J. Embryol. exp. Morph. Vol. 68, pp. 161–174, 1982
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Functional differentiation of chick gonads following depletion of primordial germ cells

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SUMMARY

The endocrine capacity of embryonic chick gonads depleted in germ cells was compared to that of controls to determine whether the somatic elements of germ-cell-depleted gonads will undergo normal functional sex differentiation. Primordial germ cells were removed from early embryos, Hamburger and Hamilton stages 7–11, by excision of the anterior germinal crescent. Embryos were sacrificed at 14 days of incubation, and their gonads were analysed for functional differentiation by: (1) electron microscopy to detect ultrastructural cellular morphology characteristic of steroid-secreting cells; (2) growth in cell culture to detect development of characteristic cell morphologies; (3) radioimmunoassay of cell-culture media to detect androgens and oestrogens (androstenedione and oestradiol 17 β) secreted by gonadal cells; and (4) measurement of steroid levels produced by cultures treated with human chorionic gonadotropin (hCG) to detect the ability of gonadal cells to increase steroid production in response to gonadotropin stimulation. As a bioassay of gonadal endocrine activity, a gross morphological analysis was performed on the genital ducts, the development of which is ovary-dependent in females and testis-dependent in males.

This study demonstrated that both male and female embryonic gonads exhibit normal functional differentiation following a significant reduction in the number of primordial germ cells. These results confirm and extend our previous finding that morphological differentiation of sterile embryonic gonads is normal (McCarrey & Abbott, 1978). It is concluded from the present study that a normal complement of germ cells is not essential to either morphological or functional sex differentiation of the somatic elements of the embryonic ovary or testis, thus arguing against any inductive role for the germ cells in this process.

INTRODUCTION

Primary sex differentiation in the embryo includes differentiation of the initially indifferent gonads into either testes in genetic males or ovaries in genetic females, and differentiation of the primordial germ cells (PGC) into either spermatogonia or oogonia. The interaction between germ cells and somatic cells is essential for the initiation of the latter process, gametogenesis, in both birds and mammals (Erickson, 1974*a*, *b*; Mintz, 1968; Tarkowski, 1970; Ford *et al.* 1974; Gordon, 1977; O & Short, 1977). However, whether or not any such interaction is normally necessary for the former process, differentiation

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of the somatic elements of the gonads, remains an enigma. This question was initiated by Dantchakoff's (1933) claim that germ cells are required for normal development of the indifferent gonads in chick embryos, and has since been periodically rekindled as evidenced by the recent suggestions that germ cell–somatic cell interactions mediated by H-Y antigen may be involved in mammalian testis differentiation and possibly also in avian ovary differentiation (Ohno, 1976; Silvers & Wachtel, 1977; Wachtel, 1977; Hazeltine & Ohno, 1981). The primary approach to this question has been to analyse the morphological differentiation of gonads experimentally depleted of PGC. Thus several investigators working with chick embryos have observed development of a morphologically normal indifferent gonad by 5 days of incubation, after elimination of PGC at 1–2 days of incubation (for review see McCarrey & Abbott, 1979). We extended these results past the stage of primary sex differentiation, demonstrating normal histological sex differentiation in a germ-cell-deficient testis at 15 days of incubation and in a germ-cell-deficient ovary at 20 days of incubation (McCarrey & Abbott, 1978). Similarly in mammalian species it has been shown that a significant reduction in germ cell numbers does not affect morphological differentiation of embryonic gonads either before or after the stage of sex differentiation (Coulombre & Russell, 1954; Mintz & Russell, 1957; Merchant, 1975; Vanhems & Bousquet, 1978; Handel & Eppig, 1979; Merchant-Larios & Coello, 1979; and Russell, 1979). However it is clear that morphogenesis is but one facet of gonadal differentiation, and that gonadal *function* is of at least equal importance. Furthermore it cannot be concluded *a priori* that normal morphology connotes normal function. Thus it remained to be shown whether germ-cell-depleted gonads undergo normal functional differentation.

