THE ARRANGEMENT OF GERM CELLS IN THE RAT SEMINIFEROUS TUBULE: AN ELECTRON-MICROSCOPE STUDY

P. B. MOENS AND A. D. HUGENHOLTZ
Department of Biology, York University, Downsview, Ontario, Canada

SUMMARY

The spermatogonia and early spermatocytes of 13 samples of rat seminiferous epithelium (about 0.05 mm$^2$ each) were mapped from electron micrographs of serial sections. Clones of cells, connected by cytoplasmic bridges (syncytiot of 2–100 cells), in various stages of spermatogenic development were identified. Maps of 7 separate areas are illustrated. It is concluded that, contrary to the models of spermatogonial proliferation based on light-microscope observations, regions of seminiferous epithelium which are identical in terms of spermatid and spermatocyte criteria have, in fact, quantitative and qualitative differences in their spermatogonial population. The data are interpreted that for a given epithelial area there is a periodic build-up of spermatogonia which then produce several successive quanta of spermatocytes and when the spermatogonia are depleted the process repeats.

That cell numbers less than double following a mitotic cycle has generally been attributed to systematic degeneration. Evidence from electron microscopy indicates, however, that at the mitotic peaks not all the syncytia undergo division but that some remain arrested. Similarly, within a dividing syncytium a few cells do not divide while they advance developmentally with the syncytium as a whole. The observed large size of spermatocyte syncytia further argues against systematic degeneration with its attendant fragmentation of syncytia.

INTRODUCTION

In animals with cystic spermatogenesis, such as many insects and some vertebrates, the numerical analysis of spermatogenesis is relatively simple. Once a germ cell enters the spermatogenic pathway, it becomes enclosed in a membrane and the products of subsequent divisions accumulate within a membrane-bound cyst. Eventually there are, for example, 64 synchronously developing spermatocytes within a cyst which will yield a bundle of 256 spermatozoa. Cystic spermatogenesis, however, has not been reported for mammals and the numerical analysis has been complex and frequently controversial.

In most animals, insects and mammals alike, the dividing germ cells remain connected by cytoplasmic bridges which persist through successive cell divisions (Dym & Fawcett, 1971) and thus clones of interconnected cells are formed. In cystic spermatogenesis each cyst contains such a clone of interconnected cells but in mammalian spermatogenesis the clones are not as clearly delimited. However, when rat seminiferous epithelium is examined through 3-dimensional reconstructions from electron micrographs of serial sections, the clones can be recognized and mapped (Moens & Go, 1971).
We report here an attempt to analyse rat spermatogenesis through the identification of clones of interconnected germ cells at various stages of development.

**MATERIALS AND METHODS**

Male Wistar strain rats weighing 350-450 g were killed with ether and one of the testes was infused with 5% glutaraldehyde buffered in N-collidine at pH 7.4 for 30-45 min. Release of seminiferous tubules from the testis prior to fixation causes dislocation of cells within the tubule. Tubules were dissected out carefully and left in fresh fixative for 30 more min. The tubules were washed for 45 min in buffer. Postfixation was done at room temperature in 2% osmium tetroxide in buffer for 1 h. The tissue was dehydrated in a graded ethanol and propylene oxide series and embedded in Epon. Small segments of tubules were sectioned tangentially starting at the surface of the tubule (Fig. 2 A). Series of 300-600 consecutive sections of 100 μm thickness were collected and mounted on single-hole Formvar-coated grids. The material was stained in uranyl acetate followed by lead acetate. Each grid had 10-12 sections, about 0.4 mm long and 0.2 mm wide. A low-magnification electron micrograph (about ×75) was made of every 10th section and these prints were used as maps on which all cells were identified by number and the bridges between them marked when observed with the electron microscope (Fig. 2 B). Completed maps contain the type of information shown in the Figs. 2, 3, etc. Cells within the area bordered by the solid lines have been examined in their entirety and all cytoplasmic bridges are recorded. Each bridge is visible in 10 or more successive sections, and occasional missing sections are therefore not critical and do not affect the results.

The method used to estimate the number of division cycles required to produce an observed syncytium is demonstrated in Fig. 2 C. Starting with the observed syncytium, as many cells as possible are paired off and each pair is represented by a single cell. The result is an estimate of the syncytium preceding the observed one by one division cycle. By repeated reductions the observed syncytium can be reduced to a single cell. The number of operations is a minimum estimate of the number of division cycles required to produce the observed syncytium from a single cell.

