CYCLIC VARIATIONS IN SERTOLI CELL LIPID CONTENT THROUGHOUT THE SPERMATOGENIC CYCLE IN THE RAT

J. B. KERR AND D. M. DE KRETSE}

Department of Anatomy, Monash University, Melbourne, Victoria 3168, Australia

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Summary. The formation, distribution and fate of lipid inclusions within the seminiferous tubules of the rat has been studied throughout the spermatogenic cycle. The occurrence of lipid inclusions within the Sertoli cell exhibited cyclic variation with the stages of the rat seminiferous cycle. At stage 9 of the cycle, residual bodies of maturing spermatids were phagocytosed by the Sertoli cell and released numerous lipid droplets which appeared to coalesce into large inclusions at the base of the Sertoli cell at stage 10. The Sertoli cell lipid inclusions persisted throughout the completion of meiosis (stages 11 to 14) and the formation of young spermatids (stages 1 to 2) and their numbers appeared to reach a peak at stages 12 to 13 of the cycle. The inclusions decreased markedly within the Sertoli cell cytoplasm during stage 2 and remained low until stage 9 when lipid from the residual bodies again became available to the cell. This cyclic variation of lipid inclusions within the Sertoli cell does not support previously held views that there is a gradual decline in Sertoli cell lipid during stages 10 to 14 of the spermatogenic cycle. A hitherto unnoticed finding was the presence of large lipid inclusions in the cytoplasm of late pachytene to diakinetic spermatocytes, and some observations suggest a transfer of these lipid inclusions from the Sertoli cells to primary spermatocytes.

INTRODUCTION

Previous investigations into the distribution and fate of lipid inclusions throughout the cycle of the seminiferous epithelium have yielded conflicting results. The limited histochemical data of Lacy (1960) and Niemi & Korman0 (1965) were based upon frozen or gelatin-embedded testicular tissue which gave unsatisfactory preservation of the seminiferous epithelium, and the brief nature of their observations failed to establish the rôle of Sertoli cell lipid during spermatogenesis. Both reports suggested that following phagocytosis of the residual bodies at stage 9 of the spermatogenic cycle (Leblond & Clermont, 1952), large lipid inclusions appeared in the cytoplasm of the Sertoli cell. As spermatogenesis proceeded through the final phases of meiotic maturation (stages 10 to 14), they reported that lipid inclusions gradually disappeared from the Sertoli cells and
became minimal by the end of stage 14 when a new generation of haploid spermatids is formed. Lacy (1960, 1962) postulated that during the final phases of meiosis (stages 10 to 14), the diminishing numbers of lipid inclusions in the Sertoli cells were associated with the synthesis of a Sertoli cell hormone which in turn acted as a local stimulus for the maturation of primary spermatocytes into spermatids. Posalaki, Szabó, Bácsi & Ókrös (1968), however, obtained improved preservation of the seminiferous epithelium and their histochemical data indicated that Sertoli cell lipid inclusions do not diminish after stage 9, but persist at a constant level throughout stages 10 to 14. The results of Posalaki et al. (1968) are contrary to those of Lacy (1960) and incompatible with his view that the signal for meiosis relies on the metabolism and depletion of lipid-based substrates within the Sertoli cell during stages 10 to 14.

One of the major problems in defining the distribution and fate of lipid during the spermatogenic cycle is the limited resolution obtained with the histochemical staining of gelatin sections or frozen testicular tissue. Although the histochemical tests used in previous reports have detected lipid inclusions, satisfactory preservation of the seminiferous epithelium is difficult, usually poor, prevents adequate identification of the stages of the cycle and at times prohibits the accurate cellular localization of lipid inclusions. On this basis, histochemical techniques alone have given inconclusive and conflicting results in earlier reports. While ultrastructural studies overcome the problem of localization, the limited quantity of tissue examined prevents adequate description of any fluctuations in lipid content within the spermatogenic cycle. To overcome these difficulties, tissue perfused and embedded for electron microscopy has been sectioned at 0.5 to 1 μm and examined with high-resolution light microscopy. Histochemical tests were employed to demonstrate lipid inclusions qualitatively within the seminiferous epithelium but, unlike previous studies, this technique was not used to establish the accurate cellular localization of lipid at specific stages of the cycle. This report describes the results of the re-examination of the formation, distribution and fate of lipid inclusions in the spermatogenic cycle of the rat.

