Neurosecretory Cells in the Ventral Nerve Cord of Leech (*Hirudinaria granulosa*)

Research of HAGADORN[1] on the brain of leech, *Theromyzon rude*, has shown 4 principal types of neurosecretory cells, which on the grounds of their morphological and cytochemical appurtenances, have been classified as α, β, γ and δ. The present work has been undertaken to examine the neurosecretory cells in the ventral nerve cord of the region of ovisacs of the leech, *Hirudinaria granulosa* and to strike a comparison of these cells with the neurosecretory cells found in the brain of *Th. rude* in regard to the type and distribution of neurosecretory material and also in respect of their morphological identity.

The unnarcotized leech was vivisected and the ventral hemocoelomic channel containing the ventral nerve cord of the region of ovisacs was removed and quickly fixed in Carnoy and Bouin fixatives. The sections of Bouin were stained with hematoxylin-eosin for histological purposes. Sections of Carnoy's fixative were used for the demonstration of DNA and RNA by methylgreen-pyronin Y according to the method of KURNICK (PEARSE[2]) and others were treated with Bonhag's mercury-bromphenol blue method for proteins. Figure 1 shows the arrangement of neurosecretory cells around the ventral nerve cord. Their axons end in the nerve cord whereas the perikarya project out in the ventral hemocoelomic channel. On the grounds of their morphological patterns and the presence of neurosecretory material, they can be grouped into 3 types. The first type includes tall, club-shaped cells. In these the zone between the nucleus and the apex of perikaryon has a flocculated cytoplasm, not receptive to cytoplasmic stain with methylgreen pyronin (Figure 4). This characteristic part of the cytoplasm is suggestive of the presence of neurosecretory material which in some cells will be found just above the nucleus and in others at the apical half of perikaryon along with some granules. The flakes of neurosecretory material appear to break down in the form of fine granules. These granules also appear along the axons. The second type includes cells that are quite like the first type but for the lucent secretory substance. In these types the cytoplasmic stain is intense around the nucleus and it gradually becomes pale towards the peripheral zone (Figure 2). The secretory

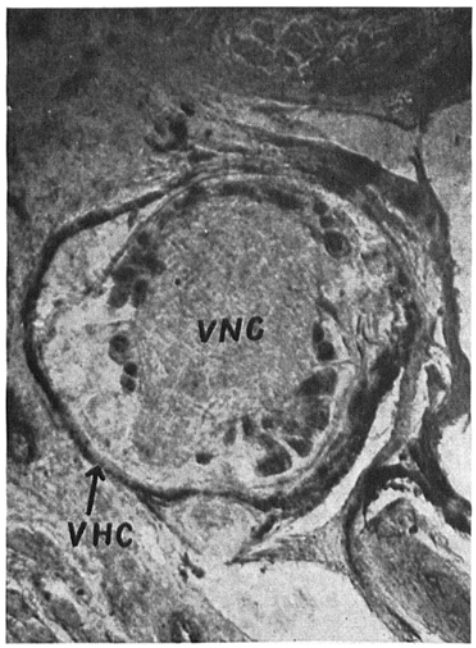

Fig. 1. Transverse section of the ventral hemocoelomic channel (VHC) showing the ventral nerve cord (VNC) surrounded by neurosecretory cells. Methylgreen pyronin Y. × 50.

Fig. 2. Part of the VNC showing club-shaped cells. Methylgreen pyronin Y. × 220.

Fig. 3. Part of the VNC showing neurosecretory cells with homogeneous cytoplasm. Methylgreen pyronin Y. × 220.

[1] I. R. HAGADORN, J. Morph. *102*, 55 (1958).
[2] A. G. E. PEARSE, *Histochemistry* (Churchill, London 1960).

substance does not appear to be proteinaceous in the types here described. These 2 types may be compared with the α cells of HAGADORN. Neurosecretory cells of the third type have deeply stained cytoplasm with methylgreen-pyronin, a central nucleus and small secretory granules evenly distributed with slight increase in number at the peripheral region. The secretory products seem to be proteinaceous (Figure 3). In the present section some of these cells appear to have retained connection with the ventral nerve cord by a thin sheet of cytoplasm. The secretion product remains in the homogeneous form throughout and the granules do not lose stainability at any time. Such cells may be compared with the β cells of HAGADORN. The extrusion of neurosecretory substance in the last type of cells is not clear. It may be that it occurs in an eccrine manner at the molecular level (KUROSUMI[3]).

