Spermatogenesis in Man: An Estimate of Its Duration

Abstract. *Testicular biopsies were obtained at various intervals after intratesticular injections of tritiated thymidine. Radioautographs revealed that the most mature labeled germ cells were the preleptotene spermatocytes at 1 hour, the midpachytene spermatocytes at 16 days, and the immature spermatids at 32 days after injection. These results indicated that one cycle of the seminiferous epithelium lasts 16 days, and the whole of spermatogenesis is estimated to consume approximately 64 days.*

In sharp contrast to other mammalian species, the arrangement of germ cells in the human seminiferous epithelium appears to be and always has been considered chaotic. No clearcut cellular association could, until recently, be identified and the "cycle of the seminiferous epithelium" in man was said to be nonexistent. This lack of organization would obviously hinder any evaluation of the rate of development of the germinal elements. Recently Clermont (*1*) determined the composition of six typical cellular associations and described the cyclic evolution of the seminiferous epithelium in man. These findings were confirmed and applied to studies on the effects of diamines on human spermatogenesis (*2*). These cellular relations have been used in the present investigation to obtain an estimate of the duration of spermatogenesis.

In a few mammals, spermatogenesis, the process by which spermatogonial stem cells proliferate and transform into spermatozoa, has been accurately timed by labeling resting (also called preleptotene) spermatocytes with a DNA precursor and radioautographing sections of testis at various time intervals thereafter (*3*, *4*). Thus, Ortavant, by injecting P^{32}-labeled phosphate into rams, estimated the duration of spermatogenesis at 49 days (*3*). The most suitable DNA precursor for radioautography, tritiated thymidine (*5*), was used in the rat to show that spermatogenesis lasts 48 days in this rodent (*4*). Parenteral injection of radioactive thymidine in man, in an amount sufficient to label nuclei, is too hazardous a procedure. Instead a technique of direct injection of thymidine-H^3 into the testis was developed for the present study.

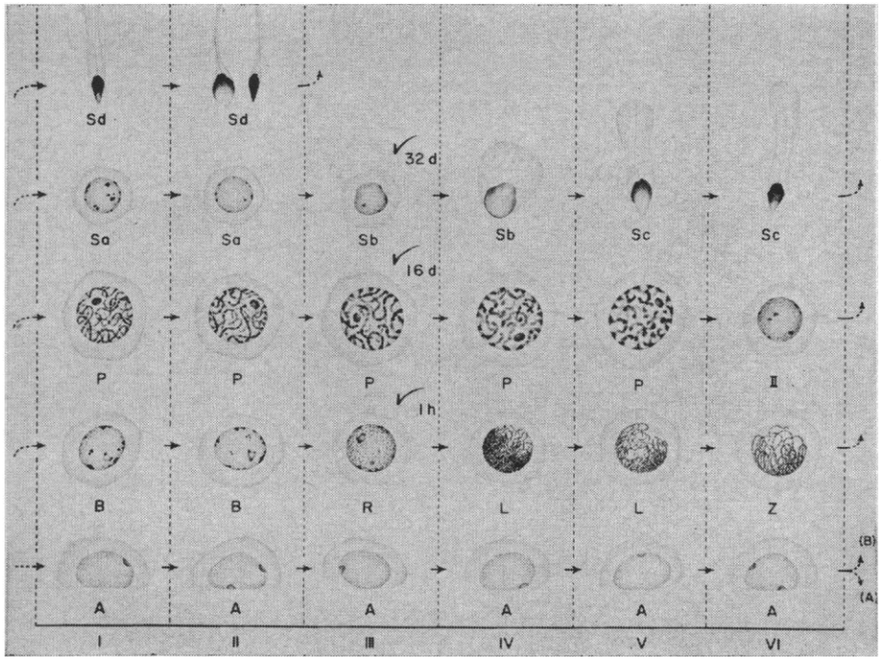
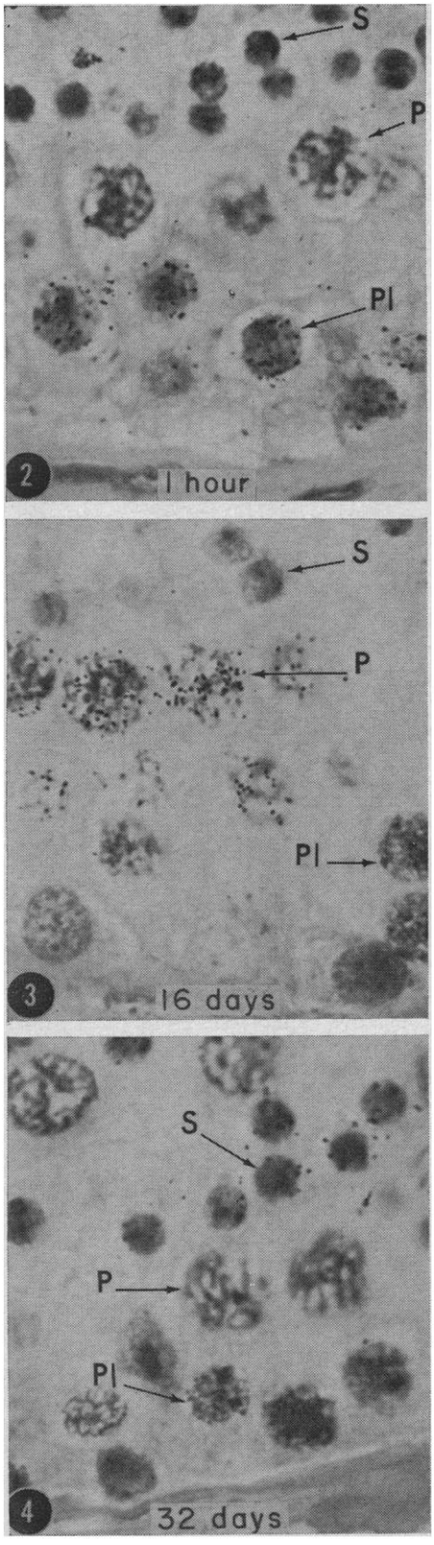


