HANDBOOK OF PHYSIOLOGY

A critical, comprehensive presentation

of physiological knowledge and concepts

SECTION 7:

Endocrinology

VOLUME V.

Male Reproductive System

Section Editors: ROY O. GREEP
EDWIN B. ASTWOOD

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Preface

In the past few decades we have witnessed the discovery of most of the modern knowledge on the male reproductive system. Spermatogenesis and its control, as well as the production of hormones by the testis, have been the focus of intensive investigation for many years. These efforts have been amply rewarded. We now understand in great detail the sequence of events that transform relatively undifferentiated spermatogonia into highly complex spermatozoa. The synthesis and secretion of steroids by Leydig cells has been well characterized, and we are approaching an understanding of the finer mechanisms of this process at the subcellular level. In contradistinction to the testis, however, research on the excurrent ducts has gained momentum only during the past few years. Fundamental observations on epididymal sperm maturation that were made in the 1920s and 1930s virtually lay dormant for a quarter of a century, until the late 1950s when new interest developed. Over the past decade or so interest in sperm maturation has quickened and, as a consequence, research into broader aspects of epididymal function has also increased. Again, the rewards have been ample, as will be shown in this volume, which is organized in a more or less classical sequence from the testis, through the reproductive tract, to the urethra.

This volume has had a long gestation; the original call for contributions was given early in 1970. However, between 1970 and 1973 a tremendous amount of work was published on the male reproductive tract, and research horizons in this field were expanded because of the availability of the powerful research tools of molecular and cellular biology. We felt that a volume on the male would not be complete without reviews of some of the new and exciting areas that these approaches afforded. In 1973, therefore, we issued invitations to new contributors for an additional nine chapters to cover the areas of physiology of the blood-testis barrier, of steroid-protein interactions (especially with respect to the new observations on androgen-binding protein), of the molecular effects of both steroid and gonadotrophic hormones at the cellular and subcellular levels, and of the effects of vasectomy. This expansion of the volume necessitated a delay of many months in publication, and we are deeply grateful for the forbearance and timely revisions of the original contributors.

We would like to extend our sincere appreciation to Harriet S. Levy and Doris L. Morton for their labors in copyediting during the early phases of the volume, and to Brenda B. Rauner for the final copyediting and integration of the completed volume. Their careful attention to detail and devotion to clarity of expression were an indispensable part of production of this volume that is appreciated by the contributors and editors and will be appreciated by the readers.

DAVID W. HAMILTON
ROY O. GREEP
Volume Editors

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CHAPTER 1

Spermatogenic function of the testis

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CHAPTER CONTENTS

THE ROLE OF THE TESTES in the reproductive process was recognized in early antiquity when observations were made on castrated men. Physiological studies of the testes actually preceded structural investigations by centuries. Aristotle (6, 7) introduced the classic technique of gonadal extirpation to the study of the physiology of reproduction as early as 300 B.C.; the first microscopic examination of germ cells was not conducted until the seventeenth century (128).

In the middle of the nineteenth century the study of the testis seriously began. The discovery that spermatozoa develop from cells residing in the testis constituted the first major advance (134), which was rapidly followed by the description of the microscopic characteristics of the interstitial cells (80) and Sertoli cells (117). The morphological classification of the various germinal epithelium cells (135) led toward comprehension of the orderly aspects of spermatogenesis (10, 132) and to the concept of the *spermatogenic cycle* (101, 133). These studies served as the basis for the present-day understanding of the kinetics of the spermatogenic process.

Most work concerned with the process of spermatogenesis has been conducted in rodents, particularly the rat. Although there are considerable species differences, the histology of the seminiferous epithelium, the morphology of the germinal cells, and the kinetics of the spermatogenic process are sufficiently close to permit a certain degree of extrapolation from the rat to other species. Because the bulk of our information is based on experiments in the rat, subsequent discussion perforce depends heavily on data obtained in this species. Other species are discussed only when necessary to illustrate possible differences or to point out specific details.

This work was supported by a grant from the Ford Foundation and Grants HD-00399 and HD-04178 from the United States Public Health Service.

BASIC STRUCTURE AND DEVELOPMENT OF THE TESTES

Structure

The testicular parenchyma is enclosed by a capsule composed of three distinct layers.

The outer layer is a serous membrane, the visceral portion of the tunica vaginalis. The parietal portion of the tunica vaginalis lines the scrotum. The space formed between the parietal and visceral layers, the cavity of the tunica vaginalis, separates the testis from the scrotal structures. Both layers of the tunica vaginalis are derived from the peritoneum and exhibit the light- and electron-microscopic characteristics of mesothelia (79).

The middle layer of the testicular capsule, the tunica albuginea, is a prominent fibrous structure composed of interlocking collagen fibers. Smooth muscle fibers have been observed in the tunica albuginea of the rabbit (66), the human being (65), and the rat [(41); Fig. 1]. It has been suggested that contraction of these muscle fibers may be responsible for sperm transport from the testicle into the epididymis (42). Recently, Davis & Langford (41) observed spontaneous contractions of the testicular capsule and demonstrated that various pharmacological agents are capable of modifying these contractions.

The third and innermost layer of the testicular capsule (the tunica vasculosa) is very thin and consists of loose areolar connective tissue rich in fine blood vessels (Fig. 1).

The testicular parenchyma is composed of seminiferous tubules and interstitial tissue (Figs. 2 and 3). The seminiferous tubules show a complex pattern of convolutions. Basically they form loops that empty at both ends into

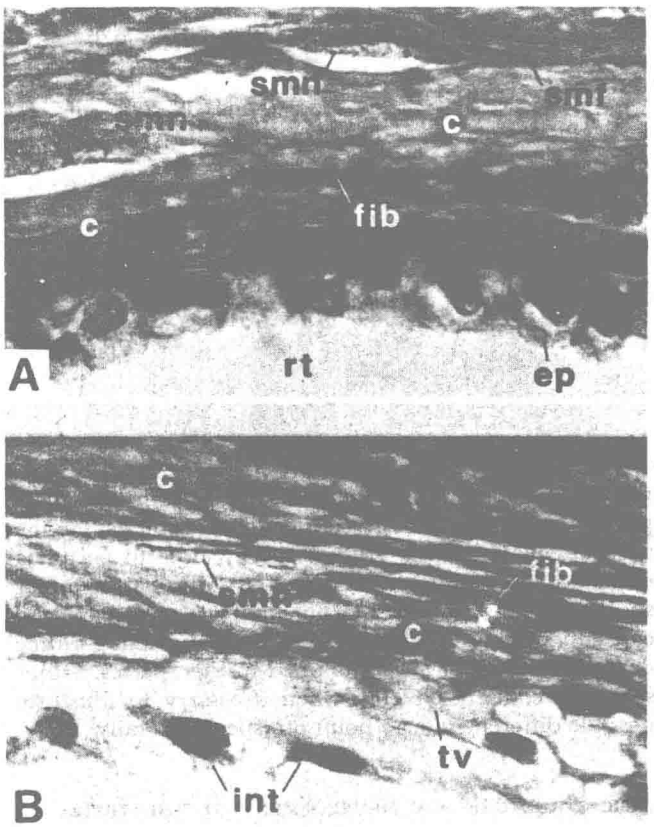


FIG. 1. Smooth muscle cells of the tunica albuginea of the adult rat. *A*: mediastinal region of the tunica albuginea surrounding a cavity of the rete testis (*rt*), lined by a cuboidal epithelium (*ep*). *B*: portion of the tunica albuginea adjacent to the mediastinum illustrating the tunica vasculosa (*tv*) and an occasional adherent interstitial tissue cell (*int*). *smn*, smooth muscle nucleus; *smf*, smooth muscle fiber; *fib*, fibroblast; *c*, collagenous tissue of the tunica albuginea. Masson's trichrome stain. × 958. [From Davis et al. (42).]

the rete testis, a structure connected with the epididymis via the ductuli efferentis (Fig. 4). The rat's seminiferous tubules, although showing a complicated pattern of convolutions, do not anastomose; in other species, for example, the human being, a series of anastomoses connecting the loops and forming blind pouches has been demonstrated (72, 81). The interstitial tissue is composed of Leydig cells, blood vessels, extensive lymphatic channels (50), and numerous macrophages. Subsequent discussion is limited primarily to the development, structure, and function of the seminiferous tubules.

General Considerations of Prenatal Development

A number of extensive reviews concerning prenatal development are available (1, 5, 11, 57, 85, 87, 115, 138). Recently, Roosen-Runge (107) and Gier & Marion (56) attempted to review the comparative aspects of testicular development, pointing out the difficulty in such a task because of the lack of systematic knowledge in this area.

The embryonal gonad is composed of three elements. Each develops from a different source. The somatic elements originate from a thickening of the peritoneal epithelium; the cortex and medulla from a condensation of the mesonephric blastema (138); the primordial cells arise outside the gonads and migrate during the embryonal to the gonadal anlage (51, 55).

