Development 117, 273-281 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

Mesonephric contribution to testis differentiation in the fetal mouse

Mia Buehr, Subin Gu* and Anne McLaren†

MRC Mammalian Development Unit, 4, Stephenson Way, London NWl 2HE, UK

*Present address: Dept. of Zoology, University of Georgia, Athens, GA 30602, USA
†Present address: Wellcome/CRC Institute, Tennis Court Road, Cambridge CB2 1QR, UK

SUMMARY

Testes from 11.5-day-old mouse embryos, with and without attached mesonephroi, were cultured for 7 days. Isolated testes failed to develop well-differentiated testis cords: however, when cultured attached to a mesonephros from either a male or a female donor embryo, testes developed cords that were normal in appearance. Testes cultured next to a mesonephric region but separated from it by a permeable filter, did not develop normal cords, nor did testes grafted to fragments of embryonic limb or heart. When testes were grafted to mesonephric regions from mice carrying a transgenic marker, the marker was found in some of the peritubular myoid cells and other interstitial cells of the testis, but not in the Sertoli cells or the germ cells. We conclude that after 11.5 days post coitum, cells can migrate from the mesonephric region into the differentiating testis and can contribute to the interstitial cell population, and that this contribution is necessary for the establishment of normal cord structure. The germ cells in all cultured testes, whether or not differentiated cords were present, were T1 prospermatogonia: no meiotic germ cells were seen.

Key words: mesonephros, testis differentiation, testis cords, interstitial cells, peritubular myoid cells

INTRODUCTION

In the mouse embryo, the gonads of both male and female develop along the inner surface of the mesonephros, a rudimentary nephric organ that lies parallel to the differentiating gonad on either side of the attachment of the dorsal mesentery. Later, as the male gonad differentiates into a testis, the mesonephric duct develops into the Wolffian duct, and is also thought to contribute to the rete testis and, in the female, to the rete ovarii. Many workers have suggested that, during the process of gonad differentiation, mesonephric cells make a substantial contribution to the structure of the ovary or testis itself, and several descriptive studies have been done that support these suggestions (Upadhyay et al., 1979, 1981; Wartenberg, 1981; Zamboni and Upadhyay, 1982). In a recent detailed light and electron microscope study of early testis differentiation in the rabbit, Wartenberg et al. (1991) illustrate the process of cell migration from both the mesonephros and the coelomic epithelium to the developing testis.

The above-mentioned authors have postulated a mesonephric origin for both Sertoli cells and interstitial cells, including peritubular myoid cells and Leydig cells. Although gonadal development and sexual differentiation can take place in the absence of the mesonephros (Merchant-Larios et al., 1984; Rodemer et al., 1986), Rodemer et al. (1986), using quail-chick chimaeras, showed experimentally that the regressing mesonephros can indeed contribute cells to the gonadal stroma. However, a direct experimental analysis of mesonephric contribution to testis structures in mammals has so far been lacking. By culturing fetal mouse testes in vitro, with and without an attached mesonephros, we have been able to examine directly some of the contribution made by the cells of the mesonephric region to the differentiating male gonad.

MATERIALS AND METHODS

Mice

Most of the embryos used in this study were of the random-bred Q strain. In some experiments, embryos from a transgenic CBA strain derived by Cecilia Lo were used (Lo, 1986). Cells of this strain contain 1000 copies of an exogenous -globin gene inserted at the telomere of chromosome 3, and not expressed.

Embryos

Embryos were collected at mid-day of the 12th day of pregnancy (the first day of pregnancy was the day on which the vaginal plug was found) and so were considered to be approximately 11.5 days old. The hindlimb bud was used as a further criterion of developmental stage. 11.5-day embryos were defined as those in which the hindlimb bud was beginning to assume a paddle shape, with slight anterior and posterior indentations proximal to the foot-plate (stages 4 and 5 of McLaren and Buehr, 1990). Embryos that did not conform to this criterion were not used. In mouse embryos of this age, the gonad lies in close apposition to the mesonephric region, and shows as yet no signs of sexual differentiation (Fig. 1A). The presence or absence of sex chromatin in amniotic cells

