Chapter 4

Pituitary - Gonadal cycle.
The role of the pituitary hormones in controlling the somatotrophic and gonadotrophic activities in the vertebrates is now well known. Despite the fact that, there is enormous literature on the amphibian pituitary, there are few contributions which provide us with a clear understanding on the structure and behaviour of the different types of cells of the anterior pituitary in Anura, such as Van Oordt (1963) on *Xenopus*, *Rana* and *Bufo bufo*; Lakshman (1965) on *Rana tigrina*, *Rana ovanoph-lyctis*, *Rana hexadactyla*, *Bufo melanostictus*, *Uperodon systoma*; Rastogi and Chieffi (1970) on *Rana esculenta* and Zysk (1975) on *Rana temporaria*. Van Oordt (1963) classified the cells in two types, the acidophils and basophils. He further classified acidophils in 2 types and basophils in 3 types. Lakshman (1965) and Rastogi and Chieffi (1970) investigated the quantitative changes in the cells of pars distalis (anterior pituitary) during annual cycle. They divided the acidophil cells into various types depending upon their staining reactions. Zysk (1975), while investigating the behaviour of cells of the pars distalis of *Rana temporaria*, described 4 types of cells: acidophils (α cells), 2 types of basophils (β cells and δ cells) and chromophobes. Many workers attempted to correlate the quantitative cytological changes with the physiological
functions of the pituitary and the behaviour of target organs in the amphibians. The basophil cells of the pars distalis have been attributed a direct relationship with ovulation and spermatogenesis (Van Oordt, 1961 and 1965; Lakshman, 1965; Rastogi and Chieffi, 1970). There is a general agreement that the acidophil cells secrete somatotrophins and basophils gonadotrophins. No definite function has been assigned to chromophobes although some workers (Zysk, 1975) believe them to be precursors of basophilic cells.

The present chapter deals with the investigation on the cytological changes of the pituitary gland and the histomorphological changes in the testis and ovary of *Rana limnocharis* during its annual cycle, with a view to understand the behaviour of the pars distalis, and the different cell types found in it, in relation to the cyclic changes in the gonads and its breeding activity. The investigation on the pars distalis has been restricted to the detailed study of behaviour of 3 major cell types: acidophils (α cells), basophils (β cells) and chromophobes during different phases of the annual cycle. The study of sub-types of cells was not considered necessary during the present investigation. Histological changes in the testis have also been studied in detail throughout the year. As the changes in the ovary were well marked even in the dissection of the females, the investigation on the
ovary has been confined only to the gross morphological and histological changes during different phases of the annual cycle.

REVIEW OF LITERATURE

Work on the pituitary gland in Vertebrates has been reviewed in the recent past by Harris and Donovan (1966) and more recently particularly in Amphibia by Banke (1974). Among histological and cytological studies, the classification and nomenclature of pituitary cell types have been subjects much debated. Two major types of cells, chromophiles and chromophobes have been described by all workers. There is still some confusion over the identification adopted by different investigators. The important contributions, in this connection are following.

As early as in 1892, Schönemann distinguished 2 types of chromophil cells in the pars distalis of human pituitary by using eosin and haematoxylin and named them 'eosinophils' and 'cyanophils'. Using his own formulated stain, Mallory, in 1900, described 2 types of chromophils, acidophils and basophils on the assumption that there were only two types of such cells in the pars distalis. Using Kresofuchsin and Heidenhain's Azan stain, Romeis (1940) did very exhaustive work on human pars distalis. He used the Greek nomenclature for different cell types and
designated ordinary acidophils as alpha cells, those stained orange and present mostly during non-pregnant state as epsilon cells, and those staining similarly as epsilon cells but present during pregnant state as eta cells, McManus (1946) prescribed PAS technique for staining glycoproteins. Using this technique Herlant (1960) identified serous cells and Pearse (1953) identified mucoid cells. Van Oordt (1961) described the gonadotrophic and other cells types in the distal lobe of the pituitary of the common frog, Rana temporaria. Purves (1961) adopted the nomenclature, acidophil and basophil for the 2 types of cells identified by PAS reaction. Due to strong PAS reaction some investigators preferred to call basophils as pasophils. By acid trichrome method Ortman (1956, 1960 and 1961) described 5 chromophil types in Rana pipiens. Van Oordt (1963) worked on Rana, Rana temporaria, Bufo bufo, newts and Salamanders and gave a tentative identification of cell types in amphibians as follows:

Carminophils = Somatotrophs
Organgeophils = Prolactin secretors
Basophils (in order of affinity for Alcian Blue)
1. Purely cyanophil = Thyrotroph
2. Cyanophil + acidophil inclusions = Folliculotrophs
3. Amphophil = Interstitiotrophs
Reyrel (1967), while working upon *Pelobates cultripes* reported by signalling colourations and histochemical reactions 6 cellular types – 3 acidophilic and 3 basophilic types. Subsequently, besides histological and histochemical, workers also followed fluorescent and bioassay techniques. Thus, Mira-Moser (1970) described acidophil I and acidophil II, basophil I, basophil II, basophil III and basophil IV. Kerr (1965), Van Kemende (1974) and Pehlemann (1974) described 2 types of acidophils (acidophil I and acidophil II) and 3 types of basophils (basophil I, basophil II and basophil III). Mira-Moser's basophil I and II correspond to the basophil II and basophil I respectively. Doerr-Schott (1974) described acidophils, basophils and amphiphils in amphibians. He reported that acidophils secrete LTH as well as STH. Earlier, Kerr (1965) reported that acidophils I, secrete STH as well as LTH and acidophils II secrete LTH. Van Kemende (1974) attributed secretion of LTH to acidophil I, STH to acidophil II, TSH to basophil I, FSH and LH to basophil II and ACTH to basophil III.

The morphology and development of anuran gonads, has been described in great detail with reference to *Rana pipiens* by Rugh (1951). There are few detailed contributions on the annual changes in the pituitary gland of Amphibia in relation to gonadal changes. Lakshman (1965) described the structural changes in the pituitary gland of female frogs and toads during different seasons of the year and reported that the
number of acidophilic cells was smallest during the period of egg laying. Rastogi and Chieffi (1970) reported similar phenomena in *Rana esculenta*. Zysk (1975) gave a detailed account of such changes in the males and females of *Rana esculenta*. He described 4 types of pituitary cells based on morphological, as well as staining characteristics, acidophil cells (α cells), 2 types of basophil cells (β and γ cells), and chromophobe cells. He described that basophilic cells were numerous soon after hibernation in the I decade of March, acidophilic cells during active period from May to September and chromophobe cells during early breeding period only. The number of chromophobe cells decreases gradually during active egg laying period in the III decade of March. He has correlated these observations with the presence of high level of gonadotrophic hormone during early breeding period and high level of somatotrophic hormone during active land life.

MATERIALS AND METHODS

The pituitary gland and gonads were processed for histological study as follows:

THE PITUITARY GLAND.

The frogs were captured from the streams during paddy season, from the paddy fields of Pologround, Shillong at regular monthly intervals. These animals were taken to the laboratory, decapitated, and the anterior pituitary gland
was taken out and fixed immediately in Bouin's Picroformol in order to avoid influence of diurnal physiological rhythm in frogs on the result (Lach, 1970). The technique of dissection of the pituitary gland has been described in Chapter V on Induced Breeding.

