III. MATERIALS AND METHODS

A. Animals used

1. Indian desert rat gerbil, *Meriones hurrianae* Jerdon: 26 (twenty six) mature male animals (body weight ranging between 175 and 200 grams) were collected by me from the arid zone of desert in the vicinity of Jaipur (Rajasthan). They were acclimatized for at least 3 weeks in the laboratory condition prior to the initiation of the experiment. 13 (thirteen) gerbils were used in each set of experiment (details in Table 9).

2. Goat, *Capra capra* (Domestic variety): 4 (four) sexually mature male animals (body weight within a range of 11-12 kg) were purchased from local markets. 2 (two) animals were used in the first set of experiment and the remaining in the second set (details in Table 10).

3. Booduga mouse, *Mus booduga*: 8 (eight) mature male mice (body weight ranging between 25 and 30 grams) were collected from the local rice field. Two sets of experiments were performed. The first and the second sets consisted of 4 (four) animals respectively (details in Table 11).
B. Two major aspects of investigation (Table 2)

(i) Cellular association study: The cellular associations in the seminiferous epithelium of the testicular tissues were studied in:
   a) Indian desert rat gerbil,
   b) Goat and
   c) Booduga mouse.

(ii) Chronology study: \(^3\)H-thymidine autoradiography with a view to estimating the duration of meiosis and spermiogenesis was conducted on:
   a) Indian desert rat gerbil,
   b) Goat and
   c) Booduga mouse.

As both these studies have certain common (overlapping) processing of the gonadal tissues, the next few pages are devoted to such methods.
(Table 2 harbours the investigational aspects on various animals used).
### TABLE 2

Various investigational methods on different experimental animals along with the major aims underlying these studies

<table>
<thead>
<tr>
<th>Studies</th>
<th>Animals used</th>
<th>Procedures performed</th>
<th>Major aims and objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>i) Gerbil (<em>Meriones hurrinae</em> Jerdon)</td>
<td>PAS - hematoxylin study of the histological sections followed by the conventional method performed by Clermont and Leblond (1959)</td>
<td>Studying the stapes (cellular associations) of the cycle of the seminiferous epithelium</td>
</tr>
<tr>
<td></td>
<td>ii) Goat (<em>Capra capra</em>)</td>
<td>Conventional autoradiographic (stripping film) method performed by Ghosal and Bandyopadhyay (1983)</td>
<td>Chronology of spermatogenesis</td>
</tr>
<tr>
<td></td>
<td>iii) Mouse (<em>Mus booduga</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*B</td>
<td>i) Gerbil (<em>Meriones hurrinae</em> Jerdon)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ii) Goat (<em>Capra capra</em>)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>iii) Mouse (<em>Mus booduga</em>)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For the amount of isotope used, vide, Table 3

+ The time of collection of testicular tissues, stages found labelled etc. in each experimental studies, A, B are displayed separately in Tables 8, 9 and 10 specifically.
C. Experimental procedure

(i) Administration of the tracer: Depending on the weight, urinary excretion and other physiological conditions of the specimens concerned, different doses of $^3$H-thymidine (Bhaba Atomic Research Centre, Trombay) were injected. In case of the desert rat, the tritiated thymidine was injected directly at various (usually 4) sites of each testis. In case of the goat, the isotope was injected directly to the different sites of the testis. Those sites were initially marked with tattoo and later tied with sterile silk thread as an additional safeguard for future identification of the sites from which biopsies were taken later, as in case of the human testis (vide, Heller and Clermont, 1963). However, in case of the booduga mouse, the tracer were injected intratesticularly. The amount and specific activity of $^3$H-thymidine injected were enlisted in Table 3.