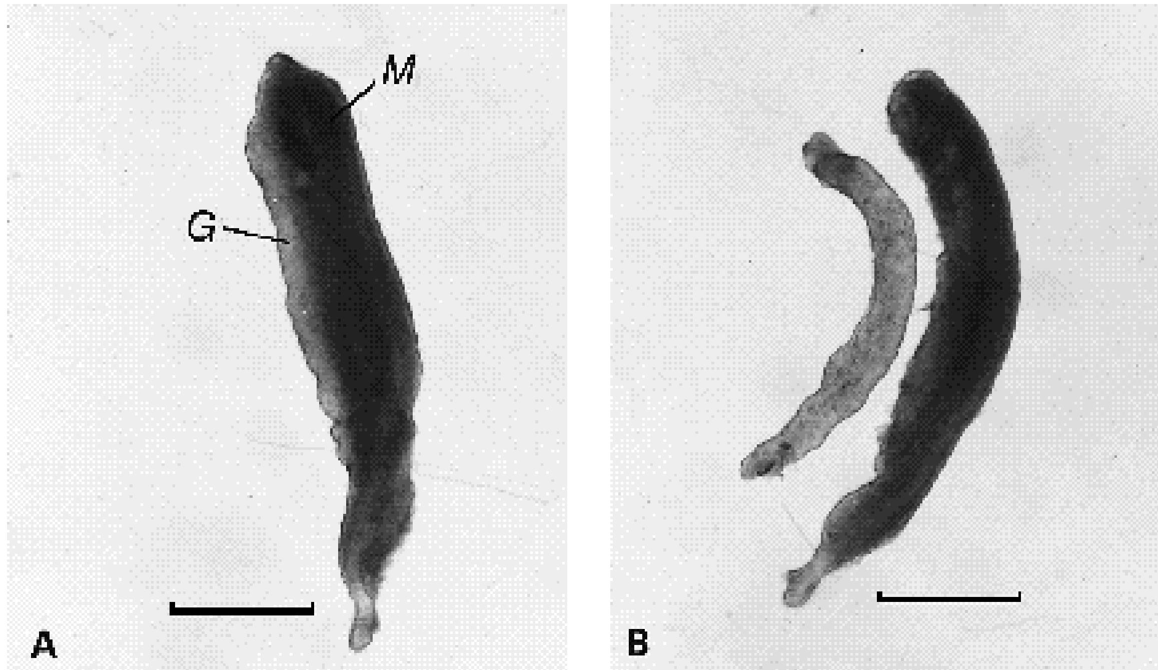

Fig. 1. (A) A urogenital ridge from an 11.5-day mouse embryo. Gonad (G), mesonephros (M). Bar, 0.5 mm. (B) A urogenital ridge from an 11.5-day embryo after dissection into gonadal and mesonephric components. Bar, 0.5 mm.

was therefore determined in order to distinguish female from male embryos. The urogenital ridges (gonads with attached mesonephric regions) were collected in Whittingham's complete PBl medium (Whittingham, 1971) and held in this medium at room temperature for about 1.5 hours while the amniotic cells were processed and examined according to the method of Burgoyne et al. (1983).

Cultures

All tissues were cultured on agar blocks (2% agar in phosphate-buffered saline: Dulbecco 'A' from Oxoid) in Falcon Petri dishes. The culture medium was Dulbecco's modification of Eagle's medium (DMEM) from Flow, with 10% fetal calf serum (Imperial Laboratories), 2 mM glutamine, 0.5 mM pyruvate, 0.1 mM -mercaptoethanol, 100 units/ml penicillin, 0.05 mg/ml streptomycin, and 1.5 μg/ml Fungizone (Gibco). The level of the medium in the dishes was adjusted so that the cultures were covered by only a thin film of fluid, and the medium was changed on alternate days. Tissues were cultured at 37°C in an atmosphere of 5% CO_2 in air for 7 days, and then fixed for microscopic examination.

Testes to be cultured without the mesonephric region were cut away from the mesonephros with a fine needle (Fig. 1B). Because of the close juxtaposition of testis and mesonephros at this stage, it was not possible to ensure that all mesonephric tissue was removed from the testis rudiment. However, any testis that carried obvious shreds of mesonephros was discarded. When mesonephric regions were isolated for grafting experiments, the cut was made sufficiently proximal to ensure that no gonadal tissue remained attached. When two tissues were to be grafted together, they were laid side by side and pressed gently into a narrow groove cut in the agar block on which the tissues rested. Any grafted pair that had not fused after 24 hours in culture was discarded, or was kept as a control. In cultures involving the maintenance of tissues on either side of a permeable filter, a piece of Millipore filter (pore size=0.42 μm) was placed vertically in the agar block, and tissues cultured on either side. To ensure their close proximity, tissues were placed in small grooves cut against each face of the filter and held in place by the surface tension of the medium over them.

Histology

Most specimens were fixed in Bouin's fluid, embedded in paraffin wax, serially sectioned at 7 μm, and stained with haematoxylin and eosin. For in situ localisation of -globin DNA sequences, tissues were fixed in cold 3:1 ethanol-acetic acid, embedded in paraffin wax, sectioned at 5 or 7 μm, and processed according to the method of Rossant et al. (1986). To improve histological preservation, some specimens were denatured in 0.15 N NaOH in 70% ethanol for 5 minutes, instead of 70% deionised formamide in 2× SSC.

RESULTS

Intact mesonephros-testis complexes (16 cultures)

After 2 to 4 days in culture, the first signs of differentiation in the cultured testes can be seen as meandering outlines of cord-like structures. The outlines of the cords become progressively more conspicuous during the culture period. After 7 days of culture, most cultures clearly show both testicular and mesonephric development. Testis cords appear in the testicular portion of the culture, while the mesonephros is usually represented by one large inflated duct and a number of smaller ones.

Histological examination (Fig. 2A,B) reveals that differentiation in these testes is good, and largely indistinguishable from normal morphogenesis in vivo. Palisades of differentiated Sertoli cells define the periphery of the testis cords, which are surrounded by a layer of flattened peritubular cells. Germ cells, all in the T1 prospermatogonial stage (Hilscher, 1981), are abundant in the centre of the cords.

