RESULTS

Histology of testis:

In *Rhacophorus maculatus*:

In mature male specimens the testes are elongated and unlobed. The average length is 3 mm and diameter is 1.5 mm. Each testis is a compact mass of tissue with firmly attached mesorchium. No distinct compartmentation is visible in the tissue (Plate I, Figure 1, p. 62). Testicular sections show a compact arrangement of the tubules, which are more or less triangular in outline and lined by thin basement membrane (Plate I, Figure 3, p. 62). Intertubular zone has a few interstitial cells (Plate I, Figures 2 and 3, p. 62).

The peculiarity of the adult seminiferous epithelium in the breeding season is that it is predominated by spermatozoan bundles (Plate I, Figures 3, 4, and 5, p. 62). A uniform (or homogeneous) distribution of the spermatogonia in the seminiferous epithelium is not found. Instead, clusters of spermatogonia are localised in certain discontinuous areas of the seminiferous epithelium (Plate I, Figure 5, p. 62). Two types of spermatogonia have been recognised. 'Primary spermatogonia' (Plate VI, Figures 1 and 2, p. 77) remain in the epithelial lining, whereas 'secondary spermatogonia' (Plate VI, Figure 3, p. 77) remain in a cystic arrangement (Roosen-Runge, 1977). In certain areas a characteristic orientation of the secondary spermatogonia has been noted. These cells remain arranged in a
PLATE I

Legend: Photomicrographs of the section (5 μ) of the testis of *Rhacophorus maculatus*.
Fixative: Bouin's fluid (aqua) and Zenker's Formol.
Stain: Haematoxylin-eosin and PAS-Haematoxylin.

Fig. 1: A section showing the compact arrangement of the seminiferous tubules.
OC: Outer covering, V: Vacuolated regions.

Fig. 2: A section of *Rhacophorus* testis showing tubular configuration and intertubular zones (ITZ) with a few interstitial cells (IC).

Fig. 3: A picture showing thin tubular lining (Tt), almost obliterated interstitial zone with a few interstitial cells (IC).

Fig. 4: This section shows spermatozoa in bundles (SPZ) and vacuolated spaces (V).

Fig. 5: This section shows the specific orientation of the secondary spermatogonia (SG^2) with a central lacuna (La). Spermatozoan bundles (SPZ^b) are also shown.

Fig. 6: A field showing the zonal arrangement of the pachytene spermatocytes (P), whose development goes on in syncytial condition.
regular circular fashion surrounding a central lacuna (Plate I, Figure 5, p. 62). This arrangement probably indicates the preparatory stages of the secondary spermatogonia for their meiotic division and lacunae provide room for the cells which would result from meiosis. Besides this, patches of synchronously dividing cells in some areas of the seminiferous epithelium comprise several clones of cells (Plate I, Figure 6, p. 62). They also remain in cyst. Due principally to this arrangement a single tubule shows different zones: one zone consisting of only spermatozoa, the other with spermatids, a third one may be with pachytene spermatocytes and another zone may be provided with any other spermatocytic stage. However, besides these lacunar spaces circumscribed by the secondary spermatogonia, other vacuolated spaces are also found in the tubular mass (Plate I, Figure 1, p. 62). This type of vacular regions is formed due to the discharge of spermatozoa. The tubular lumen is very large. Sertoli cells are prominent with roughly triangular nuclei and large cytoplasmic masses.

In Gekko gecko:

Male mature testis of this animal is oval or elliptical in shape, having an average 6 mm length and 4 mm diameter. Testicular tissue is very soft and not firmly attached to the mesorchium. Lobulation or compartmentation has not been found in the testis (Plate II, Figure 1, p. 65). Each testis is made up of channel like cords (Plate II, Figure 2, p. 65) instead of typical tubules. The channels
PLATE II

Legend: Photomicrographs of the section (5 μ) of the testis of *Gekko gecko*.

Fixative: Bouin's fluid (aquous) and Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: A portion of a testis showing compact arrangement of tubules.

Fig. 2: A portion of the testis at a higher magnification. The section shows tubular channels (Tch) and wide intertubular zones (ITZ). Each tubular channel lacks a regular shape with tubular lining (T_L), the latter not being permanent.

Fig. 3: The sections shows a part of the tubular channel and intertubular zone, both having a continuity due to the disintegration of tubular lining (T_L).

RC: Region of continuity.

Fig. 4: The section shows the orientation of the type B spermatogonia (SGB) along the irregular tubular lining (T_L) before their incorporation inside the tubule.

Fig. 5: The section shows intertubular zone (ITZ) and tubular channels (Tch), as well as the migration of type B (SGB) spermatogonia in the tubule.

Fig. 6: An additional section showing a similar migration of type B spermatonia (SGB).

Fig. 7: Autoradiography of a section showing the labelled B spermatogonia (SGB) near the tubular lining (T_L) before their migration.

Fig. 8: Section shows the early stage of dissolution of the tubular lining (T_L) facilitating the migration of type B spermatogonia (SGB) which are characteristically arranged surrounding the tubular lining (T_L).
have moderately PAS positive lining. Intertubular zones are very wide and of significance to be described below (Plate II, Diagram 2, p. 65).

Intertubular zones are occupied by compactly arranged cells (Plate II, Diagrams 2 and 5, p. 65), which are classified into four types according to the intensity of PAS staining and nuclear configuration. These cells produce a particular cell type which is identical with the only those spermatogonia which are found in the tubular channels. This finding, which indicates that these spermatogonia presumably originate from the intertubular cells, has been suggested by autoradiographic studies (Plate II, Figure 7, p. 65). The classification and characteristic feature of the cells are described in the pages 81 and 82.

The migration of the spermatogonial cells in the tubular channels from the intertubular zone was also observed (Plate II, Figures 4, 5 and 8, p. 65). At some regions, the lining of the tubular channel seems discontinuous (Plate II, Figure 7, p. 65) and the channels appear to be in close relationship to the intertubular zones at several areas. Such an association between the intertubular zone and the tubular channels may permit the entry of the certain spermatogonia which are derived from the intertubular cells (Plate II, Figures 4 and 8, p. 65).

There might be different mechanisms for entry of the spermatogonia into the tubule. Entry of the spermatogonia
Legend: Photomicrographs of the section (5 μ) of the testis of *Varanus bengalensis*.

Fixative: Bouin's fluid and Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: A section showing the compact arrangement of the tubules which are located at the peripheral region of the testis.

Fig. 2: This section shows loosely set tubules (in the middle region of testis) and intertubular zones (ITZ). STN: Newly formed small tubule.

Fig. 3: This section shows the fusion of two tubules.

FR: Fused region.

Fig. 4: A section showing the fusion of the two tubules. The fused region (FR) and the cellular 'wall' (WC) between the two lumen are visible.

Fig. 5: This section shows a further progression in the fusion of the tubules as suggested by the disintegration of the tubular lining.

$T_L(d)$: Disintegrated tubular lining.
may occur through various regions at random. A group of spermatogonia may surround any tubule whose lining shows sign of dissolution and degeneration. A new (as confirmed by PAS positive nature) lining in the meantime develops in such a manner that these groups of spermatogonia now lie in the interior of this new lining (Plate II, Figure 4, p. 65). This is apparently an 'engulfing phenomenon' which goes hand in hand with the increase in the diameter of the tubular channel and is associated with the automatic entry of the spermatogonia inside the tubular channel. Migration of a single or a very few spermatogonia may be marked by the formation of new lining enclosing the spermatogonia.

So far as the seminiferous epithelium is concerned, some sort of so called associations of cells have been observed (vide, pp. 117-123). Different types of meiotic stages along with different stages of spermatids are visible inside the tubular channels.

In Varanus bengalensis:

Of the two testes in Varanus, right one is larger than the left one. Each of them is somewhat elongated and elliptical. The length of the mature testis is about 2 cm and its diameter is about 1 cm. It is covered with firmly attached mesorchium and lacks lobulation or compartmentation. Tubules present towards the peripheral region of the mesorchium are very compact but those at the middle are provided with intertubular spaces (Plate III, Figures 1, 2, 3, 4 and 5, p. 68).
PLATE IV

Legend: Photomicrographs of sections (5 μ) of the testis of Varanus bengalensis.
Fixative: Bouin's fluid (aquadag) and Zenker's Formol.
Stain: PAS-haematoxylin.

Fig. 1: An enlarged view of the cellular "wall" (WC) between the two adjoining lumens (TLu).

Fig. 2: This section shows the dissolution of the fused linings (TL) of the two adjacent tubules.
TL(d): Disintegrated tubular lining.

Fig. 3: Enlarged view of a fused region (FR) where the dissolution is well under way.

Fig. 4: This section shows an intimate contact and fusion of the tubular linings.
TL(F): Fused tubular linings.

Fig. 5: This section shows the region of contact of the lining marking the first stage of "contact-and dissolution" phenomenon.
TL(F): Fused tubular lining.

Fig. 6: An enlarged view of the cells of the intertubular zone (ITZ). All types of germ line cells: sperms, spermatids, spermatocytes and spermatogonia are present in this region. It demonstrates the formation of a new tubule from the cells of the intertubular zone.
Legend: Photomicrographs of sections (5 μ) of the testis of *Psittacula cyanocephala* showing the tubular arrangement, tubular fusion and cell types in the tubules.

Fixative: Bouin's fluid (aquous).

Stain: PAS-haematoxylin.

Fig. 1: This section shows the compact arrangement of the tubules at this region of the testis.

Fig. 2: Two tubules at close association with small inter-tubular zone (ITZ).

\[ T_L \]: Tubular lining.

Fig. 3: The section shows the fusion of two tubules.

\[ T_{Lu} \]: Tubular lumen.

\[ FR \]: Fused region.

Fig. 4: An area showing different types of spermatogonia:

\[ SGA_1 \]: Type A₁ spermatogonia,

\[ SGA_2 \]: Type A₂ spermatogonia, and

\[ SGB \]: Type B spermatogonia.

Fig. 5: A section showing the tubular linings (\( T_L \)) and type A₁ (\( SGA_1 \)) and type A₂ (\( SGA_2 \)) spermatogonia.

Fig. 6: A view of the section showing type A₁ and type A₂ spermatogonia.
Intertubular spaces contain very few interstitial cells (Plate III, Figures 2 and 4, p. 68). The cells in the intertubular zones may proliferate and produce small tubules (Plate III, Figure 2, p. 68). One growing small tubule increases in diameter and fuses with a large tubule. Such a growing tubule remains encircled by highly PAS positive border which during fusion dissolves only at that area which fuses with the large tubule and thus become the part of large tubule. Such a fusion is frequent in the Varanus testis and takes place in the following sequence:

a) The tubules lie side by side (at certain areas, but not throughout the length) (Plate III, Figure 2, p. 68; Plate IV, Figures 4 and 5, p. 71).

b) Tubular linings fuse (The individual lumen of both tubules are yet separated by "wall" formed by the spermatogonia, spermatocyte, etc., which belong to both tubules.) (Plate IV, Figures 1 and 2, p. 71).

c) A gradual thinning and eventual dissappearance of the "wall" (caused either by the utilisation of the cell types during spermatogenesis or by the migration and reorientation of these cells in the tubules) followed by the union of both the lumens producing finally an enlarged single central lumen.