The earliest changes in peritoneal epithelium and mesonephric blastema suggestive of formation of genital ridge can be observed in a 6-mm human embryo on the ventromedial surface of the urogenital fold. The germ cells first become recognizable in the 2.5-mm human embryo; they reside in the endodermal yolk-sac epithelium, from which they rapidly migrate toward the genital ridge (Fig. 5). They are first found in the cortex and soon afterward in the medulla of the genital ridge. During the migration, germ cells behave much like ameboid cells,

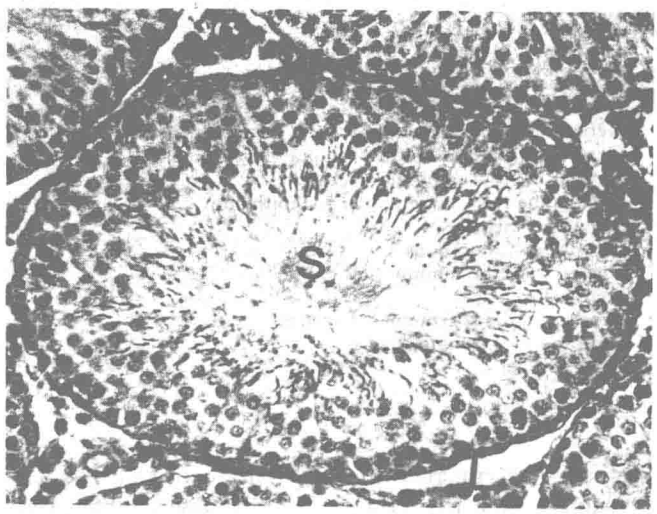


FIG. 2. Microscopic appearance of rat testis. Seminiferous tubule (*S*) contains germinal epithelium cells in a single cell association. Interstitial areas contain Leydig cells (*L*). × 175.

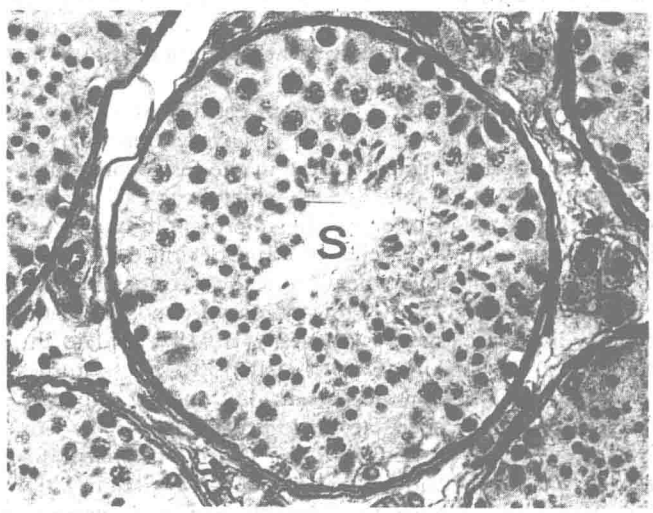


FIG. 3. Microscopic appearance of human testis. Germinal epithelium cells form several different cell associations within the seminiferous tubule cross section (*S*). Interstitial areas contain Leydig cells (*L*). × 179.

and their complicated migratory movement is thought to be directed by chemotropism (136).

During migration the primordial germ cells divide, and the mitotic activity continues after they arrive in the genital ridge. In the 6-week-old human embryo (12 mm) the gonad consists of medulla, cortex, and primordial germ cells; it is still sexually undifferentiated. The gonad is attached to the mesonephros by the broad suspensory ligament. As the gonad develops, the process of sexual differentiation continues, and, in the case of the testis, the medulla becomes organized into cords, which transform into anastomosing testicular cords.

In the course of the development of the male gonad, the medulla becomes dominant and the cortex thins out. A basement membrane forms between the cortex and the medulla; later it becomes the tunica albuginea. By this time the primitive germ cells have migrated into the medullary cords and become localized within the testicular cords, which develop into the primitive seminiferous tubules. The testes are readily distinguishable from the ovaries in an 8-week-old human fetus (57, 138). At this stage of the development, the primitive seminiferous tubules contain supporting cells that are precursors of the Sertoli cells and the primordial germ cells, the gonocytes. The mesenchyme surrounding the seminiferous tubules differentiates into connective tissue septae, which extend to the tunica albuginea and converge toward an area occupied by the rete testis. The interstitial Leydig cells acquire characteristic structural features in a 30- to 40-mm human fetus and reach the peak of growth, as well as the capacity to convert steroid precursors to testosterone, in a human fetus 12–13 weeks old (93).

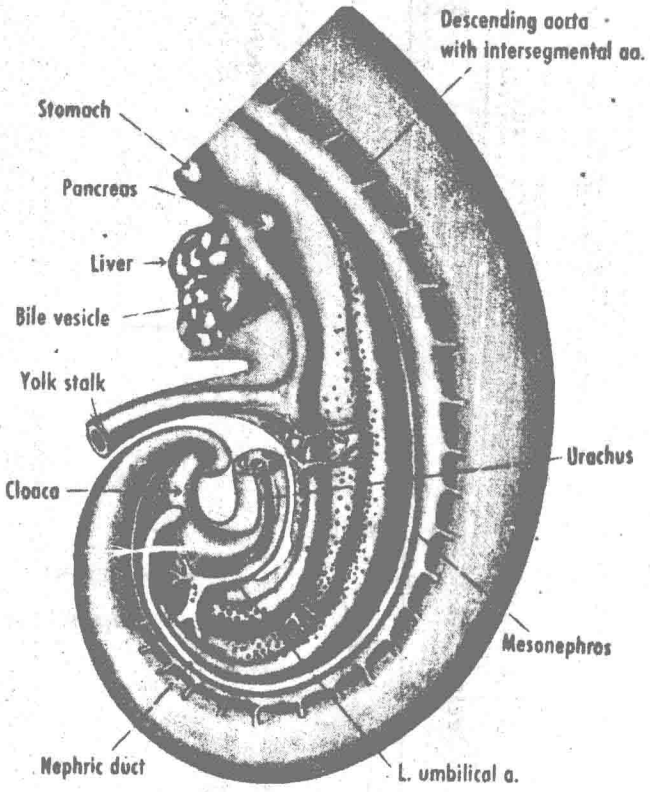


FIG. 5. Human tailbud embryo; 32 somites. Viscera; migration of germinal cells indicated by dots of various sizes. × 22. [From Witschi (136).]

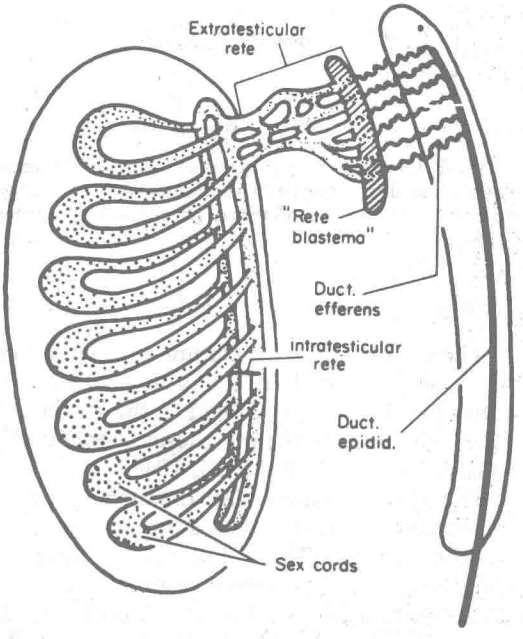


FIG. 4. Diagrammatic representation of rete testis of the rat. Seminiferous tubules are shown opening into long intratesticular portion of the rete. Intratunical portion of the rete running through the tunica albuginea connects the intratesticular rete with the extratesticular rete near the superior end of the testis. [From Roosen-Runge (105a).]

The origin of the Leydig cells is unclear. Some embryologists (5, 57) believe that they form by a process of "specialization" of the mesenchymal cells; others favor the theory that they originate from the coelemic epithelium or mesonephric blastema (137).

General Considerations of Postnatal Development

Considerable variation exists in the rate of postnatal development of the testes in different species. In certain laboratory animals, for example, the rat, the increase in testicular weight commences immediately after birth and continues until adult size is attained (Fig. 6). The weight increase is associated with increase in tubular diameter (Fig. 6) and length, as well as with the onset of spermatogenesis, the latter as early as 5 days after birth (Fig. 7). In primates an interval of several years ensues between birth and the onset of testicular growth. In the rhesus monkey the testes show only slight weight increase for the first 2½ years of age. This is followed by a growth spurt at 2½–3 years associated with the onset of spermatogenesis (Fig. 8). Similarly, in man, a quiescent period occurs between birth and the onset of the testicular growth spurt, which lasts 10–14 years (Fig. 9).