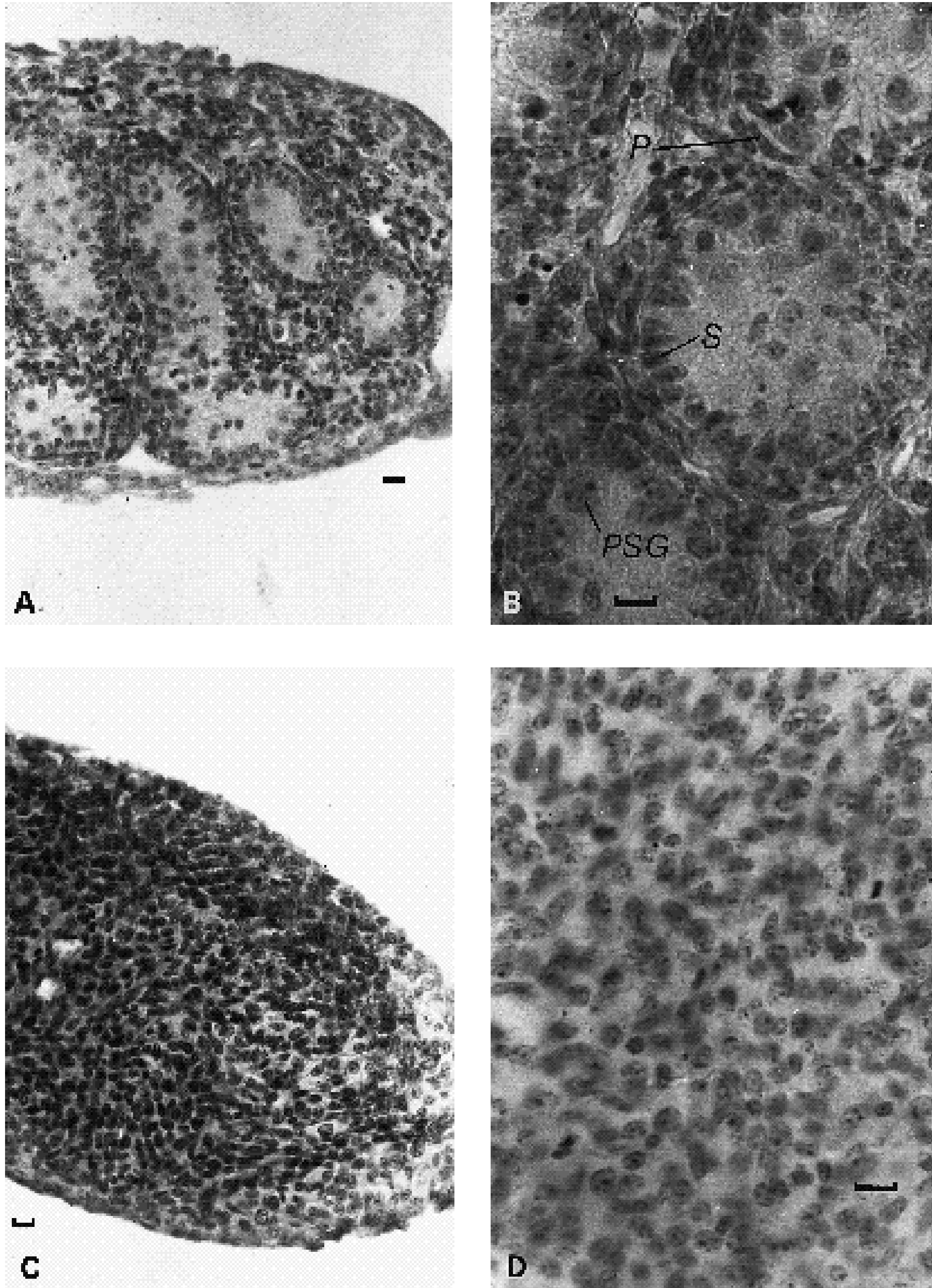

Fig. 2. (A) Low-power view (×20) of an 11.5-day testis cultured for 7 days with its mesonephros attached. The structure of the testis cords appears normal. Bar, 10 μm. (B) High-power view (×40) of an 11.5-day testis cultured with attached mesonephros. The Sertoli cells, peritubular cells and germ cells (T1 prospermatogonia) appear morphologically normal. Sertoli cells (S). peritubular cells (P), prospermatogonia (PSG). Bar, 10 μm. (C) Low power view (×20) of an 11.5-day testis cultured for 7 days without an attached mesonephros. No testis cords have formed. Bar, 10 μm. (D) High power view (×40) of an 11.5-day testis cultured for 7 days without an attached mesonephros. The cells appear morphologically undifferentiated. Bar, 10 μm.

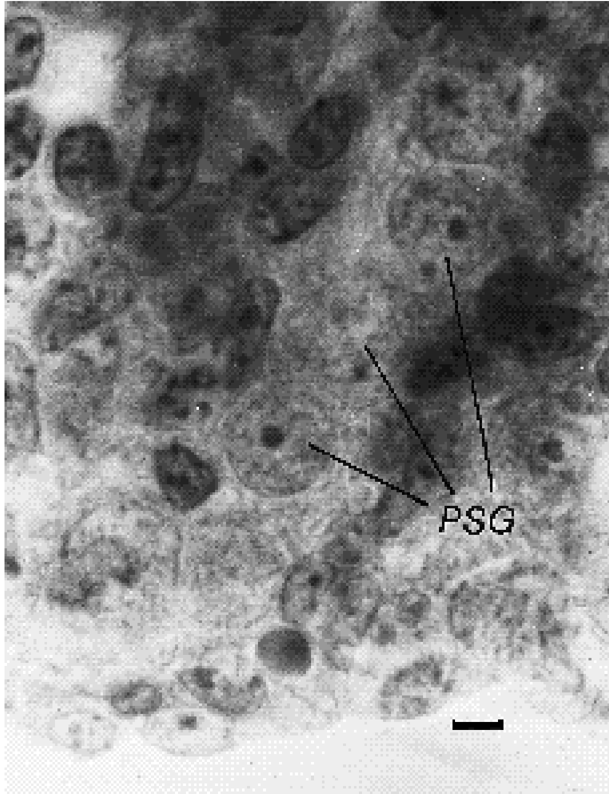

Fig. 3. A group of T1 prospermatogonia in an 11.5-day testis cultured for 7 days without an attached mesonephros. The germ cells are surrounded by morphologically undifferentiated somatic cells. Germ cells (PSG). Bar, 10 μm.

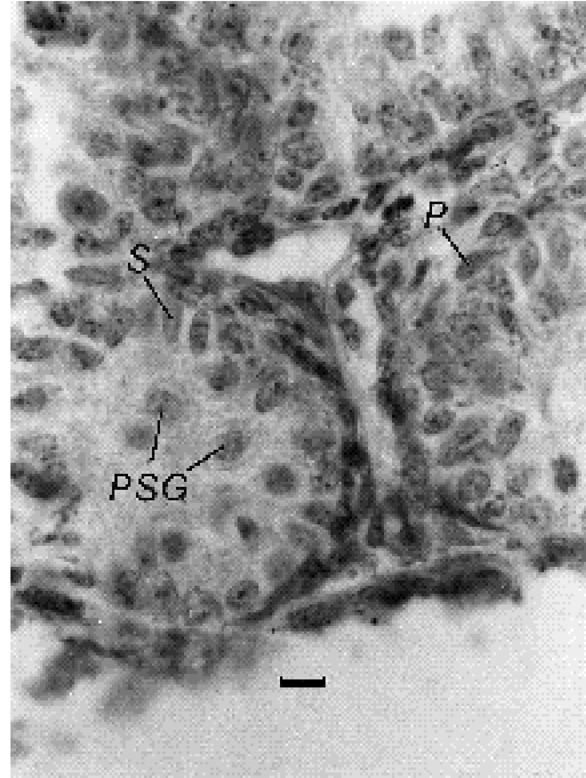

Fig. 4. A testis grafted to a female mesonephros at 11.5 days. and cultured for 7 days. The testis structure appears normal. Sertoli cells (S), peritubular cells (P), prospermatogonia (PSG). Bar, 10 μm.