The primary function of embryonic gonads, in addition to supporting the early stages of gametogenesis, is to produce and secrete hormones. These include the sex steroids, which induce secondary sex differentiation in the embryo and the adult (Jost, 1947*a*, *b*; Josso, Picard & Tran, 1977; Wolff & Wolff, 1951), and, in the case of the testis, the proteinaceous 'anti-Mullerian' hormone (Josso *et al.* 1977). A number of techniques have been used to detect the production of steroids by embryonic chick gonads. Electron microscopy has demonstrated specific ultrastructural characteristics of steroid-secreting cells, including extensive agranular endoplasmic reticulum, lipid droplets, and mitochondria with tubular cristae (Narbaitz & Adler, 1966; Carlon & Erickson, 1978). Radioimmunoassay (RIA) has been used to directly measure levels of various sex steroids both within embryonic gonadal tissue (Guichard *et al.*; Guichard, Scheib, Haffen & Cedard 1977*b*, 1979; Teng & Teng, 1977; Tanabe, Nakamura, Fujioka & Doi, 1979), and when secreted into either embryonic plasma (Tanabe *et al.* 1979), organ culture media (Guichard *et al.* 1977*a*, *b*, 1979; Teng & Teng, 1977) or cell culture media (Teng & Teng, 1979). These studies have established sex-specific patterns of production and secretion of

steroids by embryonic chick gonads. In addition, the production of steroids can be enhanced by gonadotropins, reflecting the presence of gonadotropin receptors.

A different approach to the detection of hormone production by the gonads can be achieved by analysing the development of the genital ducts, thus providing a bioassay of gonadal function. Specifically the development of the Mullerian ducts is controlled by different gonadal hormones in each sex. In females the differentiation of the left Mullerian duct into an oviduct including a shell gland in the caudal region is modulated by oestrogens secreted by the embryonic ovary (Wolff & Wolff, 1951; Hamilton, 1961). The regression of the right Mullerian duct is also ovary-dependent (Wolff & Wolff, 1951; Salzgeber, 1950). On the other hand, the regression of the Mullerian ducts in male embryos is induced by the protein anti-Mullerian hormone, which is produced by the Sertoli cells of the testis (Josso *et al.* 1977).

We have employed these tests of gonadal function to analyse the ability of gonads severely depleted of germ cells to undergo normal functional sex differentiation. The results of this study, in conjunction with our earlier morphological analysis, indicate that neither the presence of, nor any induction from germ cells is required for normal morphological or functional differentiation of the somatic elements of the gonads.

MATERIALS AND METHODS

The methods used for incubation, pre- and postoperative treatment, and excision were described previously (McCarrey & Abbott, 1978). Briefly, fertile eggs were incubated on their sides at 37·5 °C and 58% relative humidity until they reached stages 6–11 (Hamburger & Hamilton, 1951). The eggs were then windowed and the germinal crescent excised with iridectomy scissors. After surgery the windows were sealed and the eggs returned to the incubator and turned twice per day until sacrificed. Control embryos received the same treatment, except for excision of germinal crescent.

Gonads prepared for cell culture were dissected from operated and control embryos at 14 days of incubation. Each gonad, in a separate 12 × 75 mm tube, was treated with 300 μl of a 1 : 1 mixture of HEPES buffer and a cell dispersal solution consisting of 0·8% collagenase (Worthington, 144 units/mg), 2·0% bovine serum albumin (BSA), and 20 μg/ml DNAse (GIBCO, from bovine pancreas, 2100 units/mg). The tubes were incubated in a water bath at 37 °C for 1–1·5 h. During this time the gonadal tissue was dispersed by periodic pipetting with heat-drawn pipettes of progressively smaller bore. The cells were then washed 2× in HEPES buffer and counted in a haemacytometer. Separate counts were made of germ cells and of somatic cells to determine the percentage of germ cells. The cells were plated at a density of 10^5 cells/ml in McCoy's 5a medium (modified) supplemented with 2 mM L-glutamine, 100 units/ml

penicillin, and 100 μg/ml streptomycin sulphate: 50 ng of purified hCG (source: NICHHD) in 100 μl HEPES buffer was added to half the dishes and, as a control, 100 μl of plain HEPES buffer was added to the other half. The cultures were incubated for 2 days in a humidified 95% air–5% CO_2 incubator at 37 °C. Subsequently the culture media were collected and stored frozen until RIA. The cells which remained attached to the culture dish were fixed for 15 min with 2·5% glutaraldehyde in 0·1 M cacodylate buffer. The cells were then stained for 1 h in 1% OsO_4 and then for 15 min in 1% methylene blue, in preparation for viewing and photography with a Zeiss Photomicroscope II.