Fig. 1. Composition of six typical cellular associations observed in the seminiferous epithelium of man (*1*). Each column consists of the various cell types making a cellular association. The cellular associations succeed one another in time in any given area of the seminiferous tubule according to the sequence indicated from left to right in the figure. After cellular association VI, cellular association I reappears so that the sequence starts over again. The succession of the six cellular associations make up the "cycle of the seminiferous epithelium". Each cellular association (identified by a Roman numeral at the base of the diagram) is taken to characterize a "stage of the cycle". The "cycle of the seminiferous epithelium" should be clearly distinguished from "spermatogenesis," which is the complete series of changes by which a type A spermatogonium transforms into free spermatozoa. The sequence of changes is shown by small arrows to be read from left to right starting from the bottom row. Legend: *A*, type A spermatogonium; *B*, type B spermatogonium; *R*, resting (also called preleptotene) primary spermatocyte; *L*, leptotene primary spermatocyte; *Z*, zygotene primary spermatocyte; *P*, pachytene primary spermatocyte; *II*, secondary spermatocyte; *Sa* to *Sd*, steps of spermiogenesis. Check marks indicate the most advanced labeled cells at various time intervals after a single intratesticular injection of thymidine-H^3.

Inmates of the Oregon State Penitentiary volunteered for this investigation. To eliminate the possibility of eventually contaminating the population with genetically altered chromosomes, only previously vasectomized subjects (seven in all) were chosen. Thymidine-H^3 (5.2 μc/mmole) was injected directly into the testis, through the tunica albuginea, to a depth of 0.5 cm. The amount injected was 0.1 ml of saline containing 10 μc of thymidine-H^3. Two sites were injected per testis and were marked by tying a black silk suture into the tunica albuginea. At various time intervals thereafter (1 hour to 40 days), the tunica albuginea was re-exposed and a biopsy was removed from the site of injection and fixed in Cleland's solution (modified Bouin). Tissue sections, cut at 5 μ, were stained with iron hematoxylin and eosin and prepared for radioautography by the coating method of Kopriwa and Leblond (*6*). The sections were exposed from 2 to 50 days before being developed.

Before analyzing the radioautographs we will review briefly the main histological characteristics of the human seminiferous epithelium (*1*). In man, as in other mammals, generations of spermatogonia, spermatocytes, and spermatids were grouped in a few well defined cellular associations (Fig. 1). Thus, one or two generations of spermatids at given steps of spermiogenesis were always associated with one or two generations of spermatocytes and with spermatogonia at given steps of their respective development. Six such well defined cellular associations were identified and were interpreted as six stages in the evolution of the seminiferous epithelium (Fig. 1). The succession of these six stages in any one area of the tubular epithelium thus constituted the cycle of the seminiferous epithelium (*1*, *7*).

Examination of the radioautographs



Figs. 2–4. Three radioautographed portions of the seminiferous epithelium showing the same cell association: stage III of the cycle. The three pictures were taken from sections of testicular biopsies collected 1 hour, 16 days, and 32 days after a single intratesticular injection of thymidine-H^3. Legend: *Pl*, preleptotene spermatocyte; *P*, pachytene spermatocyte; *S*, spermatid. At 1 hour, *Pl* are labeled; at 16 days, *P* are labeled; and at 32 days, *S* are labeled.

revealed that within 1 hour after injection, the nuclei of some spermatogonia and preleptotene spermatocytes were labeled with thymidine-H^3 (Fig. 2). The labeled preleptotene spermatocytes were the most mature germ cells which had incorporated thymidine-H^3 at the 1-hour interval, since no other cells, more advanced in development (spermatocytes, spermatids), showed any labeling (Fig. 1). Therefore, the most mature labeled cells found in radioautographs of testicular biopsies collected at later time intervals must necessarily have arisen from such preleptotene spermatocytes labeled with thymidine-H^3.