Isolated testes (9 cultures)

Testes cultured without mesonephros remain essentially homogeneous in appearance, though after several days in culture some indication of internal structure can occasionally be seen. However, clearly defined testis cords rarely appear.

When these testes are examined histologically, organisation of the cords is seen to be poor or lacking (Fig. 2C,D). Portions of differentiated or partially differentiated cords are occasionally seen, but these are not extensive, and most isolated testes appear undifferentiated. Few cells can be identified clearly by their morphology as Sertoli cells or peritubular elements. Some germ cells, however, are present, and are identifiable as T1 prospermatogonia. Although they are most clearly seen in areas where some cord differentiation has taken place, clumps of T1 prospermatogonia often occur in the middle of undifferentiated tissue (Fig. 3). Though no counts of germ cells were made, there appeared to be fewer prospermatogonia in these undifferentiated testes than there were in the testes with well-developed cords.

Double testes (10 cultures)

Testes cultured together with the attached mesonephric region are often overgrown to some extent by mesonephric tissue and accurate measurements of testis volume are difficult to make. However, such testes do appear somewhat larger than testes cultured without mesonephros, and it is possible that normal cord differentiation can only take place within a certain minimum volume of tissue. To investigate this possibility, we grafted together two isolated testis rudiments and cultured this artificially enlarged gonad for 7 days. However, cord differentiation was no better than that seen in single isolated testes.

Testis-mesonephros grafts (11 cultures)

When an isolated 11.5-day testis is grafted to a mesonephros from another embryo of the same age, testis cords differentiate as they would in an intact testis-mesonephros complex. Of the 11 grafts performed, 5 were with a mesonephros from a male donor and 6 with a female mesonephros. However, the sex of the donor mesonephros proved to be unimportant: a mesonephros from a female embryo was as capable of supporting normal cord differentiation as one from a male. Fig. 4 shows normal testis cords in a male gonad that was grafted to a female mesonephros.

Trans-membrane cultures (7 cultures)

In an experiment designed to show whether or not a diffusible mesonephric substance is responsible for normal testis morphogenesis, isolated testes were cultured next to isolated mesonephric regions (4 cultures) or intact urogenital ridges (3 cultures), but separated from them by a permeable filter. In all cases, differentiation of the isolated

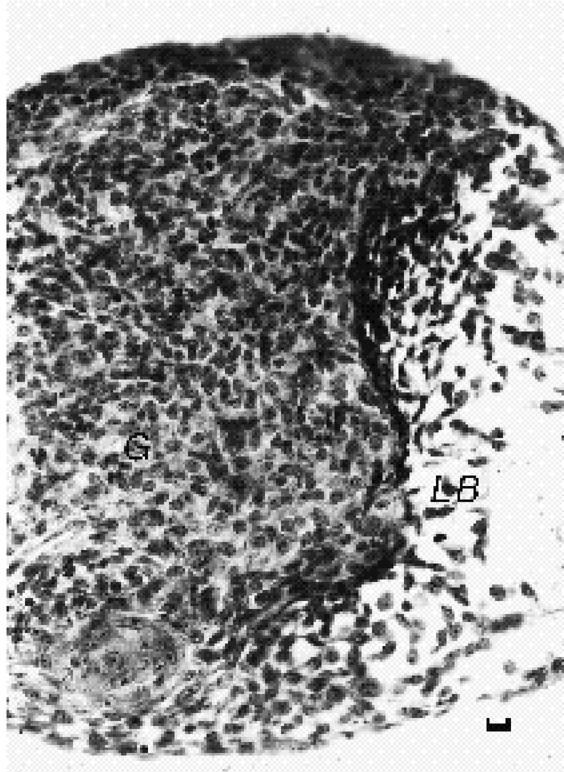

Fig. 5. A testis grafted to a fragment of 11.5-day limb bud. No normal testis structures have developed in the gonad. Gonad (G), limb bud (LB). Bar, 10 μm.

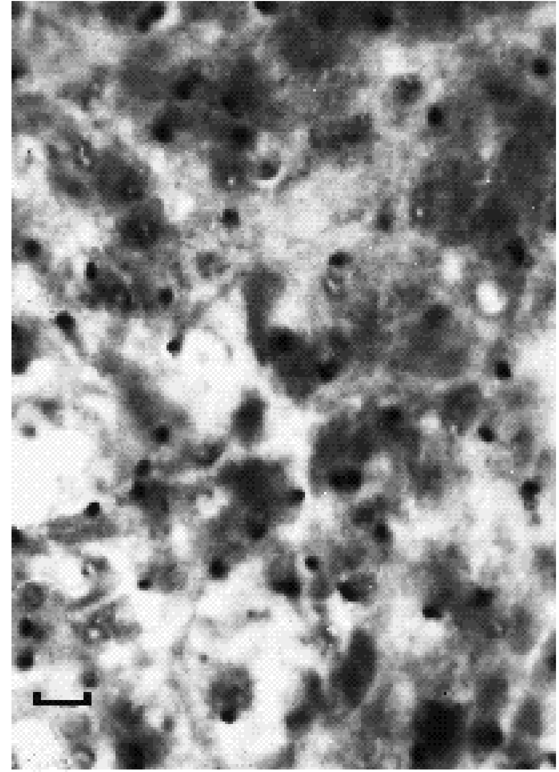

Fig. 6. A section of mesonephric tissue from a mouse carrying the nuclear marker described, stained according to the in situ localisation procedure of Rossant et al. (1986). Bar, 10 μm.

testis was poor, and cords developed no better than they had in isolated testes cultured in the absence of mesonephric influence.

