HISTOCHEMISTRY OF THE NEUROSECRETORY CELLS OF POEKILOCRUS PICTUS
A. PROTEINS:

The proteins in the 'A' type of the neurosecretory cells in the brain of *P. pictus* have been studied using the Mercuric Bromophenol Blue, (Bonhag, 1957) method. The proteins occur in the cytoplasm of the cells in the form of deeply stained bodies, some of which are larger and others small. The nucleus also contains granules of irregular shape, representing the chromatin material. The nucleoli and the nuclear membrane are deeply stained. All the types of neurosecretory cells i.e. 'A, B, C, and D' types, show a positive reaction to the stain. The concentration of the proteinaceous material seems to alter with age. The further details of the proteins at different ages are as follows:

1. Newly Moulted Adult:

The neurosecretory cells show a very strongly positive reaction to MBB. The cytoplasm contains a number of granular inclusions stained deeper than the ground cytoplasm, which also gives a positive reaction, but the intensity of the stain is much less than that in the granular elements.

The nuclei of these cells also show a positive reaction to this test. The nuclei contain small inclusions of deeply coloured bodies. The nucleoli and the nuclear membranes are also deeply stained.
The axons are traceable up to a small distance due to the presence of MBB positive granules in them.

The 'B', 'C' and 'H' types of neurosecretory cells are also positive to the reaction, though they seem to have a lesser affinity than the 'A' type of cells. The axons of these cells are also positive to MBB, but are not traceable after a short distance.

The non neurosecretory cells the perineurium and the neuropile, are moderately positive to this test. The perineurium shows the presence of a number of fine proteinaceous granules, (Fig. 25).

2. Ten-Days Old Adult:

The neurosecretory cells show a great affinity to the reaction. The cytoplasm also gives a positive reaction to the test, but the amount of proteinaceous material seems to be less than that in the newly moulted adult. The axons are traceable up to a considerable distance due to the presence of MBB positive granules in them.

The nucleus shows a positive reaction to the test. It contains a number of moderately stained bodies scattered in the nucleoplasm. The nucleoli and the nuclear membranes are deeply stained. The 'B', 'C' and 'H' types of the neurosecretory cells are also positive to the reaction.
The non-neurosecretory cells and the neuropile give a positive test. The perineurium and the connective tissue contain proteins in the form of the granules, (Fig. 24).

3. Twenty Days Old Adult:

The neurosecretory cells at this stage are positive to the test. The cellular proteinaceous materials are in the form of a number of small and large, intensely stained, granular elements, dispersed in the cytoplasm.

The nuclei also give a positive reaction. The nucleoli and the nuclear membrane are deeply stained. The axons are not traceable up to large distances.

The non-neurosecretory cells, the neuropile, the perineurium and the connective tissue are moderately stained, (Fig. 27-).

4. Thirty Days Old Adult:

These seem to be a large amount of proteinaceous material in the neurosecretory cells, which give a strong positive reaction to the test. The cytoplasm contains a number of granular element of both large and small size.

The nucleus is also positive to the test, containing a number of irregular shaped bodies, moderately stained. The nucleoli and the nuclear membrane are deeply stained. The axons are not traceable up to a large distance because of the
absence of the protein granules then.

The other types of the neurosecretory cells are also positive to the reaction.

The non-neurosecretory neurones, the neuropile and the perineurium are moderately stained. The connective tissue and the perineurium show presence of proteins in the form of fine granules, (Fig. 28).

5. Forty-Days Old Adult:

The neurosecretory cells give a strong positive reaction to MSA. There seems to be a large amount of proteinaceous material in the cells. The cytoplasm contains protein granules of large and small size.

The nuclei are also positive to the reaction. The nuclear membrane and the nucleoli are deeply stained. The nucleoplasm contains irregular bodies of chromatin material.

The axons are traceable up to a very short distance.

The 'B', 'C' and 'E' types of neurosecretory cells are also positive to the reaction. The non-neurosecretory neurones, perineurium and the connective tissue are also moderately stained, (Fig. 29).

6. Fifty-Days Old Adult:

The neurosecretory cells show a strongly positive reaction.
indicating the presence of a large amount of proteinaceous material. The protein granules of both large and small size are dispersed in the cytoplasm. These granules are more deeply stained than the ground cytoplasm.

The nuclei give a positive reaction to the test. The nucleoli and the nuclear membranes are more strongly positive than the nuclei.

The axons cannot be traced up to a large distances as they do not contain protein positive granules.