(ii) Methods for obtaining testicular tissues from the specimens concerned: In case of gerbil, the animals were killed at intervals covering a period from a few hours to 37 days following administration of the tracer and the testes were dissected out.
TABLE 3

The details regarding the amount, specific activity and mode of the injection of $^3$H-thymidine in the experimental animals

<table>
<thead>
<tr>
<th>Animals</th>
<th>Number of animals used</th>
<th>Amount of $^3$H-TdR injected in each testis</th>
<th>Specific activity of the tracer</th>
<th>Amount of carrier injected in each testis</th>
<th>Particular of testis (or sites of the testis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gerbil (Meriones hurrinae Jerdon)</td>
<td>26</td>
<td>60 μCi</td>
<td>6100 mCi/mole</td>
<td>0.6 ml</td>
<td>Tracer injected on both the testes</td>
</tr>
<tr>
<td>Goat (Capra capra)</td>
<td>4</td>
<td>160 μCi</td>
<td>7600 mCi/mole</td>
<td>2 ml (0.5 ml each site)</td>
<td>Tracer injected into the four arbitrary sites in each testis of each specimen.</td>
</tr>
<tr>
<td>Mouse (Mus booduga)</td>
<td>8</td>
<td>40 μCi</td>
<td>6100 mCi/mole</td>
<td>0.4 ml</td>
<td>Tracer injected on both the testes</td>
</tr>
</tbody>
</table>
In case of the goat, testicular tissues were collected by the conventional biopsy method (Heller and Clermont, 1963; Rowley and Heller, 1966) from each site into which the radioactive tracer had been injected.

(A) In order to relocate the site of injection while the biopsy had to be obtained later, the individual injected site was marked initially with tattoo. The very site was at first injected with local anesthesia (e.g., Xylocane). The tracer was injected at these sites (Table 3, for details). Each site was tied with sterile, black silk thread facilitating their identity.

(B) Biopses were taken at regular intervals covering a period from few hour to 41 day post-injection (d p.i.).

(C) At specific time of post-injection, sites with silk thread were identified. The tunica albuginea was excised, and about 2 mm tissues were collected in normal saline (0.9% NaCl solution). The incision site was stitched thereafter with sterile operating needles and threads (Catgut). Antiseptic and healing drugs were administered immediately and S.O.S. thereafter.
The booduga mouse were killed primarily at 2 hr then at 4 hr and lastly at 10 day 4 hr after the injection of the tracer and the testicular tissues were dissected out.

(iii) Processing the testis for cytological observations:

(a) Air drying technique: The air drying technique employed in current study is widely used for observing the meiotic stages from the mammalian testis (Evans et al., 1964; Ohno, 1965). The blood vessels, mesorchium, together with tunica albuginea were removed completely from the testicular tissues and was collected in normal saline. The tissues were then washed repeatedly with the normal saline or BSS and finally minced finely with the help of a pair of bent scissors in a cavity block (Mukherjee and Ghosal, 1969). The minced tissues were then transferred in to a centrifuge tube and flushed gently. This solution was centrifuged (1000-1200 rpm, 10 mins) and the supernatant was gently decanted. To this centrifuge tube was added any suitable hypotonic solution, such as 0.75% Kcl or 0.7% sodium citrate, and pelet was stirred gently. This cell suspension was kept at 37°C for 30 mins. Then the cell-suspension was stirred once again and recentrifuged (1000-1200 rpm; 15 minutes). The supernatent was decanted carefully and 5 ml of freshly
prepared fixative (3:1 ethanol : glacial acetic acid) was added to the remains (slowly). Fixation was done for minimum of half an hour and a maximum of sixteen hours (including overnight inside a refrigerator), latter usually for bettering the quality of fixation. The fixed tissues were treated with 45% acetic acid for 30-40 mins (Ohno, 1965), resulting in the removal of much of protein and RNA from the chromosomes (Prescott and Bender, 1964). The 45% acetic acid having consequently been discarded from the tissue by mild centrifugation (400 rpm for 15 mins), the cells were resuspended in a small amount of the same fixative (3:1 acetoalcohol) and mixed gently (1-2 mins). This mixture (in fact, a cell suspension) was drawn in a clean dropper, and two or three drops were released from a height of 40 cms on to a clean grease-free slides held in an inclined position. Then slides were air dried while each drop had been spreading on them (Evans et al., 1964) and stored in a dust free cool place (for future application of Kodak AR-10 stripping film) for autoradiographic purpose (Ghosal and Midya, 1984).

