J. Anat. (1991), **174**, pp. 171–184
With 5 figures
Printed in Great Britain

Morphological development and fate of the mouse mesonephros*

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(*Accepted* 18 *July* 1990)

INTRODUCTION

All three forms of the mammalian kidney develop from the nephrogenic mesenchyme of the intermediate mesoderm. The earliest form, the pronephros, is found in the cranial region of the embryo and is represented by a few transitory, non-functional tubules and the associated nephric (pronephric) duct. This extends by cell streaming (Overton, 1959) adjacent to the nephrogenic mesenchyme, in which it induces the formation of mesonephric tubules in a craniocaudal sequence. The induction of tubules is thought to be permissive, that is, the cells are committed to a certain pathway but require a stimulus to express the new phenotype (Saxén, 1977).

A cell may be considered to have differentiated when it begins to synthesise specific specialised proteins (Holtzer, Strahs & Biehl, 1975). The development of the mouse mesonephros raises the question of the reversibility of differentiation as its cells contribute to the developing gonadal blastema (Fraedrich, 1979; Upadhyay, Luciani & Zamboni, 1979, 1981). Have the mesenchymal cells of the mesonephros truly differentiated when they align to form tubules? This question is particularly interesting in the mouse whose mesonephric tubules are said to be non-functional (Zamboni & Upadhyay, 1981). Wartenberg (1985) holds the view that the mesonephric cells lose their differentiated characteristics (dedifferentiate) and then proceed along a completely new pathway, redifferentiating as somatic cells of the gonad.

A study of the development of a basal lamina around the developing mesonephric tubule may help to elucidate this problem. Laminin is a glycoprotein unique to basement membranes and the basement membrane-like material produced by many differentiated tissues. It is found in both the lamina densa and the lamina rara of the basal lamina (Bernfield, Banerjee, Kodu & Rapraeger, 1984). In the mouse metanephros Ekblom *et al.* (1980) found that laminin was produced by cells which were destined to become epithelial cells; the mesenchymal stroma remained devoid of laminin. More recently, Klein, Langegger, Timpl & Ekblom (1988) have shown that the two B chains of laminin are synthesised in metanephric mesenchyme before induction, while the appearance of the A chain coincides with the onset of cell polarisation at the initiation of kidney tubule formation. In the present study, immunohistochemistry has been used to examine the appearance of laminin in developing basal laminae, to support electron microscopical observations.

The mesonephros may also be involved in the development of the mammalian adrenal cortex. Adrenal cortical cells may be derived exclusively from the coelomic epithelium in the human embryo (Chester Jones, 1957), or from both the coelomic epithelium and the subjacent mesenchyme in man and in other mammals (Gruenwald,

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1942). However, both Witschi (1951) and Crowder (1957) have suggested that mesonephric glomeruli contribute cells to the adrenal cortex. More recently, Upadhyay & Zamboni (1982) reported that the mesonephros is the primary source of cells for the developing adrenal cortex in the sheep. The suggestion that gonadal somatic cells and adrenal cortical cells are of the same embryonic origin could explain functional similarities between the two cell populations. Both are involved in steroid hormone production. Moreover, adrenal-like tumours occur in the gonads (Teilum, 1976), while gonad-like tumours occur in the adrenal cortex (Neville & O'Hare, 1979).

The aim of this study is to investigate the development of the mouse mesonephros in order to assess the extent of its differentiation and its contribution to gonadal and adrenal primordia. A preliminary report on this study has been made (Smith, 1990).

MATERIALS AND METHODS

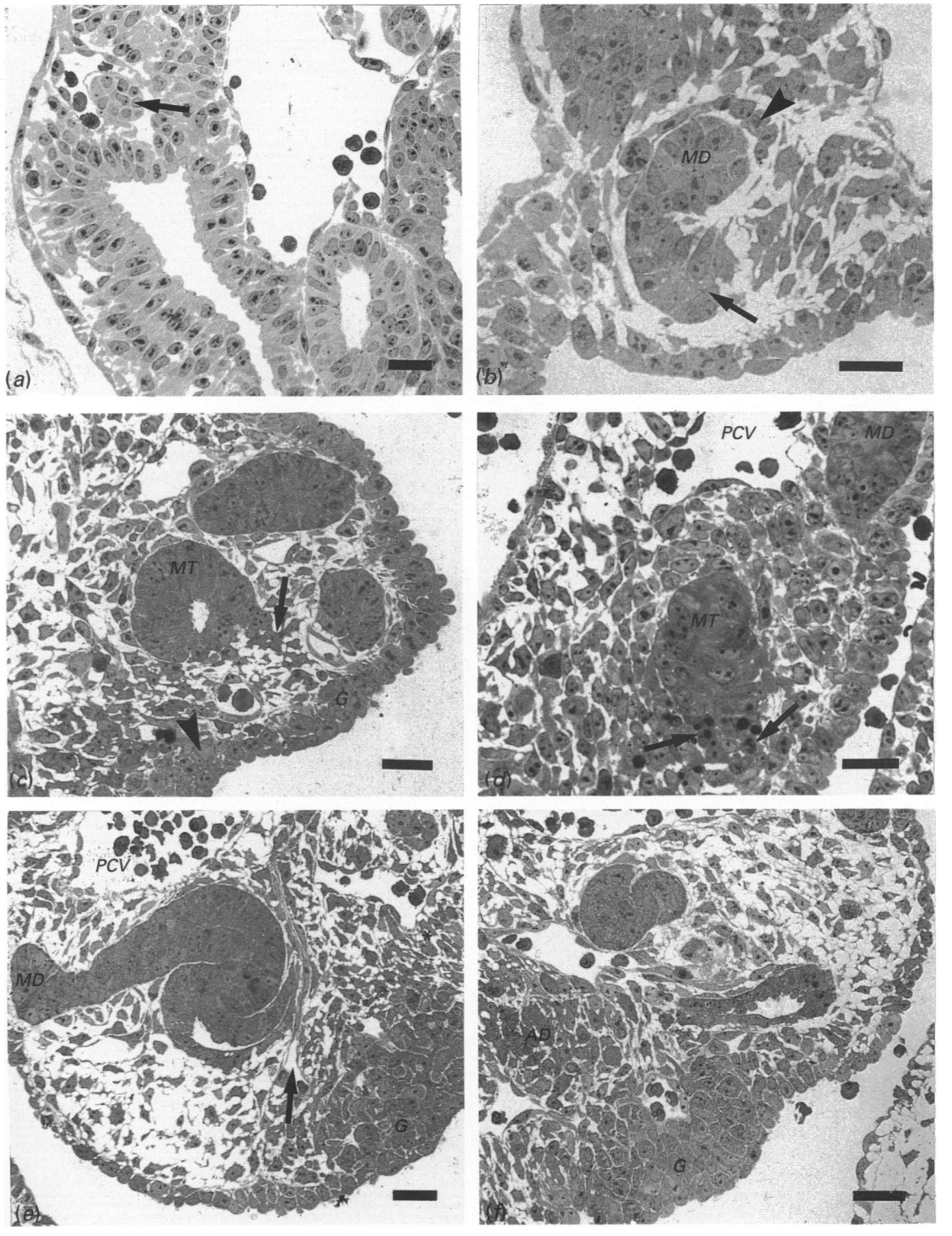
CBA strain mice from an inbred colony, maintained in a light reversal regime, were time-mated between 1200 hours and 1600 hours, at the start of the 9 hours dark period. The day of finding a vaginal plug was designated Day 0 of pregnancy. Pregnant females were killed by cervical dislocation and the embryos were removed and staged according to Theiler (1972). A total of 71 embryos was collected at Stages 14–21: Stage 14, three embryos; Stage 15, five embryos; Stage 16, seven embryos; Stage 17, ten embryos; Stage 18, thirteen embryos; Stage 19, ten embryos; Stage 20, eight embryos; Stage 21, fifteen embryos.

