

Fig. 3. Shows the close relationship between the axon (A) and the specialized cell. Note the mitochondria and agranular vesicles in the axon and the concentration of granular vesicles in the specialized cell near the axon. Lead citrate and uranyl acetate.  $\times 32,000$ .

pathways fire in phase with breathing in this species<sup>9,10</sup>, and this is consistent with mechanoreceptor activity.

Using the light microscope, FRÖLICH described 'light cells' in the bronchial epithelium of several mammals<sup>11</sup>. These cells, which were sometimes present in clumps, appeared to be innervated by fine nerve fibres extending deeply into their cytoplasm, and he suggested that they

were chemoreceptors. The presence of these cells in the normal human lung has been confirmed<sup>12</sup>, but apparently their innervation has not yet been corroborated. BENSCH et al.<sup>7</sup> suggested that their new cell type probably corresponded to FRÖLICH's<sup>11</sup> 'light cells', but did not report a close contact between these cells and nerve endings. The specialized cells which we have described very probably correspond to FRÖLICH's 'light cells', and they do have a close relationship to axons.

If our cells are chemoreceptors they suggest the possibility of a device for the rapid and direct monitoring of gases within the airway, as opposed to the relatively indirect monitoring of gases in the blood. If they are mechanoreceptors they are of interest in that it has been difficult hitherto to demonstrate convincingly any definite receptor structures within the vertebrate lung which might be sensitive to mechanical stimuli<sup>13</sup>.

**Résumé.** L'examen au microscope électronique du bronchus primaire intrapulmonaire de *Gallus domesticus* a révélé la présence d'un complexe neurite-récepteur très semblable aux cellules du glomus carotidien des mammifères, et aux cellules de Merkel qui se trouvent dans les structures épidermiques des vertébrés. La fonction possible chémorécepteur ou mécanorécepteur de ces cellules est discutée.

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<sup>9</sup> A. S. KING, V. MOLONY, J. McLELLAND, D. R. BOWSER and M. F. MORTIMER, *Experientia* 24, 1017 (1968).

<sup>10</sup> D. R. JONES, *Comp. Biochem. Physiol.* 28, 961 (1969).

<sup>11</sup> F. FRÖLICH, *Frankfurt. Z. Path.* 60, 517 (1949).

<sup>12</sup> H. VON HAYEK, *The Human Lung* (Hafner, New York 1960), p. 130.

<sup>13</sup> The electron microscope was presented by the Wellcome Foundation and the project was sponsored by the British Egg Marketing Board.

### Effect of Temperature on the Neurosecretory Activity in *Nezara viridula* Linn. (Heteroptera; Pentatomidae)

The role of temperature on the activity of neurosecretory cells has not so far been studied in detail in insects (NOVAK<sup>1</sup>). The only report is that of CLARKE<sup>2</sup>, who has studied the histological changes in the neuroendocrine system of *Locusta migratoria* at different temperature regimes. In the present work an attempt has been made to study the role of temperature on the activity of the neurosecretory cells of the brain of green cotton bug, *Nezara viridula*.

**Material and method.** The adults of *Nezara viridula* of about equal age group were collected from the plants of *Althaea rosea*. For each replicate experiment they were sorted out in 4 lots. The first lot was kept in an incubator maintained at a temperature of 35°C. The second and third lots were kept in a refrigerator maintained at 10 and 0°C for 21 days and 4 days respectively. One lot was always kept at the room temperature (28°C) as control.

The insects kept at 35 and 10°C lived for several days, but those at 0°C survived for 3–4 days only. The experimental and controlled insects were studied after staining with paraldehyde fuchsin<sup>3</sup> (PF) and performic acid victoria blue (PAVB)<sup>4,5</sup> techniques.

**Results and discussion.** In the pars intercerebralis medialis of the protocerebrum of *Nezara viridula*, there are 2 distinct groups of neurosecretory cells, situated

<sup>1</sup> V. J. A. NOVAK, *Insect Hormones* (Methuen & Co. Ltd., London 1966).