RIA of tissue culture media to detect androstenedione and estradiol 17β was done according to the methods of Erickson and Hseuh (1978). Androstenedione antiserum (titre = 1 : 2500) was obtained from Dr G. F. Erickson, University of California, San Diego. The estradiol 17β antiserum (titre = 1 : 150,000) was from Dr B. Caldwell, Yale University. Antisera were incubated overnight with ^{3}H-labelled tracer hormone, phosphate buffer, and 100 or 200 μl of culture media to be assayed. A standard curve was generated by incubating standard hormone of varying known concentrations with antisera and tracer hormone. Separation of bound from free hormone was achieved by a 30 min incubation at 4 °C in charcoal plus dextrose, followed by centrifugation at 3000 rev./min for 15 min at 4 °C. The supernatent was then decanted into scintillation vials and counted for 2 min or 10000 cpm.

Electron microscopy was performed on gonads from 14-day embryos. These gonads were fixed for 1 h at room temperature in 3% glutaraldehyde in 0·1 M phosphate buffer containing 1·5% sucrose, and then postfixed for 1 h in 2% buffered OsO_4 at 4 °C. This was followed by immersion in 2% aqueous uranyl acetate for 10 min at room temperature, dehydration in a graded series of acetone changes, and embedding in Spurr's resin (Spurr, 1969). Thick sections (1·0–1·5 μm) were cut with glass knives and stained with 1% methylene blue in 1% sodium borate, and 1% azure II according to the methods of Richardson, Jarett & Finke (1960). The blocks were retrimmed and sectioned at 60–80 nm with a diamond knife. Thin sections were stained with 4% uranyl acetate in 70% ethanol for 5–7 min and with Reynolds' lead citrate (Reynolds, 1963) for 5 min at room temperature. A Phillips EM-400 transmission electron microscope was used to view the sections at 60–80 kV.

The morphology of genital ducts was examined in both control and germ-cell-depleted embryos sacrified at 14 days of incubation. Overlying viscera were removed to expose the ducts which were then photographed *in situ* or *in vitro* with a Wild M-5 binocular dissection scope fitted with a camera on the phototube.

RESULTS

Of the 802 embryos from which anterior germinal crescent was excised, 24 (3%) survived to 14 days of incubation. Six of the eight ovaries, and five of the

Table 1. *Germ cell counts in depleted gonads*

Embryo number	Stage at sacrifice	Gonad	Germ cells (%†)	Control (%‡)
$\bar{x}$ Control§	38–40	L. Ovary	7·4 ± 0·7	—
		R. Ovary	4·8 ± 1·1	—
F-1	39	L. Ovary	10·6	142·7
		R. Ovary	9·8	203·7
F-2	39	L. Ovary	6·4	86·1**
		R. Ovary	2·2	45·7*
F-3	37	L. Ovary	1·1	14·7**
		R. Ovary	1·0	20·8*
F-4	38	L. Ovary	3·1	41·7**
		R. Ovary	2·5	52·0*
$\bar{x}$ Control‖	38–39	L. Testis	3·9 ± 0·4	—
		R. Testis	4·1 ± 0·8	—
M-1	40	L. Testis	13·4	342·7
		R. Testis	10·0	242·1
M-2	39	L. Testis	1·3	28·1*
		R. Testis	0·4	9·7**
M-3	39	L. Testis	0·4	10·7**
		R. Testis	1·8	43·6
M-4	39	L. Testis	2·3	57·5
		R. Testis	0·9	21·3*
M-5	39	L. Testis	0·1	3·3**
		R. Testis	1·8	43·6

* Germ cell reduction significant ($P < 0·05$).
** Germ cell reduction very significant ($P < 0·001$).
† In the total cell population of the gonad.
‡ Values from depleted gonads are compared to mean values of their controls.
§ Based on data from seven pairs of ovaries.
‖ Based on data from nine pairs of testes.

ten testes prepared for tissue culture showed a significant depletion of germ cells ($P < 0·05$) based on comparisons with mean percentages of germ cells calculated for similarly prepared control gonads (Table 1).