MATERIALS AND METHODS

Adult male Sprague–Dawley rats used in this study were prepared for perfusion fixation with an intravenous injection of heparin (5000 i.u. in 1 ml) and an intraperitoneal injection of 0.5 ml Novocain (2% solution). Under ether anaesthesia, the vascular system was flushed clear with a balanced saline solution (Christensen, 1965) through a cannula inserted through the left ventricle into the ascending aorta. As the testicular arteries became free of blood, a mixture of buffered glutaraldehyde, formaldehyde and trinitroresol or trinitroresorcinol (Ito & Karnovsky, 1968) was introduced at two-thirds strength. Perfusion continued for 20 to 30 min, after which the hardened testis was cut into thin pieces and immersed for a further 2 to 3 hr in the same fixative. Small pieces of tissue approximately 1 mm³ were washed overnight in buffer, post-fixed in osmium tetroxide, stained en bloc with uranyl acetate and, following dehydration, they were embedded in a 1:1 mixture of Epon and araldite. Sections, 0.5 to 1 μm
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in thickness, were stained with Toluidine blue and photographed with a Leitz Ortholux microscope using monochromatic light of 550 nm.

Preliminary ultrastructural observations were recorded from thin sections cut on a Reichert OmU3 ultramicrotome and examined with an Hitachi HU-11E electron microscope.

Microscopy and lipid histochemistry

Although the characteristics of lipid inclusions in material prepared for electron microscopy have been defined (Stein & Stein, 1971), histochemical methods were used to verify that the inclusions staining blue to black with Toluidine blue were in fact composed of lipid.

The presence of lipid inclusions within the seminiferous epithelium was confirmed using a variety of methods. The improved resolution obtained with thick plastic sections facilitates the identification of lipid material within the Sertoli cells and germinal cells. Epon–araldite sections (1 µm) were mounted onto glass slides (Grimley, Albrecht & Michelitch, 1965) and lipid inclusions were histochemically demonstrated with the use of Sudan black B dissolved in propylene glycol (Pearse, 1968). The small deposits of lipid material were clearly revealed within the cytoplasm of the spermatid and residual body at stage 7 of the cycle (Pl. 1, Fig. 1), and the morphology and distribution of lipid inclusions so demonstrated were identical to those staining deep blue to black in Toluidine blue-stained sections (Pl. 1, Fig. 2). Sudan black B-positive lipid inclusions were readily identified within the basal aspects of the Sertoli cell cytoplasm (Pl. 1, Fig. 3) although the classification of the specific stage of the cycle was not attempted. The specificity of lipid-positive inclusions in plastic sections was verified by extracting formerly Sudan-positive material with an epoxy solvent according to the method of Eurenius & Jarvskär (1970). The sites of the extracted lipids appeared as empty vacuoles within the Sertoli cell after exposure to the epoxy solvent (Pl. 1, Fig. 4). Similar results have been demonstrated for lipid inclusions within the cytoplasm of the mouse Sertoli cell and interstitial cell (Lane & Europa, 1965).

Conventional fresh-frozen or formol-calcium fixed testicular tissue was cut at 8 µm in a cryostat and the sites of lipid inclusions within the Sertoli cells and germinal cells were demonstrated with 1% Fettrot and 0.7% Sudan black B dissolved in propylene glycol (Pearse, 1968). In addition, some frozen sections were stained with 1% buffered osmium tetroxide (Adams, 1965). Lipid inclusions stained pink-red after Fettrot and brown-black with Sudan black B or osmium tetroxide (Pl. 1, Figs 5 and 6). The localization of such lipid-positive inclusions within the Sertoli cells and germinal cells was identical to that seen in testicular tissue fixed for electron microscopy, embedded in plastic and stained with Toluidine blue.

