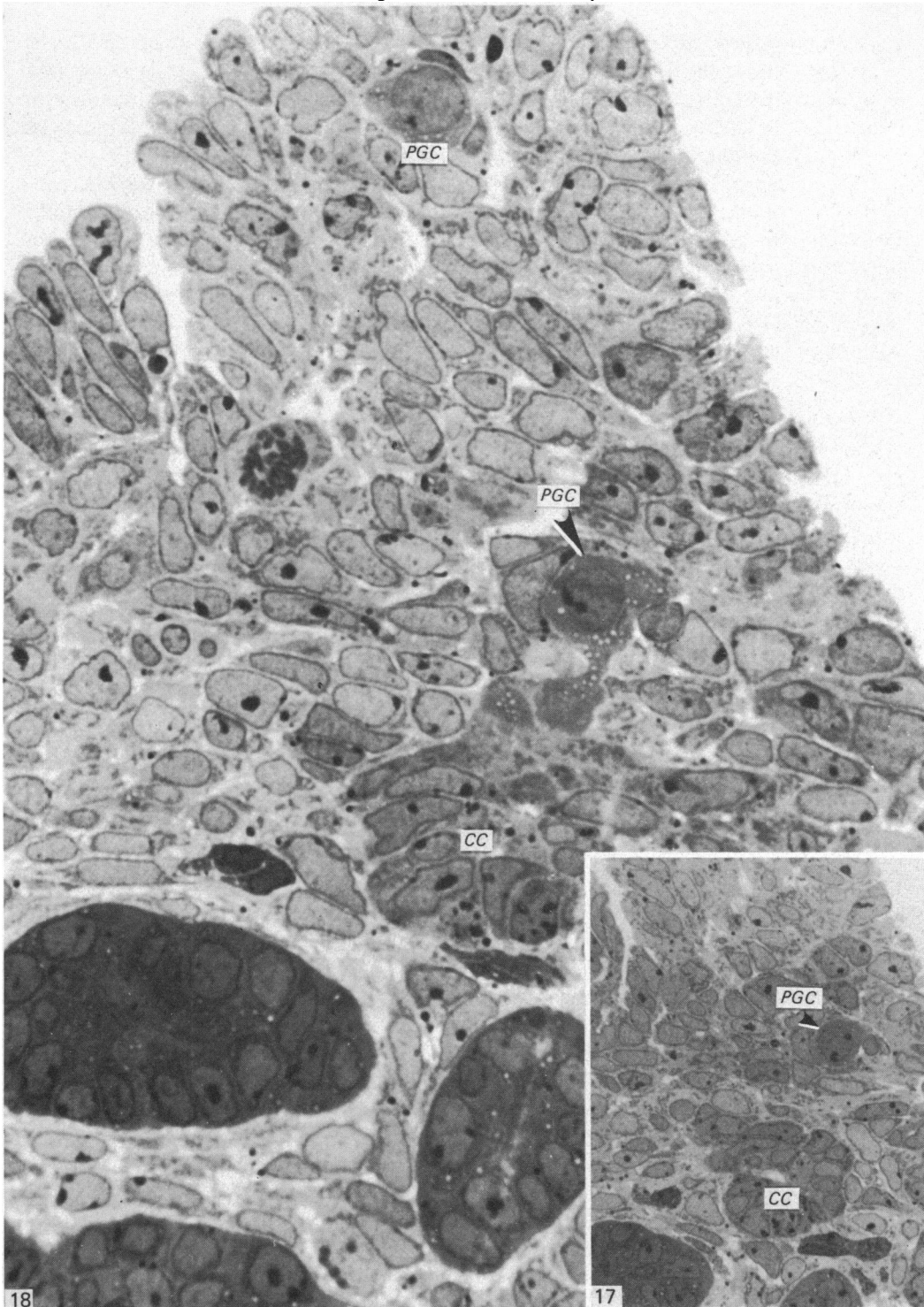
Fig. 17. Fetal gonad of a female at Day 13·5 of gestation. Some of the primordial germ cells (*PGC*) are surrounded by coelomic epithelial or mesenchymal cells (arrowhead). Clear cells (*CC*) may be seen, contiguous with a mesonephric tubule. Figures 17–19 are from closely adjacent sections. These portions correspond to those of Figure 3 in the early stages. × 200.