The radioimmunoassay results demonstrated the production of androstenedione in cultures of both control and germ-cell-depleted testes, however estradiol 17 β was not present in assayable amounts in these cultures. Detectable levels of both steroids were produced in cultures of both control and germ-cell-depleted ovaries. Steroid levels produced by germ-cell-depleted gonads in cultures with and without human chorionic gonadotropin (hCG) are shown in Table 2. Each value is also expressed as a percentage of the mean control value obtained from RIA of media from four pairs of ovaries and seven pairs of testes from control embryos. In addition, the number of germ cells showed no significant ($P < 0·05$) correlation with the level of steroid production in depleted

Table 2. *Steroid production by depleted gonads*†

Embryo number	Oestradiol 17 β (pg/ml)				Androstenedione (pg/ml)			
	−hCG	Control (%) −hCG	+hCG‡	Control (%) +hCG	−hCG	Control (%) −hCG	+hCG‡	Control (%) +hCG
				Left ovary				
$\bar{x}$ control§	87·4	—	241·5	—	47·5	—	308·1	—
F-2	105·3	120·5	276·9	114·7	72·2	151·7	273·0	88·6
F-3	156·0	178·5	304·2	126·0	136·5	286·8	453·1	146·9
F-4	76·1	87·1	226·2	93·7	68·5	143·9	245·7	79·7
CC‖	—	−0·51	—	−0·21	—	−0·47	—	−0·76
				Right ovary				
$\bar{x}$ control§	34·5	—	169·2	—	111·2	—	604·0	—
F-2	0	—	54·6	32·3	81·9	73·7	234·0	38·7
F-3	0	—	421·9	249·3	152·1	136·8	593·8	98·3
F-4	99·5	288·4	241·8	142·9	136·5	122·8	593·8	98·3
CC	—	—	—	−0·89	—	−0·52	—	−0·33
				Left testis				
$\bar{x}$ control¶	n.d.	—	n.d.	—	46·4	—	274·4	—
M-2	n.d.	—	n.d.	—	42·9	92·5	206·7	75·3
M-3	n.d.	—	n.d.	—	0	—	78·0	28·4
M-4	n.d.	—	n.d.	—	83·6	180·2	193·1	70·4
M-5	n.d.	—	n.d.	—	0	—	79·0	28·8
CC	n d.	—	n.d.	—	—	—	—	+0·82*
				Right testis				
$\bar{x}$ control¶	n.d.	—	n.d.	—	14·5	—	139·9	—
M-2	n.d.	—	n.d.	—	22·5	155·2	198·9	142·2
M-3	n.d.	—	n.d.	—	0	—	171·6	122·2
M-4	n.d.	—	n.d.	—	—	—	136·5	97·6
M-5	n.d.	—	n.d.	—	0	—	89·7	64·1
CC	n.d.	—	n.d.	—	—	—	—	−0·59

n.d., Non-detectable.

* Significant ($P < 0·05$) according to Student's t test.
† Produced in cultures of 10^5 cells/ml.
‡ 50 ng/ml.
§ Based on data from four ovaries.
‖ Correlation coefficient between percent of mean control germ cell values and percent of mean control steroid production.
¶ Based on data from seven testes.

gonads, except in the case of left testis with hCG added, which showed a significant positive correlation (Table 2).

In cultures of both control and germ-cell-depleted gonads, hCG stimulation consistently induced increased steroid production. The increase in androstenedione production was significant ($P < 0·05$) in both ovary and testis cultures, as was that in oestradiol 17 β production by ovary cultures. Where sufficient