Testes grafted to non-mesonephric tissue (9 cultures)

In order to determine if a mesonephros is specifically required to support normal cord differentiation, isolated testes were grafted to fragments of 11.5-day limb bud (6 cultures) or heart (3 cultures). Again, cord differentiation in the grafted testes was poor (Fig. 5).

Testes grafted to labelled mesonephroi

In one series of experiments, mesonephric regions of embryos carrying a nuclear marker (1000 copies of an exogenous -globin gene) were grafted onto testes from embryos of the normal Q strain. In situ hybridization of tissue sections later revealed which cells in the differentiating testis had originated in the donor mesonephric region. Fig. 6 shows the appearance of the nuclear marker in a section of mesonephric tissue from one of these donor embryos, in which all cells would be expected to show the nuclear label. The in situ staining procedure after ethanol-acetic acid fixation did not produce slides with good histological detail, but the major structural features of the testis could still be seen, and it was possible to distinguish the major cell types by their general appearance and position. Peritubular cells in particular stained more darkly than Sertoli or germ cells, and could be identified by their flattened shape. Where a tubule appeared in cross-section, it was clear that the nuclear marker was restricted to cells in the outer, flattened peritubular layer. In order to aid histological identification, some material was fixed in paraformaldehyde and stained with haematoxylin and eosin. Histological detail was good, but the nuclear marker did not stand out so clearly from other chromatin masses, especially in photographs (Fig. 7C,D). Nine grafted testes were examined (4 grafted to male and 5 to female mesonephroi) and, although individual Sertoli or germ cells were not always identifiable, the nuclear marker never appeared in cells inside the peritubular layer. In contrast, peritubular cells and other interstitial cells were frequently labelled (Fig. 7A,C,D) and, in several cases, tubules were almost completely surrounded by a sheath of peritubular and interstitial cells carrying the label that showed them to be of mesonephric origin (Fig. 7B). However, not all peritubular or interstitial cells in the grafted testes carried the marker, and indeed large numbers of both cell types were unlabelled. In one grafted testis in this series, tubules did not differentiate. However, microscopic examination revealed that, in this particular case, mesonephric cells had failed to become incorporated in the testis portion of the culture and had remained in a clump on the outside of the organ.