Tubules with different cellular associations have been noted. In the seminiferous epithelium moderately PAS positive spermatogonia are present. According to their different structural peculiarities they are conventionally divided into different types of spermatogonia (vide, pp. 85 and 86).
Cellular arrangement from spermatogonia to spermatozoa is typically similar to that in mammalian testis. Each tubule is provided with a large lumen. A growing tubule may, however, initially be without a lumen (Plate III, Figure 2, p. 68; Plate IV, Figure 6, p. 71). All the tubules do not produce sperms at the same time and hence show different degrees of cellular development. The dividing spermatogonial cells may occasionally form a syncytium.

In *Psittacula cyanocephala*:

Testis in this case is ovoid without any septation or lobulation. Each testis is about 4 mm in diameter in the breeding season. Mesorchium is loosely attached. The tissue is highly compact with closely packed seminiferous tubules. Two or more tubules at certain regions appear to be fused in the histological preparations (Plate V, Figures 1 and 3, p. 73). Intertubular spaces contain a few Leidig's cell (Plate V, Figure 2, p. 73). Tubular epithelium is highly PAS positive. Each tubule contains a large lumen.

As far as the seminiferous epithelium is concerned, it is lined with spermatogonia and sertoli cells (Plate V, Figures 4, 5 and 6, p. 73). Spermatogonia, spermatocytes, spermatids and sperms are arranged from periphery to the lumen in a typical mammalian manner. However, tubules of different cellular composition have led me to divide them in several categories *(vide, pp. 126-129)*.
PLATE VI

Legend: Photomicrographs of the section (5 μ) of the testis of *Rhacophorus maculatus*, showing spermatogonial types and several steps of spermiogenesis.

Figs. 1 and 2:
Primary spermatogonia (SG₁).

Fig. 3: Secondary spermatogonia (SG₂).

Fig. 4: Clusters of early spermatids.

Fig. 5: Early spermatids of step 1 (SPTD₁), bundles of spermatozoa (SPZ), and late spermatid at step 6 (SPTD₆).

Fig. 6: A few types of spermatids:
SPTD₂ : early spermatid at step 2
SPTD₃ : mid spermatid at step 3
SPTD₄ : mid spermatid at step 4.

Fig.: Mid spermatids at step 5 (SPTD₅).
In *Felis domesticus*:

The histological study of cat testis has been studied in great details by a great many researchers (Fawcett, 1975a). Therefore, histological study in all respects are not incorporated in this dissertation. However, the mature testis is oval measuring about 1 cm in diameter. Each testis is firmly encircled by mesorchium. The fibrous capsule, tunica albuginea, is very thick and its invaginations divide the testicular tissue into several chambers. Tissue is very tough and fibrous. Tubules are set apart with large tubular spaces (Plate XVII, Figure 1, p. 131). Intertubular space contains interstitial cells and muscle fibres (Plate XVII, Figure 1, p. 131). Seminiferous epithelium shows vividly the cellular composition as described in the Introduction (vide, pp. 4-6). Different cell association have been elucidated from the histological pictures (vide, pp.129-133).

Spermatogonial population in the seminiferous epithelium:

In *Rhacophorus maculatus*:

The nuclear morphology, topography of the nucleolus and intensity of PAS reaction helped classifying the spermatogonial population into two types, type I and type II. Type I spermatogonia have been named as the primary spermatogonia and type II, the secondary spermatogonia (Plate VI, Figures 1, 2 and 3, p. 77).

Primary spermatogonia:

These represent the stem cell population of the seminiferous epithelium. These cells undergo mitosis and
Legend: Photomicrographs of the sections (5 μ) of the testis of *Gekko gecko* showing types of spermatogonia.

Fixative: Bouin's fluid (aquous) and Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: Clusters of spermatogonia in the intertubular zone:

- SGA₁: Type A₁ spermatogonia, and
- SGA₂: Type A₂ spermatogonia.

Fig. 2: A view of the intertubular zone:

- SGA₁: Type A₁ spermatogonia, and
- SGİn: Intermediate type spermatogonia.

Fig. 3: A section showing the type B (SGB) and intermediate spermatogonia (SGİn).

Fig. 4: This section shows the continuity of the tubular and the intertubular regions formed by the desintegration of the tubular lining (TL).

- SGİn: Intermediate type spermatogonia;
- SGB: Type B spermatogonia.

Fig. 5: Pachytene spermatocytes (P).

Fig. 6: A view of the giant cell (GC) in the tubule.
give rise to secondary spermatogonia. Primary spermatogonia are characterised by the presence of oval nucleus with one to two nuclei very close to the nuclear membrane (Plate VI, Figures 1 and 2, p. 77). Nucleus is either oval or irregular in shape. Nucleoplasm contains finely granulated nuclear chromatin, the cytoplasm being moderately PAS positive. Topographically these cells remain attached with the epithelial lining of the tubules and are larger than the secondary spermatogonia. The diameter of the nucleus is 6 μm (Plate VI, Figures 1 and 2, p. 77).

**Secondary spermatogonia:**

These cells are the descendants of the primary spermatogonia. They ultimately produce spermatocytes destined to undergo meiosis. Each cell contains a round nucleus with condensed chromatin mass. The nuclear diameter is 5 μm. In histological PAS preparation the nuclei appear dark and cytoplasm faint. The nucleolus is scarcely distinguishable. These cells are smaller than the primary spermatogonia (Plate VI, Figure 3, p. 77).

**In Gekko gecko:**

The cell population in the intertubular zone and the migration of spermatogonia from the intertubular zone to the tubular channels clearly demonstrate spermatogonial variation in this reptilian species. Of four types of cells found in the intertubular zone, three comprise different spermatogonial types, and the remaining one is designated as the interstitial cell of Leidig. The three types of
spermatogonia are classified according to their distribution, nuclear morphology, nucleolar topography, chromatin nature and the intensity of PAS reaction. Conventionally they are named as $A_1$, $A_2$ and intermediate. The other type of spermatogonia, which are found in the seminiferous epithelium, represents type B. The characteristic features of spermatogonia are as under:

**Type $A_1$ spermatogonia:**

These cells are smaller in size each with a central nucleus. Nucleus is the darkly stained body having a diameter of 4 $\mu$m. The cytoplasm of these cells are highly PAS positive. (Plate VII, Figure 1, p. 80).

**Type $A_2$ spermatogonia:**

These are larger than the $A_1$ type spermatogonia. Nuclear volume is also increased with appreciable decrease in its staining intensity. The nucleus is more or less oval in shape having 5 $\mu$m length and 4 $\mu$m breadth. Cytoplasm is highly PAS positive (Plate VII, Figure 1, p. 80).

**Intermediate type spermatogonia:**

Type $A_2$ spermatogonia differentiate into intermediate type spermatogonia. These cells contain still larger nuclei with a diameter of 6 $\mu$m each. Nucleus contains a distinct nucleolus. Nucleolus is more or less eccentric in position. Coarse chromatin granules are visible in the nucleoplasm. Chromatin granules appear to remain very intimately associated with several areas of the nuclear membrane. These cells are distributed in the vicinity of the tubular channels (Plate VII, Figures 2 and 4, p. 80).
PLATE VIII

Legend: Photomicrographs of the sections (5 μ) of the testis of *Varanus bengalensis* showing various spermatogonial types.

Fixation: Bouin's fluid (aqueous) and Zenker's Formol.

Stain: PAS-haematoxylin.

Figs. 1 and 2:

Tubular portions showing type $A_1$ spermatogonia ($SGA_1$).

Fig. 3: A portion of the tubule showing type $A_2$ spermatogonia ($SGA_2$).

Fig. 4: Type $A_2$ spermatogonia ($SGA_2$) and type $A_1$ spermatogonia ($SGA_1$).

Fig. 5: A giant cell with 6 nuclei.

Fig. 6: This section shows the type $A_2$ ($SGA_2$) spermatogonia.

Fig. 7: A portion of the tubule showing type $B$ ($SGB$) spermatogonia.

Fig. 8: A magnified view of the intermediate type spermatogonia ($SGIn$).
Type B spermatogonia:

These cells are very similar in size to the intermediate type spermatogonia. Nuclear size is also identical with that of the intermediate type. The difference lies only in the stainability and the nature of chromatin. Nucleolus of type B spermatogonia is visible in the centre of the nucleus. The nuclear chromatin is flake like and seems to be attached to the nuclear membrane. Cytoplasm is faintly PAS positive (Plate VII, Figures 3 and 4, p. 80).

Type B spermatogonia are generated from the antecedent intermediate cells located in the intertubular zones. Shortly after their appearance, these B cells may remain outside tubular channels. However, as these cells later invade and enter the channels, one may come across many a channel which harbours only type B spermatogonia (Plate VII, Figure 4, p. 80).

In Varanus bengalensis:

The seminiferous epithelium of Varanus exhibits various types of the population of spermatogonia. According to the nuclear morphology, topography of the nucleolus, nature of the nuclear chromatin and PAS reaction, they are divided into $A_0$, $A_1$, $A_2$, intermediate and B type spermatogonia.

Type $A_0$ spermatogonia:

These cells are larger than other spermatogonial types. Each cell contains an ovoid nucleus with a diameter of 7 μm. Two nucleoli are visible in the nucleus and remain at opposite sides towards the nuclear membrane. Nucleoplasm is
faintly dark indicating a homogeneous chromatin distribution. Cytoplasm of these cells is moderately PAS positive (Plate VIII, Figure 1, p. 84).

**Type A₁ spermatogonia:**

These are slightly smaller than the A₀ type spermatogonial cells, but each with a nucleus which is 6 μm in diameter. Nucleus contains a single peripherally located nucleolus. Nucleoplasm is lightly stained and euchromatic in nature. Nucleolus is eccentric in position. Cytoplasm shows a higher intensity of PAS reaction as compared to A₀ type (Plate VIII, Figures 2, 3 and 4, p. 84).

**Type A₂ spermatogonia:**

The size of these cells is similar to that of the A₁ type spermatogonia. But the nuclear morphology and chromatin nature are strikingly different. The nucleus is discoidal in shape with a nucleolus eccentric in position. The nucleolus seems to be attached to the nuclear membrane with the help of a slender process (Plate VIII, Figures 3, 4 and 5, p. 84). Nucleoplasm is provided with fine chromatin granules. Cytoplasm is moderately PAS positive.

**Intermediate type spermatogonia:**

These cells are smaller in the size with a spherical nucleus having 7 μm diameter. Coarse granules show condensation at various minute areas and some are arranged at the periphery of the nuclear membrane. The cytoplasm of these cells is highly PAS positive (Plate VIII, Figure 8, p. 84).

**Type B spermatogonia:**

Type B spermatogonia are spherical in shape with a diameter of 8 μm. Nucleus is also spherical with 5 μm
diameter. Coarse granules show condensation at various minute areas and some are arranged at the periphery of the nuclear membrane. The cytoplasm of these cells are highly PAS positive (Plate VIII, Figure 7, p. 84).

Giant cells:

At some locations in the seminiferous epithelium large cells having a diameter of about 20 μm are found and each cell contains 4 to 8 nuclei. Cytoplasm is highly PAS positive. The successive karyokinesis without cytokinesis of the A₂ type spermatogonia may result in the production of these cells, whose nuclei are identical with those of the B type spermatogonia (Plate VIII, Figure 8, p. 84).

