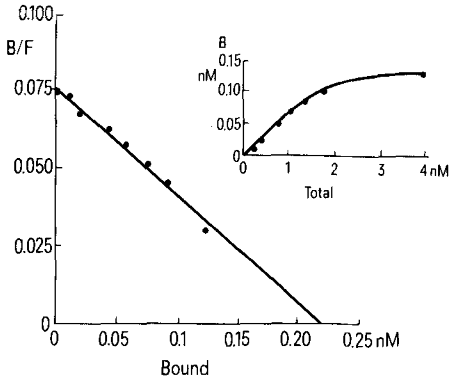
complex of the foetal plasma processed in the same experimental conditions. The number of specific progesterone binding sites after incubation of $^3$H-progesterone ($4 \times 10^{-9}$ M) with the cytosol fraction of the foetal uterus is 29.2±2.9 fmoles/mg protein at 48-52 days of gestation and increases to 66.2±3.8 at 60-65 days of gestation (4 experiments). At this period of gestation, the number of oestradiol receptors in the foetal uterus of guinea-pig is very high[2], which suggested that oestradiol could stimulate the progesterone receptors in the foetal uterus at the end of gestation. To demonstrate this, we first observed that tritiated oestradiol injected to the mother could cross the placenta and reach the foetus (0.20-0.30% of the injected radioactivity was found in each foetus, 30 min after s.c. injection of 50 μCi of $^3$H-oestradiol to the mother, from which 10-15% of the total radioactivity in the foetus was found as unmetabolized oestradiol). Following this, 1 mg/kg/day of oestradiol was injected to pregnant guinea-pigs for 3 days and the quantity of specific progesterone binding sites were evaluated in the treated and in the control animals. Table 1 indicates the effect on the weight of the foetal uterus and table 2 indicates the quantitative values of $^3$H-progesterone receptors. As is shown, there is a significant increase in the weight of the foetal uterus and the number of progesterone receptors increases 4-7 times. It is concluded that the response effect of oestradiol on the production of uterine progesterone receptors is present during foetal life.



Scatchard plot of the $^3$H-progesterone specific binding in the cytosol fraction of the foetal uterus of guinea-pig. Uterine cytosols of the foetal guinea-pig (55-64 days of gestation) containing 1.5 mg of protein (ml) were incubated with various concentrations of $^3$H-progesterone ($1.65\text{-}40.0 \times 10^{-10}$ M) in the absence or presence of $4 \times 10^{-7}$ M of unlabelled progesterone for 4 h at 4 °C. Specific binding was calculated using the charcoal-dextran method.

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## Lack of specific neurons in the ventral nerve cord for the control of prothoracic glands[1]

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*Summary.* There is a distinct serial homology in the distribution of afferent neurons in different ventral ganglia of wax moth larvae. No neurons specific to the thoracic ganglia which innervate the prothoracic glands were found. 2 peptidergic cells in the suboesophageal and 4 such cells in the prothoracic ganglion probably send axons to the glands, but seem to release their secretion also at other sites. Specific control of prothoracic glands is possible providing that liberation of neurohormones is regulated at the axonal level.

Function of the prothoracic glands (PTG) of the larvae of *Galleria mellonella* L. is stimulated by prothoracicotropic neurohormone from the brain and inhibited by unknown factor(s) from the prothoracic and mesothoracic ganglia[3,4]. It is considered that under physiological conditions this factor is transported to PTG via the peptidergic neuraxons which terminate within the glands[5-7]. The glands are innervated from the suboesophageal (SG), prothoracic ($T_1$) and mesothoracic ($T_2$) ganglia by nerves which branch to dorsal body wall musculature, stigmata, tracheae, gut, fat body, and apparently other viscera (figure 1). In an effort to find neurons innervating specifically the PTG, we compared locations of central perikarya of these nerves[8] with those of homologous nerves in the metathoracic ($T_3$) and several abdominal ganglia ($A_1$, $A_4$, $A_5$) which do not innervate PTG.