The question of whether Leydig cell development in man precedes the onset of seminiferous tubules' growth

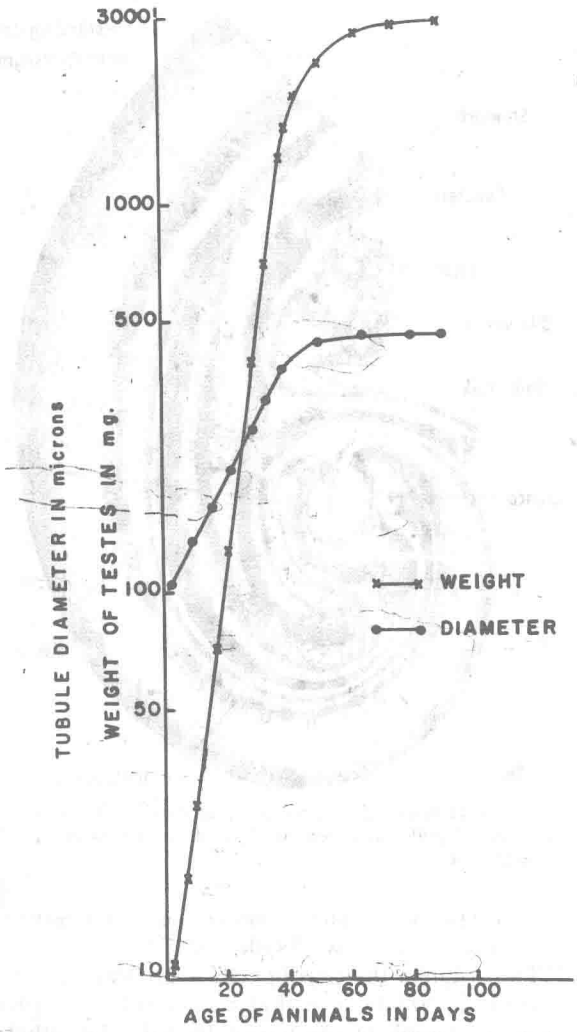


FIG. 6. Testicular weight and diameter of seminiferous tubules in the developing rat. (E. Steinberger, unpublished data.)

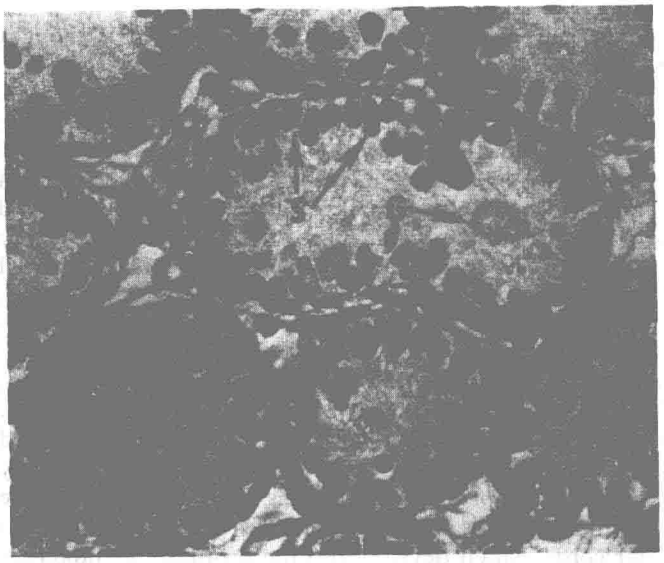


FIG. 7. Testis of 5-day-old rat showing immature Sertoli cells (*S*), primitive type A spermatogonia (*P*), and gonocytes (*G*). × 490.

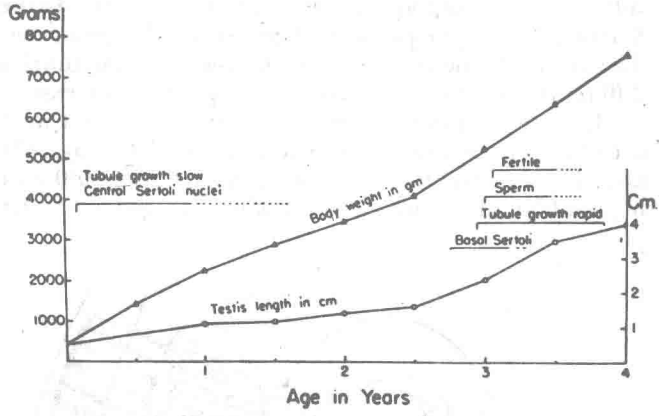


FIG. 8. Graphic representation of changes in testes of rhesus monkey during development. Coordinates are body weight and age of animals, and length of testes. [From van Wagenen & Simpson (129).]

remains unresolved. Studies on human testes dealt with randomly obtained material and are difficult to interpret (1, 15, 104, 118). The most extensive observations have been made in the laboratory rat, and most investigators conclude that Leydig cells in this species degenerate or atrophy immediately after birth (20, 108) and do not appear again until the fourth week after birth, when testicular growth has already progressed markedly.

From this morphological information a conclusion could be drawn that the onset of testicular growth, or the onset of the growth of seminiferous tubules and initiation of spermatogenesis, occurs before the appearance of morphologically demonstrable functional Leydig cells. However, recent studies on the differentiation of steroid biosynthetic pathways in developing testes indicate that interstitial tissue may be active in steroid biosynthesis in the postnatal rat and continue so without interruption until adulthood, although the type of steroids produced at different stages of testicular development is different (120).

Development of Seminiferous Tubules

The details of the development of seminiferous tubules have been studied in many mammalian species—the marsupial (43), the mouse (44), the rat (31, 105), the cat (116), the goat (139), the rhesus monkey (129), and the human being (1, 12, 15, 58, 104).

The most extensive and detailed investigation of the development of seminiferous tubules has been conducted in the rat (31). The tubules were reconstructed from serial sections of rat testes, beginning with the 16th day of embryonal life, continuing to adulthood. In each fetal testis there are 20–31 distinct sex cords. They are arranged in a series of C-shaped arches placed side by side, similar to that of tracheal cartilaginous rings, the plane of the arches forming a right angle with the long axis of the testis (Fig. 10). Two sets of arches can be observed:

one is located peripherally, forming the outer cords and running close to the tunica albuginea; the other is composed of smaller arches and located at a distance from the tunica. Most of the cords have only two connections with the rete testis. As the development of the cords progresses their longitudinal growth causes more pronounced waviness and folding of the arches. At birth the arches are extensively folded, forming about 90 tiny convolutions. Further development of the tubules is expressed by lengthening of the limbs connecting the successive convolutions, but the number of convolutions remains stable. This results in formation by the arches of a series

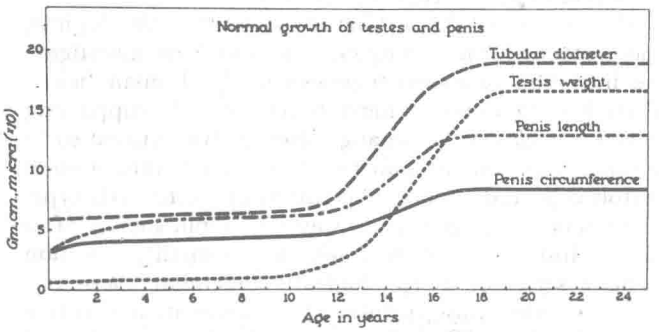
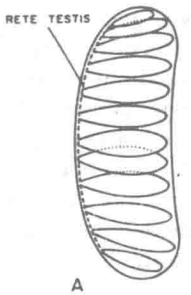


FIG. 9. Growth of testes and penis in man. Testis weight expressed in g; penis length and circumference in cm; tubular diameter in micra × 10. [From Albert et al. (1).]



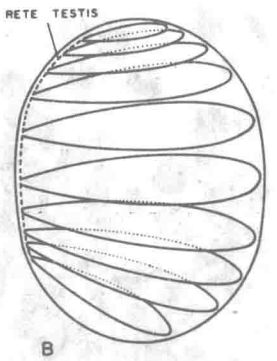


FIG. 10. Diagrammatic side view of *A*: a 17-day-old and *B*: a 19-day-old embryonic testis, showing *arches* that represent approximate distribution of outer sex cords. *Broken line* indicates position of rete testis. In *A*, paths of arched sex cords are more or less perpendicular to long axis of the organ. In *B*, as testis become more spherical, sex cords appear to fan out from the rete testis and are no longer perpendicular to the craniocaudal axis of the organ. [From Clermont & Huckins (31).]