<sup>2</sup> K. U. CLARKE, *J. Insect Physiol.* 12, 163 (1966).

<sup>3</sup> A. B. EWEN, *Trans. Am. Microsc. Soc.* 81, 94 (1962).

<sup>4</sup> H. BRAAK, *Mikroskopie* 17, 344 (1962).

<sup>5</sup> G. S. DOGRA and B. K. TANDAN, *Q. Jl. microsc. Sci.* 105, 455 (1964).

superficially on the dorsal side (Figure 1). Each group consists of 5 cells of A-type. The NSM originating in the medial neurosecretory cells of the brain is stored in the aorta wall via neurosecretory pathways and nervi corporis cardiaci-1 (Figure 1).

Under normal room temperature (28°C) the various components of the neurosecretory system, i.e. the neurosecretory cells, the course of the neurosecretory pathways within and outside the brain, and the NSM in the aorta wall are clearly visible (Figure 1). The insects subjected to a temperature of 35°C did not show any marked change in the quantity of NSM from the normal (Figure 3). The freshly collected insects from the field, where the temperature ranges from 30–35°C, were also

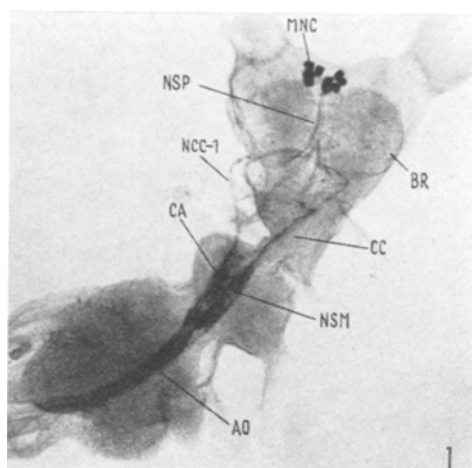


Fig. 1. Whole mount of the brain (BR) and postcerebral endocrine glands, showing the 2 groups of medial neurosecretory cells (MNC), each of them consists of 5 + 5 cells and their neurosecretory pathways (NSP). The 2 nervi corporis cardiaci-1 (NCC-1) pass over the paired corpora cardiaca (CC) of the corresponding sides and a medial corpus allatum (CA) dorsally and laterally to enter the aorta wall. The aorta (AO) loaded with neurosecretory material (NSM) at room temperature is clearly marked out. PAVB in situ,  $\times 65$ .

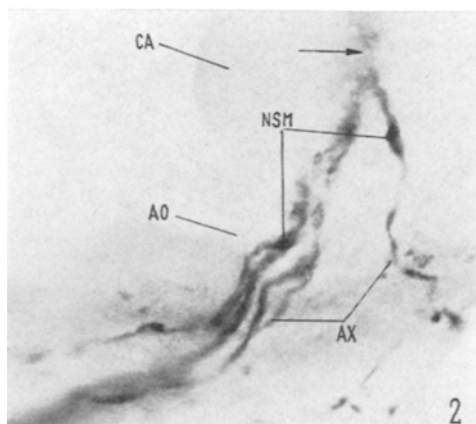


Fig. 2. Entire aorta (AO) mounted from lateral aspect clearly showing the 5 axons (AX) of the 5-medial neurosecretory cells of one side, heavily loaded with neurosecretory material (NSM) at room temperature (28°C). The entry of nervus corporis cardiaci-1 in the aorta wall is distinctly revealed (arrow). Further, the corpus allatum (CA) can be distinguished which lies ventral to the aorta. PAVB in situ,  $\times 260$ .

examined and it was found that they also show an exact similarity to those kept in the laboratory, under normal room temperature. It seems, therefore, that 28 to 35°C is the most suitable temperature range, at which the maximum amount of NSM is elaborated and discharged and all the structures maintain clear visibility. The insects kept at a temperature of 10°C for 3 weeks, show a considerable reduction in the quantity of NSM in almost all the components (Figures 5 and 6). The neurosecretory cells though quite clear, contain a small amount of NSM (Figure 5). The other components, i.e. the axons and the neurosecretory pathways, are not so well marked out and do not possess NSM. On further decrease of temperature to 0°C, in 3–4 days, no trace of NSM is noticed in the neurosecretory cells and it is difficult to trace their outlines (Figure 7). At this temperature, the activity in the neurosecretory cells is thus minimum, since no trace of NSM is seen anywhere in the neurosecretory system.