It should be noted that, at 1 hour after injection, the labeled preleptotene spermatocytes belonged to stage III of the cycle (Figs. 1 and 2). This stage includes midpachytene spermatocytes and young spermatids with a slightly irregular spheroidal nucleus. With time, the labeled preleptotene spermatocytes should successively go through leptotene, zygotene, and early pachytene steps of the meiotic prophase and reappear at stage III as midpachytene spermatocytes, thus covering the duration of one cycle (Fig. 1). An attempt was then made to find out at what time interval after thymidine-H^3 injection the midpachytene spermatocytes of stage III become labeled.

In biopsies secured 12 and 14 days after thymidine-H^3 injection, labeled pachytene spermatocytes were found, but the most advanced ones had not yet reached stage III and were found at stage II only (Fig. 1). At 16 days, however, a biopsy specimen showed labeling of the midpachytene spermatocytes of stage III of the cycle. (Fig. 3). Thus, it was concluded that the duration of one cycle must be about 16 days.

From these data one could anticipate that in an additional 16 days (that is, 32 days after thymidine-H^3 injection) the most advanced labeled cells would again appear in stage III but this time as spermatids, since the labeled midpachytene spermatocytes would have completed the maturation divisions giving rise to spermatids (Fig. 1). Therefore a biopsy specimen was obtained 32 days after thymidine-H^3 administration. The radioautographs revealed that the most advanced labeled cells were indeed the spermatids in stage III of the cycle (Fig. 4). Since, at 1 hour, at 16 days, and at 32 days after injection the most advanced labeled cells were in each case at stage III, it was concluded that each cycle of the seminiferous epithelium has a duration of 16 days.

An estimate of the duration of the whole spermatogenic process presupposes knowledge of both the beginning and the ending of spermatogenesis. The ending can be defined with reasonable precision as the time of the completion of the spermatids' metamorphosis into spermatozoa and their extrusion into the lumen of the tubule during stage II of the cycle (top row of Fig. 1). On the other hand, the point where spermatogenesis begins in man remains obscure. Mammalian spermatogenesis is considered by some (*3*, *8*) to begin with the appearance of new spermatogonial stem cells, and by others (*9*) with the first of the series of spermatogonial mitoses leading to the production of spermatocytes. In man, it is possible that, as in most mammals studied so far, spermatogonia begin their multiplication at least one cycle before they give rise to primary spermatocytes (around stage III of the cycle in man), in which case human spermatogenesis would extend over the duration of approximately 64 days (*10*). If the onset of spermatogenesis is taken to be the appearance of new spermatogonial stem cells, then this would occur before stage III, perhaps at stage I of the cycle, or possibly even earlier. Thus, spermatogenesis would last more than 64 days. Until more information is available, 64 days—the duration of four consecutive cycles—may be considered a fair approximation of the duration of human spermatogenesis (*11*).

CARL G. HELLER
YVES CLERMONT
Pacific Northwest Research Foundation
1102 Columbia Street,
Seattle 4, Washington, and
Department of Anatomy,
McGill University, Montreal, Canada

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10. At any rate the minimal duration of spermatogenesis is 48 days, or the duration of three cycles; this is the time taken by a type B spermatogonium to transform into spermatozoa (Fig. 1).

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Methotrexate: Suppression of Experimental Allergic Encephalomyelitis

Abstract. *Methotrexate inhibited the production of allergic encephalomyelitis in guinea pigs when administered between the time of sensitization and the onset of disease. If treatment was delayed until the day of onset of encephalomyelitis, the disease was still suppressed and the death rate was reduced. The protective effect of methotrexate was reversed by folinic acid.*

The antifolic acid agent methotrexate (amethopterin), has been shown to inhibit both antibody formation and the development of the delayed hypersensitive state to purified protein antigens in guinea pigs (*1*). Suppression of immune host-graft reactions have also been demonstrated with this agent (*2*). The purpose of this study was to determine what effect, if any, was produced by methotrexate on an experimental autoimmune disease. Experimental allergic encephalomyelitis (EAE) in the guinea pig was selected as the model because the disease can be produced regularly and because methotrexate has relatively little toxicity in this species.

Table 1. Effect of methotrexate (Mtx) on the incidence of EAE in guinea pigs.