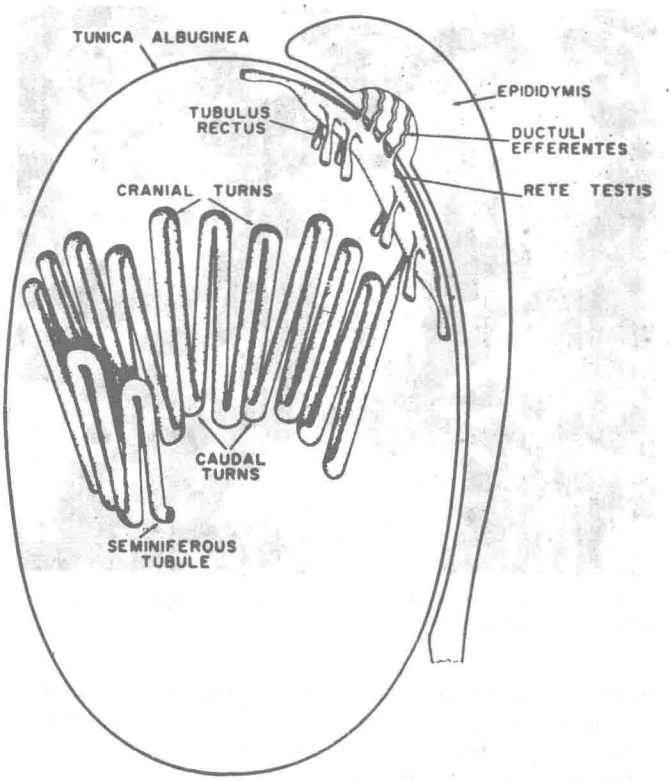


FIG. 11. Representation of a seminiferous tubule and its connection to the rete testis in a view looking into the testis from above and medially. Convoluted tubule follows a circular path within the testis and has two connections with the rete testis. Cranial portions of the palisade are closer to the tunica albuginea than are caudal ones. Several tubuli recti are seen in the lateral and internal aspects of the rete testis. [From Clermont & Huckins (31).]

of cones or funnels, with the wide portion of the cone oriented cranially and the narrow portion caudally (Fig. 11). Subsequent development of the seminiferous tubules is associated primarily with an increase in length and diameter.

Development of the Seminiferous Epithelium

The seminiferous epithelium of the fetal testis is composed of two morphologically distinct cell types—the gonocytes (primordial germ cells) and the supporting cells (precursors of the Sertoli cells) (Fig. 12). Both cell types are engaged in active mitotic activity during fetal life.

The controversy over whether gonocytes are precursors of definitive germ cells in adult testis is still not entirely settled, despite the fact that it originated as far back as the turn of the century. Many investigators have suggested that gonocytes degenerate in the neonatal testis and the definitive germinal-cell line is formed from undifferentiated, epithelial-like, supporting cells (3, 4, 52, 59, 73, 101, 114).

As recently as 1949, Allen (4) suggested that in the rat all primordial germ cells degenerate after birth, and Niemi & Ikonen (92) concluded that in the human testis

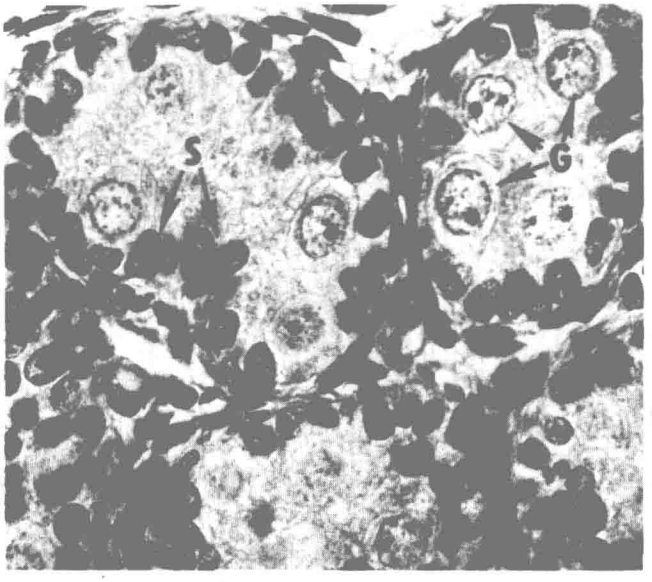


FIG. 12. Rat fetal testis showing gonocytes (*G*) and supporting cells (immature Sertoli cells) (*S*). × 560.

there probably is no continuous germ track, because the primordial germ cells dedifferentiate prior to development of permanent spermatogonia. Evidence to the contrary has been provided by a number of investigators, and a careful reevaluation of this problem in the rat by Clermont & Perey (36) provided strong evidence for the viewpoint that the definite germ-cell line does arise from gonocytes and that supporting cells are the precursors of Sertoli cells. This concept gained further support from studies by other investigators (9, 67, 106, 110, 113), and several reports concerned with the human testis also support this conclusion (1, 2, 15, 83).

The thesis that gonocytes are the precursors of the definite germ-cell line has been generally accepted; and many investigators proceeded on the basis of this premise to study the kinetics of initiation of the spermatogenic process during puberty. Most of the extensive studies were conducted in the rat, but several other species were also investigated—the ram (112), the mouse (111), the bull (8), the lamb (39), and man (1, 2, 15).

On the basis of quantitative, histological study, Clermont & Perey (36) concluded that in the rat some gonocytes degenerate soon after birth, but the remaining cells divide on the fourth postnatal day to form type A spermatogonia, which enter the spermatogenic cycle to form the basis for the definite germ-cell line. A somewhat different viewpoint was expressed by Sapsford (111, 112), who suggested that gonocytes first form a distinct cell type, the *immature* type A spermatogonia. These cells, in turn, serve as precursors of type A spermatogonia, which then enter the spermatogenic cycle. Steinberger et al. (122) observed in testes' tissue culture of newborn rats the formation of cells with morphological characteristics intermediate between gonocytes and type A spermatogonia. These cells were designated as *primitive* type A spermtogonia (Fig. 13). A similar cell type was also described in the testis of a developing rat by Beaumont & Mandl (9) and Huckins (67). Gonocytes probably do form primitive type A spermatogonia, which, in turn, divide to form either additional primitive type A spermatogonia or type A spermatogonia. The latter enter the spermatogenic process, while the remaining primitive type A spermatogonia may persist in the testes of adult animals in the form of *reserve* cells. The reserve cells are probably responsible for repopulation of seminiferous tubules after germinal epithelium atrophy secondary to an action of an injurious agent (29).

Mancini et al. (82), utilizing cytometric, histological, and histochemical techiques, attempted to investigate the initiation of spermatogenesis in the human being. These investigators concluded that in man the supporting cells (*nurse cells*) do not change after birth or during early development, but at puberty "transform" into mature Sertoli cells and do not give rise to any other cell type. The gonocytes form type A spermatogonia shortly after birth. Most of these cells degenerate; a small proportion forms a series of morphologically dissimilar spermatogonia of which most, in turn, also degenerate and only a few enter the process of spermatogenesis at the time of puberty. This observation is at variance with the conclusions of Charney et al. (15), who suggested that spermatogonia are very rare at birth and remain so until about 10 years of age, when they begin to divide actively and shortly afterward enter the spermatogenic process.

Investigations concerned with the initiation of human spermatogenesis are scarce for an obvious reason—the paucity of adequate material for study. Although the information to date provides a considerable body of knowledge, more extensive investigations are required to define the details of the kinetics of initiation of spermatogenesis in the human testis.

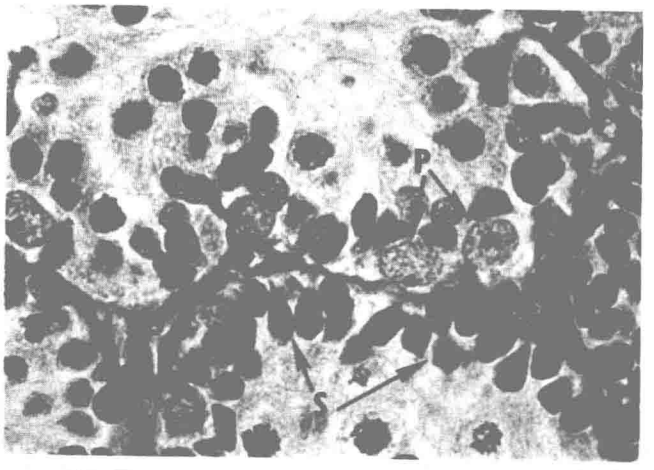


FIG. 13. Testicular tissue from 14-day-old rat, grown in culture for 3 weeks. Note Sertoli cells (*S*), and primitive type A spermatogonia (*P*). × 470.

SPERMATOGENESIS

General Considerations

The discovery that spermatozoa develop from cells residing in the testis (134) provided the initial impetus for the investigation of the morphological characteristics of the seminiferous tubules and rapidly led to the description of spermatogenesis as an orderly and well-defined process involving the development of spermatozoa from less mature cells residing within the seminiferous tubules of the testis. Once the various types of the germinal epithelium cells were classified (132, 135), it became clear that the least mature germinal cells, the spermatogonia, divide to form the meiotic cells or primary spermatocytes, which, by the process of reduction division, form haploid cells, the spermatids. The spermatids develop into spermatozoa as a result of complicated metamorphosis involving dramatic structural modification of the nucleus, formation of new organelles, and acquisition of mechanisms permitting independent directional motility.

At about the same time an observation was made that the various cell types within the seminiferous epithelium form well-defined cellular associations, which succeed one another cyclically in any given area of seminiferous tubules. Each complete sequence of changes in cellular associations was named the *spermatogenic cycle* (10, 101, 132).