Stages of the cycle of the seminiferous epithelium were identified according to the classification of Leblond & Clermont (1952).

RESULTS

Cyclic variations in the number of lipid inclusions in the Sertoli cells occurred during the spermatogenic cycle in the rat. The results of this study indicated
that the number of Sertoli cell lipid inclusions was low during the maturation of early spermatids in the first half of the cycle and it is, therefore, convenient to begin a description of the lipid cycle from stage 8 after which the Sertoli cells begin to accumulate numerous lipid inclusions in their cytoplasm.

Stages 8 to 9. Many spherical residual bodies were seen to line the luminal aspect of the seminiferous tubule and consisted of four basic components: basiphilic RNA aggregates, spherical clear vacuoles, dense lipid droplets and the excess cytoplasm of the spermatid (Pl. 2, Figs 7 and 8). In addition, small lipid inclusions were observed in the cytoplasm of early spermatids. During stage 9, the residual bodies were found within channels of Sertoli cell cytoplasm and were also present in the basal regions of the Sertoli cells (Pl. 2, Fig. 9). In this location, the residual bodies appeared to lose their well-defined margins and their lipid component was released, coalescing to form large lipid inclusions deep within the Sertoli cell.

**EXPLANATION OF PLATES**

**PLATE 1**

Fig. 1. Plastic section (1 µm) of rat testis stained with 1% Sudan black B. Individual lipid inclusions are present within the cytoplasm of the spermatids. The lipid component of the residual cytoplasm lines the luminal aspect of the epithelium (arrows). ×330.

Fig. 2. Same stage of the spermatogenic cycle as Fig. 1, but stained with 1% Toluidine blue. The localization of lipid inclusions within the residual cytoplasm and the spermatids is identical to that in Fig. 1. ×330.

Fig. 3. Plastic section (1 µm) of rat testis stained with 1% Sudan black B. Deeply staining lipid inclusions are present in the basal aspects of the Sertoli cells. An accurate classification of the stage of the spermatogenic cycle is not possible. ×330.

Fig. 4. Plastic section (1 µm) treated with an epoxy solvent was stained with 1% Sudan black B. The sites of extracted Sertoli cell lipid appear as empty vacuoles (arrows). ×330.

Fig. 5. Formol-calcium fixed perfused rat testis tissue which was frozen and stained with 1% Pettrot. Densely staining lipid inclusions appear within the Sertoli cells or within the residual cytoplasm of adjacent seminiferous tubules. ×130.

Fig. 6. Fresh frozen rat testis tissue stained with 1% buffered osmium tetroxide. Sites of lipid inclusions appear as black deposits. ×130.

**PLATE 2**

Figs 7 to 11 are of plastic sections (0.5 to 1 µm) of rat testis stained with Toluidine blue.

Fig. 7. Spherical residual bodies line the lumen of the seminiferous tubule at stage 8. The Sertoli cells do not contain large lipid inclusions. ×270.

Fig. 8. Aggregations of individual lipid inclusions (arrows) are present within the mature residual bodies. The lipid component of the spermatid cytoplasm appears as small deeply staining inclusions (arrow heads). ×870.

Fig. 9. During stage 9 of the cycle, the residual bodies are phagocytosed deep within Sertoli cell cytoplasm and can be distinguished from lipid inclusions by the loss of their well-defined spherical margins. ×300.

Fig. 10. At stage 10, it appears likely that the coalescence of many small lipid inclusions from the phagocytosed residual bodies forms large lipid inclusions deep within the Sertoli cell (arrows). ×160.

Fig. 11. Sertoli cell lipid inclusions become conspicuous towards the end of stage 10, occupying a position adjacent to the basement membrane of the tubule. S, Sertoli cell; L, lipid inclusions. ×870.

**PLATE 3**

Fig. 12. Low power survey micrograph of a stage 11 tubule in a rat testis illustrating the number and size of Sertoli cell lipid inclusions. Toluidine blue, ×320.