In Psittacula cyanocephala:

In Psittacula cyanocephala three types of spermatogonia are distinguished mainly according to their chromatin nature and nucleolar topography. Spermatogonial population has been divided into three categories. Three types of spermatogonia are conventionally named as A₁, A₂ and B type spermatogonia. They show the following characteristic features:

Type A₁ spermatogonia:

These cells are comparatively large with oval nucleus. The diameter of the cell and the nucleus is 10 μm and 5 μm respectively. The nucleus contains a peripherally located distinct nucleolus. Nuclear chromatin is homogeneously distributed in the nucleoplasm. Cytoplasm is moderately PAS positive (Plate V, Figures 4, 5 and 6, p. 73).
Type A₂ spermatogonia:

These cells are similar in size to the A₁ type spermatogonia. The diameter of the cell is also 10 μm. Nucleus is oval in shape with a diameter of 4 μm. It is eccentric in position in the nucleus. Nucleoplasm contains coarse chromatin granules. Cytoplasm is faintly PAS positive (Plate V, Figures 4, 5 and 6, p. 73).

Type B spermatogonia:

They are slightly smaller than type A₂ spermatogonia and has a diameter of 8 μm. Nucleus is spherical with 5 μm diameter. The nucleolus is centrally located in the nucleus. Chromatin granules form flakes which may sometimes form clusters close to the nuclear membrane. Cytoplasm of the cell is highly PAS positive (Plate V, Figure 4, p. 73).

In Felis domesticus:

Seminiferous epithelium of Felis domesticus is composed of five classes of spermatogonia. On the basis of their detailed nuclear morphology, and orientation of the nucleolus as well as chromatin nature, they could be conventionally classified as A₁', A₂, intermediate and B (B₁ and B₂) type spermatogonia. The characteristic features of each type of spermatogonia are as follows:

Type A₁ spermatogonia:

The cell is oval in outline and has a greater diameter of 12 μm. Nucleus is also oval (diameter 8 μm) with darkly stained nucleoplasm. The nucleolus is so peripherally located that it appears to be attached to the nuclear membrane. Cytoplasm is highly PAS positive (Plate IX, Figures 2 & 4, p. 90).
PLATE IX

Legend: Photomicrograph of the sections (5 µ) of the testis of cat showing types of spermatogonia.

Fixation: Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: This section shows type $A_2$ ($SGA_2$) and $B_2$ ($SGB_2$) spermatogonia.

Fig. 2: A view of the type $A_1$ spermatogonia ($SGA_1$).

Fig. 3: Type $B$ ($SGB_1$) spermatogonia.

Fig. 4: Type $A_1$ ($SGA_1$) and type $A_2$ ($SGA_2$) spermatogonia.

Fig. 5: Type $B_1$ spermatogonium ($SGB_1$).

Figs. 6 and 7:

Intermediate spermatogonia at different stages of development.

Fig. 8: The section shows the dividing spermatocytes and type $B_2$ ($SGB_2$) spermatogonia.
Type $A_2$ spermatogonia:

The shape and size of $A_2$ type spermatogonia resemble those of the type $A_1$ cells. Nucleus is lightly stained with a homogeneous distribution of nuclear chromatin. Nucleolus is slightly apart from the nuclear membrane, but it often appears to be connected with nuclear membrane by a slender process. Cytoplasm is moderately PAS positive (Plate IX, Figure 1, p. 90).

Intermediate type spermatogonia:

As compared to the $A_2$ spermatogonia, these cells are rather large and contain larger oval nucleus (diameter 6 μm). Nucleolus occupies central position within the nucleus. The chromatin is coarse and unevenly distributed in the nucleoplasm, which is lightly stained. Cytoplasm is moderately PAS positive (Plate IX, Figure 7, p. 90).

Type $B_1$ spermatogonia:

The nuclear volume and cell size is further increased. The nucleus, which is oval, has a diameter of 9 μm along the greater length and has an eccentric nucleolus. Flakes of chromatin are dispersed within the nucleus. Nucleoplasm is very faintly stained. Cytoplasm is moderately PAS positive (Plate IX, Figures 3 and 5, p. 90).

Type $B_2$ spermatogonia:

The shape and the size of these cells are similar to those of the type $B_1$ cells, but the nuclear volume is slightly increased (diameter 10 μm). Nucleolus is at the centre of the nucleus. Flakes of nuclear chromatin are extremely close to the nuclear membrane. Cytoplasm shows a slight increase in the PAS reaction (Plate IX, Figure 8, p. 90).
Steps of spermiogenesis in *Rhacophorus maculatus*

Eight steps of spermiogenesis are marked by Arabic numerals (1-8). The identification of the steps was done by the change in acrosomal configuration (located at the apical portion of the head) and gradual elongation of the spermatid nucleus.
Spermiogenesis:

Metamorphosis of early spermatids into spermatozoa has also been studied in all the specimens incorporated in this dissertation. PAS-hematoxylin staining clearly demonstrates the change in both the nuclear morphology and the acrosomal system along, to some extent, with the development of tail. On the basis of these morphological features classification of the spermatids and steps of spermiogenesis have been made.

Spermiogenesis in Rhacophorus maculatus:

According to the gross nuclear morphology different types of spermatids are conventionally grouped into three categories: early, mid and late spermatids. Early spermatid nucleus is spherical in appearance with a 2 µm diameter. The mid spermatid possesses an elongated nucleus ranging from 3 to 5 µm in length and nucleus of these spermatids shows a high degree of condensation. In the late spermatid, however, the nucleus is further elongated (6 µm to 9 µm) and shows progressive condensation. In mature spermatozoa nucleus is exceedingly condensed and slender. According to the change in acrosomal system, as well as the appearance and consequent development of the tail, the spermiogenesis may be divided into 8 steps.

In the 1st step acrosomal vesicle with acrosomal granules appears on the round nucleus of the early spermatid. Cytoplasm is faintly PAS positive. The nucleus is not very condensed. It rather shows large chromatin clumps (Plate VI, Figure 5, p. 77).
In the second step, acrosomal granules coalesce in the acrosomal vesicle which remains adhered to the round nucleus. Entire cell is circular with faintly PAS positive cytoplasm (Diagram 11, p. 93; Plate VI, Figure 6, p. 77).

Acrosome in the 3rd step spreads on the apical region of the slightly elongated nucleus of mid-spermatids. The cytoplasm opposite to the acrosomal side is slightly protruded outwardly indicating the first step in the development of the tail (Diagram 11, p. 93; Plate VI, Figure 6, p. 77).

In the next step, acrosome spreads further and covers approximately 1/4th of the total surface area of the nucleus which by now has been moderately elongated. Cytoplasmic protrusion also elongates (Diagram 11, p. 93).

In the 5th step of spermiogenesis, acrosome appears as a conical structure on the further elongated slender nucleus. Tail too is further elongated. The cytoplasm surrounding the nucleus is practically undetectable. A fibrillar filament appears in the developing tail of the spermatid (Diagram 11, p. 93; Plate VI, Figure 7, p. 77).

6th step of spermiogenesis is marked by a further elongation of the nucleus and the acrosome becomes firmly attached to the anterior part of the nucleus. Tail becomes slender and longer (Plate VI, Figure 5, p. 77; Diagram 11, p. 93).
PLATE X

Legend: Photomicrographs of the sections (5 μ) of the testis of Gekko gecko showing different steps of spermiogenesis.

Fixation: Zenker's Formol.
Stain: PAS-haematoxylin.

Fig. 1: Three types of spermatids:
- SPTD₁ = Early spermatid at step 1,
- SPTD₂ = Early spermatid at step 2, and
- SPTD₃ = Early spermatid at step 3.
  (Acr = Acrosome system)

Fig. 2: Early spermatids of step 1 in syncytial mass (SPTD₁).

Fig. 3: A field with mid spermatids at step 4 (SPTD₄) and 5 (SPTD₅).

Fig. 4: Mid spermatids at step 5 (SPTD₅) and 6 (SPTD₆) are present in the field.

Fig. 5: Section shows the spermatid at step 7 (SPTD₇).

Fig. 6: Late spermatids at step 8 (SPTD₈) and 9 (SPTD₉) are present in the field.

Fig. 7: Figure shows the late spermatid at step 9 (SPTD₉).
Nine steps of spermiogenesis are marked by Arabic numerals (1-9). The identification of the steps was done by the change in acrosomal configuration (located at the apical portion of the head) and gradual elongation of the spermatid nucleus. Tail is very short.
In the next step, acrosomal morphology does not show significant change, but the tail and nucleus are further elongated.

The final step of spermiogenesis is represented by the mature spermatozoa. The acrosome remains attached with the very slender nucleus and the only distinguishable part of it is the conical and pointed apical portion. Tail reaches its maximum length. (Diagram 11, p. 93). The head of the mature sperm is about 12 μm in length and the tail is about 35 μm.

Spermiogenesis in Gekko gecko:

In Gekko gecko spermiogenesis may be divided into 9 steps. Different steps are identified with the change in nuclear morphology, tail formation and acrosomal variations. Different types of spermatids are conventionally grouped into three categories: early with round nucleus, mid with slightly elongated nucleus and late spermatids with moderately elongated head or nucleus. The diameter of the early spermatid is 2-3 μm, the length of the nucleus of the mid and the late spermatid is within a range of 3-5 μm and 5-10 μm respectively. Different steps of spermatids' metamorphosis are as under:

In the 1st step, spermatid possesses a small and round nucleus of diameter of 3 μm. A thin layer of cytoplasm encircles the nucleus. A vesicle-like structure is found to be formed near the nucleus, but proacrosomic granules, which are PAS positive, are not distinctly visible (Plate X, Figures 1 and 2, p. 97; Diagram 12, p. 99).
2nd step is characterised by the appearance of a PAS positive acrosome vesicle over the round nucleus. No change in cytoplasm is visible (Plate X, Figure 1, p. 97; Diagram 12, p. 99).

In the 3rd step, nucleus is slightly elongated and the acrosome over it flattens slightly. No indication of the formation of the tail, which is discernable by a cytoplasmic protrusion, is ever present (Plate X, Figure 3, p. 97; Diagram 12, p. 99).

However, in the next step (step 4), nucleus further elongates. A slight spreading of the flattened acrosome over the nuclear surface is observed. Cytoplasm forms a little protrusion now (Plate X, Figure 3, p. 97; Diagram 12, p. 99).

In the 5th step, spermatid produces a small tail filament at the adacrosomal end. Further nuclear elongation along with the acrosomal spreading is also the usual feature of this step (Plate X, Figure 4, p. 97; Diagram 12, p. 99).

In the 6th step, nucleus further elongates and the tail filament increases in length. Certain portion of the tail filament is covered with cytoplasm. Acrosome covers almost the anterior half of the nucleus (Plate X, Figure 4, p. 97; Diagram 12, p. 99).

In 7th and 8th steps is noted the elongation of the nucleus concomitant with the further acrosomal spreading over it. A further increase of the length of the tail is not visible (Plate X, Figure 5 and 6, p. 97; Diagram 12, p. 99).
Legend: Photomicrographs of the sections (5 μ) of the testis of *Varanus bengalensis* showing different steps of spermiogenesis.

Fixation: Zenker's Formol.
Stain: PAS-haematoxylin.

Fig. 1: Early spermatids at step 1 (SPTD\textsuperscript{E1}) and 2 (SPTD\textsuperscript{E2}) with distinct acrosomal (Ac) system.

Fig. 2: Early spermatid at step 2 (SPTD\textsuperscript{E2}).

Fig. 3: Early spermatids at step 2 (SPTD\textsuperscript{E2}) and 3 (SPTD\textsuperscript{E3}) and late spermatid at step 9 (SPTD\textsuperscript{L9}).

