the front of the female she immediately rejected him by backing up and turning back and forth to avoid the head-on configuration. This rejection behaviour was duplicated exactly in courtships involving antennaeless females and normal males.

The effectiveness of female rejection behaviour is demonstrated in the Table. The mating success of glandless males or antennaeless females during a 10 min observation period was reduced to approximately $^1/_3$ that of normal moths and only 10 to 13% of the reduction was attributable to the amputation procedures (see controls). Moreover, the mating success of some of the experimentals was an artifact of the test situation. Persistant males, despite female rejection behaviour, were able to trap the females against the walls of the petri dish and achieve the head-under position. Females which were thus trapped and which had been courted repeatedly beforehand responded by raising their abdomen to the acceptance posture. This response elicited copulatory attempts from the male.

Courtship involving either of the controls was qualitatively indistinguishable from normal pairs and was quantitatively similar as well (see Table). Thus, the importance of other stimuli that might involve the male forewings or intact female antennae can be ruled out. For example, mechanical stimulation of the female antennae by the scent scales is not a likely stimulus because females with only small antennal stumps which cannot reach the glands nonetheless behave normally.

These results clearly demonstrate that the wing glands of the male Indian meal moth release a sex pheromone that is essential for successful courtship. Its function is to induce the female to remain stationary and adopt the acceptance posture. The absence of the male sex pheromone leads to a specific pattern of female rejection behaviour rather than avoidance by flight as apparently occurs in other moths[6,7].

Zusammenfassung. Die Flügeldrüsen der Männchen von *Plodia interpunctella* sondern ein Sex-Pheromon aus, das für den erfolgreichen Vollzug des Balzrituals notwendig ist. Das Pheromon bewirkt, dass das Weibchen nicht flieht und die zur Auslösung der Kopulation notwendige Bereitschaftsstellung einnimmt.

G. G. GRANT[8,9]

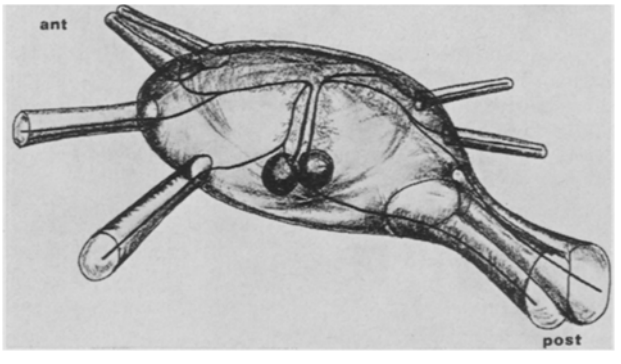
Department of Entomology, University of Georgia, Athens (Georgia, USA),
23 January 1974.

[8] This work was carried out in the laboratory of Dr. U. E. BRADY, Dept. of Entomology, University of Georgia while the author was a Postdoctoral Fellow in that department.

[9] Present address: Insect Pathology Research Institute, Environment Canada, Canadian Forestry Service, P. O. Box 490, Sault Ste. Marie, P6A 5M7 (Ontario, Canada).

Neuronal Geometry of RETZIUS Cells in *Hirudo medicinalis*

The RETZIUS cells, the two largest cells in the segmental ganglia of the leech, *Hirudo medicinalis*, have been the subject of many electrophysiological and pharmacological studies[1-5]. It has been suggested that the axons of these cells emerge only from the lateral segmental nerves and are responsible for mediating mucus release from the body wall[6,7]. LENT[6], using an intracellular Procion yellow staining technique, could not find any fibres from the RETZIUS cells in the intersegmental connectives. This was supported by the observation that high Mg^{2+} ringer blocked excitation of the cell bodies elicited by stimulation of the connectives, suggesting the presence of a synaptic input[8]. In contrast, the original results of RETZIUS, using methylene blue staining, show a fibre from each cell in the posterior connectives[1].



A 3-D reconstruction of the neuronal geometry of the paired RETZIUS cells in the segmental ganglion of the leech, *Hirudo medicinalis*. Each RETZIUS cell sends its major branches ipsilaterally, one down each of the paired lateral nerves and one down both posterior and anterior ipsilateral connectives.

