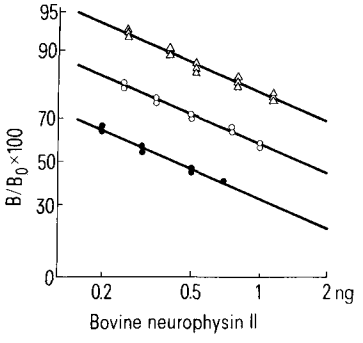
The following procedure for RIA was applied. To 0.05 M Tris-HCl buffer pH 7.5 containing 0.5% of bovine serum albumin, 10 to 100 µl of the samples and 10 µl of the antibody (final dilution 1:20,000) were added. After 2 days incubation at 4 °C the labelled [^{125}I]-bNII was added (final volume of 0.5 ml). The antibody-bound [^{125}I]-bNII was separated from free [^{125}I]-bNII by precipitation with 3% zirconyl phosphate[5].

Results. 19 media of incubated pineal cells from rat fetuses were assayed for the presence of neurophysin by RIA. We have found that these samples displaced the labelled pituitary bNII in the same manner as the homogenate of rat posterior pituitary lobe.

Expressed in pg neurophysin per mg proteins, we obtained a mean value of 442±103 (SD) pg/mg. This is significantly higher than the mean value of the control samples: 229±12 (SD) pg/mg (probably due to neurophysin present in calf serum). Some caution is required in the interpretation of the quantiative aspect of these data, as they are based on RIA with highly purified bovine pituitary neurophysin without knowledge of the relative affinity of the rat neurophysin to the antibody used. The dilution curves performed with a pool of the samples are parallel to the standard curve with bNII and the dilution curve of rat pituitary homogenate (figure).



Dilution curves of cell culture media from pineal glands of rat fetuses (△), and of crude homogenate of rat posterior pituitary gland (●), in comparison with the standard curve of bovine neurophysin II (○) determined by radioimmunoassay (logit-log plot). The quantity of culture media ranged from 0.2 to 2 mg and that of posterior pituitary homogenate from 20 to 70 µl (1 lobe in 0.5 ml).

Discussion. The demonstration by Pavel[4] that the mammalian pineal gland contained vasotocin prompted our own studies, in which we detected neurohormone carrier proteins first in the bovine[1] then in the human gland[2] and now in the media of cultured ependymal cells from rat fetuses. The present data, in which the quantity of neurophysin in incubated cell media was significantly greater than that of control, suggest that the pineal gland is capable of releasing in vitro neurophysin, which probably represent the carrier protein of vasotocin. Pavel has already demonstrated that rat and human pineal cells can synthesize vasotocin in vitro. Our demonstration that the pineal gland releases neurophysin is another piece of evidence indicating that this gland contains, releases and probably synthesizes the complete set of at least 1 neurohormone and its carrier protein or remnant of precursor form. The physiological significance of this activity remains to be established.

1 A.C. Reinharz, P. Czernichow and M.B. Vallotton, J. Endocr. *62*, 35 (1974).
2 A.C. Reinharz and M.B. Vallotton, Endocrinology *100*, 994 (1977).
3 S. Pavel, R. Goldstein, E. Ghinea and M. Calb, Endocrinology *100*, 205 (1977).
4 S. Pavel, Endocrinology *77*, 812 (1965).
5 M.C. Tissot-Berthet, A.C. Reinharz and M.B. Vallotton, Ann. N.Y. Acad. Sci. *248*, 257 (1975).
6 H. Sachs, P. Fawcett, Y. Takabatake and R. Portanova, Rec. Prog. Horm. Res. *25*, 447 (1969).
7 S. Pavel, M. Dorcescu, R. Petrescu-Holban and E. Ghinea, Science, N.Y. *181*, 1252 (1973).

Role of the nerve cord in the control of prothoracic glands in *Galleria mellonella* L.

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Summary. Certain lesions of the nerve cord in postfeeding larvae impede pupation by altering the proprioreceptive input, which modulates the prothoracicotropic activity of the brain. Direct influence of ventral ganglia on prothoracic glands is negligible.

Pupation of the wax moth is delayed or prevented after transection of the ventral nerve cord or destruction of ventral ganglia[1-4]. Edwards[2] concluded that such lesions blocked the activation of prothoracic glands (PTG) by the brain, but Alexander[3] assumed that PTG were inhibited via the suboesophageal ganglion. Since we found that also the thoracic ganglia may play a role in the control of PTG[5], we have re-examined significance of different ganglia for the onset of metamorphosis.