DISCUSSION

Our present improved culture conditions allow good devel-

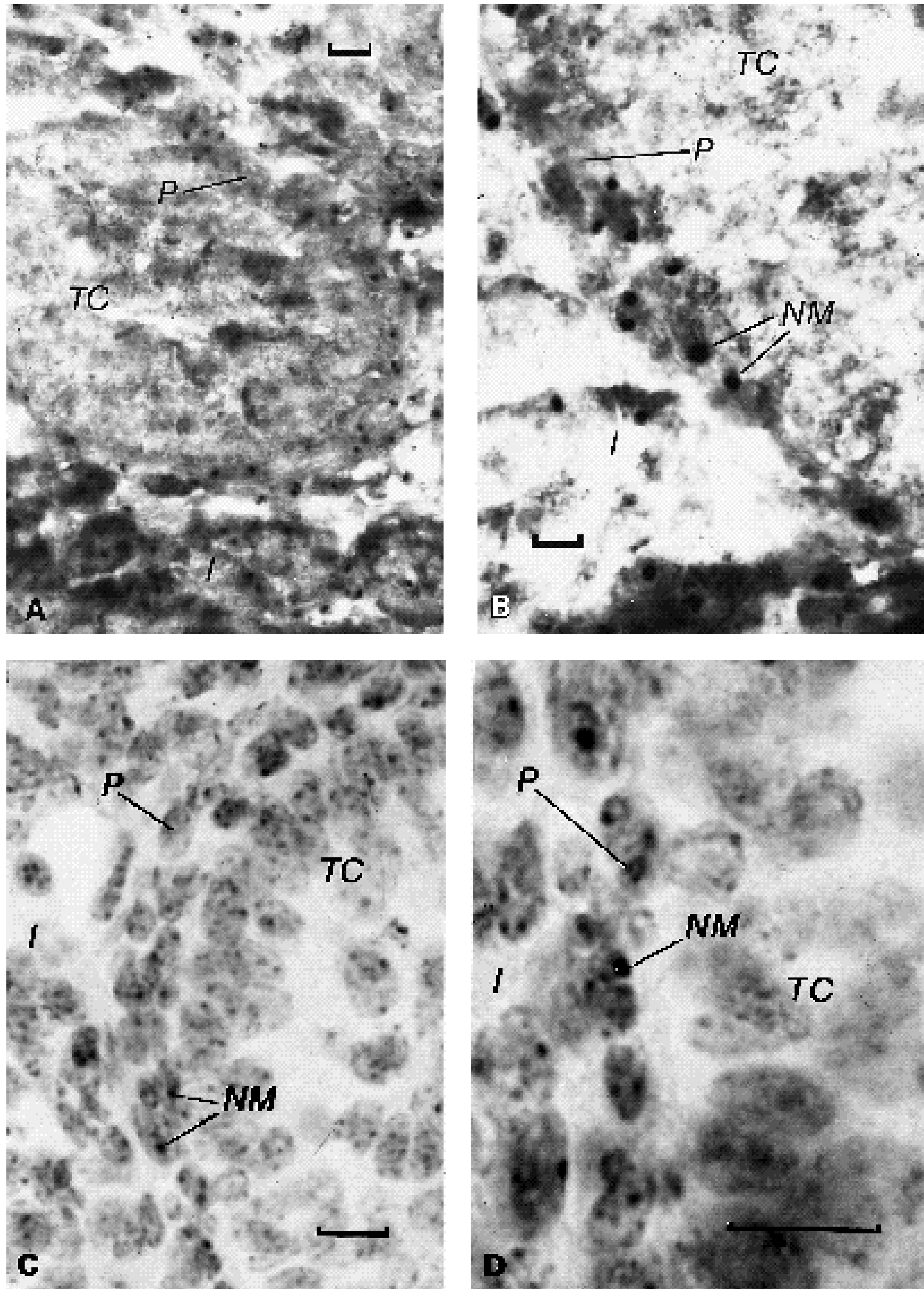

Fig. 7. (A) A section of testis cord from a Q testis grafted to a female mesonephros carrying the nuclear marker. The marker appears in peritubular elements and interstitial mesenchyme, but not in that portion of the cord where Sertoli cells or germ cells are located. (B) A testis cord from a Q testis grafted to a female mesonephros carrying the nuclear marker. The marker can be seen in the peritubular elements surrounding the cord as well as in the interstitial mesenchyme, but not in cells in the interior of the cord. Testis cord (TC), peritubular cells (P), interstitial cells (I). Bar, 5 μm. (C) As A, but fixed and stained for improved histological detail. The small black dots are chromocentres stained with H & E. Bar, 10 μm. (D) High-power view of C. Bar, 10 μm. Testis cord (TC), peritubular cells (P), interstitial cells (I), nuclear marker (NM) Bar, 10 μm.

opment of both somatic and germinal components of male fetal gonads (cf McLaren and Buehr, 1990). After 7 days in vitro, 11.5 dpc male Q-strain urogenital ridges formed testes with well-developed testis cords containing T-prospermatogonia, and no meiotic germ cells. This result is in agreement with the findings of Taketo and Koide (1981). It differs, however, from those of Byskov (1978), Byskov and Grinsted (1981) and Grinsted et al. (1979) who found that indifferent gonads, cultured in the presence of either mesonephric components or used medium from the prior culture of such components, showed disrupted cord structure and numerous meiotic germ cells. The more the cord structure deteriorated, the more meiotic germ cells were seen (Byskov, 1978). The urogenital ridges used for these cultures were from the BALB/c strain, and were probably more immature than our starting material. When we cultured 10.5 dpc male urogenital ridges, we too found that cords failed to develop and the germ cells entered meiosis rather than forming T-prospermatogonia (McLaren and Buehr, 1990).

Female genital ridges separated from the mesonephros at 11.5 dpc develop into small but normal-looking ovaries, but isolated male genital ridges show little or no differentiation (McLaren and Buehr, 1990). The results reported in the present paper confirm that the normal differentiation of testis cords in 11.5-day cultured male genital ridges requires attachment to a mesonephros. Either a male or a female mesonephros is effective, but other tissues (fragments of limb bud or heart) did not succeed in supporting differentiation. The mesonephros does not appear to exert its influence by means of a diffusible substance: direct contact between cells of the mesonephric region and those of the testis rudiment was necessary for normal tubule morphogenesis. Grafting experiments using ‘labelled’ mesonephric regions from a transgenic strain of mouse show that, after 11.5 days of development, mesonephric cells enter the developing testis and contribute to the interstitial cell population, both to the peritubular component and to the mesenchymal component that makes up more than 50% of the interstitial cell population of the fetal mouse testis, and which later gives rise to mature Leydig cells (Vergouwen et al., 1991). The mesodermal immigrants seem not to contribute to the population of Sertoli cells or to the germ cells.

The contribution of cells by the mesonephros to the developing testis is presumably a continuous process, starting from the earliest stages of gonad development at about 10 days, and continuing until about 12 days post coitum. In the present experiments, the mesonephros was removed from the testis at the arbitrarily selected age of 11.5 days, and we can know nothing of mesonephric contributions made before that time. However, two conclusions can be drawn about the role of the mesonephros in testis differentiation after 11.5 days. First, continuing mesonephric contribution is necessary for the establishment of normal testis architecture. Second, the mesonephric contribution at this time appears to be the provision of some of the interstitial components, including peritubular cells.

On the basis of histological analysis of normal development, several authors have assumed that the Sertoli cells are derived in whole or in part from the mesonephros (Upadhyay et al., 1981; Wartenberg, 1981; Pelliniemi et al., 1984; Wartenberg et al., 1991). The present experiments show that, if this is true, they originate from the mesonephros before 11.5 days, as testes grafted to labelled mesonephroi at that time appeared to contain no Sertoli cells of mesonephric origin.

Sry, now established as the testis-determining gene in mice (Koopman et al., 1991), is expressed at 10.5 and 11.5 days in the somatic tissues of the genital ridge (Koopman et al., 1990). It is believed to act in the supporting cell lineage, i.e. in the precursors of Sertoli cells since, in XX XY chimaeric mouse testes, the Sertoli cell population is made up predominantly of XY cells (Burgoyne et al., 1988; Palmer and Burgoyne, 1991; Patek et al., 1991). The interstitial cell populations of the gonad, including Leydig and peritubular cells, were found in these studies to have in general as large a contribution of XX cells as do other somatic cell lineages. The ability of XX cells from a female mesonephros to make a contribution to testis differentiation after 11.5 days is therefore hardly surprising if, as our results suggest, mesonephric contribution at this time is limited to the provision of interstitial cells only. Whether male or female mesonephroi are as interchangeable at earlier stages of differentiation, when Sertoli cell precursors may be migrating, remains an open question.

We cannot say whether the mesonephric contribution to the interstitial cell population is dependent on *Sry* expression and/or the presence of pre-Sertoli cells in the early genital ridge, as we have not combined labelled mesonephroi with female genital ridges; however, the much lesser size increase of the female genital ridge during this period, together with the rather normal ovarian development of isolated ridges that we have observed, suggests that *Sry* expression might be exerting an effect on mesonephric cells.

The possibility would be in no way inconsistent with the suggestion of Wartenberg et al. (1991) that an earlier influx of mesenchymal cells from the mesonephros induces proliferation of cells from the coelomic epithelium, which also contribute to testicular development. However, the results of Rodemer-Lenz (1989) on quail-chick chimaeras argue against a very early migration of cells from the mesonephros into the gonad. At least in birds, the indifferent gonadal primordium appears to be formed entirely from the coelomic epithelium: quail cells emanating from the mesonephros were only seen in the gonad at the end of the indifferent period.