The Cycle of the Seminiferous Epithelium

Numerous attempts have been made to clarify the details of the spermatogenic cycle. Most investigators encountered difficulties related primarily to the lack of a precise, easily recognizable marker for the identification of the various cellular associations (40, 88, 109). Leblond & Clermont (77), utilizing a special staining technique, observed characteristic morphological changes in the acrosomes of spermatids in the rat testes that were directly related to their stage of development. A detailed study of the progressive structural changes occurring in the spermatids in the course of their development and formation of spermatozoa led these investigators to the description of 19 well-defined steps of spermiogenesis (Fig. 14).

It also became apparent that in each cross section of a seminiferous tubule containing spermatids in one of the first 14 steps of spermatid development, the remaining cells of the seminiferous epithelium form a precisely defined association of specific germinal-cell types. This finding led to the extension of the original concept of spermatogenic cycle (132) to the idea of a *cycle of the seminiferous epithelium*, defined as "a series of changes in a given area of seminiferous epithelium between two appearances of the same developmental stages" (78).

In the rat the entire cycle consists of 14 stages or characteristic cellular associations, as shown in Figure 15. Utilizing this diagram, one can follow the progress of an individual germ cell through the different phases of development. For example the least mature germ cell that enters the spermatogenic cycle (type A spermatogonium) is located closest to the basement membrane. It can be traced through the mitotic divisions, formation of meiotic cells, the reduction division, and formation of spermatids. Spermatids are located close to the tubular lumen. They develop into spermatozoa, which are released into the lumen of the seminiferous tubule. The progression of the entire germinal epithelium complexes, the cellular associations, through the 14 stages of the cycle of the seminiferous epithelium can also be followed on Figure 15.

The formation of specific cellular associations and the sequence of their appearance in a given area of the seminiferous tubule are highly synchronized. Moreover the numerical relationships between the various cell types

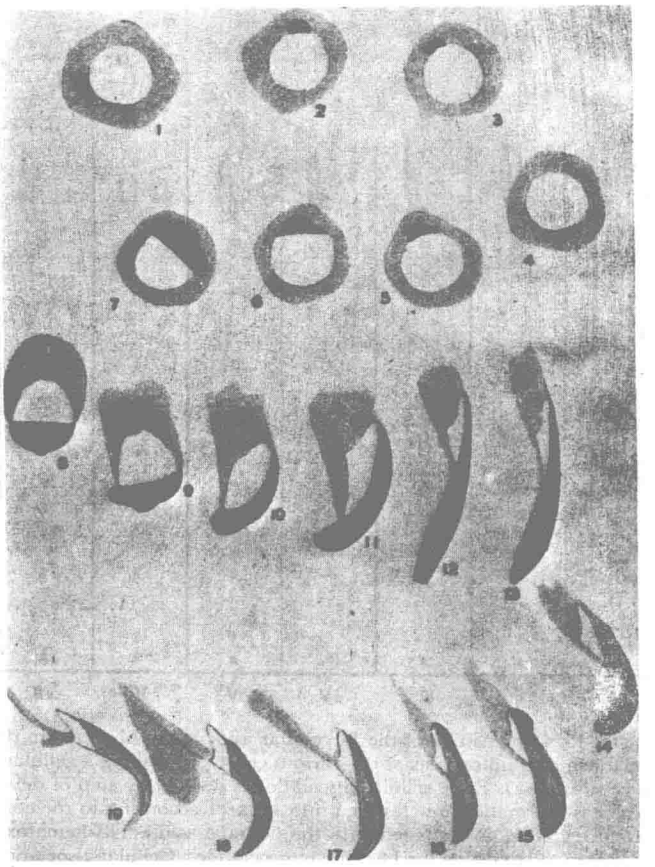


FIG. 14. Spermiogenesis in the rat. Steps *1–3*: Golgi phase. The idiosome produces two proacrosomic granules, which fuse into the single acrosomic granule. Steps *4–7*: cap phase. The acrosomic granule produces the head cap, which enlarges to cover a third of the nucleus. Steps *8–14*: acrosome phase. The nucleus and head cap elongate, whereas the acrosomic granule transforms into the acrosome. Steps *15–19*: maturation phase. Near the end of this phase the reactivity of the head cap and acrosome decreases considerably and the spermatozoon is released into the lumen. [From Leblond & Clermont (78). Copyright 1952 by The New York Academy of Sciences; reprinted by permission.]

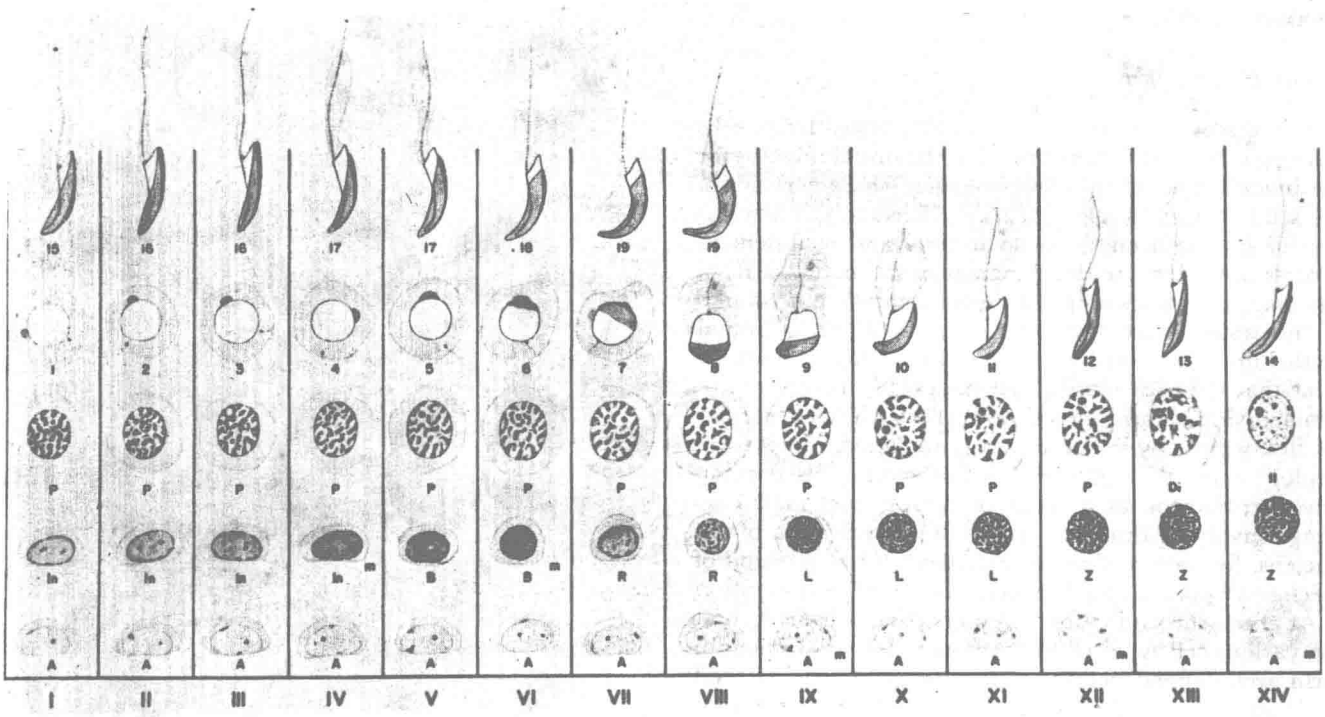


FIG. 15. Composition of the 14 cellular associations observed in the seminiferous epithelium in the rat. Each column consists of the various cell types making a cellular association (identified by *roman numeral* at base). Each cellular association is defined by step of development of spermatids present. Whereas spermatids are classified into 19 steps according to the condition of nucleus and acrosomic structures in sections stained with the periodic acid-Schiff-hematoxylin technique, only the first 14 steps are used to define the 14 cellular associations. Cellular associations succeed one another in time in any given area of seminiferous tubule according to the sequence indicated from *left* to *right* in figure. (Following cellular association XIV, cellular association I reappears, so that the sequence starts again). The succession of the 14 cellular associations comprises the cycle of the seminiferous epithelium. Cycle should not be confused with spermatogenesis, the complete series of the changes taking place in germ cells during the evolution by which type A stem cells eventually become spermatozoa. This sequence can also be seen in drawing, if one reads from *left* to *right* starting with *bottom row*. *A*, type A spermatogonia; *In*, intermediate type spermatogonia; *B*, type B spermatogonia; *R*, resting primary spermatocytes; *L*, leptotene primary spermatocytes; *Z*, zygotene primary spermatocytes; *P*, pachytene primary spermatocytes; *Di*, diakinesis of primary spermatocytes; *II*, secondary spermatocytes; *1–19*, steps of spermiogenesis. Subscript *m* next to a spermatogonium indicates occurrence of mitosis. [From Perey et al. (99).]

within a cellular association and their absolute numbers within a cross section of a seminiferous tubule are highly consistent (25, 120). The definition of these quantitative relationships of the germinal epithelium cells led to the development of exceedingly precise techniques for quantitative evaluation of cellular changes in the seminiferous tubules after damage to the germinal epithelium (17, 35, 94, 120).