(b) **Histological preparation**: Immediately after the collection of testis, it was cut into small pieces a few of which were fixed in aqueous Bouin's fluid
(24 hrs), while the remaining pieces were fixed in Zenker formol solution (24 hrs). So far as the performance of these two fixatives are concerned, acrosomic system of the spermatids is fixed better by the latter and than by the former. Zenker-formol, as such, is an excellent fixative for pinpointing distinct stages of the cycle and for calculating their relative frequency. Irrespective of the fixative used, the fixed tissues were dehydrated through graded alcohol, stored in cederwood oil for a few days for clearing and finally embeded in paraffin. Sections were cut at 4 u thickness and fixed on prealbumini­zed (with MyerI's solution) slides. These slides were stored in a dust free, cool and dry place for autoradio­graphy.

This histological procedure was formerly adopted by several workers; including Baker (1966); Beauregard et al. (1968); Lesson and lesson (1976) for the study of mammalian gametogenesis.

(iv) Staining :

(a) Air-dried slides : The slides containing the air-dried material were stained with either 2% aceto-orcin solution or 1% Delafield haematoxline solution,
prior to autoradiography. Some of those slides were also stained with Giemsa (1:20) phosphate buffer (pH 6.8).

(b) **Histological slides**: Histological sections (of tissues almost invariably fixed in Zenker-formol) were stained with periodic acid schiff (PAS) and counterstained with delafield haematoxyline (Humarson, 1967; Clermont and Leblond, 1955). The periodic acid-schiff stained the acrosomic structures (proacrosomic and acrosomic granules, acrosome, and head cap) pink while, hematoxyline outlined the nucleus in a blue shed (Clermont, 1963). Histological preparations for autoradiographic studies were stained in 1% haematoxyline (Delafield) only.

D. **Method for studying the relative frequecy of germ cell types within the seminiferous epithelium**:

The cells stained with PAS-haematoxyline in histological slides were classified according to the criteria suggested by Clermont (1963). In the experimental specimens the spermatogonia were divided into 3 types A, intermediate and B; on the exclusive basis of the nature as well as the stainability of their chromatin material. The position of the nucleolus, has, however, been used as an additional criterion for identifying the spermatogonial
type, as in the buffalo (Dhingra and Goyal 1975). Spermatocytes were separated into 4 groups depending on their nature of chromatin or more precisely the architecture of chromosomes representing the leptotene, zygotene and pachytene stages. For the sake of convenience, the diplo-tene, diakinesis and metaphase spermatocytes were usually (but not always) scored in a single group. Spermatids were classified as i) early (with PAS positive granules in the idiosome), ii) mid (cap phase) with PAS positive granules fused and spread like a cap over the nucleus and iii) late (acrosome phase) spermatids with definite acrosome. All these types of germ cells were scored in the histological sections prepared.

E. Identification and analysis of stages of meiosis and spermiogenesis:

(a) In cytological Preparations: So far as the air-dried preparation is concerned, the meiotic stages namely leptotene, zygotene, pachytene, diplotene, diakinesis and metaphases are readily distinguished by their chromosomal morphology in accordance with the conventional criteria (Ohno, 1965; Book and Kiessler, 1964; Eicher, 1966; Swanson, 1972; Sharma and Sharma, 1965; Gosalbez et al., 1979).
(b) **In Histological preparations**: In sharp contrast to the air-dried preparations, the histological preparations impose a few restrictions. Although various types of spermatogonia differ widely in their identifying characters, the spermatocytes and their meiotic stages are very similar as regards to the gross chromosomal features as revealed by the histological slides. There are some generalized features of the meiotic stages of spermatocytes. The following paragraphs are devoted to these characters as revealed in both the cytological and histological slides.