The pituitaries were fixed in Bouin's fixative, dehydrated in Butanol (normal butyl alcohol) and embedded as per the following procedure:

1. Fixation in Bouin's fixative ... ... 24 Hours
2. Washing in 70% alcohol ... ... 24 "
3. Dehydration in 70% alcohol (3/4 part) + Butanol (1/4 part) ... ... 1/2 "
4. Dehydration in 70% alcohol (1/2 part) + Butanol (1/2 part) ... ... 1/2 "
5. Dehydration in 90% alcohol (1/4 part) + Butanol (3/4 part) ... ... 1/2 "
6. Dehydration in Butanol ... ... 2 "
7. Dehydration in fresh Butanol ... ... 2 "
8. Embedding in Butanol (3/4 part) + Paraffin Wax (1/4 part) ... ... 1 "
9. Embedding in Butanol (1/2 part) + Paraffin Wax (1/2 part) ... ... 1 "
10. Embedding in Butanol (1/4 part) + Paraffin Wax (3/4 part) ... ... 1 "
11. Embedding in Molten Paraffin Wax ... ... Over night
12. Embedding in fresh Molten Paraffin wax ... ... 2–4 Hours

The paraffin wax blocks were made and microtome sections were cut in sagittal plane of the anterior pituitary,
at thickness of 4 μ. Sections were stained by 2 methods:

1. With Aldehyde-Fuchsin, Orange-G, Haematoxylin, Fast Green and (2) Heidenhain's Azan Stain from which Azocarmine was eliminated. Slidder's method, Barrett's method and PAS techniques described by Disbrey and Rack (1970) did not give satisfactory results. Other techniques could not be attempted as the specific stains could not be obtained. By the use of these two methods the main cell types of the pituitary were differentially stained. By the use of Aldehyde-Fuchsin method, the cytoplasm of the acidophil cells was stained orange and the nucleus reddish violet. The cytoplasm of the basophil cells was stained brown and the nucleus reddish violet. With the Azan stain the cytoplasm of the acidophil cells was stained orange and the nucleus bright orange. For basophil cells, the cytoplasm stained blue and the nucleus reddish blue. The cytoplasm of the chromophobe cells remains unstained or stains very lightly with both the types of stain used. Its nucleus however is stained brightly. The size of the 3 cell types and their nuclei were measured with the help of ocular and stage micrometers. The number of different types of cells per unit area (πr² = 2.113 sq. mm) has been calculated on the basis of observations in 5 unit fields under oil immersion (x 1000) of the microscope. Some cells which could not be clearly identified have not been taken into consideration.
Staining technique:

(1) **Aldehyde-Fuchsine Method.**

1. The sections were deparaffinised in xylol.
2. Hydrated the sections by passing the slides through 100%, 90%, 70%, 50%, 30% alcohol and then placing them in water.
3. Washed the slides in running water for 2 minutes.
4. Transferred the slides to 70% alcohol.
5. Stained the sections in Aldehyde-Fuchsine for 2 hours.
6. Washed in 70% alcohol.
7. Stained in *Ehrlich's Acid Haematoxylin* for 5 minutes.
8. Washed in tap water.
9. Stained in 1% aqueous Orange G for 2 minutes.
10. Rinsed in tap water.
12. Dehydrated in ethyl alcohol.
13. Transferred to xylol.
14. Mounted in D.P.X.

(2) **Heidenhain's Azan Method.**

1. Placed the slides in xylol to deparaffinise the sections.
2. Sections were hydrated.
3. Washed in distilled water.
4. Stained in Aniline Blue, Orange G mixture for 2 hours.
5. Dehydrated the slides in ethyl alcohol and xylol.
6. Mounted in D.P.X.

**THE GONADS**

The gonads (testis and ovary) were dissected out at
different periods of the annual cycle from the freshly captured frogs, fixed in small pieces in Bouin's Picroformol, dehydrated in Butanol in the same way as the pituitary gland, embedded in Paraffin Wax and sectioned at 7 μ thickness. The sections were stained in Haematoxylin and Eosin. Measurements of the various cellular structures were taken with the help of ocular and stage micrometers. The number of sections of the seminiferous tubules were counted under low power magnification of the microscope (each unit area = 7.06 sq. mm). The histological elements of each seminiferous tubule were counted under high magnification (oil immersion) of the microscope (each unit area = 2.113 sq. mm). Diagrams were made with camera lucida and photomicrographs were taken wherever needed.

OBSERVATIONS

THE PITUITARY GLAND

(A) MORPHOLOGY OF THE PITUITARY IN THE VERTEBRATES.

It would be worthwhile to have an idea of gross structure of the pituitary gland before describing its structure in Rana limnocharis. In vertebrates, the pituitary gland is located in a small bony fossa, the sella turcica, in the floor of the cranial cavity (Fig. 1). It is connected by a stalk at the base of the brain. Although it is joined to the brain, only part of the pituitary gland develops from the neural ectoderm, as a downgrowth, from
Fig. 1 - Diagrammatic longitudinal section of the pituitary gland in a vertebrate (mammal).

Abbreviations:

Atc - Anterior tuber cinereum.
Ptc - Posterior tuber cinereum.
Inr - Infundibular recess.
Ins - Infundibular stem.
Mde - Median eminence.
Ptb - Pars tuberalis.
Pdt - Pars distalis.
Pim - Pars intermedia.
Pnv - Pars nervosa.
Fig. 1
the floor of the diencephalon. This part is referred to as neurohypophysis. The larger part of the pituitary gland develops from the lining of the future oral ectoderm, the Rathke's pouch, known as the adenohypophysis. The adenohypophysis and the neurohypophysis can be divided in 3 and 2 subdivisions respectively as described below.

Adenohypophysis.

(a) Pars distalis (anterior lobe).
(b) Pars tuberalis.
(c) Pars intermedia (intermediate lobe).

Neurohypophysis.

(a) Pars nervosa (posterior lobe).
(b) Infundibulum.

(B) MORPHOLOGY OF THE PITUITARY GLAND IN RANA LIMNOCHARIS.

The morphology of the pituitary gland in *Rana limnocharis*, as observed from the ventral side of the skull after removing the palatine bone which forms the floor of the cranium is as follows: (Fig. 2).

Adenohypophysis

The adenohypophysis is situated ventro-caudal to the neurohypophysis. It is composed of the pars distalis, pars intermedia and pars tuberalis.

(a) Pars distalis (Fig. 3)

It forms the posterior lobe of the pituitary and is broad, often broader than long, bean shaped, pinkish, located
Fig. 2 - Ventral view of the brain of *Rana limnocharis* Wiegmann showing the position of the pituitary.

Abbreviations:

- Olf. Bulb = Olfactory bulb
- Tel = Telencephalon
- EyeB = Eye ball
- Optc = Optic tectum
- Opch = Optic chiasma

- Nat = Nostril
- Med = Medulla
- Cbl = Cerebellum
- Pty = Pituitary
- Dien = Diencephalon
Fig. 3 - Ventral view of the brain showing the pituitary of *Rana limnocharis* Wiegmann.

Abbreviations:

- Pro = Preoptic recess
- Oph = Optic chiasma
- Pdt = Pars distalis
- Eyeb = Eye ball
- Ptb = Pars tuberalis
Fig. 3
transversely posterior to optic chiasma. It is continuous with the pars intermedia along its antero-dorsal margin and is attached by means of connective tissue. It remains connected to the median eminence with its antero-ventral extremity. It is commonly referred to as anterior pituitary gland.

(b) Pars intermedia

It covers the postero-ventral aspect of the neural lobe and the two together form a transverse bar, the 'neuro-intermediate lobe' which is broader than the pars distalis.

(c) Pars tuberalis

It develops from the compact hypophyseal anlage, as a pair of epithelial tongue, which extend rostrally along the surface of the hypothalamus. As such, in the dissection of the brain it cannot be distinctly marked out from the hypothalamus.

Neurohypophysis

The neurohypophysis is not as well developed as the adenohypophysis. The wide succus infundibuli grows out as a pair of primary branches forming a transverse bar, situated over the anterior part of the hypophysis. The posterior wall of this bar proliferates to form the neural lobe. It stores a great amount of neuro-secretory material. The median eminence is situated antero-ventrally to the neural lobe, in contact with the antero-ventral end of the pars distalis.
Fig. 4 A - Outline diagram of the pituitary gland of Rana limnocharis Wiegmann.

Fig. 4 B - A central portion of the cross-section of the pituitary gland of Rana limnocharis Wiegmann.

Abbreviations:

- Chr - Chromophobe cells
- Sin - Sinusoids
- B - Basophil cells
- A - Acidophil cells
HISTOLOGICAL STRUCTURE

In the present investigation, the histological changes in the pars distalis (anterior pituitary) have been studied. As observed in the cross sections (Fig. 4A and B), the cells of the pars distalis are mainly arranged in cords, more or less in radiating pattern, between which are large bore of capillaries. Some cells appear to be in clusters, other in twisted cords and still others form small, well defined follicles, the lumina of which contain colloid. There is a very light meshwork of connective tissue fibre, entwined around the cells and capillaries.

There is a clear distinction in the arrangement of cells between the central and peripheral regions of the anterior pituitary. At the peripheral region, the cell cords are densely packed separated by relatively thin connective tissue and a plexus of capillaries. In the central region, the cells are less compact and larger, surrounded by large capillaries and more loosely arranged connective tissue. Three major types of cells were easily identified by the stains used: (a) Acidophil or \( \alpha \) cells (b) Basophil or \( \beta \) cells and (3) Chromophobes.

(a) Acidophil or \( \alpha \) cells.