Fig. 4: Mid spermatid at step 5 (SPTD\textsuperscript{M5}).

Fig. 5: Mid spermatid at step 6 (SPTD\textsuperscript{M6}) and late spermatid at step 7 (SPTD\textsuperscript{L7}).

Fig. 6: Mid spermatids at step 5 (SPTD\textsuperscript{M5}) and 6 (SPTD\textsuperscript{M6}), and late spermatid at step 8 (SPTD\textsuperscript{L8}).

Fig. 7: Late spermatid at step 9 (SPTD\textsuperscript{L9}).
Steps of spermiogenesis in *Varanus bengalensis*

Nine steps of spermiogenesis are marked by Arabic numerals (1-9). The identification of the steps of spermiogenesis was done by the change in acrosomal configuration (located at the apical portion of the head) and gradual elongation of the spermatid nucleus.
The final step is represented by the mature sperm. The nucleus (step 7) is moderately long, the tail being shorter than the head as regards its length. The head is anteriorly rather blunt and gradually tappers posteriorly. Acrosome covers 1/3rd of the anterior portion of the head (Plate X, Figures 6 and 7, p. 97; Diagram 12, p. 99).

Spermiogenesis in Varanus bengalensis:

Nine steps of spermiogenesis have also been observed in this species. All the steps show close resemblance to those of Gekko, but the form of acrosome and spermatozoan structure are somewhat different. On the basis of the length of their nucleus, spermatids are also classified as early, mid, and late spermatids. However, the early spermatid nucleus is 3 μm in diameter. The length of the nucleus of the mid spermatid is 4 μm - 6 μm, whereas that of the late one is 7 μm - 10 μm. Various steps of spermiogenesis are as under:

In the 1st step, the very early spermatid, characterised by flakes of nuclear chromatin, possesses a perinuclear thin film of cytoplasm with a PAS positive acrosomal vesicle on the nucleus (Plate XI, Figure 1, p. 103; Diagram 13, p. 105).

2nd step is characterised by a more condensed nucleus with somewhat flattened acrosomal vesicle (Plate XI, Figures 1, 2 and 3, p. 103; Diagram 13, p. 105).

The spermatid nucleus in the 3rd step is elongated to some extent and shows more condensation of chromatin. The acrosome becomes more flattened and spreaded. A little tail
filament appears at this stage. Cytoplasm is very pale stained and without any protrusions (Plate XI, Figure 3, p. 103; Diagram 13, p. 105).

In the 4th step, spermatid elongates further. The acrosome shows asymmetrical spreading over the nuclear surface. Tail filament is more elongated (Diagram 13, p. 105).

The 5th step of spermiogenesis is represented by a moderately elongated spermatid nucleus. Acrosome spreads further and becomes more flattened. Tail filament also elongates (Plate XI, Figure 4, p. 103; Diagram 13, p. 105).

In the 6th step spermatid nucleus is more slender and assumes somewhat curved shape. The anterior of the head is club shaped and posterior portion is pointed. The origin of the tail demarcates the junction of the pointed posterior end of the spermatid head. A greater surface of the nucleus is covered with the acrosome, but apical portion is smooth in appearance (Plate XI, Figures 5 and 6, p. 103; Diagram 13, p. 105).

In the 7th step, spermatid is almost similar in length with the 6th step spermatid. But the acrosome takes the form of a small cone at the anterior end (Plate XI, Figure 5, p. 103; Diagram 13, p. 105).

In the 8th step, the spermatid nucleus is more curved and slender. Acrosome appears as a thin film over the surface of the head and its conical anterior portion becomes prominent. The tail filament possesses a moderate length (Plate XI, Figure 6, p. 103; Diagram 13, p. 105).

Spermatids in the 9th step are extremely slender. The apical acrosomal cone elongates. Curved nature of the
PLATE XII

Legend: Photomicrographs of the sections (5 µ) of the testis of Psittacula cyanocephala showing different steps of spermiogenesis.

Fixative: Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: Early spermatids at step 1 (SPTDE1).

Fig. 2: Early spermatids at step 2 (SPTDE2).

Fig. 3: Mid spermatid at step 5 (SPTDM5).

Fig. 4: Mid spermatid at step 4 (SPTDM4).

Fig. 5: Late spermatid at step 7 (SPTDL7) and mid spermatids at step 6 (SPTDM6).

Fig. 6: Mid spermatid at step 4 (SPTDM4) and late spermatid at step 9 (SPTDL9).

Fig. 7: Figure showing late spermatid at step 9 (SPTDL9).
Nine steps of the spermiogenesis have been represented by Arabic numerals (1-9). The identification of different steps of development of spermatids was made by the change in acrosomal system (present at the apical portion of the spermatid nucleus) as well as elongation of the nucleus of the spermatid. Tail development is not well marked.
slender head is still maintained. They may now be called as mature spermatozoa. A sperm head is 14 μm in length (Plate XI, Figures 3 and 7, p. 103; Diagram 13, p. 105).

Spermiogenesis in *Psittacula cyanocephala*:

Spermiogenesis in this bird is represented by nine distinct steps. The criteria for distinguishing different stages are mainly the acrosome configuration and shape of the head, i.e., the nuclear morphology. No distinct tail is visible in the light microscopic preparation. Spermatids are conventionally divided into early, mid and late spermatids. The nucleus of the early spermatid is a 2-3 μm in diameter. The head of the mid spermatid ranges from 4-7 μm in length, and that of the late spermatids is within a range of 8 μm - 14 μm. Different steps of spermiogenesis show the following features:

1st step spermatid is round with a chromatin dot at the centre. Acrosome vesicle appears on the nuclear surface. Cytoplasm remains as thin filament (Plate XII, Figure 1, p. 109; Diagram 14, p. 111).

In the 2nd step nucleus becomes condensed. The acrosome vesicle with the prominent acrosomal granules remain attached to the nuclear surface. Cytoplasm appears as very thin filament (Plate XII, Figure 2, p. 109; Diagram 14, p. 111).

In the 3rd step, the elongation of the nucleus occurs with slight flattening of the acrosomal system. Cytoplasm is very indistinct (Diagram 14, p. 111).
PLATE XIII

Legend: Photomicrographs of the sections (5 μ) of the testis of Felis domesticus showing different steps of spermiogenesis.

Fixative: Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: Early spermatid at step 1 (SPTD_{E1}).

Fig. 2: Early spermatid at step 2 (SPTD_{E2}).

Fig. 3: Mid spermatid at step 4 (SPTD_{M4}) with spreaded acrosome.

Fig. 4: A few mid spermatids at step 6 (SPTD_{M6}) and a late spermatid at step 7 (SPTD_{L7}) the latter with distinct acrosomal system.

Fig. 5: Mid spermatid at steps 5 (SPTD_{M5}).

Fig. 6: Late spermatid at step 7 (SPTD_{L7}) with prominent "sail".

Fig. 7: Late spermatid at step 9 (SPTD_{L9}) (Acr = Acrosome).

Fig. 8: A view of several mid spermatids.

Fig. 9: A view of the spermatid at step 10 (SPTD_{L10}).

Fig. 10: A view of the spermatid at step 12 (SPTD_{L12}).
Spermatid in the 4th step show further elongation of the nucleus, the anterior end being broad and posterior end narrow. No appearance of tail is noted (Plate XII, Figure 4, P. 109; Diagram 14, p. 111).

5th step spermatid is characterised by further elongation of the nucleus and acrosome remains attached to the apical broader region (Plate XII, Figure 3, p. 109; Diagram 14, p. 111).

In the 6th, 7th and 8th steps, the spermatids do not show any significant change in their acrosomal system, but the gradual elongation clearly differentiates them (Plate XII, Figures 5 and 6, p. 109; Diagram 14, p. 111).

The final step of spermiogenesis is represented by the mature spermatozoa. Its length is now maximum and is considerably slender. The acrosome encircles the anterior portion which is club shaped. No distinct tail, as already stated, is found in the histological or squash preparation observed under light microscope (Plate XII, Figures 6 and 7, p. 109; Diagram 14, p. 111).

Spermiogenesis in the cat:

Twelve distinct steps of spermiogenesis have been found. Different steps are clearly distinguished by the change in acrosomal system and tail development. Change in the nuclear morphology is another criterion for this differentiation. However, different spermatids are grouped into early, mid and late spermatids. Unlike those in submammalian species, the spermatids of this mammal never show an incredible elongation of the nucleus. They are round in early stage and later become
somewhat elliptical or oval. The tail, which does not appear in the early spermatids, starts as a structure with cytoplasm over it at the mid spermatid stage. In the late spermatids, a distinct tail with a central filament invested by cytoplasm is observed. The different steps of spermiogenesis exhibit the following characteristic features.

In step 1, spermatids are round cells with oval central nucleus. The nucleus is provided with chromatin flakes. Moderately PAS positive cytoplasm encircles the nucleus. Acrosomal vesicle with PAS positive granules appear over the nuclear surface. In early stage a syncytial differentiation of spermatids has also been observed. An early spermatid nucleus immediately after its appearance in cat measures about 3 μm in diameter (Plate XIII, Figure 1, p. 114; Diagram 15, p. 128).

In the second step the nucleus show somewhat condensed nuclear chromatin. Acrosomic PAS positive granules form a mass in the acrosome vesicle which lie in close association with the nucleus (Plate XIII, Figure 2, p. 114; Diagram 15, p. 128).

3rd step of spermiogenesis is represented by the condensed nucleus. Acrosome system gets flattened onto the nuclear surface (Diagram 15, p. 128).

In the 4th step nucleus becomes more condensed. The coalesced acrosomal granule spreads further over the nuclear surface. Cytoplasm shows a tendency for accumulation at the adacrosomal end of the nucleus (Plate XIII, Figure 3, p. 114; Diagram 15, p. 128).

The 5th step spermatid shows no further condensation of the chromatin. Acrosome spreads further over the anterior portion of the head (Diagram 15, p. 128).
In the 6th step the nucleus becomes slightly oval and flattened. Acrosome covers the anterior third of the nucleus. The cytoplasm becomes protruded at the adacrosomal surface of the nucleus (Plate XIII, Figure 4, p. 114; Diagram 15, p. 128).

In the 7th and 8th steps, gradual elongation of the tail has been noted. Acrosome also shows further spreading (Plate XIII, Figures 4 and 6, p. 114; Diagram 15, p. 128).

In the 9th step the acrosome spread over a greater surface on the nucleus as compared to the 7th or 8th step spermatids, and becomes conical in appearance. Tail is also elongated further (Plate XIII, Figure 7, p. 114; Diagram 15, p. 128).

In the spermatids in 10th step, the acrosome becomes very distinct over the condensed nuclear chromatin. In this spermatid the nucleus is lightly stained except at the acromosal region. The tail also becomes longer (Diagram 15, p. 128).

In the 11th step, further elongation of the tail has been noted. Nucleus is reduced in size (Diagram 15, p. 128).

In the 12th step of spermiogenesis the acrosome becomes very thin and appears as a cone on the surface of the head. Tail becomes very thin due to the loss of cytoplasm. The spermatid attains the final stage of maturation around this period. Further development of the spermatid does not seem to take place within the testis (Plate XIII, Figure 10, p. 114; Diagram 15, p. 128).