Material and methods. We used our stock culture of *Galleria* and employed standard surgical techniques[5]. The age of larvae was known within ±8 h after preceding ecdysis. Most experiments were performed with wandering larvae (132 h after the last larval ecdysis). The operations involved removal of the brain, suboesophageal (SG), thoracic (T_1, T_2, T_3) and abdominal (A_1–A_8) ganglia and sections of the connectives. Control insects were subjected to injuries identical to those inflicted during operations but the nerve cord was not touched. Each experiment was performed on 15–25 larvae. The percentage of pupating insects and the length of time between operation and ecdysis (mean time ± SD in days) were recorded.

Results and discussion. Larvae with severed nerve cord often shrank anteriory to the cut whereas their posterior segments appeared normal or slightly swollen[2]. Sectioning reduced mobility and spinning of larvae, in particular when the cord was cut in the region between T_2 and A_3. In accordance with previous authors[2-4], we found that this treatment inhibited pupation in a considerable number of the larvae and delayed pupal ecdysis in the remainder

(figure). The pupae formed were externally perfect. The non-pupating insects often died after a month. Removal of one ganglion affected pupation similarly as a single cut inflicted anteriory to the respective ganglion. Extirpation of the fused A_7-A_8, however, was lethal. Section of nerves between A_8 and rectum and ligation of the body in this region were also lethal, apparently due to disturbances in excretion[4]. The sham injuries prevented or delayed pupation of control insects to a much lesser extent than the cord lesions, but in similar relation to the position of the wound on the body axis (figure). This effect propably reflected the trauma caused by integumental injuries in the adjacent portion of the nerve cord.

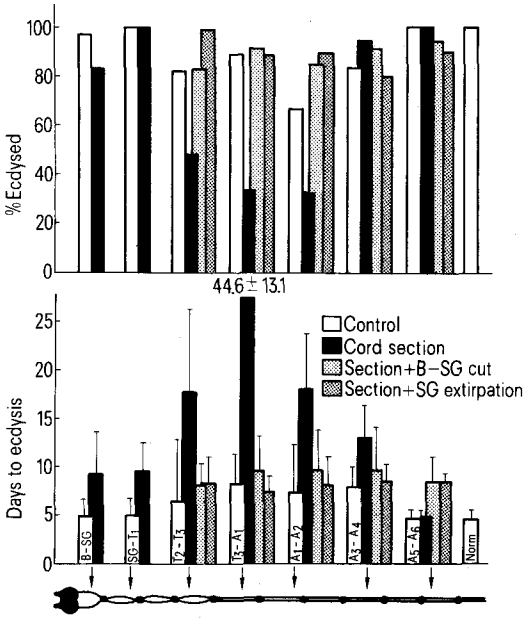
Transection of connectives between T_3 and A_1 inhibited pupation as effectively as decapitation: 84% of wandering larvae pupated in average 42.4 days after decapitation[5] and 33% of the larvae whose nerve cord was cut accomplished pupation in 44.6±13.1 days. From this it seemed that the cord section between T_3 and A_1 permanently prevented release of the prothoracicotropic hormone from the brain and that PTG were activated spontaneously as in decapitated larvae[5,6]. Other cord cuts would have only temporally inhibitory effect. This conclusion was tested by implanting brains from control larvae into those with the nerve cord severed between T_3 and A_1. The incidence of pupation increased to 67% and the ecdysis occurred in 17.6±4.4 days. These figures are comparable with the results of brain implantations into decapitated larvae (87% pupating in 17.1 days)[5] and confirm that the cord section inhibited the prothoracicotropic activity of the brain.

In order to elucidate the role of SG and thoracic ganglia in the control of PTG, we compared effects of their removal with the results of simple nerve cord severance. If PTG were inhibited by SG or thoracic ganglia, then only their removal should remedy the effect of distal cord injuries. Data summarized in the figure show, however, that both the section of connectives between brain and SG and extirpation of SG eliminated the effect of cuts between T_2 and A_3. In fact, any treatment isolating the brain from injured ganglia suppressed their adverse influence on pupation (table 1). Sham operations in the neck region did not abolish the effect of distal cord lesions.