The acidophil or \( \alpha \) cells are spherical or ellipsoidal in shape with a centric nucleus. Cytoplasm is strongly acidophilic and contains dense granules. These cells usually
Fig. 5 - Diagramatic representation of the distribution of acidophil, basophil and chromophobe cells in the pituitary gland of *Rana limnocharis* Wiegmann.

A - Distribution of cells during pre-breeding period.

B - Distribution of cells during breeding period.

C - Distribution of cells during post-breeding period.

D - Distribution of cells during hibernation period.
occupy central place in the lobule and are said to be responsible for producing somatotrophic hormone (STH).

(b) **Basophil or $\beta$ cells.**

The basophil or $\beta$ cells are the largest cells found in the gland. They are spherical, elongated, oval or angular in shape. They have eccentric nucleus. Cytoplasm is strongly basophilic. Their arrangement in the lobules is like columnar epithelium. Granulation varies depending upon the secretory phase of the cells. They are said to be responsible for producing thyrotrophic hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

(c) **Chromophobes**

The chromophobes are small or large spherical cells, with eccentric nucleus. The cytoplasm stains poorly with both acidic and basic dyes. Thus, the cytoplasm is either poorly stained or not stained at all. It may or may not possess granules in the cytoplasm depending upon the secretory condition of the cell. They occur singly or in clusters and are not clearly organized in the follicles.

(c) **Changes in the Behaviour of Different Types of Cells During Annual Cycle.**

The behaviour of acidophil cells, basophil cells and chromophobes was studied during the following different periods of their annual cycle (Table I and II; Fig. 5A, B, C and D).
### TABLE I

Average number of different types of cells per unit area (2.113 sq. mm) observed in the pituitary gland of *Rana limnocharis* during annual cycle.

<table>
<thead>
<tr>
<th>Month of Investigation</th>
<th>Acidophil or α cell</th>
<th>Basophil or β cell</th>
<th>Chromophobe cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>3.88</td>
<td>5.77</td>
<td>3.40</td>
</tr>
<tr>
<td>February</td>
<td>6.43</td>
<td>9.65</td>
<td>2.93</td>
</tr>
<tr>
<td>March</td>
<td>3.21</td>
<td>11.35</td>
<td>1.32</td>
</tr>
<tr>
<td>April</td>
<td>13.90</td>
<td>29.05</td>
<td>4.16</td>
</tr>
<tr>
<td>May</td>
<td>13.44</td>
<td>23.28</td>
<td>1.60</td>
</tr>
<tr>
<td>June</td>
<td>22.19</td>
<td>24.04</td>
<td>1.18</td>
</tr>
<tr>
<td>July</td>
<td>21.95</td>
<td>37.08</td>
<td>1.98</td>
</tr>
<tr>
<td>August</td>
<td>18.55</td>
<td>23.56</td>
<td>2.74</td>
</tr>
<tr>
<td>September</td>
<td>15.99</td>
<td>23.09</td>
<td>3.02</td>
</tr>
<tr>
<td>October</td>
<td>8.51</td>
<td>7.19</td>
<td>2.65</td>
</tr>
<tr>
<td>November</td>
<td>5.48</td>
<td>5.77</td>
<td>2.36</td>
</tr>
<tr>
<td>December</td>
<td>4.63</td>
<td>5.20</td>
<td>2.08</td>
</tr>
</tbody>
</table>
### TABLE II

Average cell size and nuclear diameter (mm) of different types of cells in the pituitary gland of *Rana limnocharis* during annual cycle.

<table>
<thead>
<tr>
<th>Month of investigation</th>
<th>Acidophil or α cell</th>
<th>Basophil or β cell</th>
<th>Chromophobe cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cell size</td>
<td>Nucleolar diameter</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Length Width</td>
<td>Length Width</td>
<td></td>
</tr>
<tr>
<td>Jan.</td>
<td>0.12 - 0.10</td>
<td>0.10 - 0.14</td>
<td>0.16 - 0.20</td>
</tr>
<tr>
<td></td>
<td>0.08 - 0.12</td>
<td>0.07</td>
<td>0.10 - 0.14</td>
</tr>
<tr>
<td>Feb.</td>
<td>0.12 - 0.10</td>
<td>0.16 - 0.13</td>
<td>0.22 - 0.16</td>
</tr>
<tr>
<td></td>
<td>0.10 - 0.12</td>
<td>0.16 - 0.18</td>
<td>0.20 - 0.28</td>
</tr>
<tr>
<td>Marc.</td>
<td>0.13 - 0.12</td>
<td>0.10 - 0.16</td>
<td>0.14 - 0.20</td>
</tr>
<tr>
<td></td>
<td>0.12 - 0.14</td>
<td>0.13 - 0.10</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td>Apr.</td>
<td>0.16 - 0.15</td>
<td>0.12 - 0.20</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td></td>
<td>0.16 - 0.20</td>
<td>0.10 - 0.12</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td>May</td>
<td>0.13 - 0.12</td>
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<td>0.10 - 0.16</td>
<td>0.20 - 0.26</td>
<td>0.12 - 0.16</td>
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<td>Jun.</td>
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<td>0.12 - 0.16</td>
</tr>
<tr>
<td></td>
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<td>0.18 - 0.20</td>
<td>0.12 - 0.16</td>
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<tr>
<td>Jul.</td>
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<td>0.12 - 0.16</td>
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<td>0.12 - 0.16</td>
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<td>Aug.</td>
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<td>0.16 - 0.18</td>
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<tr>
<td></td>
<td>0.12 - 0.20</td>
<td>0.10 - 0.08</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td>Sep.</td>
<td>0.16 - 0.13</td>
<td>0.14 - 0.18</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td></td>
<td>0.14 - 0.18</td>
<td>0.17 - 0.14</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td>Oct.</td>
<td>0.10 - 0.16</td>
<td>0.16 - 0.18</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td></td>
<td>0.12 - 0.16</td>
<td>0.16 - 0.22</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td>Nov.</td>
<td>0.22 - 0.08</td>
<td>0.10 - 0.16</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td></td>
<td>0.20 - 0.24</td>
<td>0.10 - 0.16</td>
<td>0.12 - 0.16</td>
</tr>
<tr>
<td>Dec.</td>
<td>0.17 - 0.12</td>
<td>0.12 - 0.20</td>
<td>0.15 - 0.16</td>
</tr>
<tr>
<td></td>
<td>0.10 - 0.18</td>
<td>0.14 - 0.13</td>
<td>0.12 - 0.20</td>
</tr>
</tbody>
</table>
Plate I A - A portion of the cross section of the pituitary gland of *Rana limnocharis* Wiegmann during pre-breeding period. (x 1000)

Plate I B - A portion of the cross section of the pituitary gland of *Rana limnocharis* Wiegmann during breeding period. (x 1000)

Plate I C - A portion of the cross section of the central region of the pituitary gland of *Rana limnocharis* Wiegmann during post-breeding period. (x 1000)

Plate I D - A portion of the cross section of the peripheral region of the pituitary gland of *Rana limnocharis* Wiegmann during post-breeding period. (x 1000)

Abbreviations:

Chr - Chromophobe cells  Sin - Sinusoids

α - Acidophil cells  β - Basophil cells
1. Pre-breeding Period (Late March).
2. Breeding Period (April, May, June, July and August).
3. Post-breeding Period (September and October).

1. Pre-breeding Period.

The pre-breeding period is the period starting from the time of emergence from hibernation to the time when amplexus and spawning begin. During the short duration of the pre-breeding period the distinction between central and peripheral regions as seen during post-breeding and early hibernation periods, was not observed. In comparison to the months of hibernation the cells were compact in arrangement; and few intercellular spaces were seen during pre-breeding period. There was distinct granulation in the cytoplasm of the acidophil as well as the basophil cells during this period (Plate IA). The description of the three types of cells observed during pre-breeding period is as follows:

(a) Acidophil cells: The average number of acidophil cells per unit area was 3.21. Their size was noted to be approximately 0.13 X 0.12 mm. Their nuclear diameter was measured to be approximately 0.08 mm.

(b) Basophil cells: The average number of basophil cells per unit area, approximate cell size and nuclear diameter were noted to be 11.35, 0.13 X 0.10 mm and 0.08 mm respectively.
(c) Chromophobe cells: The average number of chromophobe cells per unit area, approximate cell size and nuclear diameter were noted to be 1.32, 0.11 X 0.10 mm and 0.07 mm respectively.

