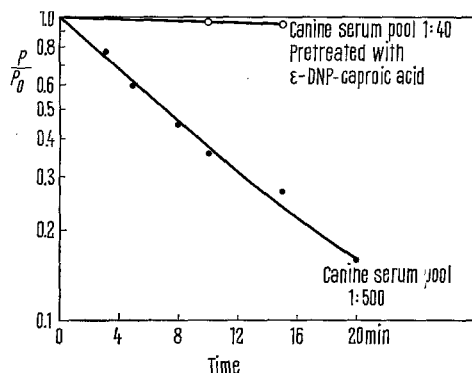


plaques at time  $t = t_0$ ;  $c$  = sample dilution factor; and  $\Delta T$  = time in min ( $t - t_0$ ).

**Results.** Following 4 intratracheal injections and one administration of antigen by aerosol, the serum pool and the bronchial secretion pool had a  $K$  of 50.5 and 0.37 respectively, with  $T_4$ -DNP. These samples each gave a  $K$  of approximately 0.02 with unconjugated  $T_4$ . Following the final bronchial administration of antigen, serum had a  $K$  of 3.9 and bronchial wash gave a  $K = 46$ . As before,  $K$ 's of approximately 0.02 were obtained with unconjugated  $T_4$ . The neutralizing activity of the anti-DNP samples could be almost completely inhibited by preincubating them with  $\epsilon$ -DNP-caproic acid (Figure).



Kinetics of DNP- $T_4$  neutralization with canine anti-DNP serum.  $P/P_0$ , ratio of plaques remaining at time  $t$  to original number of plaques. The number following the sample name refers to the dilution of antiserum used.

**Discussion.** Intratracheal and aerosol immunization of dogs with a DNP-protein conjugate resulted in circulating anti-DNP and low levels of anti-DNP in the bronchial secretions. Following the final direct instillation of DNP-KLH into the respiratory tract a definite increase in anti-DNP activity in the secretions was noted whereas the circulating anti-DNP level dropped. This may have

been due to the selective stimulation of antibody producing cells in the respiratory tract. WALDMAN et al.<sup>11</sup> have shown that the antibody response varies with particle size; the smaller particle sizes give better serum antibody responses, that larger particles give better levels of secretory antibody.

Previous authors working with dogs have demonstrated that there is a preferential secretion of IgA in canine milk, saliva and bronchial mucus<sup>6,12</sup>. Our object was to specifically purify the 'secretory' anti-DNP antibodies and to characterize them as to immunoglobulin class, valence and association constant. However, the extremely low levels of specific anti-DNP made this unfeasible. This study points out that anti-DNP antibodies can be detected in the secretions of dogs. Careful studies on the particle size, route of administration, and dose of antigen employed will be necessary in order to establish a method which will yield high levels of secretory anti-hapten antibody. Detailed physical chemical studies can then be carried out.

**Zusammenfassung.** Im Serum und in Bronchialsekreten von Hunden konnten Antikörper gegen DNP, die über die Atmungswege mit DNP-Proteinkonjugaten immunisiert worden waren, nachgewiesen werden. Die Titer der beobachteten spezifischen Antikörper waren für eine detaillierte Analyse zu niedrig.

G. A. LESLIE<sup>13</sup> and R. H. WALDMAN

Departments of Microbiology and Medicine,  
College of Medicine, University of Florida,  
Gainesville (Florida 32601, USA), 6 June 1969

<sup>11</sup> R. H. WALDMAN, R. CARROLL, E. TORRES and S. H. WOOD, Am. Rev. resp. Dis., submitted for publication.

<sup>12</sup> J. P. VAERMAN and J. F. HEREMANS, Immunochemistry 5, 425 (1968).

<sup>13</sup> This work was supported by National Institutes of Health Training Grant No. 5TI AI0128-09.

