Materials and Methods remain same in the studies of both male and female reproductive system (Chapter I & II).

Collection of Material:

Materials used in the present study consisted of adult both male and female insects of order Hemiptera and Lepidoptera collected from various localities mentioned in the table 1 below, throughout the year.

Table 1: Location data of collecting sites of materials of present study.

<table>
<thead>
<tr>
<th>Section</th>
<th>Species</th>
<th>Family</th>
<th>Places of collections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemiptera</td>
<td><em>Gerris spinolae</em></td>
<td>Gerridae</td>
<td>Ponds of various localities, Dist. Burdwan, West Bengal:</td>
</tr>
<tr>
<td>Lepidoptera</td>
<td><em>Corcyra cephalonica</em></td>
<td>Pyralidae</td>
<td>Rice fields of various localities, Dist. - Burdwan, West Bengal:</td>
</tr>
</tbody>
</table>
Materials and Methods

Experimental procedures:

i) Dissection: The adult insects were anaesthetized very mildly by chloroform or ether and both the male and female reproductive organs were dissected out in insect Ringer’s solution containing:

- Sodium chloride - 75 gms
- Potassium chloride - 0.75 gm
- Calcium chloride - 0.1 gm
- Sodium hydrogen phosphate - 0.1 gm
- Distilled water - 1,000 ml

The fat bodies and tracheae were quickly removed from the respective organs before they were transferred to the fixatives.

ii) Fixation of tissues: For histological and histochemical studies the tissues were fixed in aqueous Bouin’s fixative and Carnoy’s fixative, as per recommended time of fixation and subject to the nature of work.

iii) Whole mount: Whole mounts were made for morphological study after fixing the reproductive organs in Carnoy’s fluid and then dehydrated through graded alcohols, stained
in eosin, cleared in xylene and mounted in D.P.X. The slides were sealed with molten paraffin to avoid air bubbles.

iv) Sectioning of tissues: For normal histological and histochemical studies the tissues were embedded in full paraffin for 1 to 1.5 hours at 60°C and the serial sections of 6 u thickness were cut. All sorts of precautions were taken during preparation of sections and the tissues were affixed on the microslides, previously smeared with Myer's albumen.

v) Staining of tissues:
   a) For histological purposes following stains were used:
      1. Delafield's Haematoxylin and Eosin stain.

   b) For histochemical purposes following stains were used:
      1. Feulgen reaction [Feulgen and Rossenbeck (1924) modified by Pearse (1968)] to detect DNA.
3. The Periodic acid-Schiff technique (after McManus, 1946) to detect glycogens acid mucopolysaccharides and mucoproteins.

4. Toluidine blue stain to detect Metachromasia (Pearse, 1968).


VI) Microphotographs: Black and white microphotographs were taken by using 35 mm. ORWO films (125 ASA) and coloured ones were taken by using 35 mm KODAK VR 100 films through Pentax K 1000 camera and Olympus microscope built-in illumination.