Young primary spermatocytes are distinguished from the resting spermatogonia by their larger size. At the onset of meiosis the nucleus of each spermatocyte becomes enlarged and thin chromatin threads impart a cloudy appearance in the leptotene. The chromatin threads shortly appear more closely drawn together and are for the most part indistinguishable from each other. During zygotene the nuclear material appears condensed and is almost invariably localised at a particular site, forming a crescent, condensed zone in the nucleus, a situation described as the chromosomal polarization (Kalt, 1976). The mammalian pachytene figure is equally prominent.
The chromatin threads are well recognised as chromosomes, which are much thicker and are marked by the presence of a typical entity, called the sex vesicle (Chen and Falek, 1971; Ghosal and Mukherjee, 1971) formed by XY bivalent (Ohno, 1965). Diplotene figure is marked by the presence of so called chromosomal loops (Chen and Falek, 1969), and chiasmata. During diakinesis the chromosomes are further condensed with nearly terminalized chiasmata, which form ring-like configuration (Chen, 1973; Pathak and Hsu, 1976). At metaphase - I the chromatin appears as coarse, tangle threads interspersed with clear spaces. These three stages, viz., diplotene, diakinesis and metaphases, have thus been identified by the formation and terminalization of chiasmata (Kezer, 1964), as well as the degree of separation of the homologous chromosomes (Ghosal and Mukherjee, 1971; Markvong et al., 1976). In histological sections, however, diakinesis strands of chromosomes appear much condensed and thread-like appearance. These cells become darkly stained having rounded or dumbell shaped chromatin materials clumped near the nuclear membrane. Secondary spermatocytes, particularly in the histological slides, are much smaller than the primary spermatocytes and contain homogenously dispersed diffused chromatin throughout the
nucleus (Belleve, 1979). As the duration of the second part of meiosis is very short, the stages of this part are rarer in all the preparation of testis (Svensson et al., 1980).

Metamorphosis of a spermatid into a spermatozoon is accompanied by distinct changes in the nuclear morphology and a net loss of cytoplasm during the later stages of this event (De douve et al., 1980). Chromatin becomes more homogeneous and concurrently deeply stained as the young spermatid advances. Stages of spermiogenesis as Golgi, cap, acrosomal and maturation phases are unmistakably distinguishable in the PAS-haematoxyline stained histological sections of the testis (Clermont and Leblond, 1955). The early spermatids are identified as the round cells with light staining spherical goligizone located in juxtaposition with the nuclear membrane. They later became oval. The mid spermatids are characterized by their structural changes when their acrosomes too change from the typical cap to conical shape. The late spermatids are the elongated entities with deeply stained acrosome which is lateral in position in relation to the nucleus of each late spermatid. The cytoplasm is very scanty and the formation of tail or flagella is complete.
F. **Analysis of cellular associations**:

The stages of the cycle have been identified initially on the basis of the steps (i.e., the degree of the development) of the spermatids (Clermont, 1954; Clermont and Leblond, 1959; Leblond and Clermont, 1952 a,b and Oakberg, 1956). Of the germ cells that compose the seminiferous epithelium, the spermatids are certainly the elements that undergo the most striking series of cytological changes. These yield morphological criteria that may be used to define clear cut steps of spermiogenesis, which in turn constitute useful means for the identification of the stages of the cycle. This necessitates the study of the changes in the nucleus and the acrosome system of the developing spermatids. PAS-haematoxyline stained histological slides relating to my research were studied in accordance with the conventional system (Clermont, 1963; Roosen-Runge, 1962).

Based on the existing format (Leblond and Clermont, 1952 a,b) the developmental steps of the spermatids were identified by English (Arabic) numerals, i.e., 1,2,3,4 etc. and the different types of associations were labelled by Roman numerals (i.e., I,II,III,IV and so on) in such a manner so that association I contains step 1 spermatids.
The chronological succession of the association was deciphered on the basis of the developmental sequences of the spermatids commencing with step 1.

G. The technique for determination of duration of individual stages of meiosis and spermiogenesis:

Due to their characteristic testicular features, such as, the cellular associations, the cycle of the seminiferous epithelium, etc., the mammals form an excellent group of animals in which the duration of spermatogenic stages can very conveniently be determined from the data of the duration of the cycle of the seminiferous epithelium (Noller et al., 1977b). Different radioactive tracers like $^{32}$P-phosphate, $^{14}$C-adenine and $^3$H-thymidine can be used for nuclear labelling. $^3$H-thymidine is most commonly used for its several advantages (which are discussed later, vide, selection of the tracer).

