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Mammalian spermatogenesis

HUMAN SPERMATOGENESIS: AN ESTIMATE OF THE DURATION OF EACH CELL ASSOCIATION AND OF EACH CELL TYPE*

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In man, timing the duration of spermatogenesis and its component parts (such as the duration of the cycle, the helix, the cell associations and the germinal cells) depends upon understanding the *cytology* of the cells involved in process of spermatogenesis and the *histology* of the germinal epithelium. The attempt to establish the life cycle of each cell of the germinal series during spermatogenesis is a *cytological* endeavor. This in turn is dependent upon the present attempt to more firmly establish the duration of each of the six cell associations in man. The timing of the duration of the cell associations of the germinal epithelium is an *histological* endeavor. This timing in turn is dependent upon the length of time a single germinal cell takes to ascribe the *helix* of spermatogenesis from its position in one cell association (maturing through each of the other five) to reappear in the same cell association in which it was originally observed at the next higher level of development. The duration of the helix was originally established by labeling cells with tritiated thymidine and following their serial appearance through the transformation of the cell associations (Heller and Clermont, 1963). The duration of the cycle of the cell associations and the duration of the whole of spermatogenesis was derived from this information (Heller and Clermont, 1964).

In order to more readily understand the evolution of the present investigation, a brief review of the current status of the kinetics of the germinal epithelium and a definition of some of the terms and concepts follows.

MORPHOLOGY

CYTOLOGY

The cells

The cells of the germinal epithelium may be divided into three groups: spermatogonia, spermatocytes and spermatids. The composition of each group, in man, is as follows: (1) The spermatogonia (the most primitive of the three groups) which divide mitotically, are composed of A-dark (Ad), A-pale (Ap) and B type cells; (2) The spermatocytes (arising by mitotic division from type B spermatogonia) delineate themselves during meiotic maturation from interphase to the second maturation-division into pre-leptotene (R), leptotene (L), zygotene (Z) and pachytene (P) types, and following the first maturation-division into secondary spermatocytes (SS); (3) The spermatids, resulting from the second maturation-division, delineate themselves during spermiogenesis in order of their appearance into Sa, Sb, Sc and Sd types (Fig. 1).

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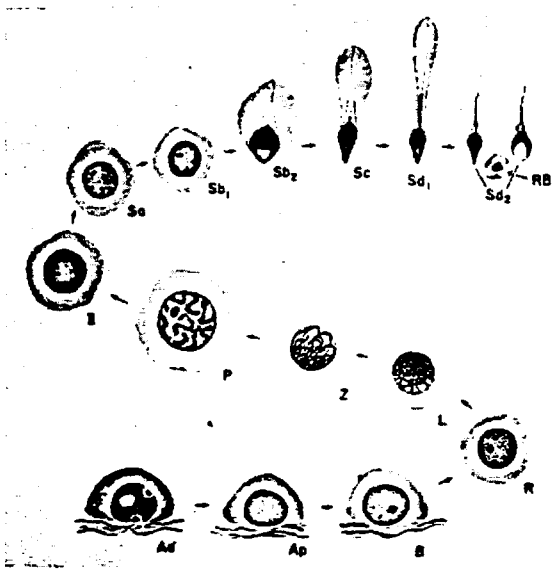

Fig. 1. Cells of the germinal epithelium, demonstrating the spermatogonia, A-dark (Ad), A-pale (Ap) and B; the spermatocytes, preleptotene (R), leptotene (L), zygotene (Z), pachytene (P) and secondary spermatocytes (II); and the spermatids, Sa, Sb, Sc and Sd. The body designated RB indicates the residual body left in the tubule when the mature sperm leaves the cytoplasm. By courtesy of the publishers of *Recent Progr. Hormone Res.* (Heller and Clermont, 1964).

HISTOLOGY

The cell associations

The germinal cells are arranged spatially within the seminiferous tubule from the basement membrane to the lumen according to their degree of maturation. Thus the spermatogonia always adhere to the basement membrane. The spermatocytes comprise the next one or two layers and the spermatids comprise the final one or two layers adjacent to the lumen.

