Evolution of the lining bodies in the embryonic chick gonad

By KULDEEP S. RAHIL and ROBERTO NARBAITZ

From the Department of Histology and Embryology, University of Ottawa

SUMMARY

Gonads from White Leghorn chick embryo of various ages ranging from 4 to 10 days of incubation, were fixed in half-strength Karnovsky’s fixative, osmicated and embedded in Araldite. Thin sections were stained with uranyl acetate and lead citrate. Our observations were especially concentrated on the germinal epithelium.

Two cytological characteristics not previously described in embryonic chick gonads were observed:

(1) A network of fine microfilaments was present in the somatic cells of the germinal epithelium. These microfilaments were especially concentrated in the apical pole of these cells and appeared to be more numerous in 5- and 6-day gonads. Since similar microfilaments have been described in other embryonic epithelia in which growth of glands or cords is taking place, their presence in the embryonic gonad may be related to the formation of sex cords.

(2) Lining bodies, organelles which are characteristic of follicular cells in the adult ovary, were found in the embryonic gonads of both sexes. They first appeared in some of the 5-day embryos and were present thereafter in all 6-day left gonads, in all left ovaries and in those left testes in which regression of the germinal epithelium had not been completed. Lining bodies were never observed in right germinal epithelia. It is classically accepted that follicular cells of the adult originate from the somatic cells of the germinal epithelium. The presence of lining bodies in the epithelial cells of young embryos appears to indicate that, despite the fact that follicles are not formed until after hatching, follicular cells initiate their differentiation much earlier. The fact that this differentiation is also present in gonads of male embryos can be interpreted in the light of current theories on sex differentiation which assume the bisexuality of embryonic gonads.

Short cytoplasmic processes, representatives of a merocrine secretory process, have been described by other authors in the germinal epithelium of young embryos. We confirmed these observations and found, in addition, that these processes were smaller, both in size and number, in the right gonads. This fact, together with the previously mentioned absence of lining bodies in right gonads, appears to be the expression, at the ultrastructural level, of the well known asymmetry of the avian embryonic gonads.

INTRODUCTION

The name of lining bodies was given by Bellairs (1965) to a type of organelle found in the ovaries of adult hens and previously described in the literature under other names (Schjeide & McCandless, 1962; Press, 1964). These rounded, ovoid or horseshoe-shaped organelles are located at the surface of follicular cells.

Authors' address: Department of Histology and Embryology, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada.
or, less frequently, in the cytoplasm of oocytes. They are formed by two concentric membranes, the inner one being much thicker. A row of large granules is found attached to the inner membrane. A third membrane surrounds the whole structure when located inside oocytes.

Schjeide & McCandless (1962) suggested that these organelles represent initial steps in the formation of mitochondria while Press (1964) postulated that their function is to stimulate the growth of cytoplasmic membranes in the oocyte. On the other hand, Wyburn, Johnston & Aitken (1965) and Bellairs (1965) simultaneously proposed that lining bodies may represent a morphological expression of the transport of material from follicular cells to the oocyte, material which would be utilized for yolk synthesis. The last mentioned author (Bellairs, 1965) even suggested that these ovarian organelles might be present in other vertebrates producing yolk-rich eggs. This suggestion has been supported by Hubert (1971) who found structures somewhat similar to lining bodies in the ovaries of the lizard *Lacerta vivipara* Jacquin.

Greenfield (1966) found that small lining bodies are already present in the ovaries from newly hatched chicks, and this observation was confirmed by one of us (Narbaitz, 1971). Preliminary observations at our laboratory showed, in addition, that lining bodies are also present before hatching. We decided to conduct the present electron microscopic study on the embryonic chick gonads with the hope that the exact knowledge of the evolution of these organelles might help to understand their functional role during the early stages of development.