Fig. 4. Neurosecretory cells, arrow pointing to the lucent secretory substance in the perikaryon. Methylgreen pyronin Y. × 220.

In the brain of *H. granulosa*, neurosecretory cells have been placed into 2 categories (NAMBUDIRI and VIJAYAKRISHNAN[4]), on the basis of the presence or absence of cytoplasmic vacuoles. But it has not been indicated how long these vacuoles persist and what eventually happens to them. The present investigation, however, has not revealed cytoplasmic vacuoles in any marked abundance. It is quite likely that the lucent secretory materials in the perikaryon have been taken for vacuoles by the authors mentioned.

The presence of neurosecretory cells in the region of the ovisac could be investigated with interest, in view of their possible bearing on the reproductive physiology of the animal[5].

Zusammenfassung. Nachweis von 3 Typen neurosekretorischer Zellen im Bauchmark der indischen Blutegel *Hirudinaria granulosa* und *Theromyzon rude*.

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Department of Zoology, Patna University, Patna 5, Bihar (India), 8 March 1967.

[3] K. KUROSUMI, Int. Rev. Cytol. *11*, 1 (1961).
[4] P. N. NAMBUDIRI and K. P. VIJAYAKRISHNAN, Curr. Sci. *27*, 350 (1958).
[5] I express my sincere gratitude to Dr. S. KESHAVA, University Professor and Head of the Department of Zoology, Patna University, Patna, for his kind encouragement and laboratory facilities provided.

On the Antitumour Activity of some Products of Wood-Decaying Fungi

In studies on the antitumour activity of some basidiomycetes, either extracts from the fruit bodies[1,2], or substances obtained by the submerged cultivation of these microorganisms on the liquid media[3], were used as the source of active substances.

In the present short communication I should like to show some results of the experiments on the antitumour activity of the red solution described by SCHÁNĚL[4]. This red solution was dissolved in physiological solution and kept at a temperature of about − 15 °C. Black mice C57B1 to which the solid Ehrlich carcinoma was implanted were injected with this solution.

The pigment was injected s.c. in amounts of 0.2 ml every 3rd or 6th day during a 50-day period. As shown in Figure 1, the prolongation of life in the group of the cured mice was ascertained beyond any doubt.

The mean time of life in the group of the cured mice was 43.7 days, while in the group of the control mice it was 33.3 days. The 95% confidence interval was given by the limits 29.2–38 days in the control group and 36.7–52.1 days in the cured group. The difference between the time

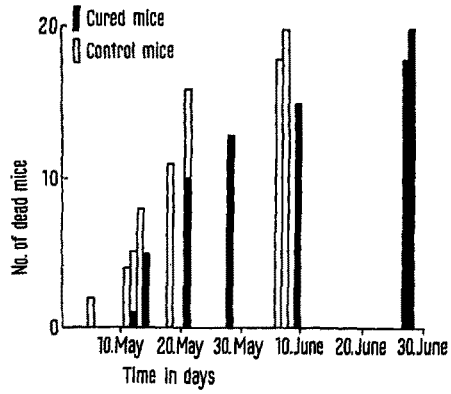

Fig. 1. Prolongation of life in the group of the cured mice.

[1] E. H. LUCAS, R. L. RINGLER, R. U. BYERRUM, J. A. STEVENS, D. A. CLARKE and C. C. STOCK, Antibiotica Chemother. 7, 1 (1957).
[2] S. PIASKOWSKI, Sylwan *10*, 5 (1957).
[3] F. J. GREGORY, E. M. HEALY, H. P. K. AGERSBORG JR. and G. H. WARREN, Mycologia *58*, 80 (1966).
[4] L. SCHÁNĚL, Experientia *22*, 517 (1966).