Fig. 13. By stage 12 of the cycle, the Sertoli cell lipid inclusions appear large and more numerous than in the preceding stages of spermatogenesis in the rat. Toluidine blue, ×320.
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Stages 10 to 11. As spermatogenesis proceeded, phagocytosis of the residual bodies was completed by stage 10, at which time large lipid inclusions lay free within the Sertoli cell cytoplasm (Pl. 2, Figs 10 and 11). These inclusions became even more conspicuous during stage 11 of the cycle (Pl. 3, Fig. 12).

Stages 12 to 14. The lipid inclusions appeared to reach their largest proportions in number and size during stages 12 to 13 (Pl. 3, Fig. 13 and Pl. 4, Fig. 14). As the meiotic maturation of the spermatocytes neared completion at stage 14, many large lipid inclusions persisted deep within the Sertoli cell cytoplasm (Pl. 4, Fig. 15) and the lipid content of Sertoli cells at this stage did not reach a particularly low level. A feature of these stages of the seminiferous cycle was the observation of large lipid inclusions apparently transitional between the Sertoli cytoplasm and the primary spermatocytes (Pl. 4, Fig. 16). Other observations revealed the presence of large lipid inclusions in the cytoplasm of pachytene, diplotene-diakinetin and dividing primary spermatocytes (Pl. 4, Figs 17 and 18). Examination of appropriate thin sections with the electron microscope confirmed the location of lipid inclusions within the cytoplasm of premeiotic primary spermatocytes (Pl. 4, Fig. 19).

Stages 1 to 7. Many large Sertoli cell lipid inclusions were evident throughout the division of secondary spermatocytes into a population of early spermatids at

EXPLANATION OF PLATES

PLATE 4

Figs 14 to 18 are of plastic sections (0.5 to 1 μm) of rat testis stained with Toluidine blue.

Fig. 14. Numerous lipid inclusions persist within the Sertoli cell as spermatogenesis progresses to stage 13 of the cycle. It is at this and the preceding stage that Sertoli cell lipid inclusions appear to reach a peak in number and size. ×120.

Fig. 15. Stage 14 of the spermatogenic cycle is identified by the older primary spermatocytes undergoing the first meiotic division. Large lipid inclusions are present deep within Sertoli cell cytoplasm (arrows). ×270.

Fig. 16. During stage 13, some Sertoli cell lipid inclusions appear to be transitional between the Sertoli cell and the cytoplasm of the primary spermatocytes. L, lipid inclusions. ×870.

Fig. 17. As meiotic division continues during stage 14 of the cycle, lipid inclusions may also be observed within the dividing primary spermatocyte cells (arrow), while other lipid inclusions remain within the Sertoli cell cytoplasm. L, lipid inclusions. ×870.

Fig. 18. Although many lipid inclusions are situated within the basal regions of the Sertoli cell during stage 12, favourable sections reveal the presence of large lipid inclusions within the cytoplasm of late pachytene-diplotene primary spermatocytes (arrows). L, lipid inclusions. ×870.

Fig. 19. An electron micrograph illustrating the location of a large lipid inclusion within the cytoplasm of a late pachytene spermatocyte. ×2900.

PLATE 5

Fig. 20. Low-power survey micrograph illustrating three adjacent seminiferous tubules in a rat testis (plastic section, 0.5 to 1 μm thick) at stage 10, 13 and 1 of the cycle. Large Sertoli cell lipid inclusions are present at each stage (arrows). The lipid content of Sertoli cells at stage 1 has not declined relative to previous stages of the cycle. Toluidine blue, ×200.

Fig. 21. The large lipid inclusions of the Sertoli cell disappear by stages 2 and 3 of the cycle. The Sertoli cell cytoplasm now contains many smaller dense inclusions, probably lysosomes and mitochondria. Small dense inclusions within the spermatid cytoplasm (arrows) are lipid droplets which form the lipid component of the residual cytoplasm later in the cycle. Toluidine blue, ×500.