The other types of neurosecretory cells i.e. the 'B', 'C' and 'E' types are also moderately stained.

The non-neurosecretory cells, the perineurium, the neuropile and the connective tissue give a positive reaction to the test, (Fig. 30).

7. Sixty-Days Old Adult:

The neurosecretory cells show a great affinity to MB, B. There are present, a number of small and large granules of proteins, dispersed in the faintly stained cytoplasm.

The nuclei give a positive reaction to the test. The nucleoli and the nuclear membrane are deeply stained.

The axons are not positive to the test as the amount of proteinaceous material in them is very small.
Fig. 25. Shaving proteins in the neurosecretory cells in *P. pictus* at newly moulted stage MBB x 450.

Fig. 26. Shaving proteins in the 'A' type of neurosecretory cells in *P. pictus* at 10 days MBB x 450.
Fig. 27. Showing proteins in 'A' type of neurosecretory cells in *P. pictus* at 20 days. MBB x 450.

Fig. 28. Showing proteins in 'A' type of neurosecretory cells in *P. pictus* at 30 days. MBB x 450.
Fig. 29.  Showing proteins in 'A' type of neurosecretory cells in *P. pictus* at 40 days. MBS x 450.

Fig. 30.  Showing proteins in 'A' type of neurosecretory cells in *P. pictus* at 50 days. MBS x 450.
Fig. 31. Showing proteins in 'A' type of neurosecretory cells in *P. pictus* at 60 days MBB x 450.

Fig. 32. Showing proteins in 'A' type of neurosecretory cells in *P. pictus* at 90 days MBB x 450.
The "A", "C" and "D" types of neurosecretory cells give a positive reaction to MBB.

The non-neurosecretory cells, the perineurium, the neuropile and the connective tissue are moderately positive to the stain. The connective tissue and the perineurium show the presence of protein in the form of fine granules. (Fig. 31).

Thus in other senile stages also the amount of proteins present in the neurosecretory cells is quite large. The neurosecretory cells give a very strongly positive reaction to the test. (Fig. 32)

Remarks:

Proteins in the median "A" type of neurosecretory cells have been studied in the brain of P. pictus using Mercuric Bromophenol Blue method. They have been studied in both young and senile stages. The neurosecretory cells are strongly positive to the test. The cytoplasm, the nuclei, the nuclear membrane and the nucleoli, all show a positive reaction. The axons show a positive reaction in the early stage, but during the senile stages the axons are not prominent, showing thereby, the absence of proteins in them. The concentration of proteinaceous material in the neurosecretory cells increases in the senile stages, as is evident by the strong positive reaction of the cells.
B. Cholinesterase:

The enzyme cholinesterase was studied histochemically in the neurosecretory cells of _P. pictus_ using the method of Gomori (1948). Acetylcholine was used as substrate. The reaction was inhibited on using 0.0001M prostigmine bromide showing thereby, that the reaction was indeed due to cholinesterase. Though the neurosecretory cells give a positive reaction to the test, the histochemical results cannot be interpreted quantitatively. According to Silver (1974), the conditions in the incubation medium, necessary for accurate localization, are themselves inhibitory.

The distribution of cholinesterase in the neurosecretory cells of _Iphita inhita_ has been studied by Nayar (1955).

The neurosecretory cells of _P. pictus_ give a strongly positive reaction to the test. The cytoplasm of the cells is stained dark brown. The nuclei give a negative reaction and are stained pink. The nuclear membranes give a positive reaction. The 'B', 'C' and 'I' types of neurosecretory cells are also positive to the reaction. The axons are stained dark brown, thus showing a positive reaction.

The non-neurosecretory neurones give a very strongly positive reaction. The brain tissue gives a negative reaction, and is stained faintly pink. (Fig. 33)
Fig. 33. Showning cholinesterase activity in the neurosecretory cells of P. nigropunctatus x 450.
Plate 18
Remarks:

The distribution of cholinesterase in the neurosecretory cells of P. pictus was studied using the Myristoyl-choline method of Gomori (1949). The neurosecretory cells gave a positive reaction to the test, thus confirming the presence of cholinesterase in them.

The method could not be used for quantitative estimation at different ages, as conditions in the incubation medium, imposed by the needs for accurate localization are themselves inhibitory. The results obtained at different ages, were nearly same and as such, they could not be used for quantitative interpretation.

That the reaction was indeed due to enzyme cholinesterase, was confirmed by using prostigmine bromide as inhibitor.