For the present investigation, $^3$H-thymidine, which is a specific precursor of DNA, was used. It was injected for labelling the DNA of the spermatocytes during their premeiotic S period and the course of migration of the labelled spermatocytes through different steps of meiosis and spermiogenesis was monitored autoradiographically in order to estimate the chronology of meiosis and spermiogenesis.
(Utakoji, 1966; Ghosal and Mukherjee, 1969; Witkin, 1980). This technique involves the intraperitoneal or direct intratesticular injection of $^3$H-thymidine, followed by the observation on the course of migration of the labelled germ cells through individual meiotic stages and spermiogenesis. The duration of any particular stage may be calculated by determining the interval of the time required for the labelled cells to appear for the first time at that very stage and the first appearance of labelling in the subsequent stages (Ghosal and Mukherjee, 1971). Suppose the pachytene figures were recorded labelled for the first time (most advanced labelled stage) at the 8th day post-injection and labelled diplotene figures were observed for the first time at 17th day post-injection, then the duration of pachytene would be approximately $(17-8=) 9$ days. Similarly if the labelled early spermatids were detected initially at 18 day post-injection and the mature spermatozoa appeared labelled at 35 day post-injection, then the duration of spermiogenesis would be close to $(35-18=) 17$ days. Actually the calculation of this time interval is done on the basis of a 'transitional phase' (details in Diagram 10). Thus the total duration of meiosis and spermiogenesis can be easily calculated from the time interval between the injection
of the tracer and the first appearance of labelled spermatozoa. However, for better resolution i.e., to determine the duration of the stages more accurately, testicular tissues should be collected at reasonably short intervals.

H. A bird's eyeview of the method of autoradiography employed in this dissertation:

(a) Selection of the tracer: A large number of precursors for labelling the DNA during its synthetic phase (S period) are present. While several earlier investigators used $^{32}$P-phosphate (Ortavant, 1958), $^{14}$C-adenine (Benett et al., 1966; Pelc and Howard, 1956), etc., the researchers at present prefer $^3$H-Thdr, i.e., tritiated thymidine for several advantages. The use of $^{32}$P is not advisable (Pollistar, 1969) as it incorporated into a variety of compounds besides DNA (Dquentl et al., 1977). $^{14}$C-adenine labels not only DNA but also RNA. On the other hand $^3$H-thymidine being supreme as it specifically labels DNA (Reichared and Esthern, 1951; Fraccaro et al., 1964; Fragese et al., 1974). It emits $\beta$ particles of very low energy (15 KeV, Perrey, 1964). The nuclear emulsion always respond to and records this type of $\beta$ particles with high efficiency (Rogers, 1967; Mukherjee et al., 1967, 1968). As a result of this a fine autoradiographic
resolution may be obtained (Taylor et al., 1957). This tracer also diffuses very rapidly throughout the ground after its injection into the animal (Utakoji and Hsu, 1975). Even when $^3$H-thymidine (of low specific activity) is administrated repeatedly (at every 4th hr prior to the depletion of the pool) for as long as 10 days in the mouse, chinese and golden hamster, neither chromosomal abberation nor any alteration in the chronology of migration of spermatocytes through stages of meiosis and spermiogenesis has been noticed (Utakoji and Hsu, 1975; Mukherjee and Ghosal, 1969). For all these advantages $^3$H-thymidine is used in this present works.

(b) Coating with stripping film: In the current investigation I used the Kodak AR-10 stripping film for studying the location of radioactivity.