Fig. 18. Fetal gonad of a female at Day 13·5 of gestation. A primordial germ cell (*PGC*) (at arrowhead), displaying an amoeboid type of movement, is in contact with the surface of the clear cell cords (*CC*). × 1000.

each mesonephric tubule located on the dorsal side was composed of dark cells (Fig. 13). The cells at the site of transition from dark to clear cells contained granules of homogeneous composition 1–3 μm in diameter, demarcated by membranes (Figs. 13, 14). These granules were observed in the immediate vicinity of the nucleus and displacement of the nuclear membrane was noted (Fig. 13).

In the clear cells composing the cell cords, there were many lipid droplets, rod-like mitochondria, granular endoplasmic reticulum, Golgi complexes, and secondary lysosome-like granules as large as 3 μm in diameter (Figs. 14, 15). In addition, degeneration was occasionally observed in cells containing these granules. Each cell cord was surrounded by a basal lamina continuous with that of the mesonephric tubule (Fig. 14), but the lamina was not observed when the cell cord was in contact with or in the immediate vicinity of the primordial germ cells.

Gonads with XX chromosomes at Day 13·5 *of gestation*

Cell cords were observed containing primordial germ cells below the coelomic epithelium which occupied the greater part of the gonads (Fig. 16). In serial sections, the number of these cell cords was six to eight, each of which was contiguous with a mesonephric tubule (as in the testis). These cell cords formed the secondary sex cords, that is, so-called 'cortical' cords (Fig. 16). The clear cells composing the cell cords had two kinds of nuclei. One type was large, irregularly circular and lightly stained with toluidine blue, while the other was darkly stained. Blood vessels and mesenchymal cells were observed between the cell cords (Fig. 16). By light microscopy, cell cords containing primordial germ cells were ill-defined immediately below the coelomic epithelium, but were defined more clearly at deeper sites. In the cells at the site of transition from mesonephric tubules to cell cords, many granules were observed which were darkly stained with toluidine blue (Fig. 16). Some germ cells were surrounded by coelomic epithelial cells (Fig. 17) and some were in contact with the surface of a cell cord, apparently displaying an amoeboid type of movement, while the majority of the germ cells were actually contained within the cell cords (Fig. 18). Primordial germ cells were also noted at deeper sites of cell cords (Figs. 19–22). In the region of transition from mesonephric tubule to coelomic epithelium, cells of the clear cell cords were easily discriminated from the cells of the coelomic epithelium by differences in their affinity for toluidine blue.

The ultrastructure of the primordial germ cells in the cell cords of the ovary was similar to that of those in the testis. Cell junctions were noted in small numbers between germ cells and clear cells of the cell cords. These were also noted between

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Fig. 19. Fetal gonad of a female at Day 13·5 of gestation. The primordial germ cell (*PGC*; at arrowhead) is located in the cell cords originating in the mesonephric tubule (*MNT*). Coelomic epithelial cells (*CE*) are easily distinguished from clear cord (*CC*) by their differences in affinity for toluidine blue *LG*, lysosome-like granules. × 400.

Fig. 20. Fetal gonad of a female at Day 13·5 of gestation. Primordial germ cells (*PGC*; arrowhead) are present in the clear cell cords (*CC*). *LG*, lysosome-like granules; *MNT*, mesonephric tubule. × 400.

Fig. 21. Fetal gonad of a female at Day 13·5 of gestation. Primordial germ cells (arrowhead) are also present at deeper sites in the cell cords. *CE*, coelomic epithelium. × 260.