The number of syncytia in a given area can be counted directly where the syncytia are smaller than the area examined, and the numbers can be converted into a per unit area estimate. The larger syncytia extend past the limits of the area examined and the numbers can only be estimated.

**Terminology**

**Syncytium.** Germ cells which are connected by cytoplasmic bridges form a group commonly referred to as a syncytium (Dym & Fawcett, 1971). Technically it is a contradiction to speak of the cells of a syncytium inasmuch as the term syncytium implies a multinucleate cytoplasm. The identity of spermatogonia, spermatocytes, and spermatids is, however, sufficiently real for them to be usually referred to as cells.

Single and twin spermatogonia are referred to as precursor cells or potential syncytia. They divide regularly and produce new single cells and new syncytia. Spermatogonia which remain connected by cytoplasmic bridges through successive mitotic cycles are said to have entered the spermatogenic developmental pathway. The syncytium is considered the logistic unit in rat spermatogenesis. In these terms syncytia of 4-80 cells represent single items at different stages of development. The number of spermatocyte syncytia produced per unit area is referred to as the quantum of syncytia for that area.

**Cytoplasmic bridge.** There are cytoplasmic connexions between pairs of germ cells, so that the cell membranes of the 2 cells are continuous. The specialized portion of the connecting membranes is referred to as the cytoplasmic bridge. Under favourable conditions, the bridges can be observed with the light microscope and J. H. McGregor, in 1899, working on spermatogenesis of the urodele *Amphiuma* postulated that 'the "bridges" are to be recognized as remnants of central spindles and when a cell contains two or more bridges, each represents a past mitotic division'. This thesis has been verified from electron-microscope studies (Dym & Fawcett, 1971; King & Akai, 1971; MacKinnon & Basrur, 1970; Mahowald, 1971). In no case have
bridges been reported to result from cell fusion. Accordingly, syncytia are always branching structures and do not contain closed loops of cells.

Stages of the seminiferous epithelium. Cross-sections of rat seminiferous tubules usually have four concentric layers of germ cells (Fig. 2a). For example, at stage I, the peripheral layer consists of spermatogonia, the next layer is composed of early spermatocytes, and more toward the lumen are 2 layers of spermatids. The layers pass through successive developmental stages in concert, so producing the characteristic 'cellular associations' (Von Ebener, 1888). Leblond & Clermont (1952) recognize 14 stages and these are indicated diagrammatically in Fig. 1. A vertical line drawn through a given stage, e.g. line (Aa) through stage I, indicates the cell types existing at that stage. Spermatids undergo the most pronounced morphological changes and they have traditionally been used to identify the different stages of development. Mitotic divisions of the spermatogonia occur at stages I, IV, VI, IX, XII, and XIV (Clermont, 1972) and they have been indicated by M in Fig. 1.

Germ cell morphology. The developmental stage of each area was determined from spermatid and spermatocyte fine-structural morphology (Susi, Leblond & Clermont, 1971; Solari, 1970). The ultimate spermatogonia (B-spermatogonia) are identified by their small size, 8-10 μm diameter, densely staining nuclei (Dym & Fawcett, 1971) and large branching syncytia. They are difficult to distinguish from their immediate product, the preleptotene spermatocytes. The spermatogonia of the smaller syncytia are designated as (1) type A, which have large cells (14-16 μm) and are less dense than the type B; (2) type pale A, with electron-transparent nuclei and cytoplasm; and (3) type dense A, which are identified as large, usually single cells attached to the basement membrane, with densely staining nuclei and cytoplasm. Type A and type pale A are probably the same kind of cell at different stages of the cell cycle.

RESULTS

Aa, stage I

The developmental stage I of this tissue is defined by the morphology of the spermatids in which the spherical Golgi apparatus is close to the nuclear envelope but does not as yet contain vesicles with proacrosomal granules and the head cap is not yet formed (Susi et al. 1971). The largest dimensions of the sectioned portion of the tubule were (in μm), base 310, top 208, width 193, with a surface of 5.00 x 10^4 μm^2. The area (Fig. 3 A, B) contained 51 spermatogonia; 2 single dense cells (a, b), one dense twin (c) and 47 less-dense cells of type A spermatogonia arranged in several chains. Chains d, e, f, h, and k have 2 or more cells, m has 4, n has 4 or more, p has 5 or more, r has 8 and q has 16 or more cells.