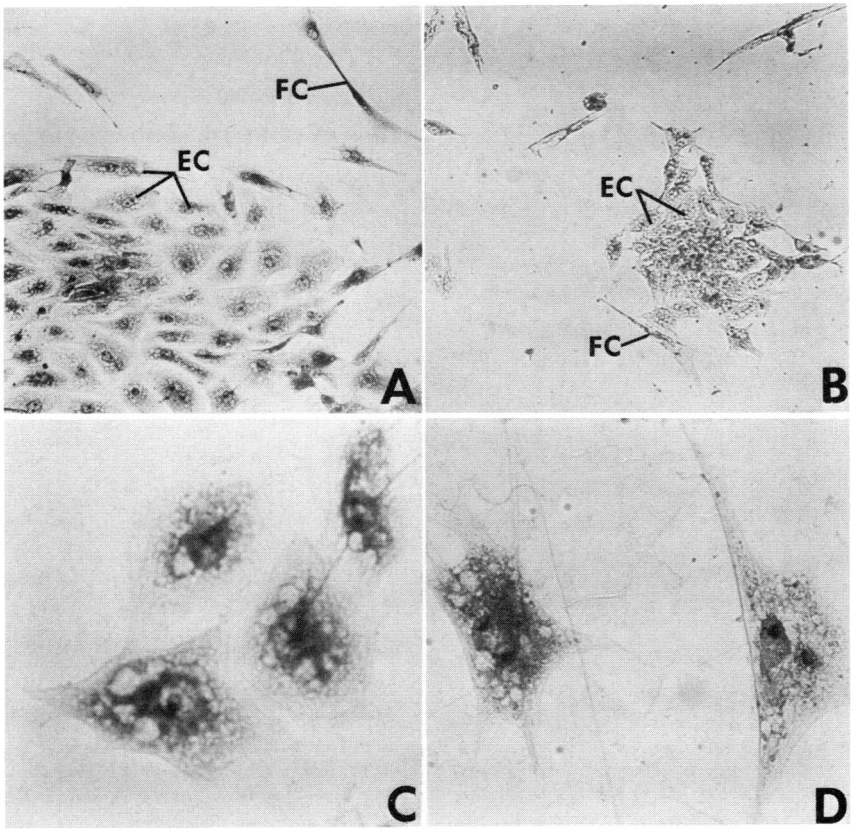

Fig. 1. Morphology of cultured gonadal cells from 14-day germ-cell-depleted embryos. (A) Ovary cultures develop large aggregates of epithelial cells (EC) in addition to individual fibroblastic cells (FC). × 72. (B) Testis cultures develop smaller aggregates of epithelial cells in addition to individual fibroblastic cells. × 72. (C) Ovary epithelial cells contain vacuolated cytoplasm, and have a rounded shape. × 261. (D) Testis epithelial cells also contain vacuolated cytoplasm, but are more rectangular in shape. × 284. All cells stained with eosin.

data were present, correlation coefficients were calculated between the number of germ cells and the degree of response to hCG stimulation (+hCG/−hCG steroid production levels) in cultures of depleted gonads. No significant correlation was found.

After removal of culture media, two cell types remained attached to the culture dishes. We have referred to these as fibroblastic and epithelial cell types, following the terminology of Teng & Teng (1979). Fibroblastic cells were elongated and contained non-vesicular cytoplasm, whereas epithelial cells were more rounded and contained vesicular cytoplasm. Both cell types were consistently found in all cultures, and no difference in their appearance was observed between left and right gonads in either sex. In addition, cultured cells from germ-cell-depleted gonads appeared identical to those of the controls.