Fig. 22. Fetal gonad of a female at Day 13·5 of gestation. *PGC*, primordial germ cell; *CC*, cell cords; *LG*, lysosome-like granules. × 800.
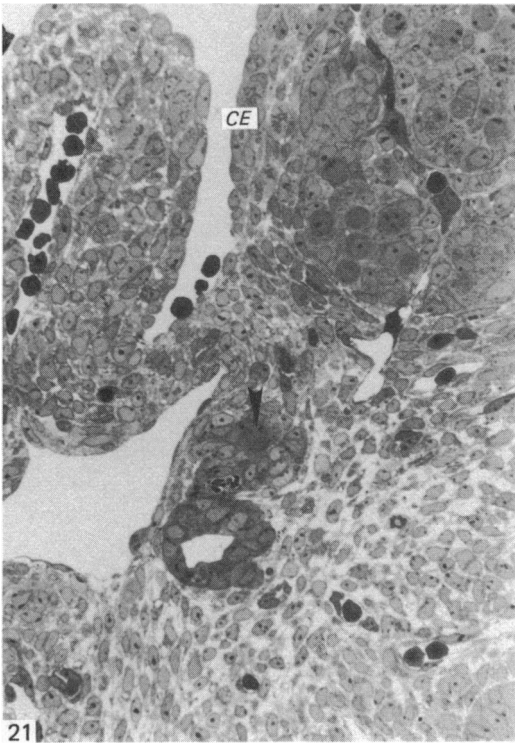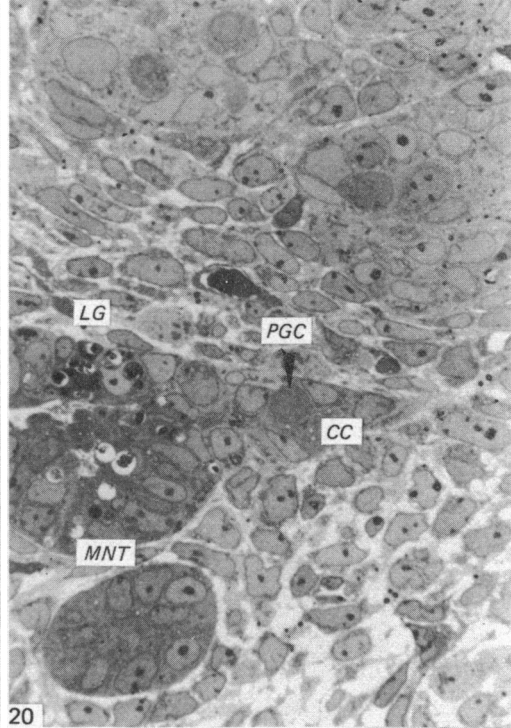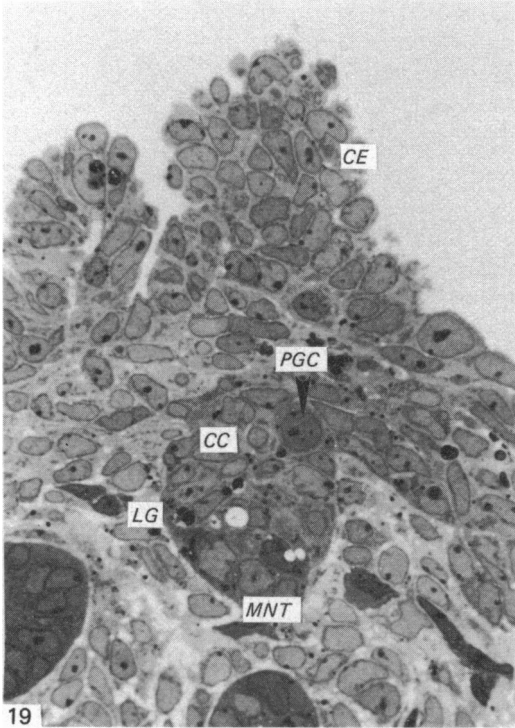