

Fig. 1. Duct cells fixed in pyroantimonate and glutaraldehyde.  $\times 6800$ . Arrows point to pyroantimonate deposits in both figures.

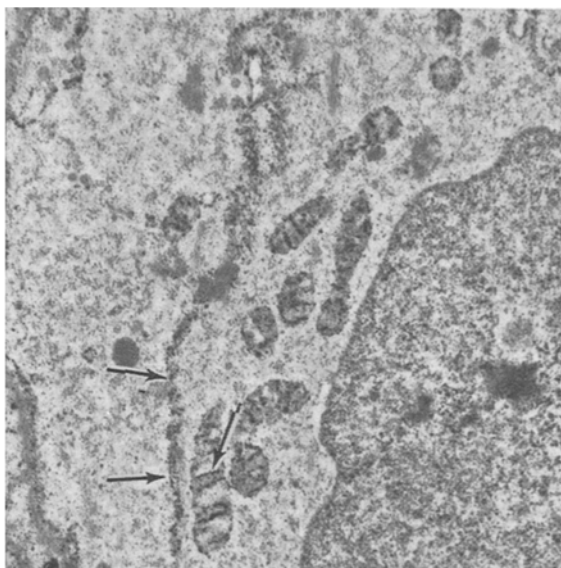


Fig. 2. Duct cells fixed in pyroantimonate and osmium.  $\times 12,800$ .

cellular precipitate particles than were noted after glutaraldehyde fixation. These intracellular particles were throughout the cell in osmium-fixed tissue, but there was some degree of localization to the periphery of the storage granules.

When duct cells were fixed in glutaraldehyde opaque precipitates were seen primarily in the interstitium, the lumen, and between lateral membranes of the adjacent cells (Figure 1). The deposit in the lateral space was distributed along the entire length of the area of tight junctions. The precipitate in this space appeared to be associated either with the external surface of the cell or to fill the area between cells. The precipitate pattern in osmium fixed duct cells was more diffuse. In addition to the pattern described above for glutaraldehyde fixed cells, deposits could also be found along the membranes of mitochondria, in the nucleus, and throughout the cell sap (Figure 2). In contrast to the extracellular localization noted in glutaraldehyde-fixed material, the deposits appeared to be primarily on the intracellular border of the cell membranes. No differences were noted between buffered and unbuffered fixative in either acinar or duct cells.

The pyroantimonate deposits were localized to the lumen of the ducts when glands were first perfused with 2% potassium antimonate and initially fixed by perfusing with either osmium or glutaraldehyde. Tissue treated in this manner did not have pyroantimonate deposits in gland cells or the interstitial space.

**Discussion.** These results using submandibular glands are similar to those reported for rat cerebral cortex<sup>17</sup> and rat kidney<sup>16</sup> in that the localization of pyroantimonate precipitate appears to vary according to the method of fixation and type of fixative employed. Furthermore,

under appropriate conditions precipitation reaction between pyroantimonate and divalent ions and biological amines can occur<sup>15, 18, 19</sup>. Therefore, although the technique may indicate the presence of sodium ions, we believe that definition as to the actual *in vivo* location of sodium using the pyroantimonate technique must be made with extreme caution<sup>20</sup>.

**Résumé.** Des glandes sousmaxillaires de chiens ont été coupées en morceaux et mises dans un fixatif composé de 2% KSB(OH)<sub>6</sub> et soit de 6.25% d'aldéhyde glutarique, soit de 2% d'osmium et préparées pour l'examen au microscope électronique. Quand les tissus sont fixés à l'aldéhyde glutarique, le précipité est principalement extracellulaire. Par contre, avec le fixatif à l'osmium, le précipité est plus diffus et se trouve partout dans la cellule. Cependant, on ne peut pas se fier à cette méthode pour localiser des ions de sodium *in vivo*.

R. BOWMAN and I. A. SIEGEL

*Center for Research in Oral Biology and Departments of Oral Biology and Pharmacology, University of Washington Seattle (Washington 98105, USA), 11 September 1972.*

<sup>18</sup> S. SHIN-ICHI, V. MIZUHARA, T. AMAKAWA and Y. FUTAESAKO, J. Histochem. Cytochem. 20, 65 (1962).

<sup>19</sup> R. L. KLEIN, S. YEN and A. THURESON-KLAIN, J. Histochem. Cytochem. 20, 65 (1972).

<sup>20</sup> This work was supported by grants No. DE 06200 and No. DE 01701 from the National Institutes of Health.

### On the Activity of Neurosecretory Cells in the Flesh-Fly *Sarcophaga bullata*

The hormones secreted by the neurosecretory cells of the insect brain are clearly known to be implicated in certain metabolic activities<sup>1</sup>. These hormones, it has been indicated, may also influence the egg production in the female<sup>2</sup>. Again, from their studies on female insects,

some workers<sup>3-5</sup> conclude that the hormones of the neurosecretory cells of brain and also of other ganglia appear to be involved in the maturation and production of eggs. However, to our knowledge, no information is on record about the involvement of these hormones in the

Columns 2 and 3 represent the average measurements (in mm) of neurosecretory cells

Stages	Cells	Nuclei
1-day-old	$0.0075 \times 0.006$	$0.003 \times 0.003$
3-day-old	$0.012 \times 0.009$	$0.0045 \times 0.0045$
5-day-old	$0.0165 \times 0.0075$	$0.0045 \times 0.0045$
8-day-old	$0.0195 \times 0.012$	$0.0075 \times 0.0075$
12-day-old	$0.0225 \times 0.018$	$0.0105 \times 0.0075$

production of larvae in the case of larviparous insects. The object of this brief communication is to throw some light on the correlation, if any, existing between the activity of the brains' neurosecretory cells and production of larvae in a larviparous flesh-fly *Sarcophaga bullata*.