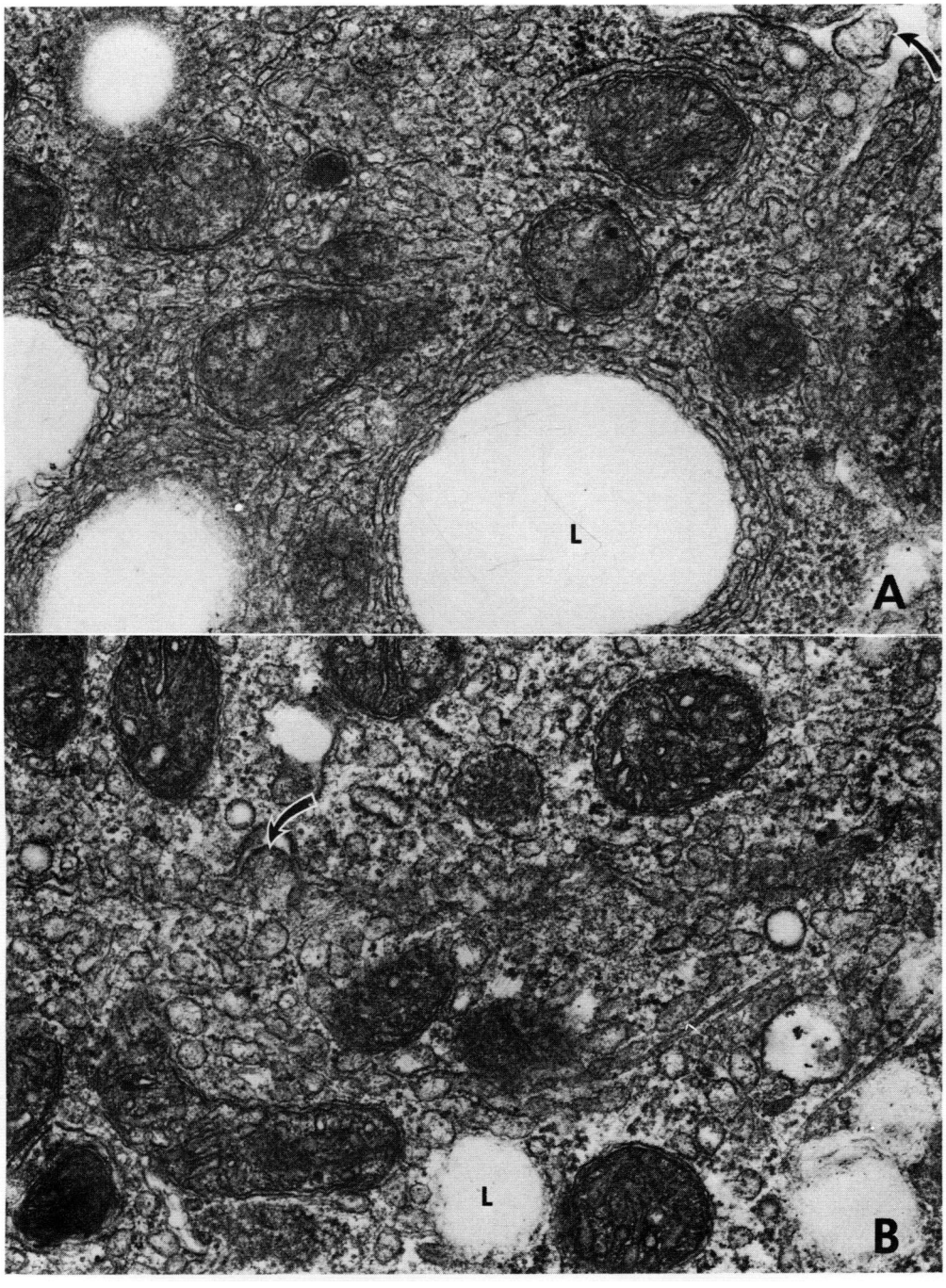

Fig. 2. Ultrastructure of steroid-secreting cells in 14-day embryonic germ-cell-depleted gonads. (A) At high magnification ovary steroid-secreting cells can be seen to contain abundant SER, lipid droplets (L), mitochondria with some tubular cristae, and protrusions at the cell surface (arrow). ×42017. (B) Testis steroid-secreting cells contain less SER than in ovary, but lipid droplets (L), mitochondria with tubular cristae, and cell surface protrusions (arrow) are equally abundant. ×42017.

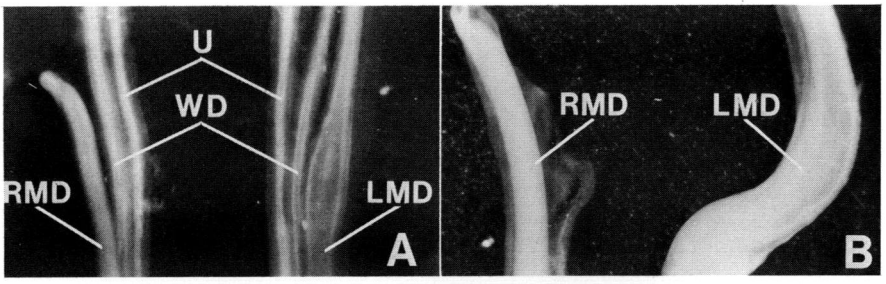

Fig. 3. Embryonic female genital ducts. (A) At 14 days of incubation control female embryos show paired sets of ureters (U), Wolffian ducts (WD), and asymmetrically developed left and right Mullerian ducts (LMD and RMD). The right Mullerian duct is in a state of regression while the left Mullerian duct is developing into the functional oviduct including a caudal swelling which represents the early shell gland. Photographed *in situ* with no fixation. × 11. (B) Germ-cell-depleted female embryos also show normal ovary-dependent asymmetrical development of the Mullerian ducts, with a regressing right Mullerian duct (RMD) and a well developed left Mullerian duct (LMD) including a differentiated shell gland. Photographed *in vitro* after glutaraldehyde fixation. × 15.