Embryos were decapitated and fixed immediately in 3% glutaraldehyde buffered in 0·1 M sodium cacodylate with 3% glucose. After postfixation in 1% osmium tetroxide in cacodylate buffer and dehydration through a graded series of alcohols, material was embedded in Spurr's epoxy resin. Semithin sections (1 μm thick) were stained with alkaline toluidine blue. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with an EM 100s electron microscope (JEOL, Tokyo).

Tissue required for immunostaining was fixed in 4% formaldehyde in 0·1 M phosphate buffer solution. The primary antibody was a polyclonal antibody which had been raised in rabbit against mouse laminin (Collaborative Research Inc., USA). The specificity of the antibody had been tested by the manufacturer using the Ouchterlony Double Immunodiffusion method. The avidin and biotinylated horseradish peroxidase complex (ABC) method was used to detect the antigen-antibody reaction. All materials used in the ABC method were supplied as part of the Vectastain Elite ABC kit by Vector laboratories, USA.

To determine the working dilution of the anti-laminin antibody a range of dilutions between 1:500 and 1:64000 was tested. The working dilution was found to be 1:2000.

Fig. 1 (*a–f*). (*a*) Cranial region of the mesonephros at Stage 14 showing the mesonephric duct (arrow). There is no distinction between intermediate and paraxial mesoderm. Bar, 25 μm. (*b*) Tubule formation at the cranial end of the mesonephros at Stage 15. Cells become attached (arrow) at the ventrolateral aspect of the mesonephric duct (*MD*) and also become aligned dorsally (arrowhead). Bar, 20 μm. (*c*) Stellate cells are continuous with the ventral edge of a cranial mesonephric tubule (*MT*) at Stage 16 (arrow); cells at the medial aspect of the genital ridge (*G*) are similar in shape. Note germ cell (arrowhead). Bar, 25 μm. (*d*) A caudal mesonephric tubule (*MT*) at Stage 17 shows poorly-defined structure and many apoptotic bodies (arrows). The mesonephric duct (*MD*) lies lateral to the posterior cardinal vein (*PCV*). Bar, 25 μm. (*e*) Mesonephric tubule at Stage 18, showing the characteristic S-shaped structure; it is continuous with the mesonephric duct (*MD*). Note arteriole (arrow) close to the ventral edge of the tubule; area of condensed mesenchyme (asterisk) at the medial aspect of the gonad (*G*) and posterior cardinal vein (*PCV*). Bar, 25 μm. (*f*) Adrenal blastema (*AD*) lying in close apposition to the medial edge of the indifferent gonad (*G*) at Stage 18. Bar, 25 μm.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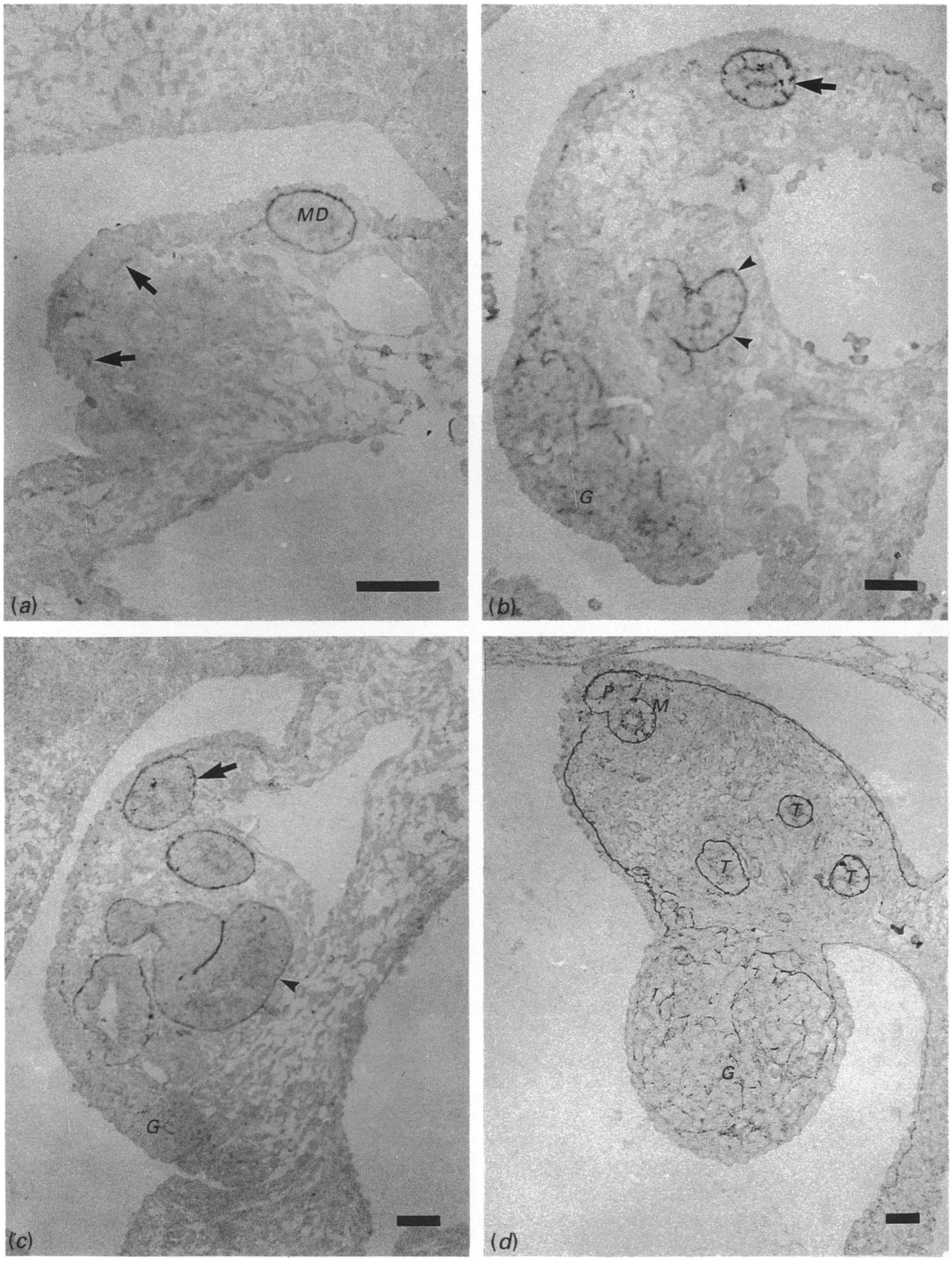
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Each slide had two adjacent serial sections mounted on it: one received a drop of anti-laminin antibody, the second acted as a control and received rabbit serum substituted in place of the primary antibody. All other steps were identical for both sections.

RESULTS

Stage 14, *equivalent to* (=) 9 *days post coitum* (*dpc*)

The mesonephric duct is apparent for the first time (Fig. 1*a*) and is continuous with the pronephric duct. Immunohistochemistry shows no laminin present at this stage and no basal lamina is visible by electron microscopy.