**MATERIALS AND METHODS**

Four-, 5-, 6-, 8- and 10-day-old White Leghorn chick embryos were used in our study. Left gonads were fixed separately and at least 10 from each age group were studied: right gonads were only studied in 6- and 8-day embryos. No effort was made to establish the sex of gonads from embryos six days old and younger.

Gonads were fixed in cold half-strength Karnovsky’s fixative (Karnovsky, 1965) for 6 h, washed with 0.1 M phosphate buffer at pH 7.4 containing 0.2 M sucrose, post-fixed in 1% osmium tetroxide, and embedded in Araldite. One-μm sections were stained with toluidine blue; thin sections were stained for 40 min with a saturated solution of uranyl acetate in 50% alcohol and with lead citrate according to Reynolds (1963) and studied with a Philips 300 electron microscope.

**RESULTS**

*Four-day gonads.* On the fourth day of incubation, the germinal epithelium contained few, large, germ cells and more numerous, smaller, somatic epithelial cells; our study was concentrated on the latter. Epithelial cells were connected at their apical end by typical junctional complexes (Fig. 1). Short and thick cellular processes emerged from their apical surfaces; their nuclei were oval
The lining bodies of chick embryos

Fig. 1. Apical portion of epithelial cells from a 4-day left gonad. Lipid droplets (L), rough endoplasmic reticulum, mitochondria, microfilaments, apical cellular processes and a junctional complex (arrows) are shown.

Fig. 2. Germinal epithelium from a 5-day left gonad. Network of fine filaments can be seen.
Figs. 3–5. Three stages in the evolution of lining bodies in the 6-day left gonads. In Figs. 3 and 4, lining bodies are seen attached to the cell of origin, while Fig. 5 shows a lining body incorporated into another cell and has a third membrane (arrow).

Shaped and their cytoplasm contained well-developed Golgi complexes, mitochondria with a dense matrix, many free polyribosomes and also numerous profiles of rough endoplasmic reticulum. Lipid droplets were a common occurrence. Bundles of microfilaments were seen in connexion with the desmosomes of the junctional complexes and, in addition, a network of fine microfilaments was found distributed through the whole cytoplasm but with special abundance at the apical pole of the cells.

Five-day gonads. During the fifth day of incubation, the microfilaments in the epithelial cells increased both in number and in size (Fig. 2). Microtubules were occasionally found among them. Small lining bodies appeared for the first time at this age, but only in a few of the gonads examined.

Six-day gonads. Fifteen left gonads of this age were examined and all of them had lining bodies in different stages of evolution. Figs. 3–5 show lining bodies from gonads of various ages and have been grouped to illustrate the changes occurring during their evolution. The smaller lining bodies (Fig. 3) were generally crescent-shaped; they were usually embodied in the plasma membrane of the epithelial cells and measured between 0.10 and 0.15 μm in length. Medium-
Fig. 6. Epithelial cells from a 6-day left gonad. Lining bodies engulfed by epithelial cells are shown (LB). Those at the left are incorporated into a lysosome-like body. An active Golgi complex is also shown.

Fig. 7. Epithelial cell from an 8-day left testis. A lining body (LB) and many microtubules can be seen.
size lining bodies (Fig. 4) were horseshoe-shaped and were either attached to the plasma membrane of epithelial cells or located free in the intercellular space. Finally, larger lining bodies (Figs. 5, 6) were rounded or oval-shaped, and were contained in the cytoplasm of epithelial cells, where they always appeared surrounded by a third thin membrane; they probably represent organelles formed by one epithelial cell and engulfed by another. We never observed lining bodies incorporated in the cytoplasm of oocytes.

In contrast with the regular presence of lining bodies in all left gonads observed, not a single one of these organelles was found in the ten right gonads studied. In addition to this, apical cytoplasmic processes described in the epithelial cells of 4- and 5-day gonads were still present at this age, being clearly shorter and less numerous in the right gonads. Microfilaments were also present at this age, but we were unable to establish differences in their distribution between left and right side.