The aorta, which is provided with abundant NSM at 28–35°C temperature range (Figure 2), shows a considerable fall at 10°C (Figure 6). At this temperature, a poor amount of NSM is seen in the aorta wall, while at 0°C it is totally lacking (Figure 4).

Further, it has been observed that at 0°C the NSM disappears from the neurosecretory cells and their pathways in 3–4 days time, while at 10°C it takes about 3 weeks to get reduced. In the latter case, no difference in NSM is observed in 3–4 days time. It indicates that the

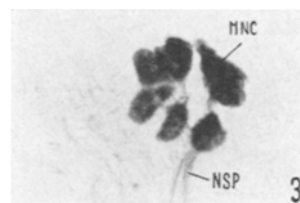


Fig. 3. Dissected pars intercerebralis of the protocerebrum showing abundant neurosecretory material in the medial neurosecretory cells (MNC) at 35°C. The neurosecretory pathways (NSP) are also clearly seen. PAVB in situ,  $\times 180$ .

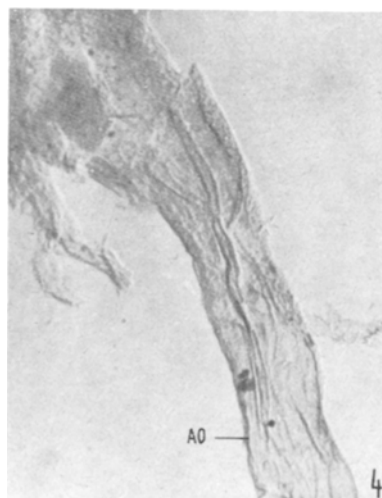


Fig. 4. Whole mount of the aorta (AO) of an individual kept at 0°C for 4 days. Note the absence of neurosecretory material in the entire organ. (Compare with Figures 1 and 2.) PAVB in situ,  $\times 65$ .

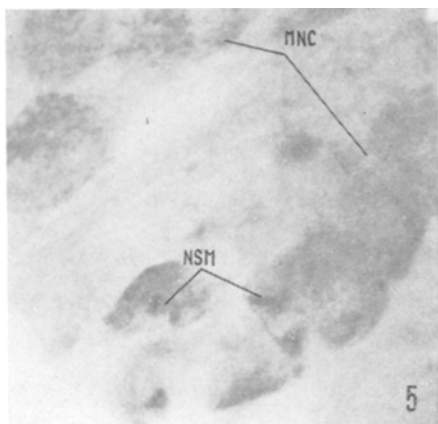


Fig. 5. Dissected pars intercerebralis of the protocerebrum. Note great depletion of neurosecretory material (NSM) in the medial neurosecretory cells (MNC) of an individual kept at 10°C for 3 weeks. PAVB in situ,  $\times 390$ .

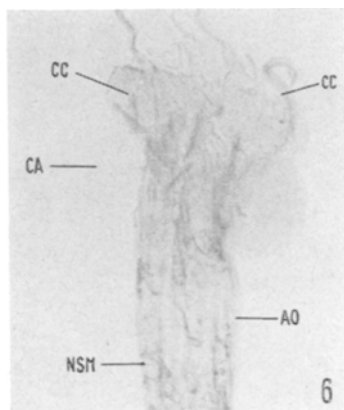


Fig. 6. Whole mount of the aorta (AO) with intact postcerebral endocrine glands. Note poor quantity of the neurosecretory material (NSM) in the aorta (AO) of an individual kept at 10°C for 3 weeks. Other structures are the 2 corpora cardiaca (CC) and corpus allatum (CA) lying below the aorta. PAVB in situ,  $\times 80$ .