2. Breeding Period.

During breeding period the central region was observed to be loosely packed with cells in comparison to the peripheral region. There was heavy granulation in the cytoplasm of the acidophil as well as the basophil cells, and their nuclei had highly dispersed chromatin indicating apparently the highly active phase of these cells. The chromophobe cells were few in number and smaller in size indicating that they were less active during this period (Plate IB). The details of the behaviour of the three types of cells observed during breeding period is as follows:

(a) Acidophil cells: Gradual increase was noted in the average number of acidophil cells per unit area during different months of the breeding period. It was recorded to be 13.9, 13.44, 22.19, 21.95 and 18.55 during April, May, June, July and August respectively. Increase in the cell size was noted in the months of April and August; in May and June, the size was less, the approximate cell size being 0.16 X 0.15 mm, 0.13 X 0.12 mm, 0.12 X 0.12 mm, 0.15 X 0.14 mm, 0.17 X 0.14 mm in April, May, June, July and August respectively. The nuclear diameter also increased.
being greatest in the month of June. It was measured to be approximately 0.08 mm, 0.07 mm, 0.09 mm, 0.08 mm and 0.08 mm in April, May, June, July and August respectively.

(b) Basophil cells: The number of the basophil cells during the breeding period was large in comparison to that observed during other periods of the annual cycle. During the breeding period the largest number of these cells was observed in July. From August onwards there was a decline in their number, the average number per unit area being 29.05, 23.28, 24.04, 37.08 and 23.56 in the months of April, May, June, July and August respectively. The cell size increased in the months of April, May, June and then showed a gradual increase during the rest of the breeding season. The approximate cell size was noted to be 0.23 x 0.13 mm, 0.18 x 0.13 mm, 0.24 x 0.13 mm, 0.22 x 0.13 mm, 0.17 x 0.16 mm in April, May, June, July and August respectively. The nuclear diameter was measured to be approximately 0.11 mm, 0.09 mm, 0.09 mm, 0.07 mm and 0.09 mm in the months of April, May, June, July and August respectively.

(c) Chromophobe cells: The number of these cells was much less in comparison to that observed during other months of the year. It started increasing from July and August. Their average number per unit area was observed as 4.16, 1.60, 1.48, 1.93 and 2.74 during April, May, June, July and August respectively. The cell size increased from April to June,
and decreased during July and August. It measured approximately 0.10 X 0.10 mm, 0.12 X 0.12 mm, 0.14 X 0.14 mm, 0.10 X 0.09 mm, 0.10 X 0.08 mm in April, May, June, July and August respectively. The nuclear diameter was found to be greatest in June, in comparison to the other months of the year. It measured approximately 0.08 mm, 0.08 mm, 0.11 mm, 0.07 mm and 0.08 mm in April, May, June, July and August respectively.

3. Post-breeding Period.

The distinction between the cells of central and peripheral region was more distinct during the post-breeding season. The cytoplasm was more dense in comparison to the earlier months of the year. The number and activity of the acidophil and basophil cells gradually decreased, whereas, that of chromophobe cells increased. (Plate I C and D).

(a) Acidophil cells: The average number of acidophil cells per unit area decreased to 15.99 in September and 8.51 in October. The size of cells also became smaller being 0.16 X 0.13 mm in September and 0.10 X 0.16 mm in October. The nuclear diameter remains approximately 0.08 mm during both the months.

(b) Basophil cells: The average number of basophil cells per unit area also decreased being 23.09 in September and 7.19 in October. The size of cells decreases in September and October, being approximately 0.17 X 0.14 mm in September
and 0.18 x 0.10 mm in October. The nuclear diameter remains approximately 0.08 mm for September and October.

(c) Chromophobe cells: The average number of cells per unit area in comparison to that of the breeding season was observed to be more, being 3.02 in September and 2.65 in October. From this period onwards gradual increase in the number of these cells was observed reaching maximum during hibernation period. The cell size also showed similar behaviour increasing approximately to 0.09 x 0.09 mm in September and 0.10 x 0.10 in October. The nuclear diameter remained similar during both the months, being 0.08 mm in September as well as in October.

4. Hibernation Period.

In the early phase of the hibernation period the anterior pituitary showed a clear distinction between its peripheral and central regions. The cells in the peripheral region were more compactly arranged in comparison to those in the central region. The cell boundaries were not very distinct and few sections of the blood vessels were observed in between them. During later phase of the hibernation period, the sections of the anterior pituitary showed a compact cellular arrangement and distinction between its central and peripheral region was not so clearly observed. Intercellular spaces in between the cells were observed. Blood vessels were large and chromophobe cells appeared to
Plate II E - A portion of the cross section of the pituitary gland of Rana limno-charis Wiegmann during early hibernation period. (x 1000)

Plate II F - A portion of the cross section of the pituitary gland of Rana limno-charis Wiegmann during late hibernation period. (x 1000)

Abbreviations:

Chr - Chromophobe cells
Sin - Sinusoids
α - Acidophil cells
β - Basophil cells
be more active than acidophil and basophil cells. The details of the behaviour of the three types of cells during hibernation period are given below. (Plate II E and F).

(a) **Acidophil cells**: The number of acidophil started decreasing in the early months of hibernation. In the last phase of the period of hibernation their number gradually rises. In comparison to other months, the number of acidophil cells during the whole period of hibernation remained less. Their number decreased from November to January. From February their number again increased. The average number of acidophiles per unit area was 5.48, 4.63, 3.88 and 6.43 during November, December, January and February respectively. The acidophil cells were also larger during hibernation period. From November onwards the cell size increased being approximately 0.22 X 0.08 mm, 0.17 X 0.12 mm, 0.12 X 0.10 mm and 0.12 X 0.10 mm in the months of November, December, January and February respectively. The nuclear diameter of the acidophil cells during hibernation period was measured to be approximately 0.06 mm, 0.08 mm, 0.07 mm and 0.09 mm during November, December, January and February respectively.

(b) **Basophil cells**: The basophil cells like the acidophil cells were less in number during the period of hibernation as compared to other months of the annual cycle. A decrease in their number was observed from November to February, the average number per unit area being 5.77, 5.20, 5.77 and 9.65
during the months of November, December, January and February respectively. The cell size was also smaller during the period of hibernation in comparison to other months of the year. However, in comparison to the acidophil cell, the cell size of the basophil cell was larger. The size of basophil cells started decreasing from November, being approximately 0.22 X 0.10 mm, 0.14 X 0.13 mm, 0.18 X 0.11 mm, 0.16 X 0.13 mm in the months of November, December, January and February respectively. There was hardly any fluctuation in the nuclear diameter. It was measured to be approximately 0.08 mm, 0.08 mm, 0.08 mm and 0.09 mm in the months of November, December, January and February respectively.

(c) Chromophobe cells: The average number of chromophobe cells per unit area was more than the number of acidophil and basophil cells during the period of hibernation in comparison to the other months of the year. The number gradually increased from November and reached maximum in the month of January and then a decline in their number was noted, being 2.36, 2.08, 3.40 and 2.93 in the months of November, December, January and February respectively. The cell size was also maximum during the months of hibernation. The size increased from November onwards, reached maximum in January being approximately 0.12 X 0.10 mm, 0.15 X 0.16 mm, 0.22 X 0.16 mm, 0.16 X 0.14 mm during the months of November, December, January and February respectively. The nuclear
diameter showed fluctuation to some extent being more during the months of January and February. It was measured approximately to be 0.08 mm, 0.06 mm, 0.10 mm and 0.10 mm from November to February.

The above observations reveals that the average number and cellular size of the acidophil and basophil cells was maximum during breeding period and minimum during hibernation period, whereas, the average number and cellular size for the chromophobe cells was maximum during hibernation period and minimum during the breeding period.

THE TESTIS

(A) MORPHOLOGY OF THE TESTIS. (Plate III C)

The testis of Rana limnocharis are paired, whitish ovoid bodies, lying ventral to and near the anterior end of each kidney. They are suspended to the dorsally placed kidney by a double fold of peritoneum known as mesorchium. The mesentry surrounds each testis and is continuous with the peritoneal epithelium which covers the ventral face of each kidney and lines the entire body cavity. The spermatozoa are produced in the seminiferous tubules, which in a section of the testis are observed as closely packed, oval-shaped sacs, separated from each other by the partitions of supporting tissue, known as interstitial tissue. The interstitial tissue is continuous with the covering of the testis known as tunica albuginea.
Plate III G - Dissection of male *Rana limnocharis* Wiegmann showing testis.

