A. COLLECTION OF MATERIAL:

Two insects were selected for the present investigation. The last stage nymphs of *Poikilocentrus pictus* Fab. were collected from the Calotropis plant, from the fields around Sagar, in the months June to October. They were kept in large cages and were fed on fresh Calotropis leaves every day. After the final imaginal moult, the newly emerged adults were kept in pairs in large glass jars and were marked for their ages.

The adults and the last stage nymphs of *Hyadecus similis* Freeman were collected from Bhindi plant (*Abelmoscus esculantus*) and cotton plant (*Gossypium hirsutum*) in the months June to December. These were then reared in the laboratory, all the year round. The insects were fed on moist cotton seeds every day. After the final larval moult, the newly emerged adults were separated, and kept in glass jars. They were kept in pairs and marked for their ages.

B. FIXATION:

The insects of both the species were selected according to their ages, and dissected in insect fixer's solution to expose the brain. The dissected brains were fixed in aqueous Bouin's fluid and cold Carnoy's fluid. Paraffin blocks were made in the usual way and 5-6 µm thick vertical sections were cut. For the histochemical study of the enzyme choline-esterase, the brains were fixed in cold acetone (4°C). The tissue
was then passed through a mixture of alcohol and ether (4°C); then into a 4% solution of colloidion in alcohol-ether mixture (4°C); and then into chloroform at room temperature. The tissue was then embedded in paraffin and 5-6 μ-thick sections were cut.

C. STAINING:

(a) For Neurosecretion:

Para-alddehyde Fuchsin (Swen 1962) and PAS (Harker, after Saini 1966) were used as the standard stains for neurosecretion. The latter gave better results.

(b) For Histochemistry:

(i) Proteins:

Mercuric Bromophenol Blue (MBB) method of Bonnag (1955, from Pearse, 1969) was used. Carnoy fixed material was stained in 1% alcoholic bromophenol blue solution, saturated with mercuric-chloride.

(ii) Cholinesterase:

For the histochemical study of the enzyme cholinesterase, Pyristoyl-choline method of Gomori (1943, from Pearse 1969) was used. Prostigmine bromide (10^-5 M) was used as inhibitor.

Biochemical Study of the Cholinesterase Activity:

For the study of activity of the enzyme cholinesterase
in the whole brain method of Ellman et al. (1961) as modified by Mehrotra and Chandra (1974) was used.

The enzyme activity was measured in the brain homogenate, prepared by homogenizing the brains of the insects in ice-cold saline-buffer (pH 8 prepared by mixing equal volumes of 0.2M NaCl, and 0.2M Phosphate Buffer, pH adjusted to 8.0). The homogenate was centrifuged at 0°C for 10 minutes at 10,000g. Supernatant was collected and used, without further purification, for the estimation of esterase activity. The reaction mixture contained in a total volume of 2 ml: 0.3 ml. homogenate, 0.6 ml. acetylthiocholine iodide ([TCh] 5×10⁻⁴M), 0.6 ml. of 5-5-dithio-
obis-2 nitro benzoic acid ([TMB] 10⁻³M), in 0.2M phosphate buffer pH 8.0.

The rate of hydrolysis of [TCh] was determined by measuring the increase in optical density at 420 nm, using molar extinction coefficient of the yellow anions produced as 1.36×10⁴.

All the optical density measurements were made with the help of a Buch and Luck 'Spectronic 20' spectrometer.

The rate of hydrolysis of [TCh] in n Moles/min/mg, was calculated by the formula: -

\[
\text{Rate of hydrolysis} = \frac{-dV}{1.36 \times 10^{-2} \times C \times Y} \times 1
\]

where \( -dV \) = change in absorbance/min.

\( C \times Y \) = total volume
\[ C = \text{concentration of the enzyme} \ \text{C mg/ml.} \]

\[ Y = \text{sample volume}. \]

For characterization of the enzyme inhibitors like eserine, sumioxon (0,0 dimethyl 0-(3-methyl 4-nitrophenyl) phosphate) were used. For eserine the incubation time was five minutes and for sumioxon the incubation time was ten minutes.