The spatial arrangement of the germinal cells between the basement membrane and the lumen is such as to allow the identification of groups of cells of different generations as being regularly associated with one another (Clermont, 1963). Thus cells at a specific stage in development in the spermatocyte group are always found associated with specific spermatids and with spermatogonia. For example, the pre-leptotene spermatocyte is always found in the company of mid-pachytene spermatocytes and Sb_1 spermatids on the lumenal side, and A-pale and A-dark spermatogonia on the basement membrane side. These groupings of cells are known as 'cell associations'. In man six such cell associations are readily identified (Fig. 2). As a given cell of a cell association matures, the associated cells mature at the same rate and gradually evolve to become the next most advanced cell association. It is assumed that the area of the basement membrane occupied by a given cell association remains the same size, and remains at the same location, as the cell association evolves to become more mature through the six successive associations. Thus no movement along the basement membrane is postulated.

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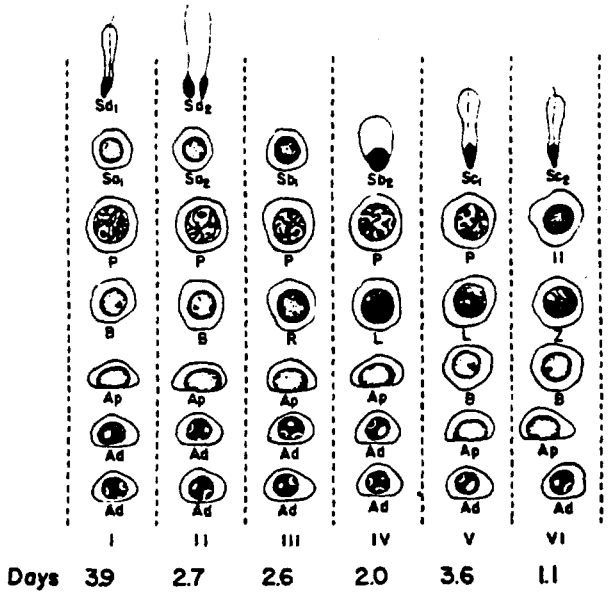

Fig. 2. The six cell associations, demonstrating the cellular composition of each. The vertical columns depict each cell association. In a histological section each cell depicted is found in association with each of the other cells depicted. Spermatogonia are represented, in a developmental order, as A-dark (Ad), A-pale (Ap) and B. Spermatocytes are preleptotene (R), leptotene (L), zygotene (Z), pachytene (P), and secondary spermatocytes (II). Spermatids are shown in order as Sa, Sb, Sc and Sd. Sd_2 are mature spermatozoa. The duration, in days, of each cell association is indicated at the bottom. Although the diagram is oriented from lumen to basement membrane, the basement membrane is not depicted. Actually each of the three spermatogonia may be in contact with the basement membrane.

THE KINETICS

CYTOLOGY

The helix

Any given cell undergoes maturation-evolution through each of the six successive cell associations to reappear in the same cell association in which it was first observed. However, it now has evolved to become a member of the next most developed generation. For example, the pre-leptotene spermatocyte evolves once through each of the other five cell associations and appears again in the same cell association as a mid-pachytene spermatocyte. During this maturation it has evolved successively through leptotene, zygotene and early pachytene to become a mid-pachytene spermatocyte. This evolution of a single cell from one cell association to the same cell association at the next most mature state is called the 'helix' of spermatogenesis (Fig. 3). The duration of one helix has been determined to be 16 days (Heller and Clermont, 1964).

HISTOLOGY

The cycle

The evolution of a single cell association, through each of the other five associations in any given area of the seminiferous tubule, is known as the 'cycle' of the germinal

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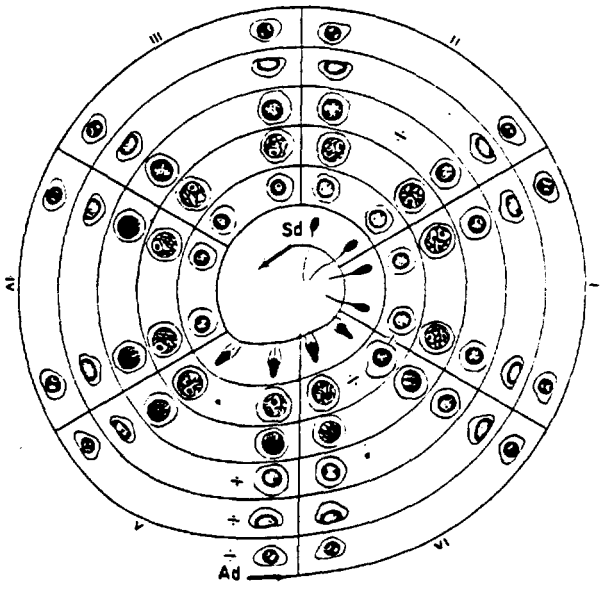