However, in both cases, ovary cultvres contained relatively larger islands of confluent rounded epithelial cells (Fig. 1 A and C), whereas testis cultures contained smaller aggregates of rectangular epithelial cells (Fig. 1 B and D).

The degree of germ-cell-depletion in testes and right ovaries prepared for electron microscopy was estimated on the basis of the proportion of germ cells among somatic cells seen in the thick sections. In left ovaries the thickness of the cortex, which is made up primarily of germ cells, provided a more direct measure of depletion. Of 22 gonads from operated embryos, 14 were depleted in numbers of germ cells.

In thick sections of both ovaries and testes, steroid-secreting cells were recognized by the presence of cytoplasmic lipid droplets. Islands of steroid-secreting cells were found throughout the medulla of both left and right control ovaries, and were equally abundant in germ-cell-depleted ovaries. At the ultrastructural level, abundant smooth endoplasmic reticulum (SER), mitochondria with tubular cristae, microvilli protruding at the cell surface, and the cytoplasmic lipid droplets were all visible in these ovarian cells (Fig. 2 A). In testes from both control and germ-cell-depleted embryos, steroid-secreting cells were found isolated or in very small groups in the interstitial tissue surrounding the seminiferous tubules. SER was present but was not abundant as in ovarian steroid-secreting cells. Other ultrastructural characteristics were similar to those observed in ovarian cells (Fig. 2 B).

Three paired sets of genital ducts were initially present in early embryos of both sexes, the ureters, the Wolffian ducts, and the Mullerian ducts. By 14 days of incubation in females, the left Mullerian duct extended from a position adjacent to the anterior end of the ovary to a posterior attachment in the caecum. A well-developed shell gland was present in the caudal portion of the

duct. The right Mullerian duct was regressing and lacked differentiated structures along its length. In male embryos both Mullerian ducts had completely regressed. These observations were consistent in all embryos studied, with no apparent differences between control and germ-cell-depleted embryos (Fig. 3).

DISCUSSION

As in our earlier study, these excision experiments produced a very low survival rate among the postoperative embryos (3% to 14 days of incubation). This has been a consistent problem experienced by all investigators who have attempted experiments employing excision or any other technique to destroy germinal crescent (Goldsmith, 1935; Dubois, 1962; Mimms & McKinnel, 1971). Undoubtedly it is this problem which normally precludes the production of completely germ-cell-free embryos capable of surviving to 14 days incubation. This is because excision of a sufficient amount of germinal crescent tissue to completely eliminate all of the germ cells almost always results in the death of the embryo. In fact our experiments represent the only successful attempt to incubate embryos *in ovo* past 5 days of incubation following excision or destruction of anterior germinal crescent at $1\frac{1}{2}$ days of incubation. The variability we observed in the degree of germ cell depletion in these experiments is probably due to excision of slightly varying amounts of germinal crescent tissue and/or some individual variability in PGC location in or around the germinal crescent area. It is also possible that an initial depletion in PGC numbers subsequently induces enhanced multiplication (to varying extents) of any remaining PGC. Thus only those gonads which remained depleted of germ cells at 14 days of incubation were considered in the various analyses of functional differentiation.

The most direct assay of endocrine activity in germ-cell-depleted gonads used in this study was radioimmunoassay of steroids produced by gonadal cells during two days in cell culture. It should be emphasized that these cultures involved the use of media that was completely defined chemically. Thus no exogenous or potentially artifactural source of contaminating steroids was present. Our approach afforded two means to investigate any relationship between germ cell numbers and steroidogenesis. First a Student's t test showed that neither the level of androstenedione nor oestradiol 17β produced by depleted gonads was significantly different from that of their controls. Second, calculation of correlation coefficients showed no relationship between the varying numbers of germ cells in each depleted gonad and the level of steroid production.

A characteristic feature of most steroid-secreting cells is their ability to respond to stimulation by gonadotropins. Our results show that somatic cells from germ-cell-depleted gonads retain this ability. Higher levels of steroidogenesis were detected in all hCG-stimulated cultures.