Once the conceptual and mathematical models for the spermatogenic process were established for the rat (78), similar models were developed for other species. Oakberg (94) defined the details of the cellular associations and subdivided the cycle of seminiferous epithelium into 12 stages in the mouse; 12 stages of the cycle were also defined in the monkey (33; Fig. 16). The spermatogenic cycle was defined as well for the hamster (22), the ram (97), the bull (63, 64, 74), the rabbit (127), and the guinea pig (24).

Defining the cycle of the seminiferous epithelium in man was quite difficult since histological examination of the seminiferous tubules failed to reveal well-defined cellular associations. A detailed reevaluation of this problem led Clermont (26) to describe the cycle of the seminiferous epithelium on a similar principle to that described for other mammalian species. He proposed that in man the entire cycle consists of six stages (Fig. 17). Although the basic concept appears to be borne out by the available information, the detailed composition of the various cellular associations, as well as the numerical relationships among the various germinal epithelium cells, remains poorly defined (126).

Stem-Cell Renewal

Germinal epithelium cells proliferate continuously throughout the adult life of a male, requiring a constant

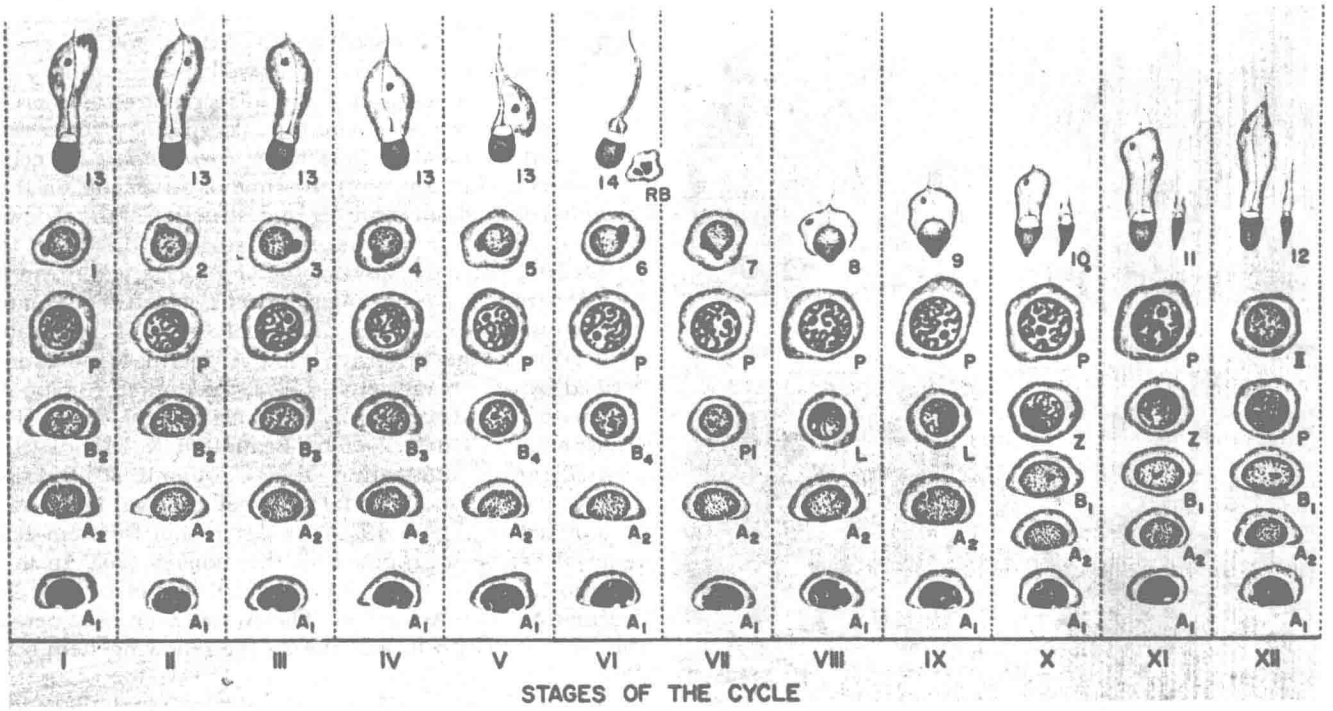


FIG. 16. Illustration of cellular composition of the 12 stages of the cycle of the seminiferous epithelium in the monkey. Each *column* numbered with a *roman numeral* shows cell types present in one of the cellular associations found in cross sections of seminiferous tubules. Cellular associations or stages of the cycle succeed one another in time in any given area of seminiferous tubule according to the sequence indicated from *left* to *right* in the figure. Following cellular association XII, cellular association I reappears, so that sequence starts over again. Stages of the cycle were identified by the first 12 of the 14 steps of spermiogenesis (no. 1–14). These steps were defined by the changes observed in the nucleus and in the acrosomic structure (acrosome and head cap seen closely applied to the surface of the nucleus) in sections stained with the periodic acid-Schiff-hematoxylin technique. Steps *10–12*: spermatids as seen from their flat surface and from the side; steps *13* and *14* as seen from their flat surface only. Associated with step *14*, the residual body (*RB*) is illustrated. A_1 and A_2, type A_1 and A_2 spermatogonia; B_1–B_4, generations of type B spermatogonia; *Pl*, preleptotene primary spermatocyte; *L*, leptotene spermatocyte; *Z*, zygotene spermatocyte; *P*, pachytene spermatocyte; *II*, secondary spermatocyte. [From Clermont (28).]

supply of germ-cell precursors. The mechanisms responsible for this process have been the topic of many investigations, and, although a number of theories have been proposed, including those that Sertoli cells are transformed into germinal cells or that cells of the wall of the seminiferous epithelium may transform into spermatogonia, the overwhelming body of evidence points toward the principle of a self-renewing germinal *stem cell*.

The early notions of bivalent mitosis (differential mitosis), a process whereby each young spermatogonium divides to form one spermatogonium and one spermatocyte, cannot be supported by the available evidence [for a review of this theory see (102)]. Using quantitative techniques for the investigation of this process, Clermont & Leblond (32) formulated the *stem-cell renewal theory*, based on the hypothesis that at the beginning of each cycle of spermatogenesis the stem-cell divisions result in formation of additional stem cells, which remain "dormant" until the subsequent cycle, and in the formation of type A spermatogonia, which enter the spermatogenic process and ultimately are transformed into mature spermatozoa. The dormant spermatogonia divide to form a new generation of stem cells and a new generation of type A spermatogonia, and the process repeats itself. This mechanism would permit a cyclic replenishment of cells for the spermatogenic process and, at the same time, would maintain the stem-cell line.

Although most of the extensive studies on the spermatogonial renewal systems have been conducted in the rat (26, 29, 32, 61) and the rat model will be used below for detailed discussion of this concept, it should be noted that a number of other species have also been studied in detail: the duck (23), the ram (97), the hamster (22), the monkey (33), and man (27).

Subsequent to the original description of the stem-cell renewal theory by Clermont & Leblond (32), it became apparent that there are several morphologically distinct types of A spermatogonia that form during four mitotic peaks at specific stages of the spermatogenic process (stages IX, XII, XIV, and I–II). These different types of A spermatogonia are designated as A_1, A_2, A_3, and A_4 (Fig. 18). In light of these findings several stem-cell-