Stage 15 (= 9·5 *dpc*)

Nephrogenic mesoderm is now clearly demarcated from the paraxial mesoderm. The mesonephric duct has extended further through the mesenchyme, although it is still distant from the cloaca. The first signs of mesonephric tubule formation are seen cranially: the mesenchyme is condensed and cells adjacent to the mesonephric duct are columnar in shape, but junctions have yet to be formed (Fig. 1*b*). Primordial germ cells can be distinguished by their intense basophilia with toluidine blue staining, lying between epithelial cells of the hindgut and genital ridge.

Stage 16 (= 10 *dpc*)

Rapid lateral and dorsoventral growth occurs at this stage. Mesenchymal cells are now separated by abundant extracellular matrix but remain in contact via long slender extensions.

The mesonephric duct reaches the cloaca in the older embryos of this stage and it is now surrounded by a basement membrane and a 'cuff' of associated mesenchymal cells. Its cells show well-developed junctional complexes at their apical ends and a few bear cilia projecting into the lumen.

Cranially, tubules are well-formed and are characteristically S-shaped. Each has a convoluted section in direct contact with the mesonephric duct dorsally; cells here show well-formed junctions. The tubule terminates with an irregular ventral edge of cuboidal cells joined only by a few desmosomes; rows of cells extend from this region towards the coelomic epithelium. Further from the ventral aspect of the tubule, the cells are stellate, like the surrounding mesenchymal cells (Fig. 1*c*). Immunohistochemistry reveals that, while the mesonephric duct is surrounded by a densely stained line of laminin (Fig. 2*a*), the tubules have only a sparse laminin covering; the more caudal the tubule, the less intense the laminin staining. Further, where present, laminin is only found on the dorsal aspect of the tubule. Some laminin staining is also found within vesicles in the mesonephric duct cells and in some tubule cells. The

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Fig. 2(*a–d*). (*a*) A complete layer of laminin is present around the mesonephric duct (*MD*) at Stage 16. Note also a thin, discontinuous layer of laminin between the genital ridge and the mesonephric mesenchyme (arrows). Bar, 50 μm. (*b*) At Stage 18, laminin is present around the mesonephric duct (arrow) and also in the apical cytoplasm of duct cells. The mesonephric tubule shows laminin at the dorsal edge (arrowheads) but not at the ventral edge. A thin, patchy distribution of laminin is seen in the gonad (*G*). Bar, 25 μm. (*c*) Laminin is present at the periphery of both the mesonephric duct (arrow) and the mesonephric tubule (arrowhead) at Stage 19. However, the intensity of laminin staining at the periphery of the tubule decreases towards the ventral aspect, where only a thin layer is present. Some staining is also seen in the gonad (*G*). Bar, 25 μm. (*d*) By Stage 21 laminin is seen at the periphery of the mesonephric (*M*) and paramesonephric (*P*) ducts, except where they are in contact. Staining is also seen within duct cells. Mesonephric tubules (*T*) show laminin peripherally and there is a diffuse laminin staining in the gonad (*G*). Bar, 25 μm.