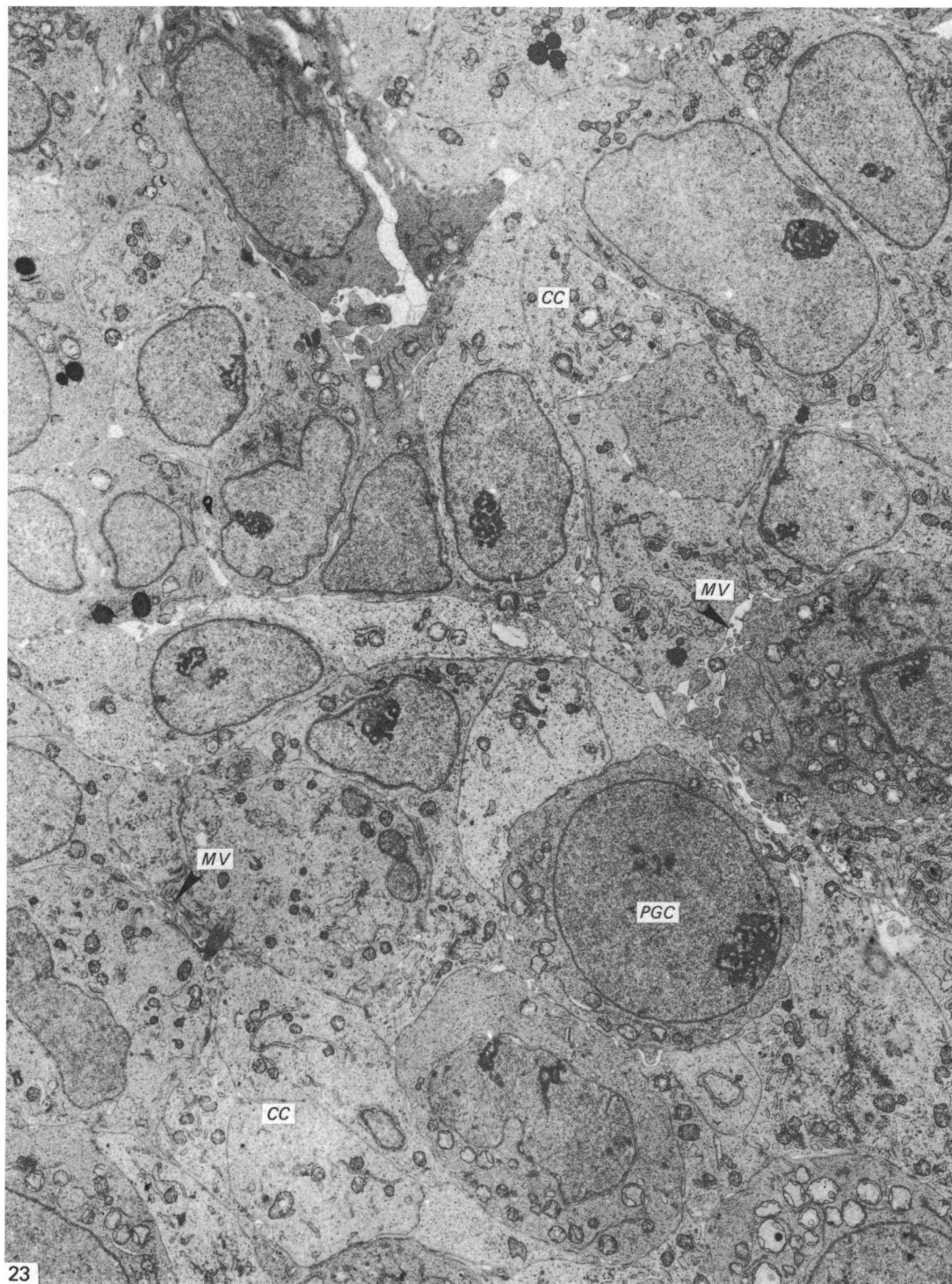
Fig. 23. Fetal gonad of a female at Day 13·5 of gestation. Microvilli (*MV*) can be seen on the surfaces of cord cells (*CC*). *PGC*, primordial germ cells. × 3600.
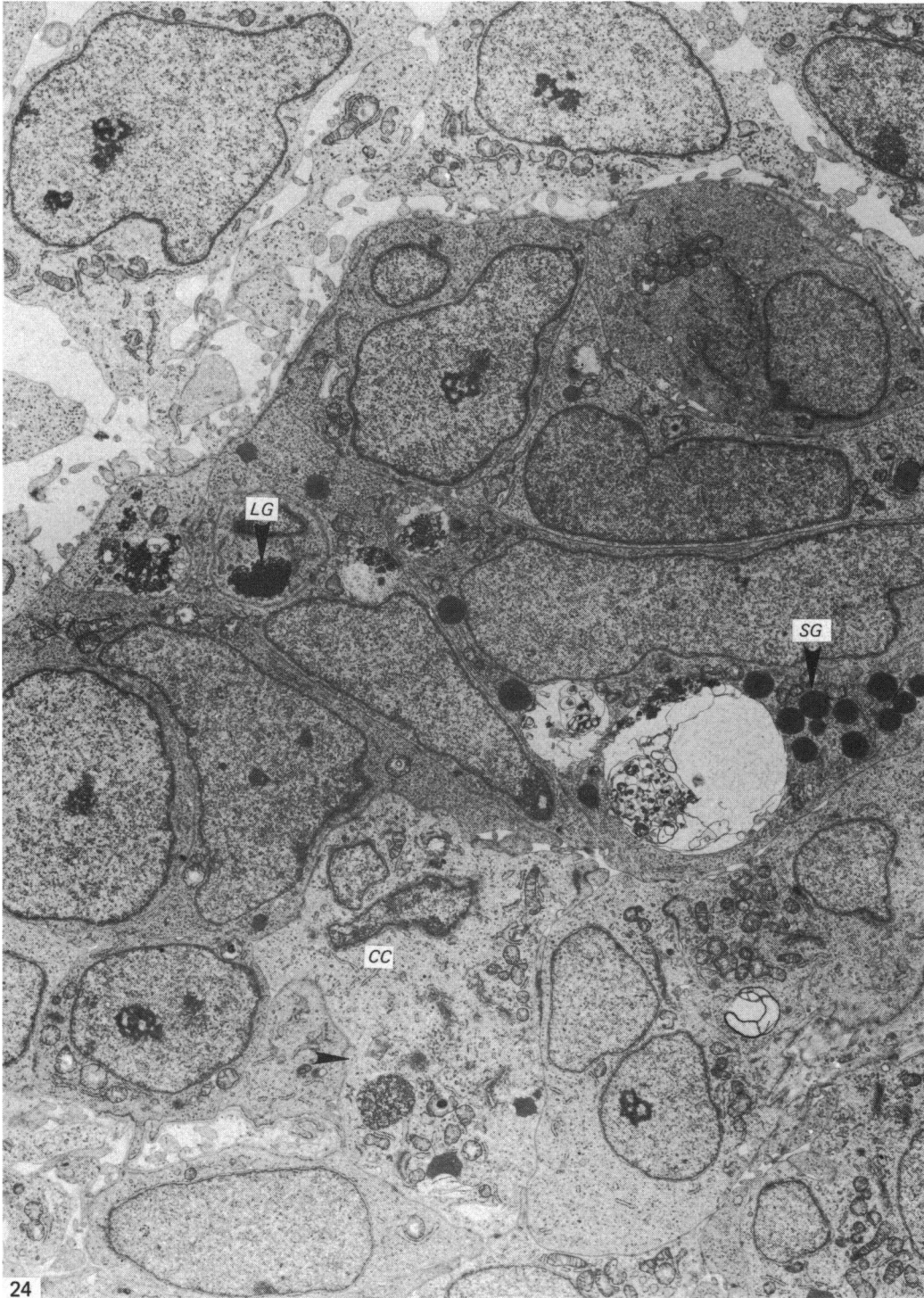
Fig. 24. Fetal gonad of a female at Day 13·5 of gestation. In the clear cells at the site of transition from dark mesonephric tubules to clear cell cords (*CC*), many small granules (*SG*) and large lysosome-like granules (*LG*) are present. × 4800.