**Germ cell association in the seminiferous epithelium:**

In *Rhacophorus maculatus*:

Typical association or grouping of cells was not found. The clonal arrangement of cells confined to various
PLATE XIV

Legend: Photomicrographs of the sections (5 μ) of the testis of *Gekko gecko* showing different cell-associations.
Fixative: Zenker's Formol.
Stain: PAS-haematoxylin.

**Fig. 1:** Stage I with only type B spermatogonia (SGB) and Sertoli cells.

**Fig. 2:** Stage IV with type B spermatogonia (SGB) and early spermatids (SPTD\textsuperscript{E}). A few mid and late spermatids are also present.

**Figs. 3 and 6:**
Stage III with type B spermatogonia (SGB), dividing spermatocytes at diplotene (Dip), diakinesis and Met-I and a few early spermatids (SPTD\textsuperscript{E}).

**Fig. 4:** Stage V with type B spermatogonia (SGB), spermatozoa (SP\textsubscript{Z}) and late spermatids.

**Fig. 5:** Stage II with type B spermatogonia (SGB) and dividing spermatocytes at leptotene (L), zygotene(Z) and pachytene (P) stages.
zones of the seminiferous epithelium, was observed (Plate I, Figure 5 and 6, p. 62).

In *Gekko gecko*:

Five distinct (stages of) cellular associations have been recognised in the seminiferous tubule of *Gekko*. The renewal of spermatogonia occurs outside the tubules. Such tubule may rather be called a tubular channels instead of a true tubules. The spermatogonial population found in a tubular channel indeed originates from the intertubular regions and later migrates to the tubular channels. Despite the similarity between the testis of the *Gekko* and that of any mammal as regards the complete repetitions of all the stages of cellular associations in any specific area of the seminiferous epithelium in course of time, an important difference lies on the cells participating at the spermatogonial renewal. Spermatogonia of type A is always in intertubular zones in *G. gecko*, this type is invariably found in the tubular epithelium in all mammals. Because of this difference these associations in *G. gecko* may be called the "stages of association" or "stages of linear waves" in sharp contrast to the "stages of the cycle of the seminiferous epithelium" in mammals.

Stage I: The tubular channel consists of spermatogonia type B (which are not in a dividing stage) and Sertoli cells. The interior lumen of the tubular channel is devoid of all other cellular elements, namely spermatocytes, spermatids and sperms (Plate XIV, Figure 1, p. 119).

Stage II: In this association besides B type spermatogonia, a large number of spermatocytes at leptotene and zygotene stages
PLATE XV

Legend: Photomicrographs of the sections (5 μ) of the testis of *Varanus bengalensis* showing different cell associations.

Fixative: Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: Stage I with type $A_0$ ($SGA_0$), $A_1$ ($SGA_1$), intermediate ($SG\text{In}$) spermatogonia, dividing spermatocytes at leptotene (L) and zygotene (Z) stages and early spermatids at step 1 ($SP\text{TDT}^1$).

Fig. 2: A view of the stage II association:

$SGA_0 = \text{Type } A_0 \text{ spermatogonia}$,
$SGA_2 = \text{Type } A_2 \text{ spermatogonia}$,
$SGB = \text{Type B Spermatogonia}$,
$SP\text{TDT}^1 = \text{Early spermatid at step 1}$,
$SP\text{TDT}^3 = \text{Early spermatid at step 3}$,
$SP\text{TDT}^4 = \text{Mid spermatid at step 4}$.

($Z = \text{Zygotene}, P = \text{Pachytene}$).

Fig. 3: A view of the stage V association:

$SGA_1 = \text{Type } A_1 \text{ spermatogonia}$,
$SGB = \text{Type B spermatogonia}$,
$SP\text{TDT}^L7 \& L8 = \text{Late spermatids at step 7 and 8}$,
$SPZ = \text{Spermatozoa}$.

Fig. 4: A view of the stage III association:

$SGA_0 = \text{Type } A_0 \text{ spermatogonia}$,
$SGA_1 = \text{Type } A_1 \text{ spermatogonia}$,
$SPTD^E3 = \text{Early spermatid at step 3}$,
$SG\text{In} = \text{Intermediate spermatogonia}$,
$SP\text{TDT}^M4 = \text{Mid spermatid at step 4}$.

Fig. 5: A view of the stage IV association:

$SP\text{TDT}^E2 = \text{Early spermatid at step 2}$,
$SP\text{TDT}^L8 = \text{Late spermatid at step 8}$. 
is present. Spermatids or spermatozoa are absent in the tubular channel (Plate XIV, Figure 5, p. 119).

Stage III: These tubular channels contain B type spermatogonial dividing spermatocytes mainly at diplotene, diakinesis and metaphase-I stages. Along with these, a few spermatids (early) are also present in the lumen (Plate XIV, Figures 3 & 6, p. 119).

Stage IV: The epithelium of the channel consists of B type spermatogonia. A few early, mid and late spermatids are observed. No dividing spermatocyte has been found in this association (Plate XIV, Figure 2, p. 119).

Stage V: This stage is represented by the presence of B type spermatogonia in the epithelium and large number of mature spermatozoa in the lumen. A few late spermatids have also been observed. No dividing spermatocyte has been found to be present in the tubular lumen (Plate XIV, Figure 4, p. 119).

From these observations it appears that with the release of spermatozoa a stage V tubule automatically results in the formation of the association of stage I. It is to be mentioned here that type B spermatogonia are invariably present in all the stages due to their continuous flow of migration from interstitial tissue into the tubular channel. Hence there is no question of spermatogonial renewal in the seminiferous epithelium. The cells show a great deal of synchronization in their differentiation process.

In Varanus bengalesis:

Five cellular associations have been found to be present in the tubules of the testis of Varanus. These
PLATE XVI

Legend: Photomicrographs of the sections (5 u) of the testis of Psittacula cumanica showing different cellular associations:
Fixative: Zenker's Formalin;
Stain: PAS-haematoxylin.

Figs. 1 and 3:
Stage I association:
SGA1 = Type A1 spermatogonia;
SGA2 = Type A2 spermatogonia, and
SGB = Type B spermatogonia

Fig. 2: Stage II association:
SGA1 = Type A1 spermatogonia;
SGA2 = Type A2 spermatogonia,
P = Pachytene spermatocytes, and
Dip = Diplotene.

Fig. 4: Stage III association.
Z = Zygotene, P = Pachytene, and
SPTDE = Early spermatid.

Fig. 5: Stage IV association:
SGA1 = Type A1 spermatogonia;
SGA2 = Type A2 spermatogonia,
SPTDE = Early spermatids, and
SPTDL = Late spermatids

Fig. 6: Stage V association:
SGA1 = Type A1 spermatogonia,
SGA2 = Type A2 spermatogonia,
SPTDE = Early spermatids, and
SPZ = Spermatozoa.
associations represent the stages of the cycle of the seminiferous epithelium. Different associations are as follows:

Stage I: Seminiferous epithelium consists of type A₀, A₁ and intermediate spermatogonia. Dividing spermatocytes present in the tubule are in leptotene and zygotene stages. The stage I is easily identified by the presence of early spermatids at steps I (Plate XV, Figure 1, p. 122).

Stage II: This association is composed of spermatogonial types A₀, A₂ and B. The dividing spermatocytes are mainly at zygotene and pachytene stages. The spermatids present are of the early type representing steps 1 to 4 (Plate XV, Figure 2, p. 122).

Stage III: These tubules contain type A₀, A₁ and intermediate spermatogonia. Pachytene spermatocytes and spermatids of steps 3, 4, 5 and 9 are present in the tubule (Plate XV, Figure 4, p. 122).

Stage IV: In this association A₀, A₁ and B types spermatogonia are present. Resting spermatocytes along with zygotene spermatocytes are present in the tubule. Only a few early (step 1 and step 2) and late (step 7 and 8) spermatids are present (Plate XV, Figure 5, p. 122).

Stage V: This association is distinguished by A₀, A₁ and B type spermatogonia along with spermatocytes in diakinesis and metaphase-II. Only late spermatids of step 6, 7 and 8 are present in the tubule (Plate XV, Figure 3, p. 122). Mature spermatozoa are also visible.

In Psittacula cyanocephala:
The cell association in the seminiferous tubule is of five categories.
Stages of the cycle of the seminiferous epithelium in the cat

The vertical columns, represented by Roman numerals (I - X), depict cellular associations at each of the 10 stages of the cycle of the seminiferous epithelium in the cat testis. Twelve steps of spermiogenesis were identified and represented by Arabic numerals (1-12). At any specific area of the seminiferous tubule one stage succeeds the remaining stages in course of time. After stage X, the stage I is repeated and the cycle is completed.

Abbreviations:

$A_1$, $A_2$, In, $B_1$ and $B_2$ = Type $A_1$ spermatogonia, type $A_2$ spermatogonia, intermediate type spermatogonia, type $B_1$ spermatogonia and type $B_2$ spermatogonia respectively.

$L$ = Leptotene; $Z$ = Zygotene; $P$ = Pachytene;

Dip = Diplotene; M-II = Metaphase-II.
Stage I: Tubules of this association contain only spermato­
gonia of type $A_1$, $A_2$, and B. Neither dividing spermatocytes,
nor spermatids, are present (Plate XVI, Figures 1 and 3, p. 125).

Stage II: Tubule contains $A_1$ and $A_2$ type spermatogonia.
Spermatocytes at different dividing stages are present whereas
spermatids or spermatogonia are absent (Plate XVI, Figure 2,
p. 125).

Stage III: This association is characterised by the presence
of $A_1$ and $A_2$ spermatogonia. Zygotene and pachytene spermato­
cytes are predominant in contrast to very few diplotene and
diakinesis spermatocytes. The tubule contains early spermatids
too.

Stage IV: The seminiferous epithelium contains $A_1$ and $A_2$
spermatogonia. No dividing spermatocytes are found. Early
and late spermatids are present (Plate XVI, Figure 5, p. 125).

Stage V: This association is characterised by the presence
of $A_1$ and $A_2$ types spermatogonia in the seminiferous epithe­
lium. No dividing spermatocytes are present in the tubule.
Early spermatids and mature spermatozoa are found (Plate XVI,
Figure 6, p. 125).

In *Felis domesticus*

Nucleus and acrosomic system undergo several
striking morphological changes during differentiation of the
cat spermatids' metamorphosis into spermatozoa. An initial
identification of different steps of spermatid metamorphosis
fascilitates the association study. According to the format
Legend: Photomicrographs of the sections (5 μ) of the testis of *Felis domesticus* showing testicular histology and cellular associations:

Fixative: Zenker's Formol.

Stain: PAS-haematoxylin.

Fig. 1: A portion of the testis showing seminiferous tubules (T) and intertubular space (ITS). MF = Muscle fibre. ISTC = Interstitial cells.

Fig. 2: A view of the stage association.

Fig. 3: A view of the stage III association.

Fig. 4: A view of the stage VI association.

(Fig. 5: A view of the stage IV association.

Fig. 6: A view of the stage II association.

(P = Pachytene, SPTD<sup>L11</sup> = Spermatids at step 11).
of Leblond and Clermont (1952), spermatids have been identified with Arabic numerals (1 to 12) and the stages of the cycle with Roman numerals (I to X) (Diagram 15, p. 128) (Leblond and Clermont, 1952). The different stages of associations have the following features:

Stage I: Seminiferous epithelium is lined by $A_1$ spermatogonia. The $A_2$ and intermediate type spermatogonia are also present in the tubule. Pachytene spermatocytes represent the only meiotic stage found. Presence of early (round) spermatids at step 1 and the advanced late spermatids at step 11 is very characteristic (Diagram 15, p. 128).