Our experiments show that the interstitial cells derived from the mesonephros after 11.5 days of development are necessary for normal testis cord differentiation. How these cells help to establish testis structure is for the moment a matter of conjecture. The answer may, however, lie in the role of interstitial cells in the synthesis of components of the extracellular matrix, and in the organisation of the basal lamina surrounding the testis cords. There is general agreement that peritubular myoid cells are involved in the elaboration of the basal lamina and the extracellular matrix (Tung and Fritz, 1980; Tung et al., 1984; Grund et al., 1986), and that Sertoli cells are dependent upon the lamina and extracellular matrix for normal differentiation (Tung and Fritz, 1984; Taketo et al., 1984; Hadley et al., 1988).

The failure of any testicular development to take place

in the genital ridges of XX XY chimaeric embryos containing less than about 20% XY cells suggests that Sertoli cell differentiation and cord formation require aggregation of *Sry*-expressing supporting cells, and that below a certain threshold concentration of such cells, aggregation does not occur. We suggest further that a threshold number of myoid cells are required if these aggregations are to be maintained, and that separation from the mesonephros at 11.5 days reduces the myoid cell population below this threshold. Insufficient production of essential matrix proteins may result and testis cord differentiation could be inhibited as a consequence.

The culture method described above successfully maintained male germ cells that were seen in abundance inside the cords of testes which had been cultured with an attached mesonephros. These germ cells could be identified as T1 prospermatogonia (Hilscher, 1981); i.e., they had reached the same stage of male germ cell differentiation in vitro as they would have in vivo. Similar success in maintaining the first stages of male germ cell differentiation in culture has been reported before (Taketo and Koide, 1981; MacKay and Smith, 1986). However, even in our cultures of isolated testes in which cord morphogenesis was poor or lacking, clumps of T1 prospermatogonia could be seen, none of them in well-differentiated cords and many in areas of undifferentiated tissue. No meiotic germ cells were seen. This suggests that the presence of fully differentiated testis cords is not required to inhibit entry of germ cells into meiosis. Similar conclusions were reached by Taketo et al. (1984), who prevented cord differentiation in fetal mouse testes by adding cyclic AMP analogues to the culture medium, and found that germ cells differentiated as prospermatogonia in spite of the absence of testis cords. In vivo, the inhibitory effect is exerted not only on germ cells within the cords, but also on those germ cells inside the testis but outside the cords, and even on some of those outside the testis, in the mesonephric region (see McLaren, 1984). Since germ cells in the adjacent adrenal primordium are not inhibited from entry into meiosis, we presume that a short-range diffusible molecule is involved.

Although cord formation is not essential for the first stages of male germ cell differentiation, an important role may be played by the Sertoli cells. In our cultures of isolated testes, the Sertoli cells are not morphologically well differentiated, but they may nonetheless be capable of producing the hypothetical meiosis-inhibiting substance. The grafting experiments show that Sertoli cells or their precursors must be present in the 11.5-day testis before we removed the mesonephros, and Magre and Jost (1983) found that Sertoli cells capable of producing anti-Mullerian hormone (AMH) differentiated in rat testes in which the development of testis cords had been prevented. AMH itself is unlikely to be the meiosis-inhibiting substance: its addition to cultures of rat fetal ovaries does not inhibit entry into meiosis but is associated with loss of germ cells around the time of entry into meiosis (Vigier et al., 1987). Any germ cells entering meiosis in our fetal testes might therefore have degenerated, though no excess cell death was seen. When urogenital ridges were taken 24 hours earlier (10.5 dpc) and maintained in culture, testis cords were not formed and the total number of germ cells was greatly reduced, but many of those remaining entered meiosis (McLaren and Buehr, 1990). This suggests (a) that Sertoli cell precursors must reach a certain stage of differentiation in vivo if they are to inhibit entry into meiosis and thus induce germ cells to enter the male rather than the female pathway of development, and (b) that our culture conditions from 10.5 to 11.5 dpc were not adequate to support this differentiation.

We thank Stephen Palmer for doing the initial in situ hybridizations.

REFERENCES

Burgoyne, P. S., Buehr, M., Koopman, P., Rossant, J. and McLaren, A. (1988). Cell-autonomous action of the testis-determining gene: Sertoli cells are exclusively XY in XX-XY chimaeric mouse testes. *Development* **102**, 443-450.

Burgoyne, P. S., Tam, P. P. L. and Evans, E. P. (1983). Retarded development of XO conceptuses during early pregnancy in the mouse. *J. Reprod.Fert.* **68,** 387-393.

Byskov, A. G. (1978). The meiosis inducing interaction between germ cells and rete cells in the fetal mouse gonad. *Ann. Biol. Anim. Biochim. Biophys.* **18,** 327-334.

Byskov, A. G. and Grinsted, J. (1981). Feminising effect of mesonephros on cultured differentiating mouse gonads and ducts. *Science* **212,** 817-818.

Grinsted, J., Byskov, A. G and Andreason, J. (1979). Induction of meiosis in fetal mouse testis in vitro by rete testis tissue from pubertal mice and bulls. *J.Reprod.Fert.* **56,** 653-656.

Grund, S. K., Pelliniemi, L. J., Paranko, J., Muller, U. and Lakkala-Paranko, T. (1986). Reaggregates of cells from rat testis resemble developing gonads. *Differentiation* **32,** 135-143.

Hadley, M. A., Byers, S. W., Suarez-Quian, C. A., Djakiew, D. and Dym, M. (1988). *In vitro* models of differentiated Sertoli cell structure and function. *InVitroCell.Dev.Biol.* **24,** 550-557.

Hilscher, W. (1981). T1-prospermatogonia (primordial spermatogonia of Rauh): the 'ameiotic' counterpart of early oocytes. *Fortschritte der Andrologie* **7**, 21-32.