In the present investigation the neuronal geometry has been studied using the technique of PITMAN, TWEEDLE and COHEN[9] which enables specific neurones to be stained black with cobalt sulphide. The electrophysiology of the cells, with special reference to their behaviour in high Mg^{2+} ringer, has confirmed these results.

Materials and methods. Both staining and electrophysiological techniques were carried out using intracellular microelectrodes made from 4 mm O.D. glass tubing.

For the staining method, electrodes were filled with 1.25 *M* cobalt chloride and had a resistance of 5–20 MΩ. Cobalt was injected into the cells by applying an 18V hyperpolarizing clamp. The injected preparation was bathed in a dilute solution of ammonium sulphide in ringer, whereupon the cobalt sulphide precipitated as a black stain. Preparations were fixed in buffered paraldehyde/glutaraldehyde, and mounted in creosote.

For the electrophysiological studies, microelectrodes were filled with 1 *M* potassium acetate (buffered to to pH 6.5) and had a resistance of 20–40 MΩ. Potentials were monitored using a conventional cathode follower and bridge circuit, and displayed on an oscilloscope or pen

[1] S. RETZIUS, *Biologische Untersuchungen*, Neue Folge II. (1891), p. 13.
[2] S. HAGIWARA and H. MORITA, J. Neurophys. *25*, 721 (1967).
[3] V. D. GERASIMOV, Symp. on Neurobiol. of Inverts (1962), p. 285.
[4] C. A. MARSDEN and G. A. KERKUT, Comp. Biochem. Physiol. *31*, 851 (1969).
[5] R. J. WALKER and P. A. SMITH, Comp. Biochem. Physiol. *45A*, 979 (1973).
[6] C. M. LENT, Comp. Biochem. Physiol. *44A*, 35 (1973).
[7] C. M. LENT, Science *179*, 693 (1973).
[8] C. M. LENT, Comp. Biochem. Physiol. *42A*, 857 (1972).
[9] R. M. PITMAN, C. D. TWEEDLE and M. J. COHEN, Science *176*, 412 (1972).

recorder. Lateral nerves and connectives were stimulated by means of tightly fitting glass suction electrodes. The normal ringer used was that of KUFFLER and POTTER[10]. High Mg^{2+} ringer contained 20 mM Mg^{2+}, and the osmolarity was maintained by removing an appropriate amount of sodium.

Results and discussion. The Figure is a reconstruction of a pair of RETZIUS cells in a segmental ganglion as seen in the cobalt-stained preparations. This technique showed that a major fibre of each RETZIUS cell is found in all the ipsilateral nerves, i.e. one in each lateral segmental nerve and one in both anterior and posterior ipsilateral connectives. Cobalt-staining shows no sign of the connection between the two cells reported by LENT[6], but shows that there is an extensive dendritic tree within the neuropile. Thus the point of electrotonic coupling described by various authors[2,3,11] may be contained within this tree rather than at a clearly-defined connection.

Stimulation of any of the lateral nerves or connectives was followed by an excitatory event in both RETZIUS cells. These events showed the characteristics of antidromic stimulation[12], i.e. they failed to fatigue, were blocked by hyperpolarization, followed the stimulation 1:1 with constant latency up to 20 Hz, and were composed of A and S spike components. These results therefore confirm that the RETZIUS cells are electrotonically coupled, and support the presence of axon branches in the lateral nerves and the anterior and posterior connectives.

The excitatory events elicited by stimulation of both lateral nerves and connectives were often blocked by high Mg^{2+} ringer, especially at low stimulus intensities. As the connection to the lateral nerves is certainly not synaptic[7] it seems that high Mg^{2+} ringer may, in addition to its effect on synaptic transmission[13], also block nerve conduction. To test this possibility, a section of connective assumed to contain no synapses was stimulated using a suction electrode. The compound AP produced was recorded extracellularly a short distance away by a second suction electrode. This AP was also reduced in the presence of high Mg^{2+} ringer, indicating a block in conduction probably associated with an effect on sodium conductance[14].

The results of cobalt injection are in contrast to the results of Procion yellow studies performed by LENT[6], who found no axon branches from the RETZIUS cells in the connectives. The electrophysiological confirmation of this depended on evidence obtained in high Mg^{2+} ringer. However, as magnesium appears to block nerve conduction as well as synaptic transmission, it is not an adequate criterion for the determination of the synaptic or antidromic nature of excitation.