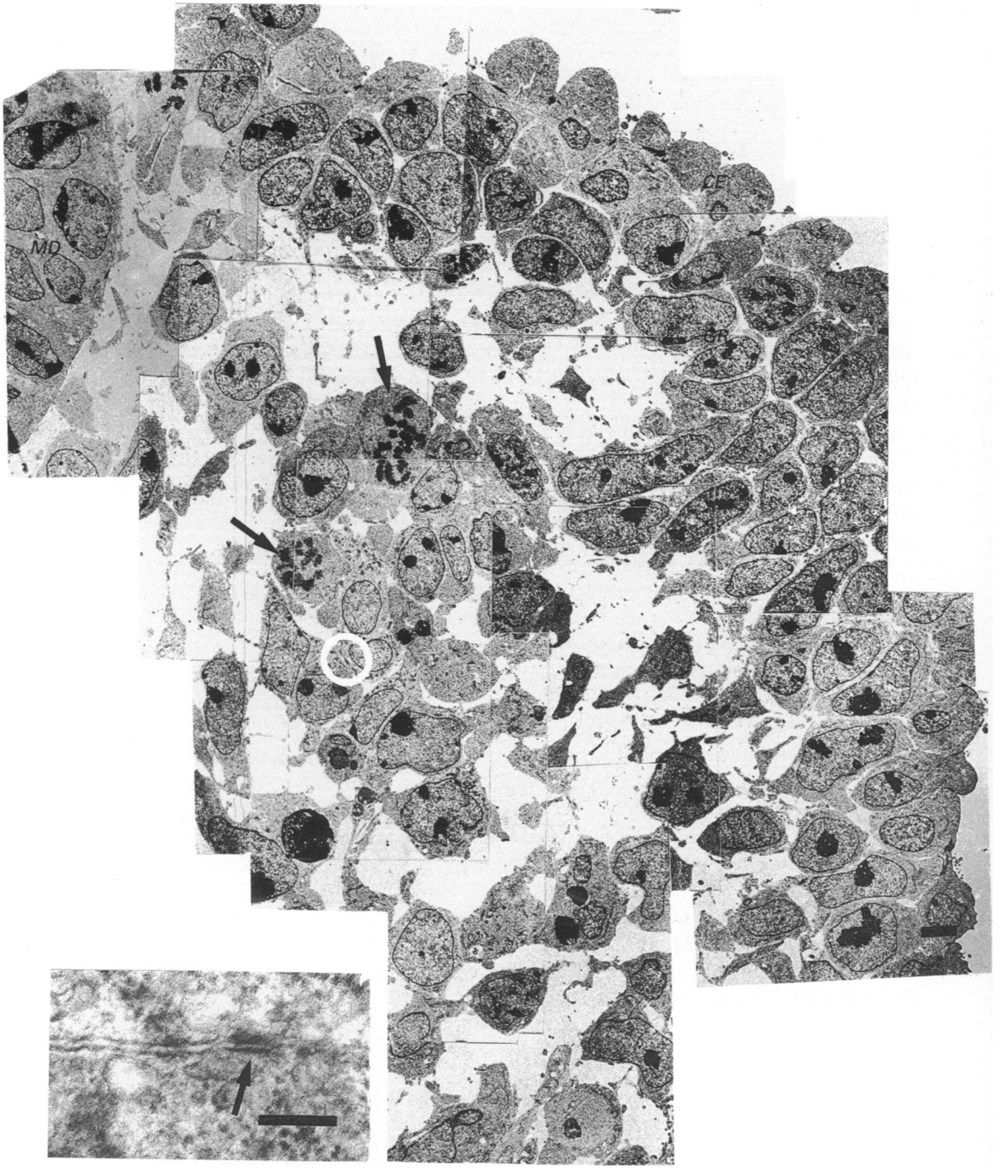


Fig. 3. The first signs of tubule formation are shown here at Stage 16. Mesonephric mesenchymal cells are grouped together near to the mesonephric duct (*MD*). Mitotic cells are apparent (arrows). Cells of the genital ridge (*GR*) are in contact with those of both the coelomic epithelium (*CE*) and the condensing tubule. Bar, 5 μm. Inset: desmosome (arrow) from region ringed in main photograph. Bar, 0·05 μm.

surrounding mesenchymal cells are aligned around the dorsal aspect of the developing tubules.

A small number of apoptotic bodies is also present in the area of mesenchyme outside the developing tubule. Apoptosis is characterised by condensation of cytoplasm, clumping of condensed chromatin against the nuclear membrane and the eventual fragmentation of the nucleus and the cell (Kerr, Searle, Harmon & Bishop, 1987).

The genital ridge is thickened, especially in areas where tubules are more advanced in development. Primordial germ cells present in the ridge are larger (Fig. 1*c*) having begun a period of growth (Spiegelman & Bennett, 1973). Cells extending from the ventral edge of cranial tubules are joined by desmosomes to those of the gonadal blastema (Fig. 3). Immunohistochemistry shows a thin, patchy layer of laminin between the gonadal blastema and the general mesenchyme. No basement membrane can be seen between the coelomic epithelium and the gonadal blastema by electron microscopy (Fig. 3) or immunohistochemistry (Fig. 2*a*).