The same area (Fig. 3 B) contained 2 large, but incompletely mapped spermatocyte syncytia (C, D, 78 and 79 cells respectively) and 2 smaller groups of 11 and 14 cells. These are in the pachytene stage of meiotic prophase, identified by the presence of synaptonemal complexes in the nuclei (Westergaard & von Wettstein, 1973). Toward the centre of the tubule were spermatid syncytia which were not mapped.

On a per mm^2 basis there are 1020 spermatogonia. One hundred and twenty of these occur in 80 groups of singles or twins (a, b, c, h), 160 spermatogonia in an undetermined number of groups of 2 or more cells (d, e, f, k) while 740 spermatogonia are arranged in some 100 groups of 4 or more cells (m, n, p, r, and q). There are 3600 pachytene spermatocytes per mm^2 organized in no more than 40 syncytia (2 incompletely mapped spermatocyte syncytia C and D occupy most of 0.05 mm^2). The minimum number of cell division cycles to produce spermatocyte syncytium C from a single cell is 7 (Fig. 2 c) and it is 8 for syncytium D.
Several observations on this tissue suggest that it contains more spermatogonia and spermatogonial syncytia than are required to produce a single quantum of spermatocytes. Firstly, if all spermatogonia except a, b, and c produced spermatocytes after the next 3 mitoses (Fig. 1) there would be 384 spermatocytes rather than the observed 182. Secondly, syncytium m can form a maximum of 32 cells after 3 divisions, which is less than the number of cells in a spermatocyte syncytium. The transition, therefore, may occur at a later time. Thirdly, syncytia r and q would form overlapping spermatocyte syncytia, which is not an observed phenomenon. It is more likely that first q, and then, at the following stage VI, r becomes a spermatocyte syncytium. Finally, although the area produces about 2 spermatocyte syncytia at any one time, it contains 3 or 4 advanced spermatogonial syncytia (m, p, r, q), some other groups (d, e, f, k) and 4 potential syncytia (a, b, c, h). The area therefore has the potential to produce 3 or 4 more quanta of spermatocyte syncytia before the area becomes depleted. Position (Aa) in the diagram of Fig. 1B summarizes the germ cell composition of this tissue.

Ab, stage II

The maps for this area have been published previously (Moens & Go, 1972; Figs. 3 and 4). The spermatids in this area have a Golgi apparatus containing several vesicles with proacrosomal granules. Some of the vesicles are attached to the nuclear envelope but fusion of vesicles and granules has not yet taken place and there is no head cap. The area measures $4.5 \times 10^4 \mu m^2$ and contains 2 single spermatogonia, 2 groups of 6 spermatogonia, and incompletely mapped groups of 2, 3, 4, 5, 6, 7, 7, 10, and 13 spermatogonia. Most of the area was covered by 2 large spermatocyte syncytia of 57 and 78 cells. There were 55 spermatocytes belonging to partially observed syncytia along the borders of the area.

---

**Fig. 1. Diagrammatic representation of sperm production.**

A, a base population of spermatogonia continues while periodically some cells of the population enter the spermatogenic developmental pathway. Such cells pass through successively more advanced spermatogonial stages, then become spermatocytes, which pass through S-phase, S; leptotene, L; zygotene, Z; pachytene, P; and the meiotic divisions, Mei. Each spermatocyte produces 4 spermatids which undergo pronounced morphological changes, some of which have been sketched in. The developmental stages are indicated in Roman numerals and the meiotic peaks indicated by M (Clermont, 1972; Huckins, 1971b). Vertical lines connect the cell types found at the various stages.