Koopman, P., Munsterberg, A., Capel, B., Vivian, N. and Lovell-Badge, R. (1990). Expression of a candidate sex-determining gene during mouse testis differentiation. *Nature* **348,** 450-452.

Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P. and Lovell-Badge, R. (1991). Male development of chromosomally female mice transgenic for *Sry*. *Nature* **351,** 117-121.

Lo, C. W. (1986). Localization of low abundance DNA sequences in tissue sections by *in situ* hybridization. *J. Cell Sci.* **81,** 143-162.

MacKay, S. and Smith, R. A. (1986). The differentiation of mouse gonads *invitro*: a light and electron microscopical study. *J.Embryol.Exp.Morph.* **97,** 189-199.

McLaren, A. (1984). Meiosis and differentiation of mouse germ cells. In *38th Symposium of the Society for Experimental Biology, Controlling Events in Meiosis* (eds C. W. Evans and H. G. Dickinson), pp. 7-23. Company of Biologists, Cambridge.

McLaren, A. and Buehr, M. (1990). Development of mouse germ cells in cultures of fetal gonads. *CellDiff.Dev.* **31,** 185-195.

Magre, S. and Jost, A. (1983). Early stages of the differentiation of the rat testis: relations between Sertoli and germ cells. In *Current Problems in Germ Cell Differentiation* (eds A. McLaren and C. C. Wylie), pp. 201-214. Cambridge University Press.

Merchant-Larios, H., Popova, L. and Reyss-Brion, M. (1984). Early morphogenesis of chick gonad in the absence of mesonephros. *Develop. Growth Diff.* **26,** 403-417.

Palmer, S. and Burgoyne, P. S. (1991). *Insitu* analysis of fetal, prepuberal and adult XX-XY chimaeric mouse testes: Sertoli cells are predominantly, but not exclusively, XY. *Development* **112,** 265-268.

Patek, C. E., Kerr, J. B., Gosden, R. G., Jones, K. W., Hardy, K., Muggleton-Harris, A. L., Handyside, A. H., Whittingham, D. G. and Hooper, M. L. (1991). Sex chimaerism, fertility and sex determination in the mouse. *Development* **113,** 311-325.

Pelliniemi, L. J., Paranko, J., Grund, S. K., Frojdman, K., Foidart, J-M.

and Lakkala-Paranka, T. (1984). Morphological differentiation of Sertoli cells. *INSERM* **123,** 121-140.

Rodemer, E. S., Ihmer, A. and Wartenberg, H. (1986). Gonadal development of the chick embryo following microsurgically caused agenesis of the mesonephros and using interspecific quail-chick chimaeras. *J. Embryol. Exp. Morph* **98,** 269-285.

Rodemer-Lenz, E. (1989). On cell contribution to gonadal soma formation in quail-chick chimaeras during the indifferent stage of gonadal development. *Anat. Rec.* **179,** 237-242.

Rossant, J., Vijh, K. M., Grossi, C. E. and Cooper, M. D. (1986). Clonal origin of haematopoietic colonies in the postnatal mouse liver. *Nature* **319,** 507-511.

Taketo, T. and Koide, S. S. (1981). *Invitro* development of testis and ovary from indifferent fetal mouse gonads. *Dev.Biol.* **84**, 61-66.

Taketo, T., Thau, R. B., Adeyemo, O. and Koide, S. S. (1984). Influence of adenosine 3 :5 -cyclic monophosphate analogues on testicular organization of fetal mouse gonads *invitro*. *Biol. Reprod.* **30,** 189-198.

Tung, P. S. and Fritz, I. B. (1980). Interactions of Sertoli cells with myoid cells *invitro*. *Biol. Reprod.* **23,** 207-217.

Tung, P. S. and Fritz, I. B. (1984). Extracellular matrix promotes rat Sertoli cell histotypic expression *invitro*. *Biol. Reprod.* **30,** 213-229.

Tung, P. S., Skinner, M. K. and Fritz, I. B. (1984). Cooperativity between Sertoli cells and testicular peritubular cells in the production and deposition of extracellular matrix components. *J. Cell Biol.* **100,** 1941-1947.

Upadhyay, S., Luciani, J. M. and Zamboni, L. (1979). The role of the mesonephros in the development of indifferent gonads and ovaries of the mouse. *Ann.Biol.anim.Biochim.Biophys.* **19,** 1179-1196.

Upadhyay, S., Luciani, J-M. and Zamboni, L. (1981). The role of the mesonephros in the development of the mouse testis and its excurrent pathways. In *Development and Function of Reproductive Organs* (eds A.G. Byskov and H. Peters), pp. 18-27. Excerpta Medica, Amsterdam.

Vergouwen, R. P. F. A., Jacobs, S. G. P. M., Huiskamp, R., Davids, J. A. G. and de Rooij, D. G. (1991). Proliferative activity of gonocytes, Sertoli cells and interstitial cells during testicular development in mice. *J. Reprod.Fert.* **93,** 233-243.

Vigier, B., Watrin, F., Magre, S., Tran, D. and Josso, N. (1987). Purified bovine AMH induces a characteristic freemartin effect in fetal rat prospective ovaries exposed to it *invitro*. *Development* **100,** 43-55.

Wartenberg, H. (1981). Differentiation and development of the testes. In *The Testis* (eds H. Burger and D. de Kreiser), pp. 39-79. Raven Press, New York.

Wartenberg, H., Kinsky, I., Viebahn, C. and Schmolke, C. (1991). Fine structural characteristics of testicular cord formation in the developing rabbit gonad. *J. ElectronMicroscop. Techn.* **19,** 133-157.

Whittingham, D. G. (1971). Culture of mouse ova. *J. Reprod.Fert.* suppl. **14,** 7-21.

Zamboni, L. and Upadhyay, S. (1982). The contribution of the mesonephros to the development of the sheep fetal testis. *Am. J. Anat.* **165,** 339-356.

(Accepted 30 September 1992)