Stage 17 (= 10·5 *dpc*)

The mesonephric area appears similar to the previous stage. Caudally some condensation is still under way but a large number of the cells contain apoptotic bodies (Fig. 1*d*). A basal lamina is now visible dorsally and at the sides of the tubules; the more cranial the tubule, the better developed the basal lamina.

The genital ridge has proliferated and is about 7–8 cells deep in the thickest central area. Cells of the coelomic epithelium are indistinguishable from those of the gonadal blastema and there appears to be no barrier between the two.

The adrenal blastema is now visible as an area of condensed spherical cells surrounded by diffuse mesenchyme.

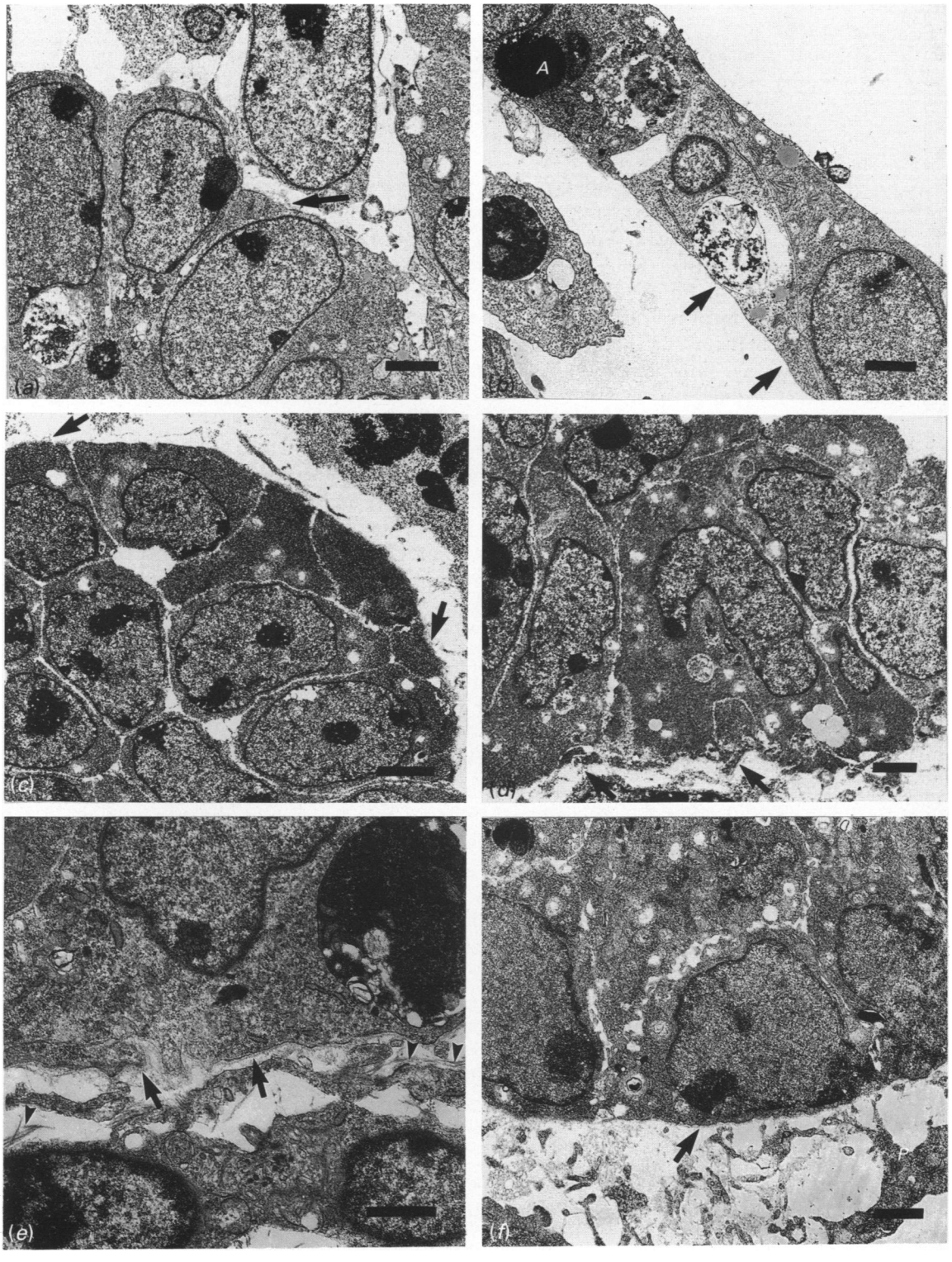
Stage 18 (= 11 *dpc*)

Mesenchymal extracellular matrix is increased and the mesonephric duct now lies directly against the coelomic epithelium. The duct cells extend cytoplasmic processes basally and are supported by a thick basement membrane, surrounded by an acellular space crossed by mesenchymal cell processes which abut directly onto the basement membrane. Immunohistochemistry also reveals laminin-stained vesicles within the mesonephric duct cells.

Tubules are found in an area virtually co-extensive with the indifferent gonad, though fewer are present caudally. Cranially, they are larger and, in older embryos of this stage, more convoluted. Epithelial cells at the ventral border of tubules have numerous cytoplasmic processes in contact with those of mesenchymal cells. A large number of these ventral cells contain lysosomes and apoptotic bodies (Fig. 4*b*). Immunohistochemistry (Fig. 2*b*) and electron microscopy (Fig. 4*a*) confirm the presence of a developing basal lamina surrounding the tubule, except on the ventral aspect (Figs. 2*b*, 4*b*); ventral epithelial cells are directly continuous with cells on the outer borders of the indifferent gonad. The mesonephros is now very well-vascularised. Occasionally blood vessels are seen in close apposition with the ventral edge of a tubule (Fig. 1*e*).