Plate III H - Dissection of female *Rana limnocharis* Wiegmann showing ovary and oviduct.

Abbreviations:

- Fb - Fat body
- K - Kidney
- E - Egg
- T - Testis
- L - Lung
- Od - Oviduct
HISTOLOGICAL STRUCTURE.

The detailed histological structure of the seminiferous tubules of *Rana limnocharis* is as follows:

1. **Seminiferous tubule**: The wall of the convoluted seminiferous tubule consists of (i) an outer capsule or tunica propria of fibro elastic connective tissue and flattened fibroblasts, which closely invests the tubule (ii) a basement membrane and (iii) a lining of complex stratified epithelium which consists of two kinds of cells: (a) the sertoli cells and (b) the spermatogenic cells.

(a) **Sertoli cells**: These are tall, irregularly arranged columnar cells, that extend from the basal lamina to the lumen. Their sides are marked by uneven showing pits and depressions, into which fit the adjoining cells. The location of the nucleus varies in different sertoli cells, from the basal position to position located at a considerable distance from the basal lamina. The nucleus is ovoid and pale staining with finely dispersed chromatin, and it usually contains one or more nucleoli.

(b) **Spermatogenic cells**: They lie in between the sertoli cells in an orderly manner, with 4 to 8 layers occupying the space between the basal lamina and the lumen, representing all stages of differentiation viz., primitive germ cell or spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and spermatozoa, according to the sexual maturity.
**Primitive germ cells or spermatogonia**: These cells are located directly inside the basal lamina and give rise to spermatozoa ultimately. They are spherical or cuboidal in shape. Nucleus is spherical with granular chromatin. They divide so as to maintain their own number and to give rise to the cells that differentiate into spermatocytes.

**Primary spermatocyte**: These cells are formed from the innermost layer of the spermatogonia. They are larger cells, larger than the spermatogonia and have large vesicular nuclei showing variable appearance of chromatin depending upon the functional state of the cell. It may be in the form of either elongated spiremes or condensed chromosomes, preparatory to cell division.

**Secondary spermatocytes**: These cell arise from primary spermatocytes, each primary spermatocyte giving rise to 2 secondary spermatocytes. They are smaller than the primary spermatocytes and lie internal to them.

**Spermatids**: These cells are derived by the division of the secondary spermatocytes and are located adjoining the lumen of the tubule. They are easily recognized by their small size and location. These cells form the last generation in the spermatogenic process. They undergo no further division, but by profound changes in their structure they become transformed into mature spermatozoa. Groups of maturing spermatids can be seen in close association with the sertoli cells.
Spermatozoa: The spermatozoa consist of a distinct head and an elongated tail. These are slender, motile and flagellate bodies. Nearly mature spermatozoa are frequently observed with their heads in close association with the cytoplasmic processes of the sertoli cells and their tails extending out into the lumen of the tubule. Mature spermatozoa are seen in bundles at the centre of the lumen.

(B) CHANGES IN THE TESTIS DURING ANNUAL CYCLE.

Histological sections of the testes prepared regularly at different periods of the annual cycle were studied. The data on the measurements of various histological structure (mean of 5 observations in each case) has been given in Tables III and IV. Following major changes were observed in the testes through different periods of the annual cycle.

1. Pre-breeding Period.

Immediately after the period of hibernation, the frogs enter a short pre-breeding period in the month of March. During this period the average number of sections of seminiferous tubules under low power of the microscopic field was 9.40. The details of different structural elements of the seminiferous tubules studied under high power of microscopic field were as follows. (Fig. 6; Plate IV: J, & K)

(a) Sertoli cells: The average number of sertoli cells were 2.55 per unit area. The approximate cell and nuclear diameter of these cells was 0.67 mm and 0.07 mm respectively.
Fig. 6 - A portion of the cross-section of the testis during pre-breeding period.

Fig. 7 - A portion of the cross-section of the testis during breeding period.

Abbreviations:
Ser = Sertoli cell
Sptg = Spermatogonia
P.Sptc = Primary spermatocyte
S.Sptc = Secondary spermatocyte
Sptd = Spermatid
Sptz = Spermatozoon
Plate IV I - A portion of the cross section of the testis of *Rana limnocharis* Wiegmann during late hibernation period. (x 450)

Plate IV J - A portion of the cross section of the testis of *Rana limnocharis* Wiegmann during early pre-breeding period. (x 450)

Plate IV K - A portion of the cross section of the testis of *Rana limnocharis* Wiegmann during late pre-breeding period. (x 450)

Plate IV L - A portion of the cross section of the testis of *Rana limnocharis* Wiegmann during breeding period. (x 450)

Abbreviations:

- Sertoli cell
- Spermatogonia
- Primary spermatocyte
- Secondary spermatocyte
- Spermatid
- Spermatozoa
Average number of Seminiferous tubules per unit area (7.06 sq. mm) and the average number of different structural elements of the Seminiferous tubules per unit area (2.113 sq. mm) observed in the testes of *Rana limnocharis* during annual cycle.

<table>
<thead>
<tr>
<th>Month of investigation</th>
<th>Seminiferous tubules</th>
<th>Sertoli cell</th>
<th>Spermatogonial cell</th>
<th>Primary spermatocyte</th>
<th>Secondary spermatocyte</th>
<th>Spermaticids</th>
<th>Spermatzoa</th>
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<td>7.36</td>
<td>2.27</td>
<td>10.69</td>
<td>23.94</td>
<td>15.52</td>
<td>6.62</td>
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<td>February</td>
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<td>2.36</td>
<td>7.85</td>
<td>14.86</td>
<td>16.75</td>
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<td>9.40</td>
<td>2.55</td>
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<td>13.25</td>
<td>17.79</td>
<td>8.61</td>
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<td>11.45</td>
<td>17.88</td>
<td>11.54</td>
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<td>1.79</td>
<td>8.32</td>
<td>9.74</td>
<td>14.86</td>
<td>20.44</td>
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<td>June</td>
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<td>1.41</td>
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<td>9.46</td>
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<td>22.40</td>
<td>453.6</td>
</tr>
<tr>
<td>July</td>
<td>4.84</td>
<td>1.32</td>
<td>3.40</td>
<td>9.65</td>
<td>6.90</td>
<td>18.93</td>
<td>481.4</td>
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<td>August</td>
<td>4.27</td>
<td>1.32</td>
<td>9.46</td>
<td>9.74</td>
<td>11.73</td>
<td>16.63</td>
<td>424.5</td>
</tr>
<tr>
<td>September</td>
<td>4.53</td>
<td>1.04</td>
<td>19.87</td>
<td>14.43</td>
<td>12.77</td>
<td>7.09</td>
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</tr>
<tr>
<td>October</td>
<td>4.58</td>
<td>1.32</td>
<td>19.97</td>
<td>14.67</td>
<td>13.06</td>
<td>6.53</td>
<td>298</td>
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<tr>
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<td>16.37</td>
<td>13.62</td>
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<tr>
<td>December</td>
<td>11.81</td>
<td>1.98</td>
<td>13.72</td>
<td>17.41</td>
<td>14.67</td>
<td>6.53</td>
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</table>
Average cell and nuclear diameter (mm) of the structural elements of the seminiferous tubules of *Buna limnochoris* during annual cycle.