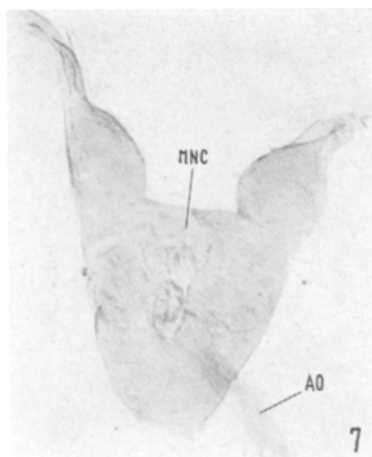


Fig. 7. Whole mount of the brain of an individual kept at 0°C for 4 days. Due to the absence of neurosecretory material, the neurosecretory cells (MNC) are not easily discernible. The aorta (AO) is also devoid of NSM. PAVB in situ,  $\times 80$ .

fall in the activity gradient in the neurosecretory cells at lower temperature is more rapid than at higher temperatures.

HIGHNAM<sup>6</sup> has shown that the neurosecretory cells at lower temperature become inactive in *Mimas tiliae*. The present study also reveals that the neurosecretory cells and the aorta at a temperature range of 28–35°C show abundant NSM, which gets considerably reduced at 10°C in 3 weeks. On further lowering the temperature to 0°C, in 4 days, no trace of NSM is found in them. HIGHNAM<sup>6</sup> recorded in the diapausing pupa that at 3°C, after 4 weeks, the NSM is produced either in slow rate or by few cells only. He is of the opinion that the production of NSM ceases abruptly during the fourth week at low temperature. In *Nezara viridula* at 10°C, after 3 weeks, NSM is found in the neurosecretory cells and aorta wall in small quantity, but at 0°C it disappears in 3–4 days time. The present observations are in agreement with the view of HIGHNAM that at low temperature the neurosecretory activity decreases. It clearly indicates that the low temperature inhibits the secretory activity of the neurosecretory cells.

Since the elaboration of NSM takes place through the mediation of alkaline phosphatase (TEWARI and AWASTHI)<sup>7</sup>, perhaps the low temperature also inhibits the enzyme activity. And, therefore, poor quantity of NSM is produced at low temperatures. ANDERSON<sup>8</sup> has clearly mentioned that the majority of enzymes are inactive at 0°C. It has been pointed out by the author (AWASTHI)<sup>9</sup> in *Gryllodes sigillatus*, that during the hibernation (winter season), the neurosecretory activity decreases and the neurosecretory cells are greatly reduced in number, concomitant with the reduction in the quantity of NSM.

Further CLARKE<sup>2</sup> has recorded significant change of NSM in the corpora cardiaca of *Locusta migratoria* at different temperature regimes, which he failed to find in the neurosecretory cells of the brain. ABRAHAM<sup>10</sup> has recorded for *Dytiscus marginalis* the maximum production of NSM at noon at 14.00 h, while in the morning at 09.00 h it is minimum. Though he has not discussed the reasons for such a variation, obviously it must be due to the decrease of temperature in the morning hours. It may be thus concluded that temperature is one of the prime factors influencing the neurosecretory activity in insects<sup>11</sup>.

**Zusammenfassung.** Die neurosekretorischen Zellen und die davon ausgehenden Nerven von *Nezara viridula* Linn enthalten bei Zucht in niedriger Temperatur bedeutend weniger Neurosekret als in Normaltemperatur.

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<sup>6</sup> K. C. HIGHNAM, Q. Jl. microsc. Sci. 99, 73 (1958).

<sup>7</sup> H. B. TEWARI and V. B. AWASTHI, Gen. comp. Endocrin. 10, 330 (1968).

<sup>8</sup> A. K. ANDERSON, *Essentials of Physiological Chemistry*, 4th edn. (John Wiley & Sons Inc., New York, London 1953).

<sup>9</sup> V. B. AWASTHI, Anat. Anz., in press (1969).

<sup>10</sup> A. ABRAHAM, Acta anat. 65, 435 (1966).

<sup>11</sup> The author is grateful to Prof. H. S. CHAUDHRY for providing the laboratory facilities and encouragement.