B, whereas the model of A predicts that given stages have comparable spermatogonial populations, the observations in this report show quantitative and qualitative differences in the spermatogonia of stages which are identical in terms of spermatid and spermatocyte morphology. The diagram attempts to accommodate such differences in spermatogonial composition while leaving the general model of spermatogenesis unaltered. The thickness of a single bar represents about 40 syncytia. There are, for example, at (Ba) 40 single and twin spermatogonia (black bar at the bottom of the figure). Then there are 120 syncytia of more advanced spermatogonia which can supply 3 successive quanta of spermatocytes (broad black band). Next are the B spermatogonia which are just dividing to produce spermatocytes. Further towards the interior of the tubule are pachytene spermatocytes and next are 2 layers of spermatids, one early, one further developed.
On a per mm² basis there are 1666 spermatogonia arranged as 44 singles and some 130–150 groups of more than 2 cells. There are 4222 pachytene spermatocytes per mm² arranged in about 40 syncytia. The 2 observed spermatocyte syncytia can be produced from a single cell in a minimum of 7 division cycles.

The testicular material was fixed differently from the procedures reported in Methods. Among the defects caused by poor fixation were 2 broken intercellular bridges. The separated cells had sealed off at the site of breakage, with one of the cells carrying the cytoplasmic limb and the bridge (Moens & Go, 1972; Fig. 2). Similar configurations were observed in hormonally treated rats (see section Abnormal syncytia) and in spermatocytes artificially separated for sedimentation experiments.

The structure and numbers of syncytia are similar to (Aa) so that the same conclusions can be drawn about the numbers of spermatogonia and spermatogonial syncytia relative to the numbers of spermatocyte syncytia. The position of (Ab) in Fig. 1B is therefore next to (Aa).

**Ba, stage VI**

The stage is characterized by the mitotic activity of the ultimate (type B) spermatogonia. Accordingly, the spermatids have a large head cap, hc, over the nucleus with the Golgi apparatus, g, off-centre (Fig. 4c). The area examined measures in μm, base 387, top 303, width 145, that is 5.00 x 10⁴ μm² or 0.05 mm².

Most of the spermatogonia are type B which are about to divide judging by the condensed chromosomes, lack of a nuclear envelope, and the formation of lamellae in the cytoplasmic bridges (Dym & Fawcett, 1971). Syncytium q has 12 cells, s, 22 cells, p, 37 cells, and there are 24 cells in smaller groups. A number of syncytia with type A spermatogonia are undergoing division simultaneously (d, h, c, e, and smaller groups). For example, syncytium h has just increased from 4 cells to 8 cells, the midbodies are still present between the telophase cells (black circles, Fig. 4A) while one of the ‘old’ bridges (bridge from a previous division cycle) still has lamellae in it. The 3 upper cells of syncytium c have just divided to give 6 cells, while the lower 4 cells are still in metaphase. All old bridges have lamellae.

The cells of two syncytia (m = 11 or more cells, and n = 3 cells) are distinctly different from the B or the A spermatogonia. These ‘pale’ A spermatogonia are in interphase and give no indication of entering or of just having completed division. Finally there is one single cell (a) and a pair of dense A spermatogonia (b) which are not dividing. Further towards the lumen of the tubule are the spermatocyte syncytia shown in Fig. 4B. The larger syncytium (F) covers over half the total area and has 70 or more cells with smaller, incompletely mapped groups along the edges (A = 9, B = 12, C = 11, D = 12, E = 37, G = 8 and 10 cells in smaller groups), a total of 169 spermatocytes in 0.05 mm².

On a per mm² basis, there are 3380 spermatocytes which are arranged in no more than 40 syncytia. The largest syncytium has 70 cells and can be reconstructed from a single cell in 8 division cycles.

There are 71 dividing type B spermatogonia in the larger syncytia and 24 in smaller groups. After division these 95 cells will produce about 190 spermatocytes in 0.05 mm².
or 800 per mm$^2$, arranged in 40–60 syncytia. This conforms with the spermatocyte numbers observed in this and other sections. The 30 dividing type A spermatogonia will produce about 60 cells (in the absence of cell losses); on a per mm$^2$ basis this is 600 and 1200 cells respectively, arranged in some 80 syncytia.

Non-dividing syncytia consist of 11 pale A cells (m) and 3 pale A cells (n). This corresponds to 280 cells in about 40 syncytia per mm$^2$. Single and twin precursor cells amount to 60 per mm$^2$ in 40 potential syncytia.