<table>
<thead>
<tr>
<th>Month of investigation</th>
<th>Sertoli cell</th>
<th>Spermatogonial cell</th>
<th>Primary spermatocyte</th>
<th>Secondary spermatocyte</th>
<th>Spermatids</th>
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<tbody>
<tr>
<td></td>
<td>Cell diameter</td>
<td>Nuclear diameter</td>
<td>Cell diameter</td>
<td>Nuclear diameter</td>
<td>Cell diameter</td>
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<tr>
<td>January</td>
<td>0.68</td>
<td>0.10</td>
<td>1.03</td>
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<td>February</td>
<td>0.80</td>
<td>0.26</td>
<td>0.64</td>
<td>0.10</td>
<td>0.90</td>
</tr>
<tr>
<td>March</td>
<td>0.67</td>
<td>0.07</td>
<td>0.53</td>
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<tr>
<td>April</td>
<td>0.64</td>
<td>0.07</td>
<td>0.51</td>
<td>0.07</td>
<td>0.60</td>
</tr>
<tr>
<td>May</td>
<td>0.62</td>
<td>0.05</td>
<td>0.95</td>
<td>0.08</td>
<td>0.58</td>
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<tr>
<td>June</td>
<td>0.57</td>
<td>0.06</td>
<td>0.56</td>
<td>0.07</td>
<td>0.48</td>
</tr>
<tr>
<td>July</td>
<td>0.50</td>
<td>0.05</td>
<td>0.54</td>
<td>0.08</td>
<td>0.62</td>
</tr>
<tr>
<td>August</td>
<td>0.48</td>
<td>0.07</td>
<td>0.51</td>
<td>0.10</td>
<td>0.79</td>
</tr>
<tr>
<td>September</td>
<td>0.50</td>
<td>0.07</td>
<td>0.92</td>
<td>0.10</td>
<td>0.80</td>
</tr>
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<td>October</td>
<td>0.53</td>
<td>0.07</td>
<td>0.96</td>
<td>0.12</td>
<td>0.82</td>
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<td>November</td>
<td>0.60</td>
<td>0.07</td>
<td>1.46</td>
<td>0.13</td>
<td>0.84</td>
</tr>
<tr>
<td>December</td>
<td>0.66</td>
<td>0.07</td>
<td>1.04</td>
<td>0.11</td>
<td>0.87</td>
</tr>
</tbody>
</table>
(b) Spermatogonial cells: The average number of spermatogonial cells was 6.62 per unit area and the cell and nuclear diameter of these cells was noted to be approximately 0.53 mm and 0.08 mm respectively.

(b) Primary spermatocytes: The average number of primary spermatocytes per unit area was 13.25 and their cell and nuclear diameter was approximately 0.79 mm and 0.08 mm respectively.

(d) Secondary spermatocytes: The average number was 17.70 per unit area and their cell and nuclear diameter was approximately 0.90 mm and 0.07 mm respectively.

(e) Spermatids: The average number per unit area was 8.61 and their cell and nuclear diameter was approximately 0.24 mm and 0.05 mm respectively.

(f) Spermatozoa: The average number of spermatozoa per unit area was 296.6.

2. Breeding Period.

The active breeding period was observed during April, May, June, July and August. The average number of sections of the seminiferous tubules per unit area was 6.11 in April, 5.69 in May, 5.04 in June, 4.84 in July and 4.27 in August. Various cellular types of each tubule showed following changes. (Fig. 7; Plate IV-L; V-M, M, O & P).
Plate V M,N,O and P - Portions of the cross section of the testis of *Rana limnocharis* Wiegmam during different months of the breeding period. (x 450)

Abbreviations:

- Ser - Sertoli cell
- Sptg - Spermatogonia
- P.Sptc - Primary spermatocyte
- S.Sptc - Secondary spermatocyte
- Sptd - Spermatid
- Sptz - Spermatozoa
(a) **Sertoli cells**: The average number of sertoli cells per unit area was observed to be 1.41, 1.79, 1.41, 1.32 and 1.32 in the months of May, June, July and August respectively. The diameter of these cells during these months was approximately 0.64 mm, 0.62 mm, 0.57 mm, 0.50 mm and 0.48 mm; and their nuclear diameter was approximately 0.07 mm, 0.05 mm, 0.06 mm, 0.05 mm and 0.07 mm respectively.

(b) **Spermatogonial cells**: The average number of spermatogonial cells per unit area was 8.80, 8.32, 3.69, 3.40 and 9.46; their diameter was approximately 0.51 mm, 0.95 mm, 0.56 mm, 0.54 mm and 0.51 mm; and their nuclear diameter was approximately 0.07 mm, 0.08 mm, 0.07 mm, 0.08 mm and 0.10 mm during April, May, June, July and August respectively.

(c) **Primary spermatocytes**: The average number of primary spermatocytes per unit area was 11.45, 9.74, 9.46, 9.65, 9.74; their diameter was approximately 0.60 mm, 0.58 mm, 0.48 mm, 0.62 mm and 0.79 mm; and their nuclear diameter was approximately noted to be 0.08 mm, 0.07 mm, 0.07 mm, 0.08 mm and 0.08 mm during April, May, June, July and August respectively.

(d) **Secondary spermatocytes**: The average number of secondary spermatocyte per unit area was 17.88, 14.86, 5.48, 6.90 and 11.73; their diameter was approximately 0.69 mm, 0.58 mm, 0.25 mm, 0.33 mm, 0.40 mm during April, May, June, July and August respectively; and their nuclear diameter was approx-
imatively 0.06 mm throughout the breeding period, except for the month of June when it measured to be approximately 0.04 mm.

(e) **Spermatids**: The average number of spermatids per unit area was 11.54, 20.44, 22.40, 16.93 and 16.63; their diameter was approximately 0.12 mm, 0.11 mm, 0.08 mm, 0.09 mm, 0.08 mm and their nuclear diameter was approximately 0.04 mm, 0.04 mm, 0.04 mm, 0.04 mm and 0.03 mm for April, May, June, July and August respectively.

(f) **Spermatozoa**: The average number of spermatozoa per unit area was observed to be 308.4, 445.4, 453.6, 481.4 and 424.5 during April, May, June, July and August respectively.

3. **Post-breeding Period**.

During post-breeding period, the average number of the sections of seminiferous tubules per unit area was 4.53 and 4.59 in the months of September and October respectively. The following changes in the histological structures of each tubule were observed during this period. (Fig. 8; Plate VI Q).

(a) **Sertoli cells**: The average number of sertoli cells was 1.04 and 1.32 per unit area during September and October, their diameter being approximately 0.50 mm and 0.53 mm respectively. Their nuclear diameter remained approximately 0.07 mm for both the months.
Fig. 8 - A portion of the cross-section of the testis during post-breeding period.

Fig. 9 - A portion of the cross-section of the testis during hibernation period.

Abbreviations:
Ser - Sertoli cell
Sptg - Spermatogonia
P.Sptc - Primary spermatocyte
S.Sptc - Secondary spermatocyte
Sptd - Spermatid
Sptz - Spermatozoa
(b) **Spermatogonial cells**: The average number of spermatogonial cells per unit area was 19.87, 19.97; their diameter approximately was 0.92 mm and 0.96 mm, and their nuclear diameter approximately was 0.10 mm and 0.12 mm during the months of September and October respectively.

(c) **Primary spermatocytes**: The average number of primary spermatocytes per unit area was 14.43 and 14.67; their diameter was approximately 0.80 mm and 0.82 mm and their nuclear diameter was approximately 0.08 mm and 0.07 mm during September and October respectively.

(d) **Secondary spermatocytes**: The average number of secondary spermatocytes per unit area was 12.77 and 13.06; their diameter was approximately 0.40 mm and 0.44 mm and their nuclear diameter was approximately 0.065 mm and 0.06 mm during September and October.

(e) **Spermatids**: The average number of spermatids per unit area was 7.09 and 6.53; their diameter was approximately 0.06 mm and 0.07 mm and their nuclear diameter was approximately 0.04 mm and 0.05 mm for September and October respectively.

(f) **Spermatozoa**: The average number of spermatozoa per unit area was 302 and 298 for the months of September and October respectively.
Plate VI Q - A portion of the cross section of the testis of *Rana limnocharis* Wiegmann during post-breeding period. (x 450)

Plate VI R - A portion of the cross section of the testis of *Rana limnocharis* Wiegmann during different months of the hibernation period. (x 450)

Abbreviations:

- Ser - Sertoli cell
- Sptg - Spermatogonia
- P.Sptc - Primary spermatocyte
- S.Sptc - Secondary spermatocyte
- Sptd - Spermatid
- Sptz - Spermatozoon
4. Hibernation Period.

During this period the average number of sections of seminiferous tubules per unit area was 8.98, 11.81, 7.36, 7.56 in the months of November, December, January and February respectively. The following changes were observed in the structures of the tubules. (Fig. 9; Plate UV-I., VI R and S).

(a) Sertoli cells: The average number of sertoli cells per unit area was 1.89, 1.98, 2.27, and 2.36; the diameter of the cells was approximately 0.60 mm, 0.66 mm, 0.68 mm and 0.80 mm and their nuclear diameter was approximately 0.07 mm, 0.07 mm, 0.10 mm and 0.26 mm during November, December, January and February respectively.

(b) Spermatogonial cells: The average number of spermatogonial cells per unit area was 14.19, 13.72, 10.69 and 7.85, their diameter was approximately 1.46 mm, 1.04 mm, 1.03 mm and 0.64 mm and their nuclear diameter was approximately 0.13 mm, 0.11 mm, 0.12 mm and 0.10 mm during November, December, January and February respectively.