Laminin is present between the coelomic epithelium and the general mesenchyme, but there is none between the coelomic epithelium and the indifferent gonad, in which laminin is widely distributed at this stage (Fig. 2*b*).

The adrenal blastema (Fig. 1*f*) has now proliferated greatly and occupies an area between the dorsal aorta and the cranial end of the gonad; it can be recognised by its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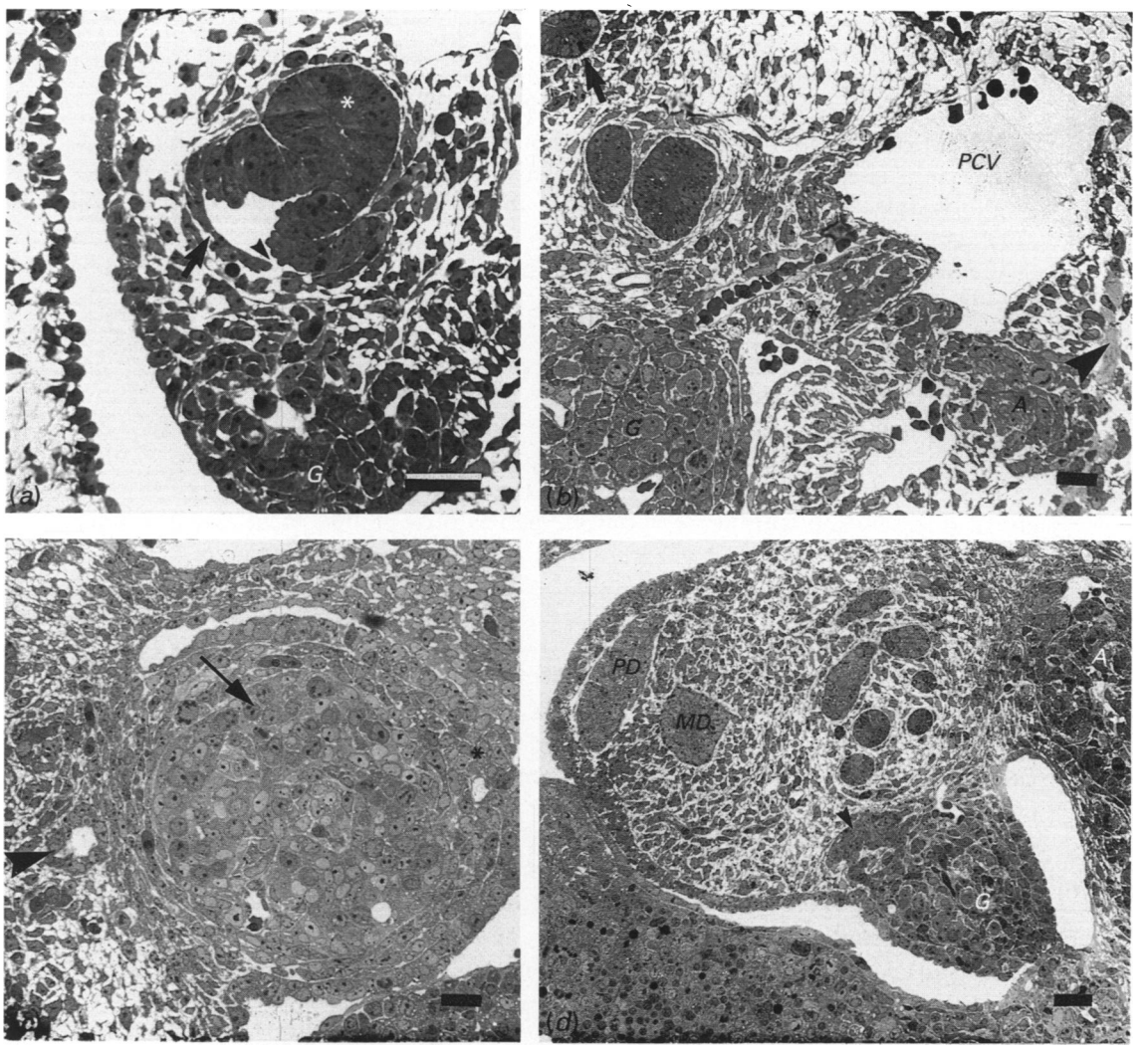

Fig. 5(*a–d*). (*a*) Tubule at Stage 19 showing flattened ventral edge cells (arrow) and columnar dorsal cells (asterisk). The ventral edge is incomplete (arrowhead). Note gonad (*G*) ventral to tubule. Bar 25 μm. (*b*) The adrenal blastema (*A*) is seen at Stage 20 ventral to the posterior cardinal vein (*PCV*). Note nerve fibres (arrowhead), condensed mesenchyme (asterisk) between gonad (*G*) and adrenal blastema, and acellular area around mesonephric duct (arrow) Bar, 20 μm. (*c*) At Stage 21 early testicular cords (arrow) are seen in male gonads. Note a mesonephric tubule with a cuboidal ventral edge (arrowhead) terminating in the region of the developing tunica albuginea and continuity between coelomic epithelium and the medial pole of the gonad (asterisk). Bar, 20 μm. (*d*) The female embryo at Stage 21 shows a well-developed paramesonephric duct (*PD*) and degenerating mesonephric duct (*MD*) with mesenchymal cells condensed around it. A tubule is shown terminating (arrowhead) in the dorsolateral aspect of the gonad (*G*). Note also the adrenal blastema (*A*). Bar, 25 μm.