Eight-day gonads. Left ovaries from 8-day embryos showed a very thick cortex, the number of both epithelial and germ cells having increased greatly. Cytoplasmic characteristics of epithelial cells were similar to those described for earlier ages; the number of microfilaments had, however, decreased, while lining bodies had increased both in size and in number. A careful search for lining bodies in other regions of the gonad was conducted and not a single one of these organelles was found in the medullary zone.

The cortical zone of right ovaries from 8-day embryos showed characteristics which were similar to those described for 6-day embryos. Left testes of this age showed a very thin germinal epithelium consisting practically only of epithelial cells. These cells contained few microfilaments and microtubules, and the number of profiles of rough endoplasmic reticulum had also decreased greatly. Lining bodies in different stages of development were, however, constantly seen (Fig. 7).

Ten-day gonads. While the cortex of left ovaries had continued to grow, the germinal epithelium of right ovaries was now reduced to a squamous layer. The cortex of left testes had also diminished in thickness. However, in those places in which it kept its original thickness, lining bodies were still observed.

DISCUSSION

Several electron microscope studies on various aspects of the differentiation of the chick embryo gonads are available in the literature (Simone Santoro, 1965; Narbaitz & Adler, 1966; Dubois & Cuminge, 1967; Cuminge & Dubois, 1969, 1971). Our observations on the germinal epithelium of 4- and 5-day embryos agree in general with those of Cuminge and Dubois (1969, 1971). These authors described characteristic short processes which they called ‘boursoufles’ and which, emerging from the somatic cells of the epithelium would represent a secretion process of merocrine type. According to the above-
The lining bodies of chick embryos

mentioned authors these cellular processes are eliminated at first at both poles of the cells, while in 4- and 5-day embryos, a basement membrane is formed at the basal pole and secretion stops at this site. Our present findings show that 'boursouflures' are present also in older embryos. We have thus found them at the apical pole of epithelial cells from all 6-day gonads and in both ovaries and testes of 8-day embryos. In addition, we have found that the distribution of these cellular processes is asymmetric, being larger both in size and in number in left gonads.

A network of fine microfilaments, especially concentrated at the apical poles of epithelial cells has been observed by us. Similar microfilaments have been described in other embryonic cells as being contractile and playing a role in the changes of cell shape that accompany several morphogenetic processes (see review by Wessels et al. 1971). Their presence in the embryonic gonad may be related to the formation of sex cords, but we have no experimental evidence to back this assumption.

We believe that our most interesting observations refer to the presence and evolution of lining bodies in the embryonic gonads. We have established that these organelles appear for the first time in 5- and 6-day embryos, and are thereafter present in all left ovaries and in all those left testes in which the germinal epithelium has not completed its atrophy. Lining bodies, were, on the contrary, consistently absent in all right gonads examined.

The production of lining bodies is one of the characteristics of the differentiated follicular cell (Bellairs, 1965; Wyburn et al. 1965). Since follicular cells are derived from the somatic cells of the germinal epithelium, the presence of lining bodies in the epithelial cells of 5- and 6-day embryos appears to indicate that the initial steps in the differentiation of follicular cells begin at this early age. The fact that this differentiation starts in the germinal epithelium of male gonads, as well as in those of females, can be understood in the light of the classical view that maintains that undifferentiated gonads are bisexual in nature and that, in embryos of both sexes, the cortex is potentially female and the medulla potentially male (see review by Burns, 1961). Our finding that lining bodies are not formed by the germinal epithelium of the right gonads, together with the previously commented unequal distribution of 'boursouflures' are, at the ultrastructural level, expressions of the asymmetry of embryonic avian gonads, which has been repeatedly described and analysed experimentally (Wolff & Pinot, 1961).

The authors wish to thank Dr Leonard F. Bélanger for reading the manuscript and making important suggestions and Dr Sohan S. Jande for advice in technical electron microscope problems.
REFERENCES


(Manuscript received 3 January 1972)