The type A spermatogonia of syncytium ε are estimated to be passing through their 4th division cycle, producing 14 cells. Only 3 or 4 more division cycles are required to produce a spermatocyte syncytium. There are, however, 6 mitotic peaks before the area reaches the next stage VI. It seems therefore likely that the cells of the syncytium need not divide at all of the subsequent mitotic peaks. Fig. 4A gives direct evidence that at the mitotic peak of stage VI the B spermatogonia divide, some A spermatogonia divide (together about 90% of all spermatogonia) while some A spermatogonia and single and twin cells do not divide at the same time.

The information on the spermatogenic development of tissue (Ba) is summarized in Fig. 1b. Going from the periphery of the tubule towards the lumen there are, per mm$^2$, 40 groups of single or twin cells, some 40 syncytia of early type A spermatogonia, which are not dividing, some 80 syncytia of dividing type A spermatogonia, about 40 syncytia of dividing type B spermatogonia, at most 40 syncytia of spermatocytes and finally uncounted early and late spermatids. The 120 syncytia of type A spermatogonia are shown as a broad black band which will produce 3 successive quanta of spermatocytes.

**Bb, stage VII**

Two areas of seminiferous epithelium taken from different rats were similar in germ cell composition and they are grouped together here. The stage is characterized by the presence of advanced spermatocytes with synaptonemal complexes – not shown – and preleptotene spermatocytes. The spermatids are very similar to those shown for stage VI in Fig. 4c with the large head cap and the Golgi apparatus off-centre (Fig. 5c). Fine-structural evidence (microtubules, midbodies, and lamellae) suggests that the type of mitotic divisions shown in (Ba) has only just been completed in tissues (Bb-1) and (Bb-2). The respective areas are 2.85 x 10$^6$ μm$^2$ (Fig. 5A) and 3.10 x 10$^6$ μm$^2$ (Fig. 5B), a total of 5.95 x 10$^6$ μm$^2$. There are 276 preleptotene spermatocytes and 11 spermatogonial cells arranged in 4 single, 3 twins, and 1 triple.

On a per mm$^2$ basis there are 4640 spermatocytes. Because both areas are small relative to the size of spermatocyte syncytia, it is in this case difficult to estimate the number of syncytia per mm$^2$. The two large syncytia (64 and 65 cells, Fig. 5A, A, B) can be reconstructed from single cells in a minimum of 7 division cycles. There are 7 groups of single and twin cells in 0.06 mm$^2$ which corresponds to 120 groups per mm$^2$.

Although tissues (Ba) and (Bb) are at very nearly the same stage of development when judged by spermatid morphology and spermatocyte composition, they are notably different in composition of germ cells. The composition of (Bb) indicates that, unlike (Ba) all spermatogonia but the single and twin cells have become spermatocytes,
whereas in (Ba) a large population of advanced spermatogonial syncytia is still present at the end of stage VI. Comparisons between tissues (A) and (C) also suggest that similarities in spermatocyte composition and spermatid morphology of several separate tissue samples do not guarantee that the spermatogonial composition will also be similar in the samples. These differences involve small percentages in terms of cell numbers (10%) but large variation (factors of 1-2) in terms of syncytial numbers.

The position of tissue (Bb) in Fig. 1B reflects the proliferation of single and twin precursor cells, while all B spermatogonia have just become preleptotene spermatocytes. The presence of some 120 potential syncytia per mm\(^2\) indicates that several quanta of syncytia can be produced from this population.

C, stage XIII

At stage XIII the advanced spermatocytes are in the diplotene stage of meiosis. The nuclei contain separated lateral elements of the synaptonemal complex as single cores (Solari, 1970) (Fig. 6c). The mitochondria are distributed throughout the cytoplasm and have not yet moved to the periphery of the cell. The younger spermatocytes are in pachytene as judged by the completed chromosome pairing with synaptonemal complexes. The area examined measures in \(\mu\)m, base 225, top 154, and width 137, total of 2.66 x 10\(^4\) \(\mu\)m\(^2\). There are 106 early pachytene spermatocytes. The spermatogonia are arranged in one branched chain of at least 10 cells (h), one chain of at least 7 cells (f), one group of 4 dense cells (e), a pale A twin (d), a single pale A (c) and two single dense A cells (a, b). Chain f is unusual in that the last 3 cells are of the dark type which usually occur as single or twin cells.