*Material and methods.* The neurons were visualized by means of cobalt filling technique[9,10]. Ganglia dissected from fully grown larvae were placed in cold Grace's medium or *Galleria* saline (20 mM NaCl, 30 mM KCl, 15 mM $MgCl_2$, 8 mM $CaCl_2$, 5 mM $NaHCO_3$, 5 mM $KH_2PO_4$, 220 mM sucrose) and the nerve to be filled was dipped across a fine oil barrier into 0.3 M $CoCl_2$ in saline. After 14-24 h incubation, the preparation was washed with saline and immersed for 10-30 min in 1% glutaraldehyde solution made by dilution of 3% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.2) with the saline to which was added 2% sucrose. Preparation was then treated with 5-10% $(NH_4)_2S$ in saline, fixed in 3% glutaraldehyde for 2 h, transferred in cacodylate buffer to room temperature, dehydrated through ethanol solutions (30, 50 and 70% solutions were adjusted to pH 7.2 with phosphate buffer (0.1 M) and 90 and 96% solutions with solid TRIS) and propylene oxide, and embedded in Durcupan. No detectable loss of CoS occurred in blocks stored for several months. Preservation of the tissue was sufficient also for EM studies.

*Results and discussion.* The cervical nerve of SG (figure 1) contains axons of 3 ventromedial neurons (group 1 in figure 2) and centripetal axons terminating ipsilaterally in SG. No connection between right and left cervical nerves

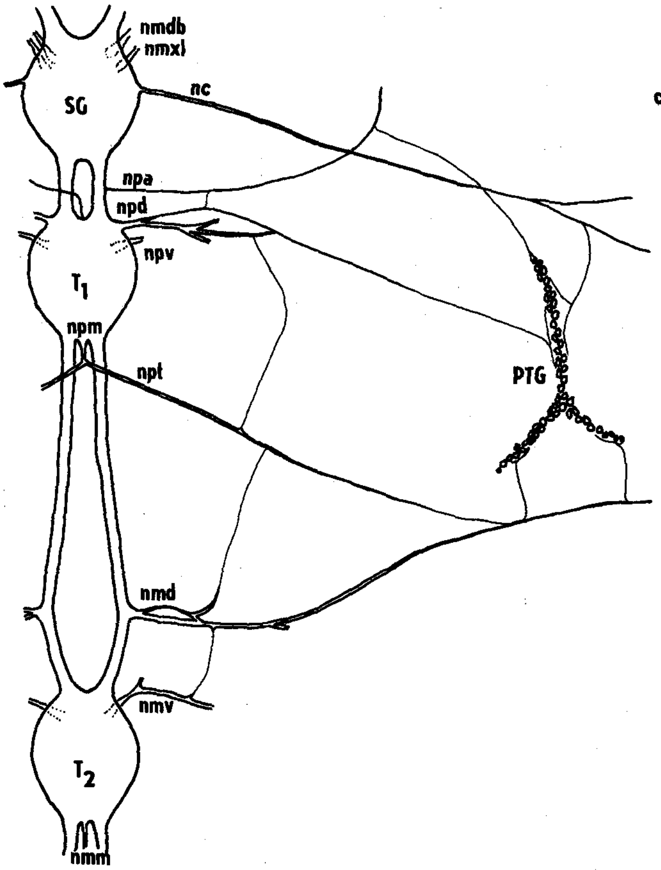

Fig. 1. Nerve roots of the suboesophageal (SG), prothoracic ($T_1$) and mesothoracic ($T_2$) ganglia and the nerve branches to prothoracic glands (PTG). nmdb: nervus mandibularis, nmxl: n. maxillaris, nc: n. cervicallis, npa: n. prothoracalis anterior, npv: n.p. ventralis, npd: n.p. dorsalis, npm: n.p. medianus, npt: n.p. transversus, nmd: n. mesothoracalis dorsalis, nmv: n.m. ventralis, nmm: n.m. medianus. Nerve ramifications in different specimens vary greatly from the situation shown on the picture.

was found. The neurons of group 1 send axonal branches to $T_1$ where they either enter the median nerve or end in close contact with axons of cells of groups 2 and 3.

The median nerves of all thoracic ganglia contain axons from 1 or 2 ventromedial (group 2) and 2 ventrolateral (group 3) neurons in each hemisphere (figure 2). The axons bifurcate into both transverse nerves. Abdominal ganglia lack group 3 and group 2 is usually reduced to 1 cell in each hemisphere.