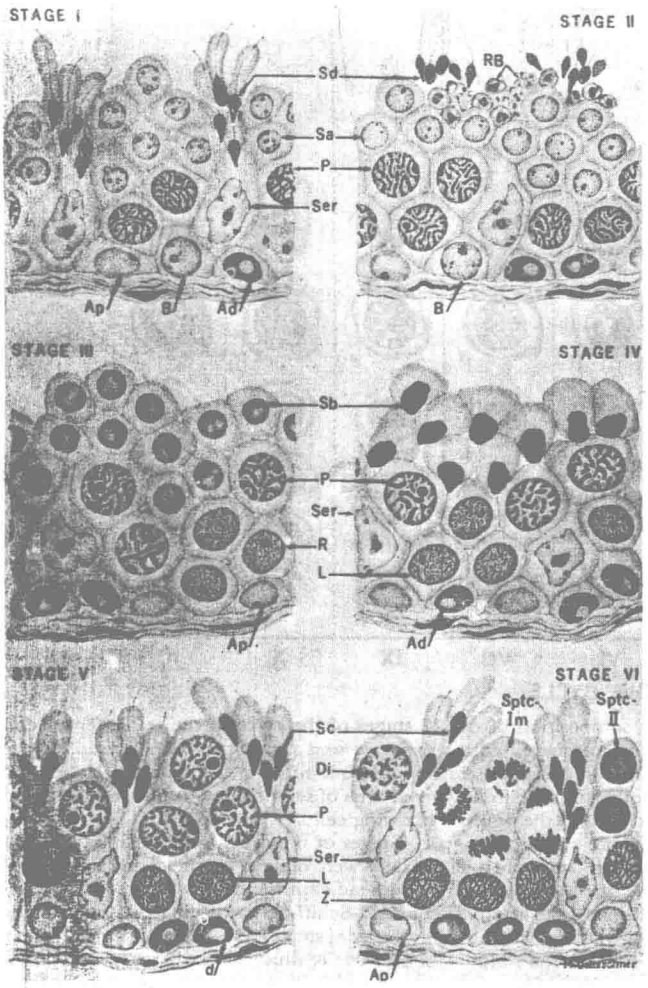


FIG. 17. Representation of cellular composition and topography of the six typical cellular associations found repeatedly in human seminiferous tubules. These cell associations, corresponding to stages of the cycle of the seminiferous epithelium, are numbered with *roman numerals*, stages I–VI. *Ser*, sertoli nuclei; *Ap* and *Ad*, pale and dark type A spermatogonia; *B*, type B spermatogonia; *R*, resting primary spermatocytes; *L*, leptotene primary spermatocytes; *Z*, zygotene primary spermatocytes; *P*, pachytene primary spermatocytes; *Di*, diplotene primary spermatocytes; *Sptc-Im*, primary spermatocytes in division; *Sptc-II*, secondary spermatocytes in interphase; *Sa*, *Sb*, *Sc*, *Sd*, spermatids at various steps of spermiogenesis; *RB*, residual bodies. [From Clermont (26).]

renewal hypotheses were proposed. Monesi (86) attributed the role of a stem cell to the A_1 spermatogonium; Hilscher et al. (61) attributed it to the Intermediate A spermatogonium. Clermont (25) modified his original hypothesis and suggested the A_3 spermatogonium for the role of stem cell. Recently, Clermont & Bustos-Obregon (29), using a new investigative technique, provided evidence that the A_4 spermatogonium is the stem cell. A similar hypothesis was also proposed by DeRooij & Kramer (45).

The Clermont & Bustos-Obregon (29) hypothesis for stem-cell renewal in the rat is based on the existence of five distinct classes of type A spermatogonia—A_0, A_1, A_2, A_3, and A_4. It is proposed that types A_1–A_4 are actively engaged in the proliferative activity leading to the formation of more mature germ cells, as well as in the process of renewal of the stem cells. Cells in this series are considered to be *renewing stem cells*. The type A_0 spermatogonia are considered by these authors to be *reserve stem cells*, cells that remain dormant until the time of an assault on the germinal epithelium resulting in destruction of the renewing stem cells. The type A_0 spermatogonia then start to proliferate, enter the spermatogenic process, and form a new generation of renewing stem cells, which repopulate the seminiferous tubules. This cell type is probably analogous to the primitive type A spermatogonia described by Steinberger et al. (122), the immature type A spermatogonia described by Sapsford (111, 112), or the transitional cell described by Beaumont & Mandl (9).

A diagram illustrating the Clermont & Bustos-Obregon (29) hypothesis for stem-cell renewal in the rat is depicted in Figure 19. A similar model for stem-cell renewal has been proposed for the monkey (28). In this species, however, there are only two classes of type A spermatogonia—A_1 and A_2. The A_1 has been considered the reserve stem cell, and the A_2 the renewing stem cell (Fig. 20).

Recently, Huckins (69) proposed a new hypothesis for a stem-cell-renewal model in the rat on the basis of quantitative reevaluation of whole mounts of seminiferous tubules. This hypothesis dispenses with the concept of a reserve stem cell (A_0) and with the assumption that type A_1 spermatogonia must arise in the course of one of the spermatogonial division peaks. It is proposed that all spermatogonia fall into one of the following categories: *1*) stem cells (A_s); *2*) proliferating cells (A_{pr}, A_{al}); and *3*) differentiating cells (A_1, A_2, A_3, A_4, A_{in}, and B). It is suggested that the stem cells (A_s) occur isolated rather

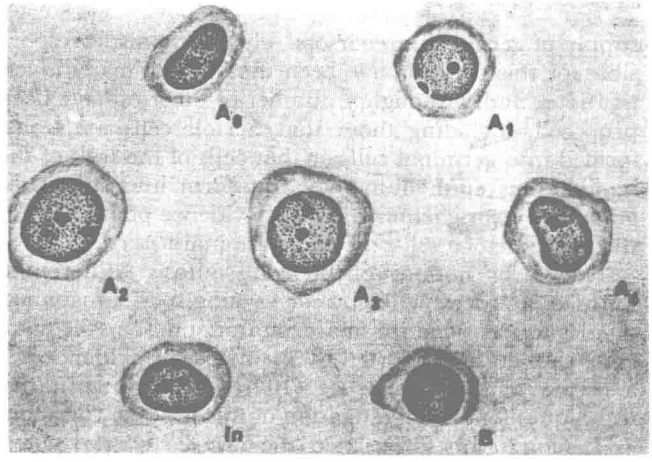


FIG. 18. Illustration of the various types of spermatogonia as seen in dissected tubules fixed in Carnoy solution, stained with hematoxylin, and mounted in toto. These cells were found at the following stages of the cycle of the seminiferous epithelium: A_0, at stages I–XIV; A_1, at stages I–IX; A_2 at stages IX–XII; A_3, at stages XII–XIV; A_4, at stages XIV–I; *In*, at stages I–IV; *B*, at stages IV–VI. [From Clermont & Bustos-Obregon (29).]

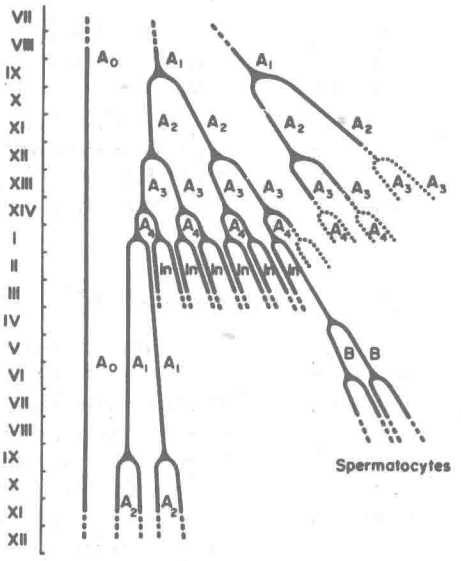


FIG. 19. Representation of the mode of proliferation and renewal of spermatogonia in the rat based on observations of dissected tubules mounted in toto and on quantitatation of the spermatogonial population. *Roman numerals*, stages of the cycle. A_0, A_1–A_4, various classes of type A spermatogonia; *In* and *B*, intermediate and type B spermatogonia. According to this model, two main categories of type A spermatogonia exist, the reserve stem cells (A_0) and the renewing stem cells (A_1–A_4). Type A_0 spermatogonia are represented as a *straight unbranched line* (*left*) to indicate that they do not proliferate to any significant extent in adult rats. In the renewing series (*right*), a pair of initial stem cells (A_1) start proliferating in stage IX of the cycle to give, successively, types A_2, A_3, and A_4 spermatogonia, each one of these dividing, respectively, in stages XII, XIV, and I of the cycle. In stage I, dividing type A_4 spermatogonia produce a new pair of type A_1 spermatogonia and 12 intermediate spermatogonia (*In*). Some type A_2, A_3, and A_4 spermatogonia (*right*) are shown to disappear by degeneration at the time of division, *dotted lines*, thus explaining the lower-than-expected ratio of intermediate spermatogonia to the initial stem cell (A_1: In = 1.6). Each intermediate spermatogonium divides to produce type B spermatogonia, which in turn gives rise to spermatocytes. No degeneration takes place during these last two spermatogonial divisions. [From Clermont & Bustos-Obregon (29).]

than in groups and are distributed randomly throughout the length of the seminiferous tubule rather than concentrating in any specific stage of spermatogenesis. These cells are regarded as the "true" stem cells that form the *stem-cell compartment*. The A_s spermatogonia divide periodically, but randomly, and either form additional stem cells, which remain scattered singly, or their daughter cells retain their proximity and form pairs of spermatogonia (A_{pr}). These cells enter a series of synchronous divisions leading to formation of chains of cells or *aligned* spermatogonia (A_{al}), which comprise the *proliferative compartment*, and ultimately are transformed to the A_1 spermatogonia. The A_1 spermatogonia enter the *differentiating compartment* of spermatogonial development characterized by a series of synchronous divisions in specific stages of the spermatogenic cycle, which result in formation of A_2, A_3, and A_4, Intermediate and B spermatogonia, in that order. The last divide to form meiotic cells (spermatocytes). (For schematic representation of this hypothesis, see Figs. 21 and 25.)

The duration of the mitotic cycles of the various types of differentiating spermatogonia have been found to be similar, except for A_1 spermatogonia (16, 70). The cycle duration was 41–42.5 hr, and the G_1 phase 11–13 hr.