The area contains about 4000 spermatocytes per mm\(^2\) but the size of the area is small relative to the size of the syncytium so that it is not feasible to estimate the numbers of syncytia per mm\(^2\). There are 2 groups of advanced spermatogonia, which corresponds to 80 syncytia or less per mm\(^2\) and the 7 single and twin cells count as 150 groups per mm\(^2\). The 4 dense A spermatogonia (e) are difficult to classify. The number suggests that the group is committed to the spermatogenic developmental pathway, but the morphology of the cells is of the type usually found in single and twin precursor cells.

The germ cell composition of this tissue is intermediate between (Bb) and (Ba) and it has been placed between the two in Fig. 1B. Spermatogonial syncytium h has undergone at least 4 division cycles and it, as well as f, could provide the next groups of spermatocytes at stage VII, following 4 more mitoses.

Abnormal syncytia

Syncytia were mapped from seminiferous epithelium taken from rats injected with 0.1 mg of testosterone propionate per day for 40 days prior to sacrifice. The preleptotene and pachytene spermatocytes of stage VII tissue had degenerate cells (Fig. 2B, arrows) and syncytial fragments. Extensive degeneration of pachytene spermatocytes was also observed while mapping a stage IX tissue of another treated rat. In addition to degenerate cells and syncytial fragments, cells were found with closed cytoplasmic
bridges – that is, bridges not leading to other cells. The same structures were observed in poorly prepared material and artificially separated cells.

Because advanced spermatogonia and spermatocytes are always part of a syncytium it follows that degeneration of cells leads to alterations in the syncytium. Direct evidence of degeneration is the presence of degenerate cells and indirect evidence includes closed bridges, fragmented syncytia, syncytial numbers other than 2", and syncytial branches where the subterminal cells have 3 bridges. The latter two phenomena, but not the first three, may also be caused by arrest of one or more cells of a syncytium during a given division cycle. It is therefore possible to differentiate between the effects of degeneration as seen in D and the effects of arrest (Fig. 3B, syncytium D, arrow).

DISCUSSION

The resolution of the methods used to produce the cell maps brings early spermatogonial development (single cells, twins, chains of cells and their connecting bridges) into particular focus. Unlike the spermatocytes and spermatids which follow a single developmental pathway, the spermatogonia appear to have several options. Single cells may produce more single cells or enter spermatogenesis or remain dormant. Having entered spermatogenesis, a clone of spermatogonia may continue to advance or delay development. Which option is exercised seems to be correlated with the supply of spermatogonia available to replace maturing spermatocytes. The observations reported here can be integrated with the existing information on rat spermatogenesis (Clermont, 1972; Huckins, 1971b) in the manner shown in Fig. 1B. The model implies that for a given area there is a periodical build-up of spermatogonia which then supply several quanta of spermatocytes and when the spermatogonia are depleted, a new build-up takes place.

Tissue (Bb) has a classical stage VII morphology of spermatids (Fig. 5c) and composition of preleptotene spermatocytes and early spermatogonia. Noticeable, however, is the large number of single and twin spermatogonia present here but not in another tissue of about the same stage (Ba) (Figs. 4, 1A). If the spermatocyte syncytia are not much larger than the partially observed syncytia reported here, then there are 40 syncytia or less per mm². Tissue (Bb) has, on a per mm² basis, 150 groups of 1, 2, or 3 spermatogonia. Not all of these can enter and continue the spermatogenic pathway or else there would be 150 spermatocyte syncytia per mm² when, after 6 division cycles, the tissue enters stage VII the next time. It follows that only some of the spermatogonial groups will enter and continue the spermatogenic developmental pathway while others will delay development or follow later. Maps of tissue (C) and particularly tissue (Ba) give further evidence for differential rates of spermatogonial development.

Tissue (C) (Fig. 6), at stage XIII, can be viewed as a later stage in the development of tissue (Bb). Two groups of spermatogonia have enlarged into long chains, while other groups did not expand in that manner. It is assumed that chains f and h will produce the next spermatocyte syncytia, while groups e and d may mature in the following period. These 2 syncytia as well as the single cells, however, are not expected to divide at each of the 10 mitotic peaks in the intervening time.
Differential spermatogonial development is particularly evident in tissue (Ba) (Fig. 4A). The type B spermatogonia are about to divide, while some syncytia of type A spermatogonia are dividing and some are not. In addition, there is a small number of non-dividing single and twin spermatogonia. The different sizes of the type A spermatogonial syncytia furthermore indicate that they are not at the same stage of development: \( n \) has 3 cells, \( m \) has 11 or more cells. After division, \( c \) has 14 cells, \( h \) has 8 cells, and \( f \) has 4 cells.

