of both castrated and non-castrated male adults. These facts indicate that part of the BH accumulated in the female pupal body may be consumed for egg maturation and/or yolk deposition.

Discussion. Generally speaking, the amount of BH we obtained was much higher than ISHIZAKI's[10]. Besides his races employed were different from ours, and he dialyzed the BH preparations. As nearly $^1/_3$ of the active fractions of BH would escape from the cellulose tubing after dialyzation[9], we did not dialyze our BH preparations. These are some reasons why our BH titer was generally much higher than his.

The present experiments showed that the amount of BH in the thorax-abdomen was highest in the young pupae regardless of races. Thereafter it gradually decreased through the newly emerged adult stage and markedly diminished after mating. On the other hand, according to ISHIZAKI[10] the striking increase of BH quantity appears only in the females thorax-abdomen during adult development and reached a maximum level at the time of adult emergence. Although our silkworms were of different races from his, it seems unlikely that liberation of BH from the brain into the body continues during the pupal period. It is quite probable that shortly after the pupation, liberation of both BH and ecdysone[12] from the endocrine organs reach a maximum level, and thereafter, prothoracic glands degenerate and the brain maintains its secretory activity in the neurosecretory cells without liberation of BH until emergence. This hypothesis is also supported by the result of the debraining experiment in which the BH titer of moths newly emerged from the debrained pupae was exactly the same as the normal control.

Neurohormone from the brain appears directly or indirectly to control the egg maturation in some insects[13]. This is also supported by the present castration experiment in which the large amount of BH accumulating in the young female pupal body was consumed by the egg maturation and/or yolk deposition processes.

Male pupae had a considerable amount of BH in their bodies even if the titer was $^1/_3$ of the female one and activity of BH disappeared at the time of emergence. This fact may suggest that some BH is consumed during the adult development of both males and females.

The hormone(s) secreted in the neurosecretory cells of the brain is said to have a multiplicity of effects, such as moult inhibiting[14], tanning[15], and melanotropic[16] effects, besides prothoracotrophic and gonadotropic ones. However, whether the so-called 'Brain Hormone' is a hormone having various hormonal actions, or is a hormone complex, remains unanswered. At present, it seems hardly possible make any definite conclusions because no hormone has yet been isolated. The facts that the brains of developing adults secrete (or at least reserve) a hormone having ecdysiotropic action after the prothoracic glands have degenerated and a hormone having the same ecdysiotropic activity is liberated into and accumulated in the body before or just after pupation and consumed during egg development and copulation, suggest that BH at least as the thoracotrophic hormone has diversified actions.

In adult lepidoptera, juvenile hormone activity is higher in the male body[17] and ecdysone activity[12] as BH is higher in the female body; these hormones have no apparent hormonal roles in the adult stage. At present, we can only suggest, without clear proof, what is actually happening within the insect body.

Summary. The present report is concerned with the comparison of the brain hormone titers contained in the head and thorax-abdomen of both sexes of 3 races of silkworms, *Bombyx mori* during and post imaginal differentiation and also with the effects of debraining and castration on the brain hormone titer in the body.

J. NISHIITSUTSUJI-UWO and M. S. NISHIMURA[18]

Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka, 553 (Japan), 20 March 1975.

[12] E. SHAAYA and P. KARLSON, Devel. Biol. *11*, 424 (1965).
[13] W. W. DOANE, in *Development Systems* (Eds. S. J. COUNCE and C. H. WADDINGTON, Academic Press, New York 1973), vol. 2, p. 291.
[14] D. B. CARLISLE and P. E. ELLIS, Nature, Lond. *220*, 706 (1968).
[15] G. FRAENKEL and C. HSIAO, J. Insect Physiol. *11*, 513 (1965).
[16] A. GIRARDIE, Bull Biol. Fr. Belg. *101*, 79 (1967).
[17] L. I. GILBERT and H. A. SCHNEIDERMAN, Gen. comp. Endocr. *1*, 453 (1961).
[18] Acknowledgments. We are grateful to Dr. M. MEZAKI of Gunze & Co., Ltd. and Dr. T. HIRASAKA of Shinei & Co., Ltd. for supply of the special races of silkworms. We wish to acknowledge Miss A. OSHAWA for her technical assistance.