Fig. 3. Helical arrangement of spermatogenesis. Roman numerals represent the six cell associations. The development of cells begins on the outer layers as Ad spermatogonia and progresses towards the center. The Sd_2 spermatid completes the development. One helix is the maturation of any one cell through the six cell associations to reoccur in the same cell association one generation more mature. Spermatogenesis, beginning at the division of an A-dark spermatogonium into two A-pale spermatogonia, requires 4.6 helices.

epithelium. The duration of the cycle is 16 days (Heller and Clermont, 1963; Heller and Clermont, 1964; Heller *et al.*, 1963).

Duration of spermatogenesis

The duration of the whole of spermatogenesis in man may be assumed as beginning with the division of A-dark spermatogonia into two A-pale spermatogonia and ending in the release of mature spermatozoa into the tubule lumen. This process has been estimated to have a duration of 4.6 helices (and therefore 4.6 re-occurrences of the cycle). Since each helix is 16 days in duration, the duration of spermatogenesis is 74 ± two days (Heller and Clermont, 1964).

MATERIALS AND METHODS

The estimation of the duration of each cell association was approached in two ways: (1) quantitating the total number of germinal cells in each cell association, (2) quantitating the total number of Sertoli cells in each cell association. In each approach a total of 30 cell associations were quantitated and the percentage of cells occurring for each cell association was calculated. The time of the duration of one cycle of the germinal epithelium — 16 days — was divided by the percentage of each cell association to arrive at the time in days for each.

Testicular biopsy specimens were obtained from 44 normal healthy subjects by surgical incision, as described by Rowley and Heller (1966). Each had normal seminal fluid examinations and normal output of urinary gonadotropins, estrogens, and/or testosterone.

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Quantitation was performed on Zenker-formol fixed tissues stained with Harris' hematoxylin and eosin or on Cleland's fixed tissues stained with iron hematoxylin and eosin. At least 100 four-micron serial sections were cut from each biopsy.

The sections were scanned in the same manner as differential blood smear counts are performed. Only those cell associations that were complete in all respects were counted. In order to achieve statistical validity, it was determined that 30 cell associations must be quantitated for each subject. Care was taken to eliminate areas of mixed cell associations, tangentially cut tubules or areas of the section with artifacts (such as the section edge). Only those cells cut through the center of the nucleus or through a fixed point such as a nucleolus were considered to avoid over-counting due to variance in cell volume. To avoid counting the same cell twice every ninth section was utilized, thus separating cells by 36 microns. The maximum nuclear diameter was 15 microns. It required two to seven sections to find the requisite of 30 cell associations.

RESULTS AND DISCUSSION

Quantitative data determining the duration of the six cell associations are represented in Table I and Table II. It is assumed that the duration of each cell association (C.A.) is proportional to the percentage of cells it contains at any one given point in time. The duration, in days, of each cell association is the result of the percentage of cells in one cell association multiplied by 16 (the duration of one cycle). Since 24.4% of the cells are found in C.A. I it has a duration of 3.9 days. Cell association II has 17.1% of the cells and is 2.7 days in duration; C.A. III has 16.6% of the cells and lasts 2.6 days; C.A. IV has 12.6% of the total cells and is 2.0 days long. C.A. V has 22.6% of the cells and is 3.6 days in duration; C.A. VI has 6.8% of the cells and endures 1.1 days (Table I).

TABLE I

Statistical derivation of the duration of the cell associations based on total cell numbers of 44 biopsy specimens

	Cell association					
	I	II	III	IV	V	VI
Mean cells per biopsy	228.3	159.9	156.0	117.8	211.8	63.6
S.E. ±	4.9	6.5	5.1	5.3	7.9	3.7
Per cent of total cells	24.4	17.1	16.6	12.6	22.6	6.8
Duration in days (% × 16)	3.9	2.7	2.6	2.0	3.6	1.1
S.E. ± in days	0.08	0.11	0.08	0.08	0.13	0.06

The Sertoli cell can be used as a countercheck on the method by determining the number of Sertoli cells per cell association. It is generally accepted that Sertoli cells exist in direct proportion to the number of germ cells they maintain (Clermont, 1962; Clermont and Morgentaler, 1955; Oakberg, 1957; Roosen-Runge, 1955). Therefore, a count of the Sertoli cells (counting only those cut through the nucleolus) found in each cell association should reveal the same percentages and the same duration as the total cell count. That this is the case is demonstrated in Table II.