Résumé. Les cellules de RETZIUS de la sangsue (*Hirudo medicinalis*) ont été teintes par injection intracellulaire de sulphide de cobalt. Chaque cellule fait émet une branche majeure dans tous les nerfs segmentaux connectifs (antérieur et postérieur) ipsilatéraux. Il y a aussi des ramifications dendritiques nombreuses dans la neuropile. Ces découvertes furent confirmées par des enregistrements électrophysiologiques en présence ou non de magnésium.

A. J. SUNDERLAND[15], P. A. SMITH[16], L. D. LEAKE[15] and R. J. WALKER[16]

Department of Biological Sciences, Portsmouth Polytechnic, Portsmouth PO1 2DY (England), and Department of Physiology and Biochemistry, Southampton University, Southampton S09 3TU (England), 8 January 1974.

[10] S. N. KUFFLER and D. D. POTTER, J. Neurophysiol. *27*, 290 (1964).
[11] R. ECKERT, J. gen. Physiol. *46*, 573 (1963).
[12] L. TAUC and G. M. HUGHES, J. gen. Physiol. *46*, 533 (1963).
[13] D. A. BAYLOR and J. G. NICHOLLS, J. Physiol., Lond. *203*, 591 (1969).
[14] Work in preparation.
[15] Department of Biological Sciences, Portsmouth Polytechnic, Portsmouth P01 2DY (England).
[16] Department of Physiology and Biochemistry, Southampton University, Southampton S09 3TU (England).

Cerebral Cortical Blood Flow in the Rat. Effect of Furosemide

An effect of furosemide on vascular smooth muscle is known to exist in the renal vascular bed[1-4] and the isolated portal vein[5]. Furthermore, the antihypertensive effect of furosemide seems to depend on a systemic vascular action. Desensitization of vascular smooth muscle to noradrenaline by furosemide is also well known[6]. The effect of the drug on blood flow and vascular resistance of cerebral cortex was tested, after topical or systemic administration, on a preparation where blood flow was measured by means of the hydrogen clearance method in rats under urethane anaesthesia.

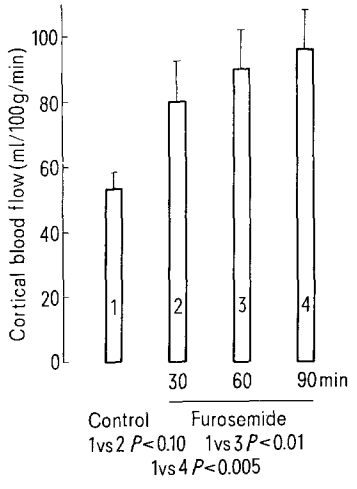


Fig. 1. Cortical blood flow before (1) and at different times after (2, 3, 4) topical application of furosemide (10 mg/ml).

Material and methods. Rats were anaesthetized with i.p. urethane (1.5 g/kg) traqueostomized and fixed to a nose clamp. The left frontoparietal cortex was exposed and a platinized-platinum electrode inserted there. The platinum electrode, and an indifferent silver-silver

[1] J. B. HOOK, A. H. BLATT, M. J. BRODY and H. E. WILLIAMSON, J. Pharmac. exp. Ther. *154*, 667 (1966).
[2] A. G. BIRTCH, R. M. ZACKEIM, L. G. JONES and A. C. BARGER, Circulation Res. *21*, 869 (1967).
[3] J. H. LUDENS, J. B. HOOK, M. J. BRODY and H. E. WILLIAMSON, J. Pharmac. exp. Ther. *163*, 456 (1968).
[4] C. VORBURGER, A. M. HARVEY and R. L. MALVIN, Arch. Pharmak. exp. Path. *261*, 346 (1968).
[5] J. R. BLAIR-WEST, M. J. MCKINLEY and J. S. MACKENZIE, J. Pharm. Pharmac. *24*, 442 (1972).
[6] M. F. LOCKETT and T. E. NICHOLAS, Br. J. Pharmac. Chemother. *33*, 136 (1968).