Group I control	Group II Mtx begun day 1 to 5	Group III Mtx begun day 6 or 8
	EAE by day 19	
17/22* (77%)	1/32 (3%)†	4/18 (22%)
	EAE by day 39	
17/22 (77%)	11/32 (34%)	8/18 (44%)
	Death	
13/22 (59%)	8/32 (25%)	4/18 (22%)

* Ratio of the number of animals with EAE to the total number of animals given the emulsion containing spinal cord and adjuvant. † Developed EAE on day 19, 2 days after last injection of methotrexate.

Guinea pig spinal cord was ground in a "Tri-R Teflon" tissue homogenizer, suspended in a solution of 0.25 percent phenol in water, and emulsified in an equal volume of complete Freund's adjuvant containing 4 mg of heat-killed *Mycobacterium tuberculosis* per milliliter. Male Hartley guinea pigs were injected intradermally; each guinea pig received 0.4 mg of mycobacteria in a total volume of 0.2 ml of emulsion. The concentration of spinal cord in the emulsion varied from 5 to 12.5 percent, but in each trial the control and methotrexate-treated guinea pigs to be compared were injected with the same preparation. With this procedure, the incidence of disease in any control group was not less than 60 percent.

Methotrexate was injected intraperitoneally in a dose of 5 mg, usually daily, sometimes on alternate days. Drug administration was begun on day 0, 2, 4, 6, or 8 of the experiment; day 0 represents the day of sensitization. Drug treatment was terminated on either the 17th or the 23rd day.

The diagnosis of EAE was based on the appearance of grossly obvious, definite paralysis or paresis which was always associated with sphincter disturbances and weight loss, and was usually associated with a wet chin, tremor, or convulsions. No histologic studies were made.

The pattern of response was similar in each of the separate trials and the results are therefore combined in Table 1. Animals that received methotrexate treatment beginning on day 0, 2, or 4 are tabulated together (group II) as are those started on day 6 or 8 (group III). Days 19 and 39 were chosen as reference points because no control animals developed EAE later than day 19 while none of the treated animals developed it after day 39.

In group I, the untreated controls, 17 of 22 animals developed EAE by day 19. In group II, which received methotrexate beginning on day 0, 2, or 4, no animals developed the disease during treatment, and only one of 32 developed it by day 19. In group III, methotrexate administration was begun on day 6 or 8, and four of 18 guinea pigs developed the disease by day 19. Many of the treated animals developed it from 2 to 22 days after cessation of methotrexate injections. This temporal sequence is illustrated in Fig. 1.

Some of the animals lost weight temporarily or failed to gain normally without showing definite signs of EAE after the methotrexate injections were stopped. In those animals there may have been an attenuated form of the disease, and the true incidence of late disease may be higher than that illustrated. Histologic studies will be required to deal with this question. The mortality was reduced in both methotrexate-treated groups (Table 1).

Table 2. Reversal of methotrexate (Mtx) effect by folinic acid (FA) in guinea pigs.

Controls	Mtx day 5-18	Mtx plus FA day 5-18
	EAE by day 14	
6/9*	0/9	7/9
	EAE by day 39	
6/9	6/9	7/9

* Ratio of the number of animals with EAE to the total number of animals given the emulsion containing spinal cord and adjuvant.

Methotrexate exhibited little if any gross toxicity in this experiment. One animal died on the last day of an 18-day course of 5 mg daily with no evidence of EAE, and this was therefore considered a toxic death. Two other animals with no evidence of disease failed to gain weight while receiving methotrexate. The remainder of the treated animals that were protected gained weight and looked outwardly normal while receiving the drug.

Because folinic acid (citrovorum factor) prevents the other known effects of methotrexate, an effort was made to determine whether it would alter the protective effect of methotrexate. In one trial, a control group was compared with a methotrexate-treated group, while a third group was injected daily with the same dose of methotrexate plus 20 mg of folinic acid subcutaneously. The results are shown in Table 2. The addition of folinic acid completely reversed the protective effect of methotrexate.

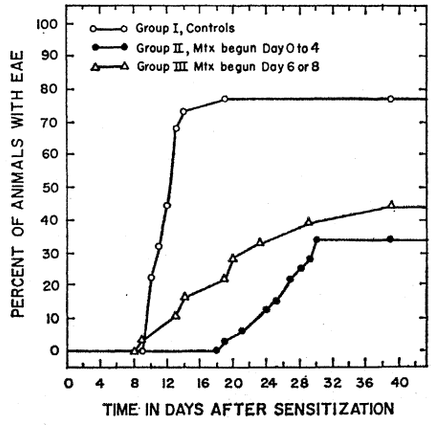

Fig. 1. Effect of methotrexate (Mtx) on the development of EAE.