Fig. 4(*a–f*). (*a*) Electron micrograph shows dorsal aspect of mesonephric tubule at Stage 18. Note patchy distribution of basal lamina material (arrow). Bar, 2 μm. (*b*) Ventral aspect of tubule seen in (*a*). Little evidence of a basal lamina is seen basally (arrows). Note apoptotic body (*A*). Bar, 2 μm. (*c*) Electron micrograph of cranial mesonephric tubule at Stage 19. Note patchy basal lamina (arrows). Bar, 2 μm. (*d*) Ventral aspect of tubule seen in (*c*); note patchy basal lamina (arrows). Bar, 2 μm. (*e*) Electron micrograph of ventral aspect of a mesonephric tubule at Stage 20. Note basal lamina (arrows) and reticular layer (arrowheads) forming basement membrane. Bar, 1 μm. (*f*) Ventral aspect of a mesonephric tubule at Stage 21. Mesenchymal cell processes (*P*) are seen adjacent to basal lamina (arrow). Bar, 2 μm.

intense basophilia. Adrenal cells lie in close proximity to those of the gonad, in some areas appearing to be in continuity, in others separated by a band of elongated mesenchymal cells.

Stage 19 (= 11·5 *dpc*)

Tubules extend from a region slightly cranial to the genital ridge for about three quarters of its length. Cranially, they are highly convoluted; the most cranial tubules have no visible lumen and terminate close to the gonad with a flattened ventral epithelium, which may show discontinuities (Fig. 5*a*). Only the most cranial one or two tubules have laminin covering their entire surface and a developing basal lamina apparent by electron microscopy (Fig. 4*c*), although the immunoreactivity at the ventral aspect is weak suggesting a thin, patchy laminin distribution (Fig. 2*c*). Again, a patchy basal lamina is seen here by electron microscopy (Fig. 4*d*).

The more caudal tubules are much less convoluted and also terminate with a flattened ventral edge epithelium whose cells extend into the general mesenchyme and into the dorsal aspect of the indifferent gonad. Laminin is present laterally.

All of the tubules beyond approximately the midpoint of the genital ridge show signs of degeneration with many apoptotic bodies present. The most caudal tubules show an advanced stage of degeneration; no laminin is present around them. The adrenal blastema lies medial to the cranial part of the mesonephros and the gonad, only two to three cells from its dorsal edge.

Stage 20 (= 12 *dpc*)

The mesonephric duct has degenerated to a level slightly caudal to the cranial tip of the indifferent gonad. The paramesonephric duct is now present, lateral to the mesonephric duct; it develops from the coelomic epithelium in a region slightly caudal to the cranial limit of the mesonephric duct.

Mesonephric tubules are highly convoluted and found in a region stretching from the cranial tip of the gonad to just beyond its midpoint; those outside this area have degenerated completely. The majority of the tubules terminate very close to the lateral edge of the gonad, with loosely organised cells similar in appearance to gonadal somatic cells. Cells of the ventral border of the most caudal tubules are now cuboidal and supported by a basement membrane (Fig. 4*e*).

In the older embryos of this stage gonadal sex can be determined by the presence of testicular cords in the male. The coelomic epithelium is separated from the gonad laterally and medially, but is apparently continuous with the central part of the gonadal edge. The adrenal gland is well-innervated and contains both light and dark-staining cells (Fig. 5*b*). Ultrastructurally, the cells have conspicuous nucleoli, abundant ribosomes, numerous mitochondria, some RER and occasionally a Golgi apparatus.

Stage 21 (= 13 *dpc*)

Sex differences are now apparent, but some features are common to both. The mesonephric duct extends from an area slightly caudal to the mid-point of the gonad to the cloaca; the paramesonephric duct lies lateral to it. A continuous layer of laminin surrounds both ducts but is absent at their opposing surfaces (Fig. 2*d*). Laminin vesicles are again present within the mesonephric duct cells, most being situated apically.

The male mesonephric duct is slightly longer than the paramesonephric duct. In older male embryos of this stage the paramesonephric duct shows early signs of degeneration; fewer junctions are found between its cells and some apoptotic bodies

are found within the cells. In females, the paramesonephric duct is larger and has a lumen.

Mesonephric tubules are very convoluted, having increased in length, and are found at the level of the cranial end of the gonad. No lumen is visible and the tubules terminate blindly within the outer edge of the gonad. A basement membrane supports the tubule cells (Fig. 4*f*). Laminin is present as a continuous layer around the tubules before they enter the gonad and is widely distributed within the gonad. In males the outer edge of the gonad has a thick layer of flattened mesenchymal cells, the presumptive tunica albuginea. In females this layer is much thinner (1–2 cells thick). The developing testis is rounded and now shows testicular cords (Fig. 5*c*). A clear boundary separates it from the coelomic epithelium, except in one centralised area. There is an incomplete layer of laminin between these two regions, although none is present in the central area (Fig. 2*d*). Laminin is widely distributed around the testicular cords. The ovary is much less rounded and mesonephric tubules are clearly continuous with cells inside the gonad (Fig. 5*d*). Only a thin layer of mesenchyme lies between the mesonephros and the ovary. The adrenal gland is now quite rounded and lies in an area close to the medial border of the gonad, separated from it by an area of condensed mesenchymal cells.

DISCUSSION

Tubule formation

Mesonephric tubules develop within the intermediate mesenchyme: cells condense, form junctions and then align together, forming tubular structures which become delimited by basal laminae. Tubule formation is first seen at Stage 15, in accordance with the findings of Fraedrich (1979), and is not completed until the end of Stage 21.