For mathematical evaluation of our results, we cumulated data obtained with larvae whose nerve cord was severed at T_2-T_3, T_3-A_1, and A_1-A_2, respectively. When the cord was simultaneously cut between brain and SG, 86% of the larvae ecdysed as pupae in 9.1±3.0 days; removal of SG induced 90% of the larvae to pupate in 8.1±2.3 days, and removal of T_1 and T_2 caused 92% of them to ecdyse in 8.3±3.0 days. The difference in time of pupal ecdysis between the 1st and 2nd groups was on the margin of significance according to the t-test ($\rho = 0.05$), other differences were insignificant. The calculations thus confirmed that brain is the major controlling centre of PTG whereas

Table 1. Influence of different operations on the rate of pupation of larvae with the nerve cord cut between the metathoracic and first abdominal ganglion

Operation	Number of larvae	Pupated (%)	Days to pupal ecdysis $\bar{X} \pm SD$
None	21	33	44.6± 13.1
Cut between brain-SG	22	96	9.6± 3.6
SG removed	18	89	7.8± 1.8
Cut between SG-T_1	19	95	9.8± 2.7
T_1 and T_2 removed	20	95	8.2± 2.8
SG, T_1 and T_2 removed	17	82	11.2± 1.7
T_3, A_1 and A_2 removed	18	67	19.1± 2.9



Incidence and time of pupal ecdysis in wandering larvae subjected to integumental injuries (empty columns) or to operations at the nerve cord: the cord was either cut only at the points indicated with arrows (black columns), or at these points and between brain and suboesophageal ganglion (columns with tiny dots), and/or it was cut at indicated points but the suboesophageal ganglion was removed (columns with large dots). The thin bars indicate SD from the mean times of ecdysis.

Table 2. Moulting of penultimate (VI) and last (VII) instar larvae with the nerve cord injured at indicated h after ecdysis

Operation	Time	Number	Ecdysed (%)	Days to ecdysis $\bar{X} \pm SD$	Remark
SG removed	VI/6	15	0	-	Dead in 4.4 days
T_3-A_1 cut	VI/12	16	75	13.1± 5.7	Larval ecdysis
			54	35.9± 8.1	Extra larval ecdysis
SG removed	VI/36	16	0	-	Dead in 6.1 days
Brain-SG cut	VI/36	15	0	-	Dead in 5.0 days
None	VI/0	15	100	3.8± 0.5	Last larval ecdysis
SG removed	VII/6	21	0	-	Dead in 4.7 days
T_3-A_1 cut	VII/6	20	70	29.2± 10.2	Pupal ecdysis
A_5-A_6 cut	VII/6	19	95	9.4± 2.0	Pupal ecdysis
SG removed	VII/36	20	50	11.4± 3.4	Pupal ecdysis
Brain-SG cut	VII/36	21	57	9.9± 1.8	Pupal ecdysis
SG-T_1 cut	VII/36	27	45	12.3± 5.9	Pupal ecdysis
None	VII/0	20	100	8.6± 0.8	Pupal ecdysis

SG and thoracic ganglia play only a negligible and little understood role[5,7].

We can conclude that release of the prothoracicotropic hormone occurs in response to integration of stimuli transmitted largely via the nerve cord. For example, immobilization of wandering larvae caused that only 22% of the insects pupated in 17.8±3.3 days, but when the connectives between brain and SG were cut, 50% of the insects pupated in 11.3±3.6 days. Significance of individual ganglia changes during the development. The complex of brain and SG is indispensable for the food intake and its damage before the larvae finish obligatory feeding is lethal[4]. Removal of SG or cut between brain and SG were lethal also when performed at the end of the feeding period in the penultimate instar (table 2). We have no explanation for this effect. Transection of the nerve cord between T_3 and A_1 inflicted during the penultimate or at the start of the last larval instar prolonged the respective interecdysial period less than when inflicted to wandering larvae. Larvae subjected to this operation in the penultimate instar mostly underwent a supernumerary larval ecdysis, apparently as a result of suppressed body growth; starvation readily induces extra larval moults in *Galleria*[8]. High importance of the integrity of the last thoracic and the 1st few abdominal ganglia in wandering larvae may be related to the fuse of these ganglia during the larval-pupal transformation[9].