Stage II: This stage contains $A_1$, $A_2$, and intermediate spermatogonia. Pachytene spermatocytes persist. The early spermatids which is present in this tubule are at step 2. Late spermatids at step 11 are still present (Plate XVII, Figure 6, p. 131; Diagram 15, p. 128).

Stage III: All cell types are the same as those belonging to stage II, except the fact that in this stage step 3 spermatids are found instead of step 2 ones (Plate XVII, Figure 3, p. 131; Diagram 15, p. 128).

Stage IV: This association shows $A_1$, $A_2$ and $B_1$ spermatogonia. The intermediate spermatogonia are wanting. Pachytene stage persists. In addition to the step 11 spermatids, the early spermatids belonging to step 4 are present (Plate XVII, Figure 5, p. 131; Diagram 15, p. 128).

Stage V: In this stage spermatogonial population and dividing spermatocytes are same as those in the stage IV. But the
spermatids in step 5 and step 12 (Diagram 15, p. 128) are observed.

Stage VI: Besides $A_1$, $A_2$ and $B_1$ spermatogonia and pachytene spermatocytes, one may find spermatids at step 6 at this stage (Plate XVII, Figure 4, p. 131).

Stage VII: $A_1$ and $A_2$ spermatogonia as well as pachytene spermatocytes are yet present. But the $B_1$ spermatogonia have been replaced by $B_2$ type spermatogonia. The step 6 spermatids have been transformed into the step 7 spermatids (Diagram 15, p. 128).

Stage VIII: These tubules are also provided with $A_1$, $A_2$ and $B_2$ spermatogonia. Pachytene spermatocytes also exist in the tubule. No other meiotic stage has been noticed. Spermatids reach the 8th step of their metamorphosis (Diagram 15, p. 128).

Stage IX: This is characterised by spermatogonia $A_1$ and $A_2$. The spermatocytes are at leptotene and diplotene stages. The spermatids have now progressed to step 9 (Diagram 15, p. 128).

Stage X: The $A_1$ and $A_2$ spermatogonia are still found. Besides their being at zygotene, a few spermatocytes at diakinesis and metaphase-I are also noticed. Spermatids represent their 10th step during their metamorphosis. This stage eventually merges to stage I and the cycle is repeated thereafter (Diagram 15, p. 128).
Legend: Autoradiograms of the testicular preparations showing labelling of several stages of meiosis and spermiogenesis of *Rhacophorus maculatus* injected with $^3$H-thymidine (Squash preparations).

Fixative: Aceto alcohol (1:3).

Stain: Aceto orcein (2%).

Fig. 1: Pachytene appeared labelled first at 2.20 d p.i.

Figs. 2 and 3:
Labelled diplotene stages appeared at 7.00 d p.i.

Fig. 4: Labelled diakinesis appeared at 7.16 d p.i.

Fig. 5: Metaphase-I appeared labelled at 7.25 d p.i.

Fig. 6: Metaphase-II appeared labelled in the testicular preparation of the animal (R 21 A) sacrificed at 7.50 d p.i.

Figs. 7 and 8:
Early spermatids appeared labelled at 7.50 d p.i.

Fig. 9: Labelled spermatozoon appeared for the first time at 17.25 d p.i.
### TABLE 5

Chronology of the appearance of the most advanced labelled stages of meiosis and spermiogenesis at different days (d p.i.) following the $^{3}H$-thymidine labelling of the spermatocytes of *Rhacophorus maculatus*

<table>
<thead>
<tr>
<th>Serial no. and experimental set</th>
<th>Days post injection (d p.i.)</th>
<th>Most advanced labelled stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>R 1 A</td>
<td>0.04</td>
<td>Leptotene</td>
</tr>
<tr>
<td>R 2 A</td>
<td>0.25</td>
<td>Leptotene</td>
</tr>
<tr>
<td>R 3 A</td>
<td>0.50</td>
<td>Leptotene</td>
</tr>
<tr>
<td>R 4 A</td>
<td>1.00</td>
<td>Leptotene</td>
</tr>
<tr>
<td>R 5 B</td>
<td>1.50</td>
<td>Leptotene</td>
</tr>
<tr>
<td>R 6 A</td>
<td>1.76</td>
<td>Leptotene</td>
</tr>
<tr>
<td>R 7 B</td>
<td>2.00</td>
<td>Zygotene</td>
</tr>
<tr>
<td>R 8 A</td>
<td>2.20</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 9 B</td>
<td>2.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 10 A</td>
<td>2.68</td>
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<td>Pachytene</td>
</tr>
<tr>
<td>R 12 A</td>
<td>4.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 13 A</td>
<td>4.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 14 A</td>
<td>5.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 15 A</td>
<td>6.16</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 16 B</td>
<td>6.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>R 17 A</td>
<td>6.75</td>
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</tr>
<tr>
<td>R 18 B</td>
<td>7.00</td>
<td>Diplotene</td>
</tr>
<tr>
<td>R 19 A</td>
<td>7.16</td>
<td>Diakinesis</td>
</tr>
<tr>
<td>R 20 B</td>
<td>7.25</td>
<td>Diakinesis &amp; Metaphase-I</td>
</tr>
<tr>
<td>R 21 A</td>
<td>7.50</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>R 22 A</td>
<td>7.75</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>R 23 A</td>
<td>8.04</td>
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<td>Early spermatid</td>
</tr>
<tr>
<td>R 28 A</td>
<td>14.50</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>R 29 B</td>
<td>16.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>R 30 A</td>
<td>17.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>R 31 A</td>
<td>17.25</td>
<td>Late spermatid &amp; Sperm</td>
</tr>
<tr>
<td>R 32 B</td>
<td>17.50</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 33 A</td>
<td>18.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 34 A</td>
<td>21.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 35 A</td>
<td>24.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 36 A</td>
<td>26.00</td>
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<td>R 37 A</td>
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<td>Sperm</td>
</tr>
<tr>
<td>R 40 A</td>
<td>36.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 41 A</td>
<td>38.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 42 A</td>
<td>40.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 43 A</td>
<td>43.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>R 46 A</td>
<td>46.00</td>
<td>Sperm</td>
</tr>
</tbody>
</table>

Explanation: R indicates *Rhacophorus*, 1,2,3 etc. indicate the serials of the animals sacrificed and A & B indicate experimental set.
Chronology of meiosis and spermiogenesis

In Rhacophorus maculatus (Table 5, p. 136):

The course of migration of labelled spermatocytes through various stages of meiosis and spermiogenesis analysed in accordance with the conventional technique to calculate the individual stages of meiosis and spermiogenesis in this animal. The first appearance of labelled meiotic stages, spermatids and spermatozoa following $^{3}$H-thymidine injection has been recorded in the table 5 (p. 136). Leptotene was labelled in the testis of the 1st frog (R 1 A) sacrificed at 0.04 day post injection (d p.i.). However, the same remained 'hot' until 1.76 days. In the specimen (R 7 B) sacrificed at 2 d p.i. zygotene was found to be the most advanced labelled stage. At 2.20 d p.i., pachytene appeared to be labelled for the first time (Plate XVIII, Figure 1, p. 135). Now assuming the absence of any G$_2$ period, it may be said that the duration of leptotene is anywhere in between 1.76 days and 2 days. Zygote duration is very short and presumably not more than 0.54 day (2.20 - 1.76). The pachytene stage, which was observed labelled for the first time at 2.20 d p.i., continued to remain radioactive upto 6.75 days (Table 5, R 17 A, p. 136), while labelled diplotenes (Plate XVIII, Figures 2 and 3, p. 135) were detected at 7 d p.i. (Table 5, R 18 B, p. 136). It is apparent that at any time between 2.00 and 2.20 day post injection zygotenes reached the pachytene stage and also that these pachytenes became converted to diplotenes sometime between 6.75 and 7.00 d p.i.
Therefore, the duration of pachytene would neither exceed \((7.00 - 2.00 =) 5\) days, nor be less than \((6.75 - 2.50 =) 4.55\) days. One may either (a) conclude simply that the pachytene duration ranges between 4.55 and 5 days or (b) conventionally state that the pachytene duration is \(4.78 \pm 0.23\) days. Hence pachytene has a relatively longer duration. Diakinesis (Plate XVIII, Figure 4, p. 135) was found labelled initially at 7.15 d p.i. and metaphase-I at 7.25 d p.i. (Table 5, R 19 A, p. 136; Plate XVIII, Figure 5, p. 135). These findings obviously suggest not only that duration of the individual stages like diplotene, diakinesis and metaphase-I is very short, but also that the succession of these stages is incredibly rapid. The total duration of these stages is incredibly rapid. The total duration of these three stages appears to be less than a day. Labelled early spermatids having characteristically circular outline were detected as early as 7.50 d p.i. (Table 5, R 21 A, p. 136; Plate XVIII, Figure 7, p. 135).

Mature spermatozoa, that were yet unlabelled at 17.00 day, exhibited labelling at 17.25 day (Table 5, R 31 A, p. 136) and continued to be the most advanced elements later (Plate XVIII, Figure 9, p. 135). The duration of spermiogenesis could never be more than 10.00 days, nor less than \((17.00 - 7.50 =) 9.50\) days. The meiosis and spermiogenesis must have been completed any time in between 17 and 17.25 days.

In Gekko gecko (Table 6, p. 139):

Following the administration of \(\text{H}^3\)-thymidine in the testis of Gekko, biopsies were taken covering a period
TABLE 6

Chronology of the appearance of the most advanced labelled stages of meiosis and spermiogenesis at different days (d p.i.) following the $^3$H-thymidine labelling of the spermatocytes of *Gekko gecko*

<table>
<thead>
<tr>
<th>Serial no. and experimental set</th>
<th>Days post injection (d p.i.)</th>
<th>Most advanced labelled stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1 A</td>
<td>0.04</td>
<td>Leptotene</td>
</tr>
<tr>
<td>G 2 A</td>
<td>0.40</td>
<td>Leptotene</td>
</tr>
<tr>
<td>G 3 A</td>
<td>0.50</td>
<td>Leptotene</td>
</tr>
<tr>
<td>G 4 B</td>
<td>2.00</td>
<td>Leptotene</td>
</tr>
<tr>
<td>G 5 A</td>
<td>2.25</td>
<td>Leptotene</td>
</tr>
<tr>
<td>G 6 A</td>
<td>2.41</td>
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<tr>
<td>G 7 B</td>
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<tr>
<td>G 8 A</td>
<td>2.75</td>
<td>Zygotene</td>
</tr>
<tr>
<td>G 9 A</td>
<td>2.83</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 10 A</td>
<td>3.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 11 A</td>
<td>3.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 12 A</td>
<td>4.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 13 B</td>
<td>4.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 14 B</td>
<td>6.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 15 B</td>
<td>7.25</td>
<td>Pachytene</td>
</tr>
<tr>
<td>G 16 A</td>
<td>7.50</td>
<td>Diplotene</td>
</tr>
<tr>
<td>G 17 B</td>
<td>7.75</td>
<td>Diakinesis &amp; Metaphase-I</td>
</tr>
<tr>
<td>G 18 A</td>
<td>8.00</td>
<td>Metaphase-I</td>
</tr>
<tr>
<td>G 19 A</td>
<td>8.25</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>G 20 A</td>
<td>8.50</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>G 21 B</td>
<td>12.00</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>G 22 B</td>
<td>14.00</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>G 23 A</td>
<td>17.50</td>
<td>Mid spermatid</td>
</tr>
<tr>
<td>G 24 B</td>
<td>18.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>G 25 B</td>
<td>19.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>G 26 B</td>
<td>21.50</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>G 27 A</td>
<td>22.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>G 28 A</td>
<td>24.00</td>
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</tr>
<tr>
<td>G 29 A</td>
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<td>30.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>G 31 A</td>
<td>32.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>G 32 A</td>
<td>36.00</td>
<td>Sperm</td>
</tr>
</tbody>
</table>

Explanation: G indicate *Gekko*; 1, 2, 3, 4 etc. indicates serial no. of the specimen sacrificed; and A & B indicate the set of the experiment.
Legend: Photomicrographs of the labelled stages of meiosis and spermiogenesis from the testicular preparation of *Gekko gecko* injected with $H^3$-thymidine.