(c) Primary spermatocytes: The average number of primary spermatocytes per unit area was 16.37, 17.41, 23.94 and 14.86; their approximate diameter was 0.84 mm, 0.87 mm, 0.93 mm and 0.90 mm and their nuclear diameter was approximately 0.09 mm, 0.10 mm, 0.09 mm and 0.10 mm respectively.
(d) **Secondary spermatocytes** : The average number per unit area for these cells was 13.62, 14.67, 15.52, 16.75, their cellular diameter was approximately 0.54 mm, 0.50 mm, 0.54 mm and 0.60 mm and their nuclear diameter was approximately 0.06 mm, 0.06 mm, 0.07 mm and 0.07 mm during November, December, January and February respectively.

(e) **Spermatozoa** : The average number of spermatozoa per unit area was 4.63, 6.53, 6.62 and 8.51; their diameter was approximately 0.06 mm, 0.08 mm, 0.10 mm and 0.12 mm and their nuclear diameter was approximately 0.04 mm during November, December, January and February.

(f) **Spermatozoa** : The number of spermatozoa per unit area was observed to be 75, 99, 129 and 203 during November, December, January and February respectively.

The above observations reveal that the number of cross sections of the seminiferous tubules per unit area was maximum during the hibernating period and minimum during the breeding period; their size being largest during breeding period and smallest during hibernation period. The number of sertoli cells was maximum during early breeding period and minimum during post-breeding period; their cellular size being maximum in late hibernating period and minimum during late breeding period. The number of spermatogonial cells was greatest during post-breeding period and lowest during
late breeding period; their cellular size being maximum during early hibernation period and minimum in breeding period. The number of primary spermatocytes was maximum during hibernating period and lowest in the active breeding period; their size was also maximum during hibernation and lowest in the breeding period. The number of secondary spermatocyte was maximum and their size largest during pre-breeding and early breeding period; while during late breeding period, their number as well as the size both were minimum. The number of spermatids was maximum during breeding period and minimum in the post and early hibernating months; their cellular size being largest during and early breeding period. The number of spermatozoa was observed to be largest during breeding period and smallest in the early hibernating period.

THE OVARY

(A) MORPHOLOGY OF THE OVARY. (Plate III R)

The ovaries of the frog are paired, multi-lobed organs, attached to the dorsal body wall by a double layered extension of the peritoneum, known as mesorchium. This peritoneum continues around the entire ovary as the theca externa. Each lobe of the ovary is hollow and its cavity is continuous with the other lobes. The size of the ovary varies with the seasons. During early breeding period, the paired ovaries fill the whole of the body cavity and distend the abdomen. The mature eggs are highly pigmented on
the surface of the animal pole, so that the ovary has a speckled appearance of black pigment and white yolk, representing the animal and vegetal poles of the mature egg.

HISTOLOGICAL STRUCTURE. (Fig 10 A, B & C)

As seen in a section, the ovary is surrounded by peritoneal covering the theca externa, from which arise internally a number of individual sacs, each made up of another membrane known as the theca interna or cystwall, in which smooth muscle fibres can be observed. The theca interna surrounds each egg except for the limited area bulging towards the body cavity, where it is covered by only the theca externa. This region ruptures during ovulation to allow the egg to escape from its follicle into the body cavity. The theca externa and the follicle cells together comprises the ovarian follicle. This has the supply of both blood vessels as well as nerves. Within each follicle are found follicle cells, with their oval and granular nuclei, derived originally from oogonia. These follicle cells surround the developing oocyte and are found in close association with it throughout the process of maturation which occurs within the follicle. Enclosed within each follicle cells, and closely applied to each mature egg, is the non-cellular transparent vitelline membrane, probably derived from both the ovum and follicle cells. This membrane is developed and applied to the egg.
Diagrammatic representation of:

**Fig. 10 A** - Cross section of the growing oocyte of *Rana limnocharis* Wiegmann.

**Fig. 10 B** - Cross section of the primary oocyte with pigment of *Rana limnocharis* Wiegmann.

**Fig. 10 C** - Cross section of the secondary oocyte of *Rana limnocharis* Wiegmann.

Abbreviations:

- **Te** - Theca externa
- **Tl** - Theca interna
- **Flc** - Follicle cell
- **Vp** - Vegetal pole
- **Blv** - Blood vessel
- **Pfr** - Point of follicular rupture
- **Ncl** - Nucleus
- **Nole** - Nucleolus
- **Ap** - Animal pole
- **Pgm** - Pigment
- **Ykg** - Yolk globules
during the maturation process so that it is not seen around the younger oogonia. As the oocyte enlarges and matures, the follicle cells and membranes are so stretched and flattened that they are not easily distinguished.

The mature egg, as seen in the sections of the ovary is a large sac of yolk, the heavier and larger granules of which are concentrated at the vegetal pole. It has a thin outer layer of cytoplasm, which is more concentrated towards the animal hemisphere and in the region surrounding the egg entire is covered by a pigmented coat which has been described in text books as non-living coat.

(B) CHANGES IN THE OVARY DURING ANNUAL CYCLE.

During early breeding season in April, the ovary was observed full of mostly mature ova. As soon as the spawning started, different developing stages of the ovum were observed. Arising from the germinal epithelium large number of oogonia with or without pigment and devoid of any yolk were observed in the sections of the ovary. Each oogonium was observed to be surrounded with a number of follicle cells. The growing oogonia were distinguished by the accumulation of yolk and the displacement of nucleus to one side, towards the animal pole. The chromatin material becomes achromatic and large number of nucleoli are seen in the nucleus. Nucleus become germinal vesicle with wavy outline. Varying sizes of primary oocyte were seen according
to the amount of yolk accumulated. Secondary oocyte were distinguished as fully grown oocyte with normal nucleus. Maturation of secondary oocyte was not studied but as described by other workers such as Rugh (1951) occurs just at the time of ovulation. Gross changes in the ovary during different periods of annual cycle were observed as described below. (Table V; Plate VII T, U, V, W and Plats X, y, & Z)

1. Pre-breeding Period.
   The ovaries were observed to contain large number of fully grown oocyte filling the whole of the body cavity.

2. Breeding Period.
   During early breeding period the ovaries were observed still occupying the whole of the body cavity. As spawning occurred, the size of the ovary was seen to have become smaller; and by the end of the breeding period in August, the ovaries were much reduced in size. Females were spent and histological sections of the ovaries showed very few well developed oocytes. Large number of developing oogonia were observed in the sections of the ovary during April when the spawning follows and in the active breeding period such as June.

3. Post-breeding Period.
   During post-breeding period the sections of the ovaries showed a very large number of primary oocytes in different stages of growth. Mature oocytes were rarely seen.
Plate VII T - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing growing oocyte. (x 60)

Plate VII U - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing growing oocyte and pigment cell. (x 60)

Plate VII V - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing primary oocyte. (x 60)

Plate VII W - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing secondary oocyte. (x 60)

Abbreviations:

- **Te** - Theca externa
- **Ncl** - Nucleus
- **Ti** - Theca interna
- **Pgm** - Pigment cell
- **Gdo** - Growing oocyte
- **Nclo** - Nucleolus
- **Po** - Primary oocyte
- **Flc** - Follicle cell
- **So** - Secondary oocyte
- **Ap** - Animal pole
- **Ykg** - Yolk globules
- **Vp** - Vegetal pole
Plate VIII X - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing growing oocyte and peripheral region of mature oocyte. (x 100)

Plate VIII Y - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing pigment cell and primary oocyte. (x 100)

Plate VIII Z - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing secondary oocyte. (x 100)

Abbreviations:

Go = Growing oocyte  Ncl = Nucleus
Po = Primary oocyte  Nclo = Nucleolus
So = Secondary oocyte  Flc = Follicle cell
Ti = Theca interna  Pgm = Pigment cell
### TABLE V

Size of Oocytes in the ovary of *Rana limnocharis* Wiegmann during different periods of annual cycle.

<table>
<thead>
<tr>
<th>Period</th>
<th>Month</th>
<th>Size range (Diameter) of Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hibernation</td>
<td>February</td>
<td>0.08 - 0.43 mm</td>
</tr>
<tr>
<td>Pre-breeding</td>
<td>March</td>
<td>0.08 - 0.93 mm</td>
</tr>
<tr>
<td>Breeding</td>
<td>April</td>
<td>0.15 - 0.90 mm</td>
</tr>
<tr>
<td>Breeding</td>
<td>May</td>
<td>0.10 - 0.93 mm</td>
</tr>
<tr>
<td>Post-breeding</td>
<td>October</td>
<td>0.03 - 0.05 mm</td>
</tr>
</tbody>
</table>
4. **Hibernation Period.**

During early hibernation period large number of well grown primary oocytes were observed. Dissection of females revealed gradual increase in size of the ovaries during November, December, January and February. Study of the sections of the ovary at the end of the hibernation period revealed the presence of large number of mature oocytes and several oocytes in different maturing stages.