Inset: Spermatids and residual cytoplasm stained histochemically with Sudan black B to reveal lipid inclusions within spermatid and residual cytoplasm.
stage 1. A comparison of adjacent seminiferous tubules at stages 10, 13 and 1 of the spermatogenic cycle (Pl. 5, Fig. 20) illustrated that the lipid content of the Sertoli cells at stage 1 did not decline below the levels found during stage 10 or 13 of the cycle. As spermatogenesis progressed beyond stage 1, the Sertoli cell lipid inclusions disappeared and were not seen during stages 2 and 3 of the cycle (Pl. 5, Fig. 21). The small lipid inclusions observed within the spermatid cytoplasm at stages 2 or 3 formed the lipid component of the excess residual cytoplasm at stage 7. The lipid inclusions present in primary spermatocytes during stages 12 to 14 were not observed subsequently.

DISCUSSION

The value of the techniques used in this study has been shown by the demonstration of large lipid inclusions in primary spermatocytes late in the meiotic prophase, an observation hitherto unnoticed by other investigators. While transfer of such inclusions between the Sertoli cell and primary spermatocytes appears likely in some 1 µm sections, this phenomenon requires further ultrastructural confirmation. The rôle of such a transfer is not clear but it may be involved in the local regulation of the spermatogenic cycle.

In the rat, the local co-ordination of spermatogenesis by the Sertoli cell has been proposed by Lacy (1960, 1962) who suggested that a cyclic supply of lipid from the residual bodies to the Sertoli cell serves as a substrate for steroid biosynthesis in this cell. Biochemical studies by Christensen & Mason (1965), Hall, Irby & de Kretser (1969) and Bell, Vinson & Lacy (1971) have shown that isolated rat seminiferous tubules are capable of steroid conversions and the Sertoli cell has been suggested as the site of this activity. Lacy (1962) has proposed that a decline in the number of Sertoli cell lipid inclusions before meiosis corresponds to the production of a signal which promotes meiotic maturation during stages 10 to 14 and thus regulates the cycle. While the present results have shown that the lipid content of the Sertoli cell is not depleted over these stages (see Lacy, 1960; Niemi & Korman, 1965), the suggested incorporation of lipid inclusions into premeiotic spermatocytes supports the hypothesis of Collins & Lacy (1969) that these spermatocytes may utilize Sertoli cell lipid inclusions to complete the meiotic process.

The present observations suggest that although the phagocytosed residual bodies probably release their lipid content into the Sertoli cell during stage 9 of the cycle, the relative number of lipid inclusions within the Sertoli cell appears to increase as spermatogenesis proceeds through the meiotic maturation phases (stages 10 to 14). It seems likely therefore that the increase in lipid content following stage 10 may represent new synthesis of lipid by the Sertoli cell, but the techniques used in this study and those used by Lacy (1960) have not permitted further elucidation.

An alternative rôle for the cyclic variation of the numbers of lipid inclusions in the Sertoli cell may be in the control of FSH secretion from the pituitary. The variation in the number of lipid inclusions in the Sertoli cell has been shown to be related to the seminiferous cycle and serum FSH levels have also been shown to be related to the germinal cell complement, particularly spermatogonial.
numbers (de Kretser, Burger & Hudson, 1974). Irradiation and local heat applied to the testis are known to disrupt spermatogenesis and cause an increase in the lipid inclusions in the Sertoli cells of the rat (Lacy, 1960; Lacy & Rotblat, 1960; Collins & Lacy, 1969), and to be associated with elevated levels of FSH (Swerdloff, Walsh, Jacobs & Odell, 1971), presumably resulting from a decrease in the feedback signal from the testis. It is conceivable that the lipid increase may result from the decreased utilization of substrate necessary for elaboration of the feedback signal. This is further supported by the observation that, in the rat, resumption of spermatogenesis is associated with a decrease in the number of lipid inclusions, and, in man, resumption of spermatogenesis after irradiation is accompanied by a fall in FSH levels (Lacy & Lofots, 1965; Collins & Lacy, 1969; C. A. Paulsen, personal communication). The changes in lipid content may directly represent substrate stores but, if not, they may reflect variations in the synthetic mechanisms of the Sertoli cells involved in elaborating the feedback signal.

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J. B. Kerr and D. M. de Kretser