Specimens of *S. bullata* were reared on mammalian kidney, sugar and water in the laboratory. The larvae pupate after 3 days of larval life. The pupal period lasts for 12 days. The adult females on their 11th/12th day of emergence from pupae lay larvae on the flesh. Brains from females of different ages were dissected out, fixed and processed for histological details, as also for the detection of nucleic acid(s), by standard methods<sup>6,7</sup>.

The neurosecretory cells occur in many places in the brain of this insect but those in pars intercerebralis are more distinct. These cells, along with their nuclei, increase in size gradually as the fly becomes old (see Table).

The cellular activities, as observed during different stages of growth of the fly, can be inferred from the figures. In a 1-day-old fly the cytoplasmic granules (CG) are perinuclear in position, leaving a clear gap along the periphery of the cell (Figure). These become uniformly distributed in the extranuclear zone (Figure 2) in a 3-day-old fly. In a 5-day-old fly, these granules tend to accumulate on the periphery of the cell leaving a perinuclear gap (Figure 3). However, in a 8-day-old fly these granules, amidst which have meanwhile developed a few vesicles, appear to be uniformly distributed (Figure 4). The progressive increase in the number of vesicles, presumably at the expense of the granules, is marked hereafter, so much so that the whole of the cytoplasm of the neurosecretory cell becomes crammed with vesicles in the 12-days-old flies (Figure 5).

The nucleus (N) is without nucleolus in the first day of the emergence of the fly but in due course the nucleoli (NU) appear in variable number in different stages

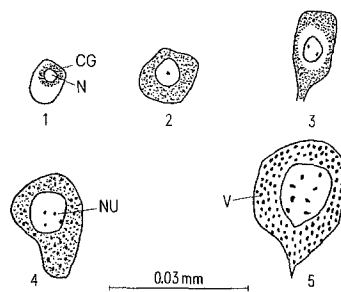


Fig. 1-5. Camera lucida diagrams of neurosecretory cells from the brain of female *S. bullata* at different stages.

(Figures 2-5). Intense reaction for RNA in cytoplasmic constituents and nucleoli, and DNA in nuclear chromatin have been observed in all the stages.

In as much as the adult fly initiates and completes larvalaying during the 11th-12th day of its emergence from the pupal phase, the observed activity of neurosecretory cells is seemingly correlated with the development and laying of larvae. The persistence of vesicles in the cells beyond the 12th-day stage indicates their implication in yet other metabolic activities.

The abundance of RNA in the neurosecretory cells of *S. bullata* suggests that synthesis of neurosecretory material is coupled with a high rate of protein synthesis. Similar results have been derived from work with *Periplaneta*<sup>5,8</sup>.

**Résumé.** L'activité des cellules neurosécrétrices dans le cerveau de la femelle de *Sarcophaga bullata* est en corrélation avec le développement des larves dans la femelle et leur position.

G. P. VERMA, C. C. DAS and N. K. TRIPATHY

Post-Graduate Department of Zoology,  
Berhampur University, Orissa (India), 4 May 1972.

- 1 B. SCHARRER, J. comp. Neurol. 74, 93 (1941).
- 2 E. THOMSEN, Nature, Lond. 161, 439 (1948).
- 3 P. KAISER, Ann. Acad. Brasl. Sci. 26, 283 (1954).
- 4 M. LÜSCHER and F. ENGELMANN, Revue suisse Zool. 62, 649 (1955).
- 5 U. S. SRIVASTAVA and O. PRASAD, Proc. natn. Acad. Sci. India, B. 35, 399 (1965).
- 6 R. FEULGEN and H. ROSSENBECK, Z. phys. Chem. 135, 203 (1924).
- 7 N. B. KURNICK, Stain Tech. 30, 213 (1955).
- 8 K. K. NAYAR, Proc. Indian Acad. Sci. B. 47, 233 (1958).

## A Radioautographic Study of Neural Induction in the Chick Embryo

GALLERA and OPRECHT<sup>1</sup> and LAVARACK<sup>2</sup> used histochemical technique to study the correlation between RNA content and morphogenesis in early chick embryos. They showed that the content of basophil cytoplasmic material was always high in morphologically active regions, e.g., Hensen's node and neural folds. LAVARACK<sup>2</sup> further showed that there was an increase in basophilia in the ventral portion of the neural groove adjacent to the notochord and that the intensity of the staining here was much greater than in the underlying notochord. These findings together with those obtained from studies on

amphibian embryos have led BRACHET<sup>3,4</sup> to suggest that induction involves a rather massive passage of ribonucleo-protein-containing particles (microsomes) from the inducer into the reacting tissue.

The present work was undertaken to examine radioautographically whether there is any marked transfer of

- 1 J. GALLERA and E. OPRECHT, Revue suisse Zool. 55, 243 (1948).
- 2 J. O. LAVARACK, J. Embryol. exp. Morph. 5, 111 (1957).
- 3 J. BRACHET, Acta biol. belg. 2, 16 (1949).
- 4 J. BRACHET, Symp. Soc. exp. Biol. 7, 207 (1947).