**DISCUSSION**

The pituitary gland plays a key role in the life processes of the vertebrates. The different cell types of anterior pituitary (pars distalis) secrete various hormones which play an important role in the growth, regulation and maintenance of the vertebrate body. Although the pituitary gland is a most important endocrine gland, there are still controversies regarding the nomenclature and functioning of the different cell types in the pituitary gland. Since 1892, when Schöenemann for the first time described two chromophil cell types, eosinophiles and cyanophiles, several workers tried to distinguish the different cell types by the use of various staining techniques. Not only their nomenclature but also the hormones they secrete is still somewhat controversial especially in Amphibia. In two recent exhaustive reviews, one by Purves (1966) on "The cytology of
the adenohypophysis" and another by Hanke (1976) on "Frog
Neuroendocrinology", only three major cell types - the
acidophiles, basophils and chromophobes have been described
as commonly accepted by many workers. And now sub-types of
the acidophils and basophils (Kerr, 1965; Mira-Moser, 1970;
Van Kemende, 1974; Pehlemann, 1974) have also been described.
In the present investigation, behaviour of only three major
types of cells has been studied during different periods of
the annual cycle of Rana limnocharis.

The histological study of the testis and ovary of
Rana limnocharis shows distinct phases of spermatogenesis
and oogenesis in relation to the different periods of the
annual cycle. The spermatogenic activity was maximum during
breeding period from April to August when largest number of
spermatozoa ranging from About 308.4 to 424.5 per unit
area were observed in each section of the seminiferous
tubule in contrast to hibernation period from October to
January when their number in each section ranged from 298
to 129 per unit area. It was observed to increase from
February (203 per unit area) which is the last month of
hibernation. The ovary also showed changes having largest
number of mature ova during early breeding season. Their
number decreased during active breeding period obviously
due to spawning. During late breeding and post-breeding
period, active oogenesis was observed. The growth of these
oocytes was observed during the hibernation period, so that
the ovary was full of mature ova in February during the last phase of the hibernation period. Detailed histological changes in the ovary could not be studied as it was difficult to get the frogs regularly during this period. The role of pars distalis of the pituitary of *Rana limnocharis* in controlling these different phases of gametogenesis and breeding activity are clearly correlated with the activity of the three cell types (acidophil cells, basophil cells and chromophobes) found in it.

The acidophil (α) cells characterized by their spherical to ellipsoidal shape, centric nucleus and acidophilic cytoplasm were observed to be maximum in number (22.19 per unit area) during the egg laying period in June. After the breeding or egg laying period is over, it gradually decreased and became minimum (3.88 per unit area) during the hibernating period in January. Immediately after the period of hibernation, the number started increasing again at the pre-breeding period reaching maximum during the breeding season. The size of the acidophil cells was also maximum (0.16 - 0.15 mm) during the breeding period and minimum at hibernation (0.12 - 0.10 mm). These findings conform those of Zysk (1975), who also describes increase in the number of acidophil cells in breeding period and decrease after the egg laying period up to hibernation. The present findings, however, do not corroborate with the observations of Lakshman (1965) and Rastogi and Chieffi (1970),
who reported smallest number of these cells during the period of egg laying in amphibians. The increase in the number of acidophil cells during late breeding season of *Rana limnocharis* correlates with its active life and active feeding activity. Juszczyk et al (1966) and Krawczyk (1970) have reported increased weight of intestinal mucosa and high lipid content in the walls of gastrointestinal tract of frogs during such active periods. High level of STH (Somatotrophic hormone) required for synthesis during active gametogenesis. This appears to be a reason why acidophil cells were observed to be in the active phase in *Rana limnocharis* during later half of the breeding period.

The basophilic (\(\beta\)) cells, identified by their spherical or elongated shape, eccentric nucleus and basophilic cytoplasm, show an increase in their number and high secretory granules in their cytoplasm during the most active phase of the gonads. The number of basophilic cells started as soon as they come out of hibernation in March and became maximum (37.08 per unit area) in July during breeding period. Soon after the breeding period, when egg laying and active phase of spermatogenesis was over, the number started decreasing both in males and females becoming minimum (5.20 per unit area) in the hibernation period. Along with the increase in number, the size of the cells was also observed to be largest during the breeding period, reaching the maximum (0.24 - 0.13 mm) in June and again becoming minimum
(0.14 - 0.13 mm) in December. These observations support Zuber-Vogeli (1953), Öröst (1960, 1961) and Zysk (1975) who reported that the basophilic cells are actively involved in the production of gonadotrophins, such as FSH (Follicle-stimulating hormone) and LH (Luteinizing hormone) controlling the gonadal function as well as the development of secondary sex characters. Increase in the activity of the basophilic cells during breeding season show high level of gonadotrophins. This correlates with the breeding activity including the release of eggs in females and spermatozoa in males. The basophilic delta (Δ) cells are supposed to be the source of TSH (Thyreotropic hormone) and ACTH (Adrenocorticotrophic hormone). As the present investigation was not aimed to find out the relationships of pituitary cells with thyroid activity, the investigation on the basophilic delta (Δ) cells was not carried out.

The third type of cells, the chromophobic cells, characterized by a centric nucleus, poorly staining cytoplasm having no definite shape, showed greatest reduction in number (1.98 per unit area), when the number of the basophilic cell was maximum in the breeding period. The size of these cells was observed to be maximum during hibernation (0.22 - 0.16 mm) and minimum during the breeding period (0.10 - 0.08). Similar observations have been made by Zysk (1975). Ackermann, Nowicki and Sarneka-Kellar (1971) suggested these cells to be precursors giving rise to other
cell types. These workers have also reported that their secretion stimulates the thyroid and they also produce ACTH. Rastogi and Chieffi (1970) felt that the chromophobe are degranulated basophil cells, which produce gonadotropins. The function of chromophobe is thus, still not clear as also reported by Zysk (1975).

On final analysis, it was seen that the three types of cells, 2 chromophil types (acidophils and basophils) and chromophobes in the pars distalis of *Rana limnocharis* showing distinct phases of activity throughout its annual cycle. The acidophils cells did not show much fluctuation in comparison to the number of basophil cells. The number of acidophil cells increased from pre-breeding to breeding season and started decreasing by the time the frogs started undergoing hibernation. The number of the basophil cells was always found to be more than acidophil cells except during post-breeding period. They were almost double in number than the acidophil cells during pre-breeding period; and about $\frac{1}{2}$ times more than acidophil cells during breeding season. The cyclic behaviour of the acidophils, basophils and chromophobes correlates well with the Pre-breeding, Breeding, Post-breeding and Hibernation periods of the annual cycle of the animal.
SUMMARY

The changes in the three main types of cells of the pars distalis of the pituitary, viz., acidophils, basophils and chromophobes and the corresponding histomorphological changes in the testis and ovary of Rana limnocharis Wiegmann during different periods of the annual cycle have been investigated. The number and size of acidophil cells increased during pre-breeding and breeding periods and decreased when the frogs entered hibernation. The basophil cells were always more in number than the acidophil cells except during the post-breeding period. Their size was also larger during the pre-breeding period. The size of the basophil cells was larger and their number was almost double than that of the acidophil cells and it reduced to $\frac{1}{2}$ times during the breeding period. The number of chromophobe cells was maximum during the hibernation period and minimum during the breeding period. These cyclic changes in the cells of the pars distalis were distinctly correlated with the cyclic changes in the gonads. The spermatogenic activity in the testis was maximum during breeding period from April to August when the largest number of spermatozoa were observed in the seminiferous tubules. The ovary contained the largest number of mature ova during early breeding period although this number decreased during the breeding period due to spawning. Active oogenesis was observed during the late breeding and post-breeding periods. Large
number of the basophilic cells (gonadotrophic) during pre-breeding and breeding periods indicates high level of gonadotrophic hormone and correlates well with the onset and progress of breeding activity; and large number of acidophilic cells (somatotrophic) during the late breeding period and post-breeding period indicates high level of somatotrophic hormones and correlates well with high gametogenetic activity.
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