Three cell associations are approximately equal in duration (C.A. II, III and IV), C.A. I and V are slightly longer in duration. Cell association VI is shortest (1.1 days) as was to be expected because the reduction-division occurring here is a rapid process.

The definition of the cell associations in animals was arrived at by identifying the degree of development of the spermatids (Leblond and Clermont, 1952; Oakberg, 1956).

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TABLE II

Duration of cell associations based on Sertoli cells

	Cell association					
	I	II	III	IV	V	VI
Per cent of total cells	23.0	17.1	19.0	12.4	21.8	6.6
Duration in days (% × 16)	3.7	2.7	3.0	2.0	3.5	1.1
S.E. ± in days	0.11	0.11	0.11	0.09	0.13	0.06

In animals the entire tubular cross-section is occupied by a single cell association (Perey *et al.*, 1961; Roosen-Runge, 1962; Oakberg, 1956). Those cells subtending a particular group of spermatids were usually included in that cell association.

The definition of the cell associations in man was arrived at by taking into consideration the degree of maturation of each of the cell types comprising a cell association. Thus the limits of a given cell association are not necessarily defined by the maturation of the spermatid but by the maturation of each cell comprising the cell cluster.

Earlier estimates of the relative duration of each of the six cell associations were based upon percentages obtained by the 'paper-cutout' method on four subjects (Clermont, 1963). When later the duration of one cycle of the germinal epithelium was derived, the estimated duration of each cell association was expressed in days (Heller and Clermont, 1964). Their estimates were reasonably close approximations of the data presented here derived from 44 subjects.

Once the duration of each cell association was established, attention was turned to the individual cells involved. Estimating the duration of each cell type produced the following cell results: The spermatogonia, Ad, Ap and B were found to exist 16+, 16+ and 9.5 days, respectively. Spermatocytes R, L, Z and P, last 2.6, 5.6, 1.1 and 15.0 days, respectively. The spermatids, Sa, Sb, Sc and Sd are 6.6, 4.6 and 5.3 days, respectively (Table III).

TABLE III

Estimated duration of each germinal cell type

	Cell types										
	Ad	Ap	B	R	L	Z	P	Sa	Sb	Sc	Sd
Duration in days	16+	16+	9.5	2.6	5.6	1.1	15.0	6.6	4.6	4.7	5.3
S.E. ± in days	—	—	0.14	0.08	0.15	0.06	0.21	0.13	0.11	0.15	0.13

The cells which have a duration that can be estimated within narrow limits are the R, L, Z, P, Sa, Sb, Sc and Sd. For these cell types, this estimate is accurate, varying only by the standard error of the association or associations in which the cells are found. The leptotene spermatocyte, for example, comes into existence as a leptotene at the beginning of C.A. IV and matures into a zygotene at the end of C.A. V. Therefore, it has a duration of 2.0 days (duration of C.A. IV) plus 3.6 days (duration of C.A. V) with a standard error of 0.15 days.

The duration of a few cells that do not come into existence at the beginning of a cell association was approximated by estimating the time the cell appeared in the association. These cells (Ad, Ap and B) can only be estimated within wide limits. For example, the Ap spermatogonium divides to form B spermatogonia within the 3.6 days duration of

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C.A. v, and the B then in turn divides at the end of C.A. II. Thus the duration of the B spermatogonium may vary from about 8.3 days to 11.3 days depending upon when in C.A. v it appears. We estimate the duration at 9.5 days. How long the Ad or Ap exist remains in large part conjecture as they appear in all six cell associations. They exist for at least one full cycle (16 days).

SUMMARY

The time in days that each of the six cell associations (C.A.) endures in man has been established. The procedure was to count the number of cells in each association, then determine the percentage for a given cell association and multiply this by the duration of the cycle of the germinal epithelium. Calculations based on the study of 44 normal human testicular biopsy specimens show the following durations in days for each cell association: C.A. I - 3.9, C.A. II - 2.7, C.A. III - 2.6, C.A. IV - 2.0, C.A. V 3.6 and C.A. VI - 1.1. The duration of each cell type was estimated from these figures. Since this test was prepared a fourth spermatogonium (AL) believed to be the least differentiated has been discovered (Rowley *et al.*, 1969).

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