Fixative: Aceto alcohol (1:3).

Stain: Aceto orcein (2%).

Fig. 1: Labelled leptotene appeared for the first time at 0.04 d p.i. (Squash preparation).

Fig. 2: Pachytene appeared labelled for the first time at 2.75 d p.i. (Squash preparation from animal G 9 A).

Fig. 3: Labelled diplotene from the animal sacrificed at 7.50 d p.i. (Air dried preparation).

Fig. 4: Labelled diakinesis appeared for the first time at 7.75 d p.i. (Animal, G 17 B, air dried preparation).

Fig. 5: A labelled diakinesis from the same (G 17 B) animal, but from the squashed preparation.

Fig. 6: A labelled metaphase-I obtained from the air dried preparation of testicular tissue of the animal (G 17 B) sacrificed at 7.75 d p.i.

Fig. 7: A labelled metaphase-II obtained from the squash preparation of the testis of animal sacrificed at 8.25 d p.i.

Fig. 8: Labelled early spermatid (SPTD$^E$) obtained from the squashed preparation of the animal sacrificed at 8.25 d p.i.

Fig. 9: Labelled pachytene from the histological preparation of the testicular tissue of cat.

Fig. 10: Labelled early spermatid (SPTD$^E$) appeared for the first time at 8.25 d p.i. (Air dried preparation).

Fig. 11: Labelled late spermatid appeared at 19 d p.i. (Air dried preparation).

Fig. 12: Labelled spermatozoa appeared first at 22.00 d p.i. Obtained from the testis of gecko sacrificed at 22.00 d p.i. (Air dried preparation).
from 0.04 day to 36 days post injection (d p.i.) (Table 3, p. 51). The leptotene continued to be the only labelled stage in all the biopsies (G 1 A to G 6 B) up to 2.41 d p.i. (Table 6, p. 139; Plate XIX, Figure 1, p. 141), while zygotene appeared labelled at 2.50 d p.i. (Table 6, G 7 B, p. 139). Pachytene appeared labelled for the first time at 2.83 d p.i. (Table 6, G 9 A, p. 139; Plate XIX, Figure 2, p. 141). This suggests that leptotene duration must be somewhere in between 2.41 and 2.50 days, whereas zygotene duration is very short and within the range of a period of (2.75 - 2.50 =) 0.25 day to (2.83 - 2.41 =) 0.42 day. Pachytene, which first appeared labelled at 2.83 d p.i., continues to remain labelled up to 7.25 d p.i. Diplotene appeared labelled at 7.50 d p.i. (Table 6, G 16 A, p. 139; Plate XIX, Figure 3, p. 141).

Hence, the duration of pachytene seems to be in between (7.25 - 2.83 =) 4.42 days and (7.50 - 2.75 =) 4.75 days. Labelled diakinesis and metaphase-I (Plate XIX, Figure 4, 5 and 6, p. 141) were detected in the biopsies at 7.75 and 8.00 d p.i. (Table 6, G 17 B and G 18 A, p. 139). The early spermatid having a round appearance was scored labelled at 8.25 d p.i. (Table 6, G 19 A, p. 139; Plate XIX, Figures 8 and 10, p. 141). Therefore, the duration of diplotene, diakinesis and metaphase-I would jointly be less than a day. The spermatids (Plate XIX, Figures 8, 9 and 10, p. 141), which were labelled in the animal (G 19 A) sacrificed at 8.25 d p.i., continued to be radioactive even at 21.50 d p.i. (Plate XIX, Figure 12, p. 141), when, however, they were transformed into late
TABLE 7

Chronology of the appearance of the most advanced labelled stages of meiosis and spermiogenesis at different days (d p.i.) following the H\(^3\)-thymidine labelling of the spermatocytes of Varanus bengalensis

<table>
<thead>
<tr>
<th>Serial no. and experimental set</th>
<th>Days post injection (d p.i.)</th>
<th>Most advanced labelled stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>V 1 A</td>
<td>0.04</td>
<td>Leptotene</td>
</tr>
<tr>
<td>V 2 A</td>
<td>0.16</td>
<td>Leptotene</td>
</tr>
<tr>
<td>V 3 A</td>
<td>1.25</td>
<td>Leptotene</td>
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<td>Zygotene</td>
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<tr>
<td>V 7 B</td>
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<td>V 8 A</td>
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<td>V 9 A</td>
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<tr>
<td>V 14 B</td>
<td>9.00</td>
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<td>V 15 A</td>
<td>9.50</td>
<td>Diakinesis &amp; Metaphase-I</td>
</tr>
<tr>
<td>V 16 A</td>
<td>10.00</td>
<td>Early spermatid</td>
</tr>
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<td>V 17 B</td>
<td>10.25</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>V 18 A</td>
<td>12.00</td>
<td>Early spermatid</td>
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<td>V 19 B</td>
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<tr>
<td>V 20 A</td>
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<td>Early spermatid</td>
</tr>
<tr>
<td>V 21 A</td>
<td>17.00</td>
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<td>21.50</td>
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</tr>
<tr>
<td>V 25 B</td>
<td>22.75</td>
<td>Late spermatid</td>
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<tr>
<td>V 26 B</td>
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</tr>
<tr>
<td>V 27 A</td>
<td>24.00</td>
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<tr>
<td>V 28 A</td>
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</tr>
<tr>
<td>V 33 A</td>
<td>36.00</td>
<td>Sperm</td>
</tr>
</tbody>
</table>

Explanation: V indicates Varanus; 1, 2, 3, 4 etc. indicate no of animals sacrificed; A & B are experimental set.
Legend: Photomicrographs of the labelled stages of meiosis and spermiogenesis from the testicular preparation of *Varanus bengalensis* injected with H³-thymidine.

Fixative: Aceto alcohol (1:3).
Stain: Aceto orcin (2%).

Fig. 1: Labelled zygotene appeared for the first time at 1.90 d p.i. (Squash preparation).

Fig. 2: Pachytene appeared labelled for the first time at 2.90 d p.i. (Squash preparation).

Figs. 3 and 4:
Labelled pachytene appeared in the testicular preparation of the animal (V 10 A) sacrificed at 5.00 d p.i. (Air dried preparation).

Figs. 5 and 6:
Labelled diplotene appeared at 9.00 d p.i.
(Air dried preparation).

Fig. 7: Labelled diakinesis appeared for the first time at 9.50 d p.i. (Air dried preparation).

Fig. 8: Labelled metaphase-II appeared at 10.00 d p.i.
(Air dried preparation).

Figs. 9 and 10:
Labelled early spermatids obtained at 10.00 and 12.00 d p.i. (Squash preparation).

Fig. 11: Spermatozoa appeared labelled for the first time at 23.25 d p.i. (Squash preparation).
spermatids. The labelled mature spermatozoa were detected for the first time at 22.00 d p.i. (Table 6, G 27 A, p. 139; Plate XIX, Figure 13, p. 141). Therefore, spermiogenesis in this animal appears to be completed in between (21.50 - 8.25 =) 12.25 and (22.00 - 8.25 =) 13.75 days. The total duration of meiosis and spermiogenesis is around 22 days.

In Varanus bengalensis (Table 7, p. 143):

Following H\(^3\)-thymidine injection the first biopsy, which was taken at 0.04 day, showed the labelled leptotene stages in squash, air dried and histological preparations. Leptotene stages remained 'hot' until 1.75 d, following which zygotene stage displayed labelling for the first time at 1.90 d p.i. (Plate XX, Figure 1, p. 145). Labelled pachytenes (Plate XX, Figure 2, p. 145) were detected at 2.90 d p.i. The duration of leptotene is, therefore, in between 1.75 and 1.90 days. It is apparent that zygotene would not have a duration shorter than (2.90 - 1.90 =) 1 day. But pachytene, which for the first time appeared labelled at 2.90 d p.i. (Table 7, V 7 B, p. 143), remained so upto 8.75 d p.i. (Table 7, V 13 B, p. 143) and possesses a longer duration, which is anywhere in between (8.75 - 2.90 =) 5.85 and (9.00 - 2.25 =) 6.75 days. The diplotene and diakinesis figures (Plate XX, Figures 6 and 7, p. 145) appeared labelled at the same time at 9.00 d p.i. (Table 7, V 14 B, p. 143), whereas diakinesis and metaphase-I (Plate XX, Figure 8, p. 145) were found to be labelled at 9.50 d p.i. (Table 7, V 15 A, p. 143). It may thus be concluded that these stages,
diplotene, diakinesis and metaphase-I, together take a transitory period of 0.75 day approximately. The early spermatid (Plate XX, Figure 9, p. 145) in this animal appeared labelled for the first time at 10.00 d p.i. (Table 7, V 16 A, p. 143). Spermatids undergoing spermiogenesis were labelled until 22.75 d p.i. (Table 7, V 26 B, p. 143). Spermatozoa were first detected labelled (Plate XXII, Figure 11, p. 145) at 23.25 d p.i. (Table 7, V 27 A, p. 143). In all the later biopsies spermatozoa consistently were labelled. Therefore, spermiogenesis in this case appears to be completed sometime in between \((22.75 - 10.00 = )\) 12.75 days and \((23.25 - 9.50 = )\) 13.75 days. Both meiosis and spermiogenesis are completed within 23.25 days.