the germ cells and the clear cells, but were smaller in number than in the testis. The incidence of the crescent-like cells which were observed in the testis was also low (Fig. 23). There were two types of cells in the cords. One had a large nucleus with fine nucleoplasm and cytoplasm containing granular endoplasmic reticulum and lipid droplets, while the other type had slightly coarse nucleoplasm and cytoplasm with rather high electron density (Figs. 16, 23, 24). These cell cords were covered with a basal lamina contiguous with that of the mesonephric tubules (Fig. 24). Rupture of the basal lamina was observed near the primordial germ cells. In the dark cells composing the mesonephric tubules, lipid droplets and a considerable number of small granules were observed (Fig. 24). In the cells at the site of transition from dark mesonephric tubules to clear cell cords, large lysosome-like granules demarcated by membranes were observed (Fig. 24), but were not so numerous as in the testis.

DISCUSSION

Some authors have related gonadogenesis to mesonephric tubules. In early studies, Kolliker (1898) proposed that mesonephric tubules were involved in the formation of the ovary and constituted the origin of follicular cells. More recently, Byskov & Linterun-Moore (1973) have indicated that the rete ovarii is involved in follicle formation in the immature mouse ovary. Merchant (1975) has indicated that the cells of the mesonephric tubules seem to be partially involved in gonadogenesis in earlier stages, though he states that the primordial germ cells are always tightly surrounded by the coelomic epithelial cells and, more often, mesenchymal cells, and that this condition remains unchanged in subsequent stages of development. Upadhyay et al. (1979) maintain that the oiiginal mesenchymal constituents of the gonadal ridge are replaced entirely by cells of the mesonephric tubules and primordial germ cells in the early stages of mouse ovarian development. These authors further consider that the ovigerous cord is formed by the gradual extension of the mesonephric tubules from the dorsal to the ventral sides, towards the subepithelial region of the coelom, and that the basal lamina of the cord is formed from the dorsal to the ventral sides.

In the present study, there was no evidence to support the view that the 'epithelial cord' is formed by the coelomic epithelium and mesenchymal cells, and that the gonad is entirely occupied by individual cells separated from the mesonephric tubules. No explanation could be found as to where and how the 'cord' is demarcated from the mesenchyme by the basal lamina. From the present observations, it is suggested that clear cells appear in mesonephric tubules that have previously ramified during the indifferent gonad stage, and that the clear cell cords emerge contiguous with the tubular structures. This is followed by their proliferation and extension to form the secondary sex cords and the basal lamina is then formed around the cell cords.

Clear cell origin from mesonephric tubules

It was observed that cell cords consisting of clear cells are contiguous with the mesonephric tubules (Figs. 7, 10, 16–22). These clear cells are of mesonephric tubule origin and form secondary sex cords, as substantiated by the following evidence. Each mesonephric tubule is directly contiguous with or very close to the coelomic epithelium in the early stages of gonadogenesis and clear cells occur in regions

which were surely mesonephric tubules during the early stages (Figs. 3, 17–19). All the secondary cords are contiguous with mesonephric tubules (Figs. 7, 10, 16–20). The clear cells which appear in the mesonephric tubules on the dorsal side (Figs. 20–22) are ultrastructurally similar to the clear cells composing the cell cords (secondary sex cords) beneath the coelomic epithelium. Also, the basal lamina covering the cell cords is extensively contiguous with that of the mesonephric tubule (Fig. 14).

On the other hand, some but not all of the mesonephric tubules are directly contiguous with the coelomic epithelium in a few parts of the early gonad (Figs. 3, 17–19). The mesonephric duct is originally differentiated from the coelomic epithelium and the cells of mesonephric tubule origin have some similarity to the coelomic epithelium. The cells of coelomic epithelial origin may possibly be converted into clear cells and involved partially in the formation of cell cords in the regions where both are contiguous or in contact. However, in the present investigation, clear cells could not be found in the coelomic epithelium, even though they are more often noted in the mesonephric tubules at deeper sites, often sporadically or in groups, as well as in the distal portions. The clear cell cords are of great length, mostly out of contact with the coelom, and occupy the greater part of the gonads. These findings could not support the view that cells of coelomic epithelial origin migrate to the cell cords and form the secondary sex cords. It is suggested that primordial Sertoli cells do not originate from the coelomic epithelium, but rather, they seem to be clear cells of mesonephric tubule origin. Leydig cells arise from original mesenchymal cells.

Entry of primordial germ cells into cell cords

It was observed that some primordial germ cells are surrounded by cells originating from the coelomic epithelium or by mesenchymal cells (Figs. 17, 18); some appear to contact the cell cords in an amoeboid fashion (Fig. 18) and some are located within the cords (Fig. 19). Moreover, since large numbers of germ cells are found even in the proximal portions of each cell cord (Figs. 19–22), it appears that they enter the cell cords from the subepithelial region of the coelom by amoeboid movement and proliferate while migrating toward the proximal portion of the cell cords. This movement of the primordial germ cells suggests that they have a strong affinity for the clear cord cells. It is not strange that the germ cells possess such an ability for migration, since they have migrated from the extragonadal region. The regions where the coelomic epithelial cells proliferate seem to play a role as sites where primordial germ cells reside temporarily during gonadal development.