1 J.J. Bounhiol, Bull. Biol., suppl. *24,* 1 (1938).
2 J.S. Edwards, J. Insect Physiol. *12,* 1423 (1966).
3 N.J. Alexander, J. Insect Physiol. *16,* 271 (1970).
4 S.D. Beck, Ann. ent. Soc. Am. *63,* 144 (1970).
5 J. Malá, N.A. Granger and F. Sehnal, J. Insect Physiol. *23,* 309 (1977).
6 D. Gibbs and L.M. Riddiford, J. exp. Biol. *66,* 255 (1977).
7 K.P. Srivastava, R.K. Tiwari and P. Kumar, Experientia *33,* 98 (1977).
8 F. Sehnal, Z. wiss. Zool. *174,* 53 (1966).
9 R.L. Pipa, Biol. Bull. *124,* 293 (1963).

Lack of relationship between Langerhans cells, epidermal cell proliferation and epidermal G1 chalone

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Summary. Mouse tail interscale epidermis contains numerous Langerhans cells, whereas the adjacent scale regions are devoid of these cells. No difference in a) proliferative activity and b) inhibitory effect of the epidermal G1 chalone can be demonstrated in both regions. A direct relationship between Langerhans cells and growth control may be excluded.

Investigations in the past years have adequately demonstrated that the high-level dendritic Langerhans cells in the epidermis constitute an active, self-maintaining cell population[1], which is not related to the pigmentary system[2,3] but whose biological significance is not yet entirely understood. Since there seems to be an inverse relationship between the frequency of Langerhans cells and the proliferative rate of the corresponding tissue[4-7], they have been supposed to be involved in the control of proliferation or tissue homoestasis in keratinizing epithelia[3].

A similar function has been attributed to a family of endogenous growth inhibitors, the chalones[8]. It has been suggested, therefore, that Langerhans cells may synthesize and secrete chalonelike epidermal growth inhibitors[5,9]. In this context, especially the Langerhans cell granule has been supposed to be the site of production of those inhibitors, since the changes in their frequency per cell could be correlated to the kinetics of cell proliferation during wound healing in epidermis[10]. According to the original chalone concept, the increased proliferative activity in the vicinity of wounds is thought to be due to a local depression of the chalone level[8]. Also, after wounding the relative frequency of Langerhans cells is transiently decreased[10] and the normal frequency is attained only when the epidermal continuity has been reestablished[11]. Furthermore, both Langerhans cells and the chalones are encountered only in keratinizing epithelia[12,13]. Mature, granule-containing Langerhans cells are located predominantly in the suprabasal layers of keratinizing tissues, and the epidermal G1 chalone which controls the G1-S transition is apparantly concentrated in the same region[14].

These considerations seemed sufficiently plausible to investigate whether a direct relationship between Langerhans cells and the epidermal G1 chalone which is thought to be the physiologically more important of the 2 epidermal chalones[8], could be proved. Since it is not possible to separate Langerhans cells from keratinocytes to check their possible chalone content, a more indirect approach had to be made. Recently, we were able to show that adult mouse tail epidermis consists of a regularly alternating pattern of parakeratotic scale resions without Langerhans cells and orthokeratotic interscale regions in which Langerhans cells are abundant[6] (figures 1-3). Provided there is any relationship between Langerhans cells and growth control, especially the chalone mechanism, one should predict a measur-

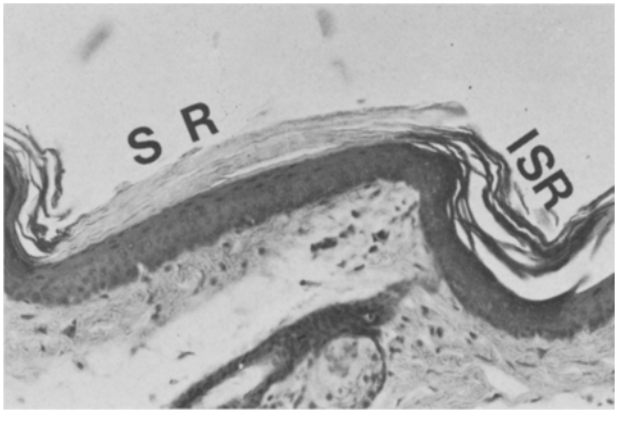

Fig. 1. Vertical section through adult mouse tail skin (H&E; × 110). SR: Langerhans cell-free parakeratotic scale region. ISR: Langerhans cell-containing orthokeratotic interscale region. Note the clear-cut transition between the 2 areas, which can be followed up to the keratin layer.