## A Note on the Neurosecretory Pathways in *Pyrilla perpusilla* Walker (Fulgoridae: Homoptera)

GABE<sup>1</sup> in his book 'Neurosecretion' mentioned: 'the microscopic anatomy of the stomatogastric system and the retrocerebral endocrine glands in Homoptera would appear to have less study than the anatomy of the same organs in Heteroptera'. This prompted me to undertake a study of the neuroendocrine system of the sugarcane leaf hopper, *Pyrilla perpusilla* Walker, the details of which will be presented elsewhere. During the course of these investigations stained material was invariably seen in the intercellular spaces of the neuropillar tissue of the brain. This material had the same staining properties as the neurosecretory material (NSM) revealed in the neurosecretory cells of the protocerebrum. In this paper the distribution and significance of the intercellular material will be described briefly.

Adults of *P. perpusilla* were collected from sugarcane fields. The required organs were fixed and stained with aldehyde-fuchsin (AF) or performic acid-victoria blue (PAVB) in the manner described by DOGRA and TANDAN<sup>2</sup>. The preparations were supplemented by histological sections stained either before (PAVB) or routinely after sectioning (AF).

Two groups of A-type cells were found in the brain. These cells stained deep purple and greenish-blue with the AF and PAVB techniques respectively. The groups are located on the ventral side of the pars intercerebralis medialis; this in contrast to their location in other insects where they are usually found in an antero-dorsal position. Each group consists of 12-16 cells. When the A-cells were loaded with NSM, their neurosecretory pathways were visible (Figure 1). In such preparations stained colloids or granules were seen on the postero-lateral margins of the A-cells (Figures 2 and 3), but were never observed in brain regions anterior to these cells. The staining intensity of the material visible in the intercellular spaces of the brain varied in preparations from different insects, but was directly proportional to the amounts of NSM in the neurosecretory cells of the same individuals (Figures 2

<sup>1</sup> M. GABE, 'Neurosecretion', Pergamon Press, London, New York (1966).

<sup>2</sup> G. S. DOGRA and B. K. TANDAN, Quart. J. micr. Sci. 105, 455 (1966).

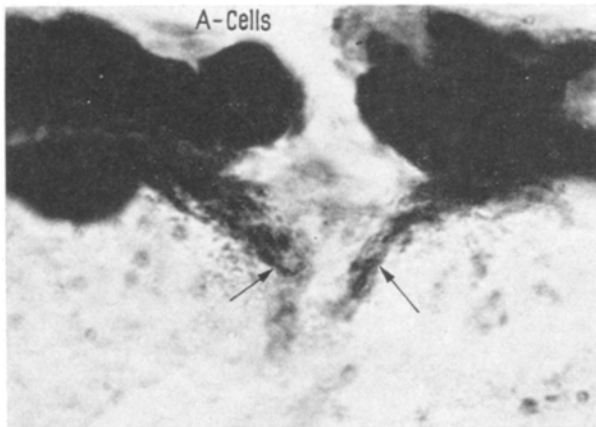


Fig. 1. Whole mount of both groups of A-cells of *P. perpusilla* and their neurosecretory pathways (arrows), AF  $\times 450$ .

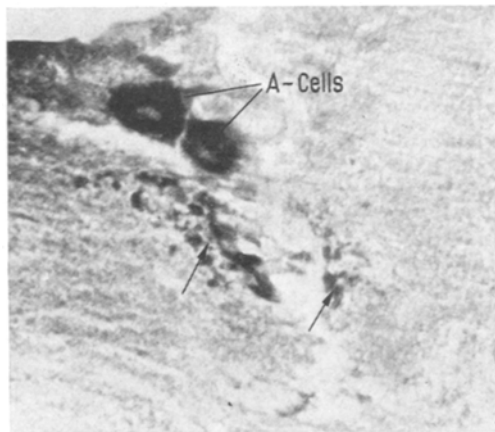


Fig. 2. Cross section passing through the pars intercerebralis region of the brain, showing 2 A-type neurosecretory cells. Note the presence of the neurosecretory colloids in the neuropillar tissues of the brain (arrows), AF  $\times 450$ .