Between Stages 17 and 20 the tubules appear to lose cells from their ventral border to the general mesenchyme and to the genital ridge; further work is necessary to confirm the direction of movement. The stellate cells present ventrally are ultrastructurally indistinguishable from the original mesenchymal cells; we suggest, therefore, that they are no more differentiated than the cells of the general mesenchyme, being furthest from the inducing influence of the mesonephric duct. By Stage 21 cells of the ventral border of the tubules resemble the other tubule cells and show a basal lamina and junctional complexes. The tubules are by now highly convoluted and extend into the gonad, thereby forming the excurrent pathway of the testis or the ovarian rete.

The fate of the mesonephros

Functional state of the mouse mesonephros

Glomerulus-like structures were found very rarely at Stages 18 and 19, and consisted of little more than capillaries apposed to the ventral aspects of the tubules. The S-shaped bodies, from which glomeruli should develop, either degenerated at Stages 19 and 20 or remained as tubules. The mouse mesonephros may therefore be considered non-functional, in agreement with Zamboni & Upadhyay (1981).

Role in gonadal development

Present results show that no structural barriers exist between the mesonephric and gonadal cell populations and that the coelomic epithelium is in direct contact with the gonadal blastema, between Stages 16 and 18 approximately. Beyond Stage 18 the area

of communication is reduced as a basal lamina appears along the lateral and medial borders of the gonad. However, an area of communication always remains at the ventral pole of the gonad, where it is impossible to distinguish between cells of the coelomic epithelium and somatic cells of the gonad. Upadhyay *et al.* (1979) state that "the mesonephros is the source of the somatic cells which establish early and permanent association with the germinal cells". Present results show that, while the major contribution to the gonadal blastema may indeed be derived from the dorsal aspect of the gonad, the potential contribution of the coelomic epithelium cannot be ignored. In human embryos, the coelomic epithelium is a generous contributor of somatic cells to the ovary (Makabe & Motta, 1986).

Cells entering the dorsal aspect of the gonad are considered to be solely derived from mesonephric tubules by Upadhyay *et al.* (1979, 1981) and by Wartenberg (1978, 1981, 1982, 1983, 1985). While present results do show that mesonephric cells are directly continuous with the gonadal blastema, the number of sections in which a direct continuity was present was, in fact, very small. Tubules generally terminated a short distance from the dorsal edge of the gonadal blastema and the cells of their ventral border were often continuous with mesenchymal cells. These undifferentiated cells may respond to a trophic factor produced within the genital ridge and move towards it, together with other mesenchymal cells in the same area. While the origin of somatic cells that are contributed to the dorsal aspect of the gonad (from mesonephric tubules, from mesenchyme or from both) is debatable, it is functionally of little interest as both cell populations are ultimately derived from the same source: the intermediate mesoderm.

Colonisation of the genital ridge by primordial germ cells begins between Stages 15 and 16 and, by Stage 16, germ cells are present within the ridge. These results support those of Theiler (1972) and Spiegelman & Bennett (1973) but not those of Upadhyay *et al.* (1979), who did not find germ cell colonisation until Day 11 (approximately equal to Stage 18).

Role in adrenal cortex development

No cords of cells were seen extending between the ventral edge of the mesonephric tubules and the adrenal blastema. However, areas of condensed mesenchyme were often present between the medial border of the gonad and the adrenal blastema at Stage 18, suggesting that mesenchymal cells, contributed from the gonadal region, may be responsible for the adrenal proliferation seen at this stage. While it is difficult to exclude a contribution to the adrenal cortex from mesonephric tubules and the coelomic epithelium, the majority of its cells appear to arise from the general mesenchyme.

Tubule degeneration

Mesonephric tubules caudal to approximately the midpoint of the gonad degenerate. Involution of tubules which have been developed to the S-shaped stage is seen from Stage 19 onwards, but at Stage 17 the most caudal tubules are condensed and also show signs of degeneration. This finding contrasts with the statement of Upadhyay *et al.* (1981) that the mesonephros of the mouse does not undergo involution. Degeneration could be recognised by the large number of intensely basophilic lysosome-like vesicles present around the tubule. Upadhyay *et al.* (1979) and Wartenberg (1985) both note these vesicles around the ventral edge of normal tubules and suggest that they are characteristic of the mesenchymal cells being mobilised from the walls of the tubules; Upadhyay and his colleagues suggest that they may be required for the enzymatic digestion of the basal lamina. This suggestion is not

supported here as we found that no basal lamina is present at this, or earlier stages, around the ventral edge of the tubule during the time of suggested cell migration. Further, although these basophilic vesicles are present around the edges of some normally developing tubules, the numbers are very small compared to those seen around the degenerating tubules. Electron microscopy showed basophilic vesicles to be either cells showing characteristic signs of apoptosis, or secondary lysosomes. The latter contained the remains of apoptotic bodies which had been phagocytosed by neighbouring tubular cells or by neighbouring mesenchymal cells.

The statement by Wartenberg (1985) that the mesonephric cells transdifferentiate is not supported by the results of this study in the mouse; there is no evidence to suggest that the cells of the ventral border of the tubules (the area from which the transdifferentiating cells are said to segregate) are any more differentiated than the surrounding mesenchymal cells. A basal lamina does not appear around the ventral edge of the tubule until after the period of gonadal colonisation.

SUMMARY

A study has been made of the development of mesonephric tubules in the mouse to investigate the possible transdifferentiation of tubule epithelial cell to gonadal somatic cells and/or adrenal cortical cells. Immunohistochemical localisation of laminin was carried out to study the development of basal laminae. Cells at the ventral aspect of mesonephric tubules did not show an epithelial phenotype during the period of somatic cell population of the gonadal blastema; a basal lamina appeared ventrally only after this period. Therefore, it is not necessary to postulate transdifferentiation of these cells.

We are grateful to Professor R. J. Scothorne for valuable discussions and to our colleagues, Drs S. W. McDonald and A. J. Todd for assistance with immunohistochemistry. We also thank Owen Reid for technical assistance and Margaret Hughes for photography.

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