Tissue (Ba) confirms that there can be an abundant supply of spermatogonia, numbers of syncytia well in excess of the numbers required to produce the following quantum of spermatocytes. Assuming that the single and twin cells will remain dormant until the supply of more advanced spermatogonia has been exhausted, there are 120 type A spermatogonial syncytia per mm², sufficient to supply 3 successive quanta of spermatocytes. As before, the syncytia could not participate at every division cycle. In Fig. 1 this is diagrammed by a broad band of 120 syncytia which produces 3 quanta of 40 spermatocyte syncytia and there is a separate thin band of 40 spermatogonial syncytia which start to increase when advanced spermatogonia become depleted. At that time the configuration of type (Bb) is restored.

Tissue (Aa) is at stage I, probably prior to the mitotic divisions which occur during this stage (Fig. 1A). The area is dominated by syncytium \( q \) of 16 or more spermatogonia (Fig. 3A). This syncytium can reach a maximum size of 128 cells over the next 3 division peaks and it will likely become the spermatocyte syncytium for this area at stage XII. Syncytium \( r \) has 8 cells and lies more towards the periphery of the tubule, partially underneath syncytium \( q \). It can reach a maximum of 64 cells in the next 3 divisions, which is smaller than observed for a spermatocyte syncytium. It is therefore not a likely candidate and it is not expected to mature until one cycle after \( q \) matures. Moreover, in no case have spermatocyte syncytia been found to overlap as would be the case here if \( q \) and \( r \) matured simultaneously. At a later period again syncytium \( m \) is expected to develop into a spermatocyte syncytium. The waiting period includes 10 mitotic peaks for \( r \) and 16 for \( m \). They are likely to remain dormant at some of these if excessive proliferation is to be avoided. In Fig. 1B, three successive quanta of spermatocytes are shown to emerge from the solid band of spermatogonia of tissue (Aa). Single and twin cells are indicated by a separate band.

Comparisons between maps of approximately the same stage, e.g. (A) vs (C) and (Ba) vs (Bb), show non-equivalence of spermatogonial populations in tissues which by criteria of spermatocyte and spermatid morphology and composition are at similar developmental levels. Although this is a limited set of observations, it cautions against the averaging of data from similar stages in studies of spermatogonial renewal and proliferation.

The delayed development of whole syncytia implies that at times when most spermatogonia divide, some of them do not undergo division. In labelling experiments (Huckins, 1971a) it was found that essentially all metaphase type A spermatogonia were labelled 18 h after treatment with \(^3\text{H}\)-labelled thymidine (Huckins, 1971a). It seems therefore likely that the non-dividing type A spermatogonial syncytia (Fig. 4A) are arrested at \( G_1 \). In addition, there is extensive evidence that within a syncytium
which progresses through the division cycle, a few cells may remain arrested. Because the cells of a type B or spermatocyte syncytium are all in the same stage of development, it is concluded that such dormant cells progress in developmental stages with the syncytium as a whole in spite of the absence of a cell division. Temporary arrest at $G_1$ must be assumed if a 2n chromosomal content is to be maintained.

The likelihood that some cells are temporarily arrested is suggested by: (1) spermatogonial syncytia of 3, 5, 6, 7, 9, etc., cells in the absence of fragmented syncytia, broken bridges, or degeneration; (2) subterminal cells of a syncytium which have 3 bridges (arrows in Figs. 3B, 4B, 5A and 6B) – here one of the terminal cells has failed to divide; (3) in the reduction of a large syncytium there are regularly cells that cannot be paired off – presumably they did not divide (arrowheads, Fig. 2C).