PRO EXPERIMENTIS

A Bipolar Stimulation Electrode for in vitro Stimulation of Small Pieces of Electric Organ of *Torpedo marmorata*

An increasing number of biological studies require qualitative and quantitative measurements of changes of vesicles in cholinergic synapses of *Torpedo marmorata* with reference to their density and diameter under the influence of drugs. To find out whether the number and size of vesicles in stimulated and unstimulated organs differ, the main nerves of the electric lobe were stimulated with repeated trains (DUNANT[1], NAEF[2]). As it is not possible to apply the drugs into the blood-circulatory system, they were poured directly into the electric organ. This method needs a large amount of drug solution and it cannot be assumed with certainty that the organ is throughly soaked with it.

In this short communication, an electrode system for direct stimulation and signal detection is described. With this system, it is possible to stimulate in vitro small (4 mm^3) as well as bigger (1 cm^3) pieces of electric organ of *Torpedo marmorata* (Figure 1).

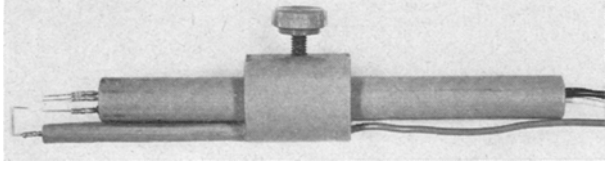
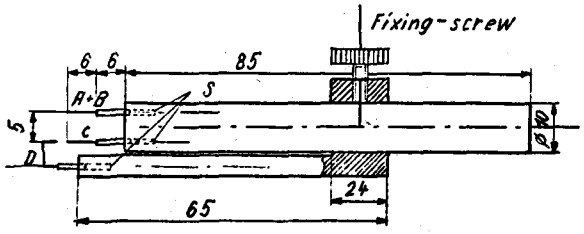


Fig. 1. Photograph of the electrode. ×0.5

[1] Y. DUNANT, M. GAUTRON, B. LESBATS and R. MANARANCHE, J. Neurochem. *19*, 1987 (1972).
[2] W. NAEF and P. G. WASER, in *Cholinergic Mechanisms* (Ed. P. G. WASER; Raven Press, New York 1975), p. 67.



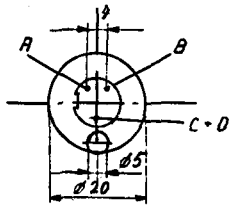


Fig. 2. Drawing of the stimulation electrode. All the dimensions are in mm. S, sockets; A, B, stimulation electrodes; B, C, signal detection electrodes.

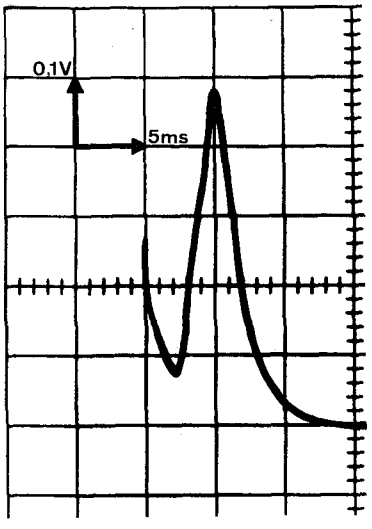


Fig. 3. Response of a piece of the electric organ stimulated directly in vitro (stimulation parameters: 100 V, 1.5 msec duration, 10 pps).

Principle. The sockets (S) of the bipolar stimulation electrodes and the signal detection electrodes are embedded with Araldite in a PVC tube and are localized on an area of 12 mm² (Figure 2). For the bipolar stimulation electrodes (A, B) and the 2 monopolar signal detection electrodes (C, D) non-insulated stainless steel electrodes with a diameter of 0.2 mm were used. The second signal detection electrode (D) connected with the mass of the organ piece can be changed with respect to its position. It has the great advantage of being able to stimulate organ pieces of different sizes in very small amounts of drug solution. All the electrodes are exchangeable by Amphenol plugs of the type 220–PO2–100. The response of a directly stimulated organ piece with this stimulation electrode is represented in Figure 3.