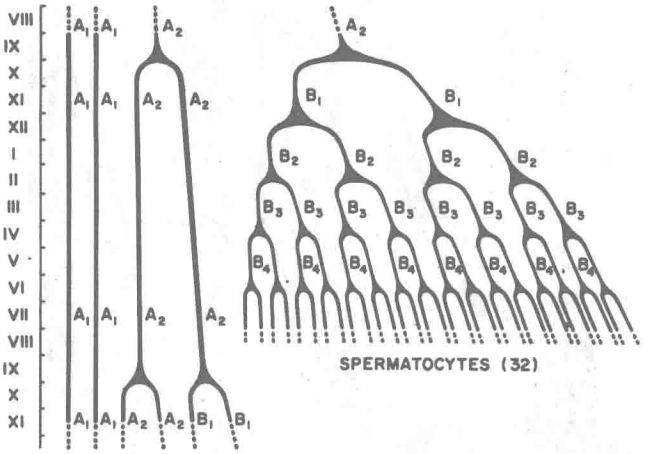


FIG. 20. Illustration of mode of proliferation and renewal of spermatogonia in the monkey. Model is based on qualitative and quantitative observations of dissected tubules mounted in toto and on radioautographed sections of a ^{3}H-labeled thymidine-injected animal. Stages of cycle are indicated on *left* by *roman numerals*. A_1 and A_2, type A_1 and A_2 spermatogonia; B_1–B_4, type B_1–B_4 spermatogonia. On *left*, a pair of type A_1 spermatogonia is represented as *two parallel, unbranched lines*, indicating these cells did not proliferate to any significant extent during the cycle of the seminiferous epithelium of normal adult animals. A pair of type A_2 spermatogonia is shown to divide at stages IX–X of the cycle; one of them would yield a new pair of type A_2 spermatogonia that would remain dormant until stages IX–X of the next cycle. The other (*right*) would yield a pair of type B_1 spermatogonia, which in stage XII would produce type B_2 cells. Type B_2 spermatogonia would give rise, during stage II, to type B_3 spermatogonia, which in stage IV would produce type B_4 cells, which finally divide in stage VI to yield spermatocytes. [From Clermont (28).]

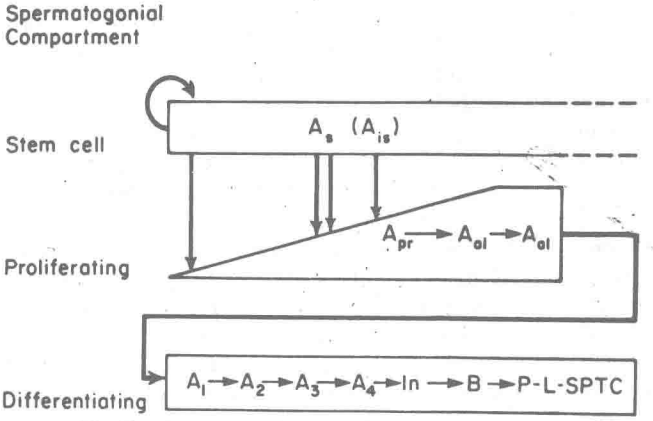


FIG. 21. Proposed model for renewal and differentiation of spermatogonia in adult rat testis. See text for discussion. [From Huckins (69).]

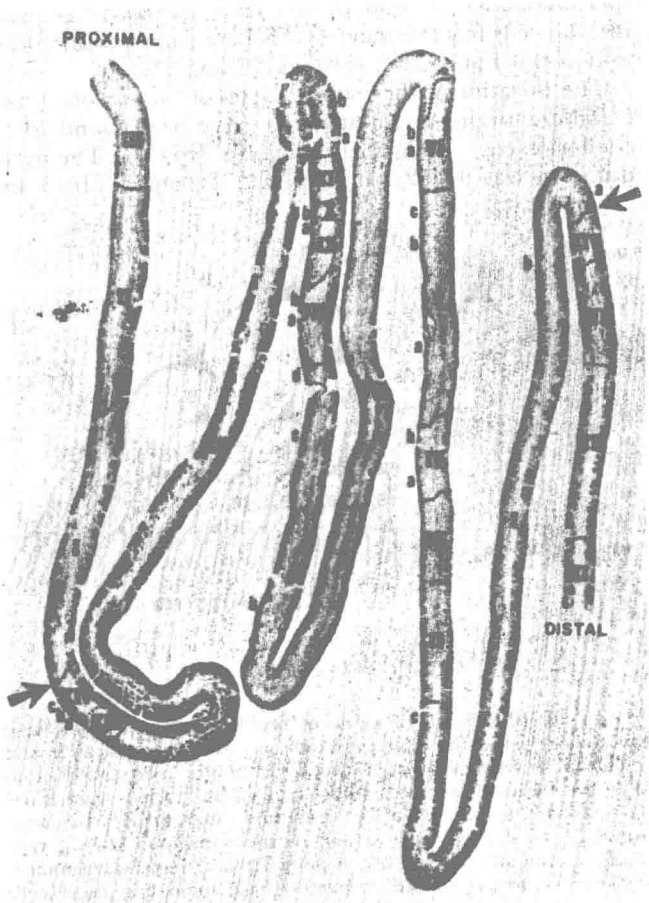


FIG. 22. Low-power photograph of longitudinally cut tubule showing a wave without modulation. Note the continuity of segmental order, the descent of the order in a distal direction, and the variability in the length of segments of a given type (compare the two segments I, also the two XII). No modulation was observed over this length of tubule. The limits of a wave may be taken to be the borderlines between segments XIV and I (*arrows*). (The small letters are not relevant.) × 25. [From Perey et al. (99).]

The duration of the S phase (DNA synthetic phase) showed progressive lengthening in the process of development of A_1 to A_4 spermatogonia. Since the S + G_2 phase has not changed, the lengthening of the S phase was at the expense of the G_2 phase (70).

The above discussed hypothesis of stem-cell renewal and spermatogonial differentiation has been supported by studies in the mouse testis (96). In these investigations, ^{3}H-thymidine was used for labeling of dividing spermatogonia, and the label was visualized by means of radioautography of histological sections of the testis. The obtained data support the concept of renewing-stem-cell compartments and do not support the concept of reserve stem cells (A_0 spermatogonia). Similarly to the rat, the mouse spermatogonia are separated into five classes on the basis of their nuclear morphology and appear to undergo mitotic divisions and morphological changes as do the rat spermatogonia.

It is quite clear that in the past 18 years considerable progress has been made in the knowledge of the kinetics and cytological details concerned with the process of spermatogonial renewal. The most recent hypothesis appears to be well supported by experimental data. However, it does not entirely answer all the questions. The mechanisms involved in inducing some stem spermatogonia to enter the proliferating compartment still remain to be solved.

Kinetics of the Spermatogenic Process

In an analysis of the kinetics of spermatogenesis the interaction between the temporal and spatial aspects of this process must be considered. Von Ebner (132) formulated the concept of the *wave of the seminiferous epithelium*, and Regaud (101) extended this concept and postulated that the "wave is in space what the cycle is in time." The existence of the wave of the seminiferous epithelium has been described in testes of a number of mammals—the mouse, bull, guinea pig, rabbit, ram, boar, dog and cat (10), and marsupials (54).

Recently the wave concept of von Ebner and Regaud has been confirmed and defined with great precision in the seminiferous tubules of rat testes (99). Extensive analysis of serial sections of the entire rat testicle, reconstruction of the seminiferous epithelium, and analysis of cell associations along segments of seminiferous tubules sectioned longitudinally revealed that specific stages of the cycle are arranged in consecutive order along the tubule so that adjacent segments are either less or more advanced by a single stage, and the development always proceeds in one direction (Fig. 22). The segments, however, may vary in length in different tubules. When a tubule is examined lengthwise in a direction distal to the rete testis, the cell associations show a descending order (toward a less advanced stage of spermatogenesis). The tubules form loops with both ends emptying into the rete testis; the sequence of stages reverses in the middle of the loop to permit the formation of the descending order in both limbs of the loop. The sequential order is broken occasionally for a short distance; such breaks in sequence have been coined *modulations of the segmental order* [(99); Fig. 23]. The cause or significance of these irregularities is not known.

The wave of the seminiferous epithelium is defined "as a series of adjacent segments which include the fourteen possible types, in addition to any segment which is involved in modulation" (99). The segments are not arranged in any specific geometric fashion, as, for example, in a spiral arrangement to form the wave, as assumed by Regaud (101). The boundaries of segments are irregular but sharply demarcated (Fig. 22).

Reevaluation of the spermatogenic wave in rat seminiferous tubules (16) utilizing radioautographic techniques applied to whole mounts of the seminiferous tubules (18) revealed orderly mitotic activity of spermatogonia arranged segmentally within the seminiferous tubules, with the segments corresponding to the stages of spermatogenesis. This finding provides additional evidence for