Degeneration of cells, fragmentation of syncytia, and broken bridges were found only in hormonally treated material (Results, Abnormal syncytia), and in poorly fixed material (results Ab), which also contained multinucleate cells. Such defects were not found in the other areas of which maps were made, including the stages reported to have maximum degeneration during the mitotic peaks (Clermont & Bustos-Obregon, 1966) of stage VI (Ba, Fig. 4), stage IV, and stage XIV (not shown here). Stage XIII of tissue C (Fig. 6) had no evidence of recent major degeneration at stage XII. While scanning tissues from normal rats with the electron microscope one was found to have large numbers of degenerating cells, suggesting some local defect. It follows that although degeneration is extensive under some conditions (Gondos, Zemjanis & Cockett, 1970; Hitier & Vodovar, 1972; Elliott, Stetson & Menaker, 1972; Chowdhury & Steinberger, 1964; Fox & Fox, 1967), it is not systematic and therefore not a necessary characteristic of spermatogenesis (Allen, 1918). At the mitotic peaks spermatogonial numbers less than double, because, among others, some syncytia do not divide and the dividing syncytia contain some cells that do not divide.

REFERENCES


(Received 22 January 1975 – Revised 10 June 1975)

Fig. 2. A, diagram of a seminiferous tubule showing the arrangement of cells as seen in cross-section and tangential section. Sertoli cells are clear, the spermatogonia dark, the spermatocytes are the small cells connected to one another by bridges, the spermatids and spermatozoa have tails. This report is based on 3-dimensional reconstructions of tangential serial sections from which maps of interconnected cells were prepared.

B, low-magnification electron micrograph of each 10th section served to identify all cells by number and their cytoplasmic bridges (dense lines between cells). Eventually all such maps were combined to produce a complete map of the area. This section was taken from a rat treated with testosterone (Results, Abnormal syncytia) and contains degenerate cells (arrows).

C, the spermatocyte syncytium (Fig. 3B, C) can be reduced to its presumptive ancestral B spermatogonial syncytium by pairing off as many cells as possible and by replacing each pair by a single cell. The procedure is repeated until the syncytium is reduced to a single cell. The number of operations, 7 in this case, is a minimum estimate of the number of divisions required to produce the observed syncytium. The arrowheads mark some of the cells that could not be paired off. The estimated number, 7, is not affected greatly if the number of cells belonging to the syncytium but falling outside the observed area is less than the observed number of cells.
Rat spermatogenesis
Fig. 3. Stage I, results Aa.

A, a map of the spermatogonial cells and their cytoplasmic bridges. Cells are represented by circles and bridges by lines. Only cells within the boundaries were examined in their entirety. Groups a, b, and c are dense type A spermatogonia.

B, the spermatocyte syncytia A, B, C, and D overlying the spermatogonia in 3A. The arrow marks a branch where one of the terminal cells has failed to divide or a cell was lost.

C, a young spermatid characteristic of seminiferous epithelium in developmental stage I. The Golgi apparatus g is attached to the nucleus n but the head cap has not formed as yet. × 9000. Scale bar is 1 μm.
Fig. 4. Stage VI, results Ba.

A, map of spermatogonia. The 2 short bars across the connecting lines represent lamellae observed in bridges of cells about to divide. Syncytia p, q, and r are the main groups of B spermatogonia which are undergoing division to become preleptotene spermatocytes. Syncytia c, d, e, f, h, and k are dividing A spermatogonia and the black circle between the divided cells represents the midbody. The remaining spermatogonia a, b, m, and n are not dividing.

B, the spermatocytes of the same area.

C, electron micrograph of spermatid to show the characteristics of developmental stage VI. a, acrosome; g, Golgi; h, head cap; n, nucleus. x 19000. Scale bar is 1 μm.
Fig. 5. Stage VII, results Bb.

A, B, the open circles represent the spermatogonia and the black circles the spermatocytes. The arrows point to configurations which cannot be formed if all cells of a syncytium divide and no losses occur.

c, spermatid morphology found at developmental stage VII. a, acrosome; g, Golgi; n, nucleus. × 16400. Scale bar is 1 µm.
Rat spermatogenesis
Fig. 6. Stage XIII, results C.

A, map of spermatogonia. Unusual are the large groups of dense spermatogonia (marked by a dot inside the circle).

B, map of spermatocytes.

C, spermatocytes in the diplotene stage of meiotic prophase identified by the single axial core in the chromatin, and the random distribution of mitochondria. Diplotene cells occur at developmental stage XIII. c, core; m, mitochondrion; n, nucleus. × 11000. Scale bar is 1 μm.
Rat spermatogenesis