In *Psittacula cyanoccephala* (Table 8, p. 148)

The leptotene was the only labelled stage (Plate XXI, Figure 1, p. 150) in the birds sacrificed at 0.04 and 1.50 days postlabelling (Table 8, P 1 A and P 3 A, p. 148). Zygotene (Plate XXI, Figure 2, p. 150) appeared labelled for the first time at 1.75 d p.i. (Table 8, P 4 B, p. 148). The pachytene figures were detected (Plate XXI, Figures 3 and 4, p. 150) labelled at 2.50 d p.i. (Table 8, P 5 A, p. 148) in the testicular preparations. The analysis of these findings reveals here that the duration of leptotene is in between 1.50 and 1.75 days whereas zygotene duration is very short (and even comparable to a few hours). It is to be mentioned here that in calculating the duration of individual stages, a post DNA synthetic gap (comparable to the G2 period, which is the gap between the termination of DNA synthesis and
TABLE 8

Chronology of the appearance of the most advanced labelled stages of meiosis and spermiogenesis at different days (d p.i.) following the $^3$H-thymidine labelling of the spermatocytes of *Psittacula cyanoccephala*

<table>
<thead>
<tr>
<th>Serial no. and experimental set</th>
<th>Days post injection (d p.i.)</th>
<th>Most advanced labelled stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1 A</td>
<td>0.04</td>
<td>Leptotene</td>
</tr>
<tr>
<td>P 2 A</td>
<td>0.50</td>
<td>Leptotene</td>
</tr>
<tr>
<td>P 3 A</td>
<td>1.50</td>
<td>Leptotene</td>
</tr>
<tr>
<td>P 4 B</td>
<td>1.75</td>
<td>Zygote</td>
</tr>
<tr>
<td>P 5 A</td>
<td>2.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 6 B</td>
<td>2.75</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 7 A</td>
<td>3.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 8 A</td>
<td>4.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 9 A</td>
<td>6.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 10 B</td>
<td>7.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 11 B</td>
<td>7.75</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 12 A</td>
<td>8.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>P 13 A</td>
<td>8.25</td>
<td>Diplotene, Diakinesis &amp; Metaphase-I</td>
</tr>
<tr>
<td>P 14 B</td>
<td>8.50</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>P 15 A</td>
<td>9.00</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>P 16 A</td>
<td>12.00</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>P 17 A</td>
<td>14.00</td>
<td>Mid spermatid</td>
</tr>
<tr>
<td>P 18 B</td>
<td>16.50</td>
<td>Mid spermatid</td>
</tr>
<tr>
<td>P 19 A</td>
<td>17.00</td>
<td>Mid spermatid</td>
</tr>
<tr>
<td>P 20 B</td>
<td>17.25</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>P 21 A</td>
<td>18.50</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>P 22 B</td>
<td>20.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>P 23 B</td>
<td>20.75</td>
<td>Sperm</td>
</tr>
<tr>
<td>P 24 A</td>
<td>21.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>P 25 A</td>
<td>22.00</td>
<td>Sperm</td>
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<tr>
<td>P 26 A</td>
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<td>Sperm</td>
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<td>P 27 A</td>
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<td>P 31 A</td>
<td>34.00</td>
<td>Sperm</td>
</tr>
<tr>
<td>P 32 A</td>
<td>36.00</td>
<td>Sperm</td>
</tr>
</tbody>
</table>

Explanation: P indicates *Psittacula*; 1, 2, 3 etc. indicate no of animals sacrificed; A & B indicate the sets of experiment.
PLATE XXI

Legend: Photomicrographs of the autoradiograms showing the labeled stages of meiosis and spermiogenesis in *Psittacula cyanocephala* at different days after the injection of H\(^3\)-thymidine.

Fixative: Aceto alcohol (1:3).
Stain: Aceto orcein (2%).
(Air dried preparation).

Fig. 1: Leptotene appeared labelled for the first time at 0.04 d p.i.

Fig. 2: Labelled zygotene stage appeared at 1.75 d p.i.

Figs. 3 and 4:
Pachytene stages appeared to be labelled for the first time at 2.50 d p.i.

Figs. 5 and 6:
Labelled diplotene stages appeared at 8.25 d p.i.

Fig. 7: Metaphase-I appeared labelled at 8.25 d p.i.

Fig. 8: Labelled early spermatid appeared for the first time at 8.50 d p.i.

Figs. 9 and 10:
Labelled spermatozoa appeared for the first time at 20.75 d p.i.
initiation of cell division in mitotic cells) before the initiation of meiosis has not been detected due presumably to either its total absence, or its excessively short duration in spermatocytes. There is ample evidence that post DNA synthesis gap does not exit in mammalian spermatocytes (Monesi, 1962; Lima-de-Faria et al., 1962; Baker, 1971; Joarder, 1977). However, pachytene figures (Plate XXI, Figures 5, 6 and 7, p. 150; Table 7, P 13 A, p. 148) were found to be the advanced labelled stages at 8.25 d p.i. From these observations it may be concluded that the duration of pachytene stage is obviously in between (8.00 - 2.50 =) 5.50 and (8.25 - 2.50 =) 5.75 days. In the successive biopsies no labelled diplotenes were traced out and diakinesis and metaphase-I exhibited labelling for a very short interval, as at 8.50 d p.i. early spermatids (Plate XXI, Figure 8, p. 150; Table 8, P 14 B, p. 148) were found to be labelled. The total, as well as the individual, duration of diplotene, diakinesis and metaphase-I, hence, seems to be very short and is difficult to ascertain. Mid and late spermatids appeared labelled for the first time at 14.00 d p.i. and 17.25 d p.i. respectively. Mature spermatozoa (Plate XXI, Figure 9, p. 150) appeared labelled at 20.75 d p.i. (Table 8, P 23 B, p. 148) when some labelled late spermatids were also found. It therefore appears that spermiogenesis must have a duration of about (20.75 - 8.50 =) 12.25 days. The entire process of meiosis and spermiogenesis jointly is completed in 20.75 day.
### TABLE 9

Chronology of the appearance of the most advanced labelled stages of meiosis and spermiogenesis at different days (d p.i.) following the $^3$H-thymidine labelling of the spermatocytes of *Felis domesticus*

<table>
<thead>
<tr>
<th>Serial no, and experimental set</th>
<th>Days post injection (d p.i.)</th>
<th>Most advanced labelled stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 1 A</td>
<td>0.04</td>
<td>Leptotene</td>
</tr>
<tr>
<td>C 2 A</td>
<td>0.30</td>
<td>Leptotene</td>
</tr>
<tr>
<td>C 3 A</td>
<td>2.75</td>
<td>Leptotene</td>
</tr>
<tr>
<td>C 4 B</td>
<td>3.00</td>
<td>Leptotene</td>
</tr>
<tr>
<td>C 5 B</td>
<td>3.75</td>
<td>Leptotene</td>
</tr>
<tr>
<td>C 6 A</td>
<td>4.00</td>
<td>Zygote</td>
</tr>
<tr>
<td>C 7 B</td>
<td>4.25</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 8 A</td>
<td>4.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 9 B</td>
<td>5.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 10 A</td>
<td>6.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 11 A</td>
<td>8.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 12 A</td>
<td>9.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 13 B</td>
<td>12.88</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 14 A</td>
<td>14.00</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 15 B</td>
<td>18.50</td>
<td>Pachytene</td>
</tr>
<tr>
<td>C 16 B</td>
<td>19.75</td>
<td>Pachytene</td>
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<td>C 17 A</td>
<td>20.25</td>
<td>Diplotene, Diakinesis &amp; Metaphase-I</td>
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<td>C 18 A</td>
<td>20.50</td>
<td>Early spermatid</td>
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<tr>
<td>C 19 A</td>
<td>21.00</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>C 20 B</td>
<td>23.75</td>
<td>Early spermatid</td>
</tr>
<tr>
<td>C 21 A</td>
<td>24.16</td>
<td>Early spermatid</td>
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<td>Mid spermatid</td>
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<td>Late spermatid</td>
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<td>C 25 B</td>
<td>33.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>C 26 A</td>
<td>35.75</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>C 27 A</td>
<td>37.00</td>
<td>Late spermatid</td>
</tr>
<tr>
<td>C 28 B</td>
<td>40.00</td>
<td>Late spermatid</td>
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<td>C 29 B</td>
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</tr>
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<td>Sperm</td>
</tr>
<tr>
<td>C 35 A</td>
<td>48.00</td>
<td>Sperm</td>
</tr>
</tbody>
</table>

Explanation: C indicates Cat (*Felis domesticus*); 1, 2, 3 etc., indicate no. of biopsies taken, and A & B indicate the experimental set.
Legend: Photomicrographs of the autoradiograms showing labelled stages of meiosis and spermiogenesis in Felis domesticus at different days after the administration of the tracer.

Fixative: Aceto alcohol (1:3).
Stain: Aceto orcein (2%).
(Air dried preparation)

Fig. 1: Zygotene stage appeared labelled for the first time at 4.00 d p.i.

Fig. 2: Labelled early pachytene stage detected for the first time at 4.25 d p.i.

Fig. 3: A labelled pachytene stage detected in the biopsy at 8.50 d p.i.

Fig. 4: Diplotene stage appeared labelled at 20.25 d p.i.

Fig. 5: Labelled diakinesis stage appeared at 20.25 d p.i.

Fig. 6: Labelled metaphase-I appeared at 20.25 d p.i.

Fig. 7: Metaphase-II appeared labelled in the biopsy at 20.25 d p.i.

Fig. 8: A labelled late spermatid appeared at 40.00 d p.i.

Fig. 9: Labelled early spermatids were detected for the first time at 20.50 d p.i.

Fig. 10: Late spermatids appeared labelled at 26.00 d p.i.

Fig. 11: The labelled mature sperm was detected at 41.25 d p.i.
PLATE XXIII

Legend: Photomicrographs of the autoradiograms of the testicular sections (5 μ) of Felis domesticus showing various labelled stages of meiosis and spermiogenesis appeared at different days after the administration of the tracer.

Fixative: Zenker's Formol.
Stain: PAS-haematoxylin.

Fig. 1: A labelled zygotene stage appeared for the first time at 4.00 d p.i.

Fig. 2: Labelled pachytene figures appeared at 4.25 d p.i.

Fig. 3: Labelled diplotene stages appeared at 20.25 d p.i.

Fig. 4: Diakinesis stage appeared labelled at 20.25 d p.i.

Fig. 5: Metaphase-I appeared labelled at 20.25 d p.i.

Figs. 6, 7 and 8:

Labelled mature spermatozoa appeared for the first time at 41.25 d p.i.
In *Felis domesticus* (Table 9, p. 152):

Labelled leptotene was first observed in the biopsy taken at 0.04 d p.i. (Table 9, C 1 A, p. 152). Until this period after administration of $^3$H-TdR, no other meiotic stages appeared radioactive. Labelled zygotenes (Plate XXII, Figure 1, p. 154) were the most progressed meiotic stage in the biopsy at 4.00 d p.i. (Table 9, C 6 A, p. 152). The pachytene (Plate XXII, Figures 2 and 3, p. 154) figures displayed autoradiographic silver grains overlying them for the first time at 4.50 d p.i. (Table 9, C 8 A, p. 152). Hence the duration of leptotene would be anywhere in between 3.75 and 4.00 days. The duration of zygotene is very short and would be nearly 0.50 day. Pachytene, having appeared labelled at 4.00 d p.i., exhibited labelling at even as late as 19.75 d p.i. (Table 9, C 16 B, p. 152). Labelled diplotene, diakinesis and metaphase-I figures (Plate XXII, Figures 4, 5 and 6 p. 154; Plate XXIII, Figures 3 and 4, p. 156) were simultaneously scored at 20.25 day. From these findings it is evident that pachytene has a longer duration ranging from 14.00 days to 15.25 days. Early spermatids (Plate XXII, Figure 9, p. 154; Plate XXIII, Figure 5, p. 156) appeared labelled at 20.50 d p.i. (Table 9, C 18 A, p. 152). Nuclear elongation in the spermatid was first noted at 24.50 days (Table 9, C 23 A, p. 152) after the administration of the tracer. The late spermatids (Plate XXII, Figure 10, p. 154) in labelled condition were observed at 26.00 d p.i. The labelled mature spermatozoa (Plate XXII, Figure 11, p. 154; Plate XXIII, Figures 6, 7, and 8, p. 156) were observed for the first time at 41.25 d p.i. Spermiogenesis in the cat is therefore completed in between 20 days and 21.00 days. Both meiosis and spermiogenesis in this species is completed any time between 41.00 and 41.25 days.