The slides (whether histological or cytological) were stained about 2 hours prior to covering them with film. They were taken to the darkroom having a deep red safelight (Filter: Wratten MB). With a sharp blade the film was cut into required number of pieces. One edge of the outlined square was held with a blunt forcep and was pulled upward slowly under a controlled temperature ($19^\circ C$) and humidity (32-34%). The stripped film was
floated with its emulsion surface downwards on distilled water for 2-3 minutes. A slide with material on its upper surface was gently raised upwards through water (beneath the floating film) in such a manner that the sticky emulsion surface of the film came inclose contact with the materials of the slide (Stevens, 1966). The film adhered firmly to the slide while it was being dried infront of a fan at the darkroom temperature. The dried slides were then kept in light-tight blackboxes containing dehydrated blue silicagel (Pfizer) as desicant and were sealed with black tape. These boxes were then kept in a refrigerator at 4°C for a period of one month for exposure, so that the emulsion was exposed only to the 3 rays from the cells had taken up ³H-thymidine. The silica-gel absorbs moisture and keeps the emulsion appreciably dry. The moisture is detrimental to the slides at this stage and must be got rid of for preparing good autoradiograms (Rogers, 1967). The silica-gel, which gradually became hydrated, was replaced by freshly dehydrated blue silica-gel every week (Ghosal and Bandyopadhyay, 1983).

(c) Preparation of autoradiograms: After a 4-weeks exposure, the slides were developed in Kodak D-19 developer (vide, Table 4; developer: distilled water 1:2) for 4 mins
(19°C). The developed slides were rinsed in distilled water (30 secs) and kept (10 mins) in the fixer (30% solution (w/v) of sodium thiosulphate). They were kept in gently running tap water for half an hour. All these processings were done at 19°C in darkness (Mercant et al., 1965). The slides were then dried in the air and kept in a dust free box. These autoradiograms were examined under microscope with the help of an oil emersion lens. The tiny silver grains, appeared as black dots on the stripping film immediately overlying the materials from which the 3 rays were being emitted. The sites of emission are indeed the integral locations of the DNA which had incorporated the tritiated thymidine (Mukherjee et al., 1968).

(d) The removal of silver grains: In some cases silver grains were deposited enormously on the stripping film. Abundant grains obscured the details of the material thereby hindering the identification of any particular stage. In such cases silver grains were removed (Bianchi et al., 1964) by passing the slides through 7.5% potassium ferrycianide solution (K₃Fe(CN)₆) for 3 mins and 20% Na₂S₂O₃ (Sodium thiosulphate) solution for 3-5 mins. The slides should be washed thoroughly in running tap water, airdried and observed under shillaber's immersion oil.
**TABLE 4**

Ingredients for preparing Kodak D-19 or D-19b developers

<table>
<thead>
<tr>
<th></th>
<th>Kodak D-19</th>
<th>Kodak D-19b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Elon (Kodak)*</td>
<td>2.00 gms</td>
<td>2.20 gms</td>
</tr>
<tr>
<td>2. Sodium sulphate (anhydrous)</td>
<td>90.00 gms</td>
<td>72.00 gms</td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium sulphite (crystalline)</td>
<td>180.00 gms</td>
<td>144.00 gms</td>
</tr>
<tr>
<td>3. Hydroquinone</td>
<td>8.00 gms</td>
<td>8.8 gms</td>
</tr>
<tr>
<td>4. Sodium carbonate (anhydrous)</td>
<td>45.00 gms</td>
<td>48.00 gms</td>
</tr>
<tr>
<td>or</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium carbonate (crystalline)</td>
<td>122.00 gms</td>
<td>130.00 gms</td>
</tr>
<tr>
<td>5. Potassium bromide</td>
<td>5.00 gms</td>
<td>4.00 gms</td>
</tr>
<tr>
<td>6. Distilled water</td>
<td>1000 ml</td>
<td>1000 ml</td>
</tr>
</tbody>
</table>

* Rhodol (synthetic metol), Rhodia, Paris is equally good.
I. Method for calculating the duration of spermatogenesis:

(a) Relative duration of the cycle stages: In order to estimate the duration of the cycle by the method elucidated by Clermont and Trott (1969) it is necessary first to determine the relative frequency with which the various stages of the cycle appear. Such frequencies (expressed as percentages of a single cycle) correspond to the relative durations of the various stages. In a large number of random sections of seminiferous tubules, a stage of short duration appears rarely, while a stage of longer duration is seen more frequently. Therefore the frequency of occurrence of the various stages obtained from a survey of a large number of tubular cross sections may be used as an index to their relative duration (Noller et al., 1977b).