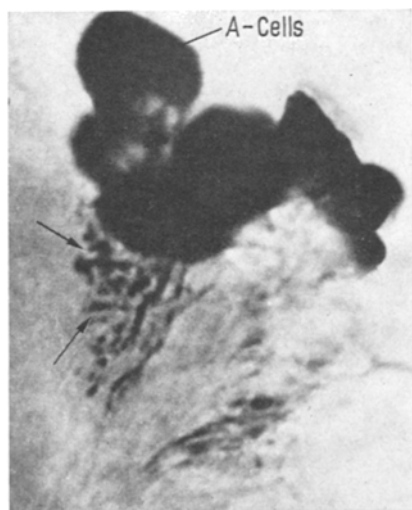


Fig. 3. Whole mount of one group of neurosecretory cells. Note the presence of neurosecretory colloidal granules in the neuropillar tissue (arrows), PAVB  $\times 450$ .

and 3). Stained granules were found in 90% of the preparations. Although this material runs close to the neurosecretory axons, it was never observed to be bound to them; it rather appears to be released directly into the blood.

It is an established fact that neurosecretory colloids elaborated in the pericaryon of the neurosecretory cells are channelled to the neurohaemal organs *via* the neurosecretory tracts. Nonetheless, many workers reported stained material, similar to that elaborated by the A-cells of the brain, in the intercellular spaces of the protocerebrum. A few of them considered this material as NSM<sup>3,4</sup>, others as gliosomes<sup>5,6</sup>. Recently, ADYODI and BERN<sup>7</sup> interpreted it as NSM in the dendritic processes of the neurosecretory cells.

The limitations of the light microscope do not permit an insight into the events taking place at the level of the plasma membrane of the neurosecretory cells. Direct evidence that the intercellular material observed in *Pyrilla* is true NSM, secreted by the neurosecretory cells cannot be given. However, the staining properties together with the observed parallelism between the amounts of the intercellular and the intracellular material suggest a very close relationship. According to electron microscopic studies by SCHARRER<sup>8</sup>, the plasma membrane of a neurosecretory cell is continuous and devoid of canaliculi, whereas NISHITSUTSUJI-UWO<sup>9</sup> had demonstrated the presence of canaliculi connecting the neurosecretory cells and the surrounding blood in *Bombyx mori*. She believed that the NSM observed in the intercellular spaces was probably discharged directly via these minute canaliculi.

On the basis of these, and the new observations in *Pyrilla*, it could well be surmised that two modes of release of NSM exist: one via the neurohaemal organ and another directly from the pericaryon into the blood.

**Zusammenfassung.** Im Gehirn der Zuckerrohrzikade *Pyrilla perpusilla* finden sich zwei Gruppen neurosekretorischer Zellen vom A-Typ. Diese Zellen liegen auf der Ventralseite der *Pars intercerebralis medialis*. In 90% der untersuchten Tiere wurde in den Interzellularräumen hinter den A-Zellen ein Material gefunden, das sich färbereichs gleich verhielt wie das intrazelluläre neurosekretorische Material (NSM) der A-Zellen, wobei die Quantität des interzellulären Materials direkt mit derjenigen des intrazellulären NSM korreliert ist. Es wird daher angenommen, dass das extrazelluläre Material NSM sei und vermutet, dass NSM nicht nur via den neurosekretorischen Trakt in die Neurohaemalorgane gelange, sondern auch direkt an das Blut abgegeben werde.

R. C. SRIVASTAVA<sup>10</sup>

Entomology Research Division,  
Agricultural Experiment Station, University of Udaipur,  
Udaipur (India), 13 May 1969.

<sup>3</sup> J. M. WHITTEN, Gen. comp. Endocrin. 4, 176 (1964).

<sup>4</sup> G. S. DOGRA, Acta Anat. 70, 288 (1968).

<sup>5</sup> E. SCHARRER and B. SCHARRER, Biol. Rev. 12, 185 (1937).

<sup>6</sup> R. L. PIPA, J. comp. Neurol. 116, 15 (1961).

<sup>7</sup> K. G. ADYODI and H. A. BERN, Gen. comp. Endocrin. 11, 88 (1968).

<sup>8</sup> B. SCHARRER, Z. Zellforsch. 89, 1 (1968).

<sup>9</sup> J. NISHITSUTSUJI-UWO, Z. Zellforsch. 54, 613 (1961).

<sup>10</sup> The author is grateful to Dr. G. S. Dogra for critically reading the manuscript and making valuable suggestions, and to Dr. B. K. Srivastava for providing laboratory facilities.