Some workers (Witschi, 1951; Pinkerton *et al.* 1961) consider that primordial germ cells are essential for gonadogenesis, while others do not (Everett, 1943; Mintz, 1960; Merchant, 1975). Disappearance of the basal lamina is observed in regions where the germ cells are in contact with or in the close vicinity of the coelomic epithelium and clear cell cords. The observations described above suggest that primordial germ cells may play a role in the disappearance of the basal lamina of the coelomic epithelium (Fig. 5), in the subepithelial intrusion of the epithelial cells, in the partial disappearance of the basal lamina of the cell cords and in entry into the cell cords. It is more reasonable to consider that the primordial germ cells partially dissolve the adjacent basal lamina and enter the clear cell cords rather than that they migrate towards defects in the basal lamina. However, this action of the germ cells seems to be of a transient nature.

Primordial germ cells which are not incorporated into cell cords (Fig. 18) may remain in contact with coelomic epithelial cells and mesenchymal cells, and although most of these germ cells degenerate, they may form primary follicles. However, if such follicles exist, they must be small in number, since the majority of primordial germ cells are contained in clear cell cords. In this connection, further investigation into the more advanced stages of gonadal development would be desirable.

In the testis, a considerable number of cell junctions are observed between primordial Sertoli cells and the crescent-like cells probably formed as a result of displacement by cellular proliferation (Figs. 10, 11). In the ovary, cell junctions and cellular deformities occur to a lesser extent and microvilli are noted on the surfaces of the cord cells (Figs. 23, 24). The cell cords are somewhat ill-defined and the basal lamina shows rather poor development and preservation, which may possibly be related to the fact that primordial germ cells and clear cells have a tendency for migration and rearrangement. It seems that in subsequent development the cord cells (primitive granulosa cells) are selectively brought into contact with the germ cells, resulting in the subdivision and fragmentation of cell cords and consequent formation of primary follicles.

The present study leads to the conclusion that the morphogenesis of the rat gonad proceeds according to the following steps. In the indifferent gonad, one of the mesonephric tubules bifurcates from the mesonephric duct at the upper end of the gonadal anlage, becomes highly tortuous and ramifies further into six to eight branches. The distal portion of each mesonephric tubule extends and becomes contiguous with or closely approaches the coelomic epithelium of the gonadal ridge. When the primordial germ cells have completed their migration into the gonadal ridge and the coelomic epithelium begins to proliferate, clear cells appear in the distal portion of each mesonephric tubule, along with the appearance of large granules, and proliferate to form cord-like structures. The primordial germ cells enter the cell cords from the subepithelial region of the coelom by amoeboid movement through defects of the basal lamina and migrate towards the proximal portion of the clear cell cord while undergoing active proliferation. The gonad is formed from six to eight secondary sex cords containing primordial germ cells, original mesenchymal cells and blood vessels which are present between these cell cords. In the testis, seminiferous tubules are formed by these cell cords, which are well maintained by the basal lamina and the cell junctions between the clear cells. The so-called 'rete' testis is formed by highly tortuous mesonephric tubules, although anastomosis of tubules is not observed. In the ovary, 'cortical' cords are formed by clear cell cords of mesonephric tubule origin. The rete ovarii originates from mesonephric tubules.

SUMMARY

Gonadogenesis was investigated using Wistar rat embryos at 12–14 days after fertilisation. In indifferent gonads, a mesonephric tubule which bifurcates from the mesonephric duct at the upper end of the gonadal anlage ramifies into six to eight branches, the distal portions of which are contiguous, in contact with, or in close proximity to, the coelomic epithelium of the gonadal ridge. After the primordial germ cells reach the gonadal ridge, the overlying epithelium proliferates and clear cells appear in the distal portion of each mesonephric tubule, proliferating and forming cord-like structures. The primordial germ cells appear to enter these cell cords by an amoeboid type of movement. The basal lamina covering the cell cord partially

disappears near the germ cells. The germ cells within the cord migrate toward the proximal portion of the cell cord and proliferate in great profusion.

From the present observations, it can be concluded that the gonad is mainly formed of clear cell cords originating in mesonephric tubules into which germ cells have entered. The original mesenchymal cells and blood vessels form the interstitial tissue of the gonad. The rete testis and rete ovarii are of mesonephric tubule origin.

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