Our results differ from those previously reported by other investigators

concerning the endocrine profile presented by the regressing right ovary in female embryos at 14 days of incubation. Guichard *et al.* (1977*a*), Teng & Teng (1977, 1979), and Woods & Erton (1978) observed asymmetrical production of steroids by the ovaries, with the left ovary actively producing oestrogens while the right ovary produced relatively more androgens, suggesting that the right ovary resembles a testis rather than the left ovary in endocrine function. However we found that at 14 days of incubation the right ovary is more like the left ovary in its endocrine activity. While the right ovary produced slightly more androgens and less oestrogens than the left, it differed from the testes which did not produce oestrogen and secreted less androstenedione than either ovary. This conclusion is supported by similarities noted both in cultured cell morphologies of left and right ovarian tissue and in histological preparations.

Our observation that ovarian cells of two distinct morphologies, epithelial and fibroblastic, attached to the culture dishes is in agreement with the findings of Teng & Teng (1979). The results presented here extend their findings by demonstrating the occurrence of similar epithelial and fibroblastic cell types in cultures of testicular cells as well. The more rounded epithelial cell morphology observed in ovarian cultures as compared to the more rectangular shape of the testicular epithelial cells is reminiscent of the histological characteristics of follicle cells and Sertoli cells observed in preparations of intact ovaries and testes, respectively.

The electron microscopy results presented here confirm and extend our previous finding that depletion of germ cells does not affect the development of histological characteristics by showing that the ultrastructure is normal as well. This not only demonstrates normal morphological differentiation, but is also an excellent measure of functional differentiation. Our ultrastructural study supports conclusions derived from work with mammalian species. Merchant (1975), Vanhems & Bousquet (1978), and Merchant-Larios & Coello (1979) showed that ultrastructural differentiation of rat gonadal cells was normal following depletion of germ cells by drug treatment. In addition ultrastructural studies of testicular biopsy tissue from human XX sterile males showed normal Leydig cell morphology (Nistal & Paniagua, 1979). Thus it can be concluded that depletion of germ cells affects neither morphological nor, to the extent that ultrastructure indicates steroidogenesis, functional differentiation of gonadal somatic cells in either birds or mammals.

Of all the approaches to test endocrine capacity employed in this study, the one least susceptible to experimental error and/or artifact was the analysis of genital duct differentiation. The normal development of the genital ducts in germ-cell-depleted embryos shows that a severe reduction in germ cell number has no effect on the production, secretion, transport, or response to either the steroidal oestrogens produced by embryonic ovaries or the proteinaceous anti-Mullerian hormone produced by embryonic testes.

The results of this study indicate that a normal complement of germ cells is

not required for primary sex differentiation, thus arguing against any inductive role for the germ cells in this process. This conclusion is supported by our own previous findings (McCarrey & Abbott, 1978), as well as those of several other investigators (for review see McCarrey & Abbott, 1979), all of which showed that eliminating the primordial germ cells prior to their migration to the early genital ridge does not prevent the development of morphologically normal indifferent gonads. Indeed our results demonstrate that a severe reduction in germ cell numbers has no effect on either morphological or functional differentiation of the somatic cells of the gonads throughout embryogenesis. This is contrary to the claim of Dantchakoff (1932, 1933) that germ cells actually induce the formation of the indifferent gonads and that no such differentiation can occur in their absence. In addition, the suggestion that germ cell–somatic cell interaction may be an important step in H-Y antigen-mediated mammalian testis differentiation (or avian ovary differentiation) (Ohno, 1976; Silvers & Wachtel, 1977; Hazeltine & Ohno, 1981) also seems unlikely in view of our results and similar results produced in mammalian species. Thus the search for the mechanism governing primary sex differentiation of the gonads must now be limited to systems capable of operating primarily in the somatic cells.

This study was supported in part by a grant-in-aid of research from Sigma Xi, and a UCD research grant. The authors are most grateful to Dr Gregory F. Erickson of the University of California, San Diego Medical School for providing many useful ideas as well as laboratory facilities for portions of this work. In addition we would like to thank Mr Bob Munn for performing the electron microscopy and Dr Jörg T. Epplen for reading the manuscript.

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(Received 9 September 1981, revised 27 October 1981)