(b) Position of most advanced labelled cells within cycle at various intervals after $^{3}$H-thymidine injection: The spermatocytes, actively busy with their DNA replication, become instantaneously labelled as soon as they receive $^{3}$H-thymidine. These cells are the most advanced cells and would represent leading meiotic stage as spermatogenesis still progress. It may please be noted that the terms, such as the 'leading', 'highly advanced', 'most advanced'
or 'maximally progressed' cells (which I shall use indiscriminately) will hereinafter refer these very spermatocytes throughout this dissertation.

Clermont and Trott’s elaborate (Clermont and Trott, 1969) paper furnishes an easy-to-conceive description in case of C3H mice. They found that at 8 d 3 hr (8 days and 3 hours) after 3H-thymidine injection the group of the most advanced labelled cells were the pachytene spermatocytes (which evolved from preleptotene primary spermatocytes which had incorporated 3H-thymidine 8 days earlier). At 10 d 3 hr p.i. (post-injection), the group of the most advanced labelled cells were the pachytene spermatocytes. Therefore, during the two intervals, the front of the group of the most advanced labelled cells had progressed from stage VII to stage XI of the cycle (vide, Diagram 7, page ).

(c) Percentage of tubular cross sections containing the most advanced labelled cells: In an attempt to determine with accuracy the position of the most advanced labelled cells within the cycle of the seminiferous epithelium, a qualitative analysis was performed. A survey was done on a large number of tubular cross sections at this stage of the cycle in which the most advanced labelled cells had been seen and the percentages of these tubules containing labelled cells was calculated.
(d) **Calculation of the cycle duration**: After looking the relative duration of the stages and comparing the position of the most advanced labelled cells of the two times, it was obvious that the fronts or tips of the two arrows were not occupying the same stage of the cycle. The tip of the second day was behind the tip of the first day of sacrificing the animal by a distance labelled 'X'. By using the data on the relative duration of the stages of most advanced labelled cells, and the percentages of tubular cross sections at these stages containing labelled spermatocytes, this distance X can be evaluated in percentage of the duration of the whole cycle. Thus -

\[ X = (100\% - a) \times b + c \times d, \]

where 'a' is the percentage of tubular cross section at that stage containing most advanced labelled spermatocytes at the second time of sacrificing of the animals; 'b' the relative duration (%) of that very stage; 'c' the percentage of tubular cross sections at the stage containing labelled spermatocytes at the first time of sacrificing the specimen after \(^3\)H-thymidine injection; and 'd', the relative duration (%) of the stage IX.

The equivalent of the portion of the cycle that remained to be travelled by the most advanced labelled cells to complete the duration of one cycle was 6.95.
The difference between the time interval (3 hrs and 8 days 3 hrs) thus corresponded to 93.05% (100 - 6.95%) of the duration of one cycle and therefore one cycle (or 100%) lasted 8.59 days.

Similar calculations can be done by comparing the position of the most advanced labelled cells at the first and at the third time of sacrificing the specimens after injection (i.e., between 3 hrs and 10.3 hrs). In this case the tip of the 10 days 3 hr arrow deep in stage XI was ahead of the 3 hr arrow by a distance Y. Using the data, this distance also can be calculated as a percentage of the duration of the cycle. Thus -

\[ Y = (100\% - a) \times b + c + d \times e \]

where 'a' is the percentage of tubular cross sections at stage IX containing labelled spermatocytes, at 3 hr after post-injection; 'b', the relative duration (%) of stage IX; 'c' the relative duration (%) of stage X; 'd' the percentage of tubular cross section at stage XI containing labelled spermatocytes 10 d 3 hr after \(^{3}\text{H}-\text{thymidine injection and;}\) 'e' the relative duration (%) of stage XI.

The portion of the cycle by which the most advanced labelled cells bypassed the duration of one cycle
was 15.14%. Therefore, the difference between two time intervals (3 hrs and 10 d 3 hr) or 10 days was the equivalent of 115.14% of the duration of one cycle. Thus one cycle or 100% would last in this case was 8.68 days. Since, approximately 4 cycles are required to complete the process spermatogenesis, the duration of the total process can be computed easily by multiplying the one cycle duration with 4 (i.e., 8.68% x 4 = 34.82 or nearly about 35 days in this case).