Zusammenfassung. Ein Elektrodensystem wird beschrieben, um kleinere und grössere Teile des Elektro-Organs von *Torpedo marmorata* direkt in vitro stimulieren und ableiten zu können.

W. NAEF, K. A. WÜTHRICH and P. G. WASER

Institute of Pharmacology, University of Zürich, Gloriastrasse 32, CH–8006 Zürich (Switzerland), 3 April 1975.

Preparation of Plant Material for Microautoradiography and Electron-Probe-Microanalysis: The Xylene Technique

Localization of ions in plant cells is usually based either on micro-autoradiography, on electron microscopy or on electron-probe-microanalysis (EPMA). In order to avoid any possible changes in the location of the examined ions during fixation and analysis, preparation must be made under such conditions as prevent their displacement.

The techniques used for preparation of plant material for electronprobe microanalysis and microautoradiography are usually freeze-drying[1–3] or freeze substitution[4,5]. Although freeze drying yields reasonable results in EPMA[6] and theoretically has an advantage over all other techniques, it is known to cause some tissue distortion[7,8], particularly at the subcellular level. On the other hand, freeze-substitution, which includes embedding of plant tissues in epoxy resin, suffers from uncertainties regarding the behaviour of ions during the dehydration procedure and is suspected of allowing some ionic movement[5,9,10]. In the present paper, a technique is described for preparation of high quality sections from frozen specimens which avoids both distortion of tissues and ion movement.

Segments of fresh leaves of *Panicum repens* L. and of *Cucurbita pepo* L. petiols were frozen and sectioned. Cross as well as longitudinal sections (8, 10, 15 20 and 40 µm thick) were cut in a cryostat at −20 °C. The frozen sections were placed on cold (−20 °C) aluminium plates precovered by chromalum glue ($CrK(SO_4)_2 \cdot 12H_2O$). Drops of cold (−20 °C) xylene were placed on top of the frozen sections. The sections were then left to dry overnight under the same conditions. The xylene drops evaporated after 2 h whereas it took a few hours before the ice sublimated. The material was deeply frozen throughout the procedure. The dried sections were then transferred to a dessicator at room temperature and left there for 1 h. Sections for EPMA were then coated with evaporated gold-paladium (60%/40%). Electron-probe X-ray microanalysis was conducted with a JXA-3A X-ray microanalyzer[6].

Displacement of soluble ions or their diffusion out of treated tissues during treatment with xylene was tested

[1] A. LÄUCHLI and U. LÜTTGE, Planta *83*, 80 (1968).
[2] A. J. TOUSIMIS, Proc. 2nd A. SEM Symp. IITRI, Chicago (Ed. O. JOHARI; Illinois Inst. of Technol. Res. Inst., Chicago 1969), p. 217–229.
[3] F. D. INGRAM, M. J. INGRAM and C. A. M. HOGBEN, J. Histochem. Cytochem. *20*, 716 (1972).
[4] A. LÄUCHLI, A. SPURR and R. W. WITTKOPP, Planta *95*, 341 (1970).
[5] C. K. PALLAGHY, Austr. J. biol. Sci. *26*, 1015 (1973).
[6] A. RAMATI, A. ESHEL, N. LIPHSCHITZ and Y. WAISEL, Experientia *29*, 497 (1973).
[7] R. L. BIELESKI, Pl. Physiol. *41*, 455 (1966).
[8] P. TRIP and P. R. GORHAM, Can. J. Bot. *45*, 1567 (1969).
[9] D. B. FISHER, Pl. Physiol. *49*, 161 (1972).
[10] D. B. FISHER and T. H. HOUSLEY, Pl. Physiol. *49*, 166 (1972).