Most axons from the dorsal nerve terminate or originate in the proper or the next proximal ganglion, but a few pass further up and a few further down the nerve cord, and 1 axon enters the contralateral dorsal nerve (figure 2). The axons from the proximal ganglion come from 2 or 3 dorsal, 1 lateral, and 2 ventral groups of neurons. The central dorsal group 4 and the posterior dorsal group 5 each consist of 1 cell in all ganglia, whereas 2 neurons designated as group 7 occur only on the ipsilateral dorsal side of $A_4$. The contralateral group 6 contains either 1 (SG, $T_1$, $T_2$) or 2 ($T_3$, $A_4$) neurons. The prominent ventral posterior group 8 contains 7-10 cells divided in 2 subgroups. 1 or 2 neurons of group 9 in the central ventral region are occasionally in the immediate vicinity of group 8.

In the proper ganglion of the dorsal nerve, some axonal branches form a network within the ipsilateral hemisphere and the perikarya are arranged in 4 distinct groups (10-13).

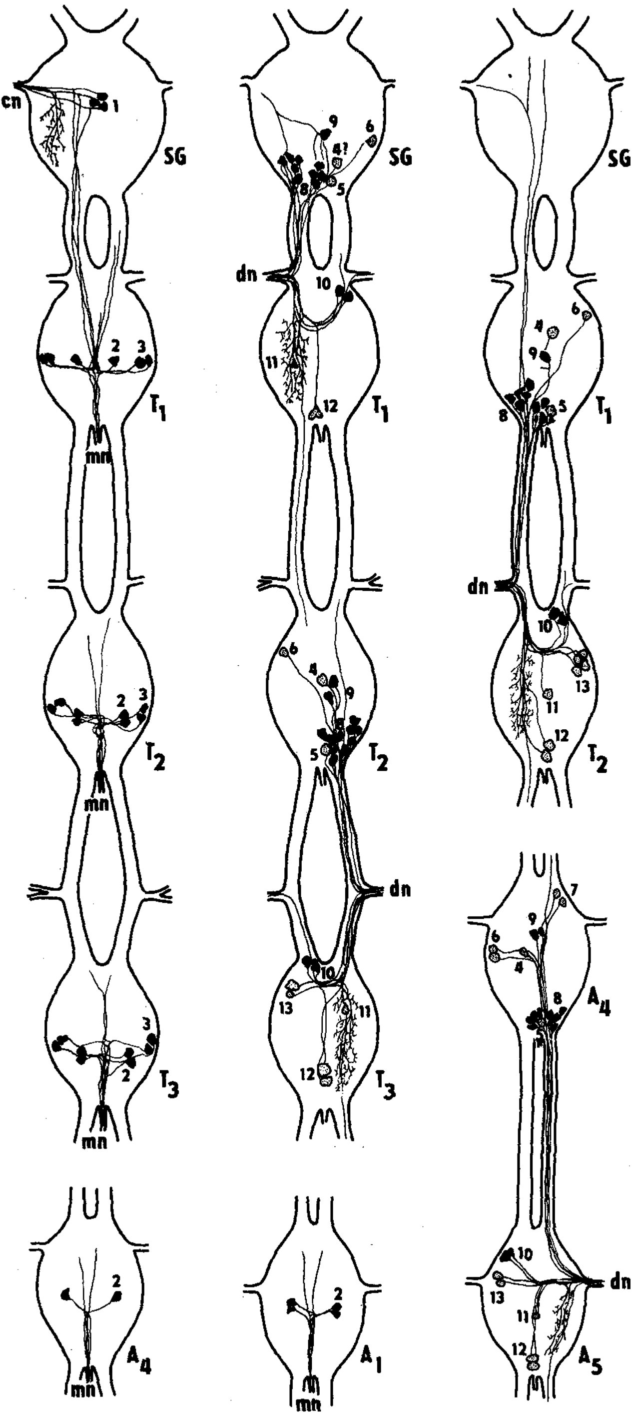

Fig. 2. Neurons and axonal pathways in ventral ganglia as visualized by cobalt filling of the cervical nerve (cn) of SG and of the dorsal (dn) and medial (mn) nerves of the thoracic ($T_1$, $T_2$, $T_3$) and abdominal ($A_1$, $A_3$, $A_4$) ganglia. Neurons located dorsally within the ganglia are stippled, those located ventrally are black. Homologous neurons are marked with identical numbers.

Their distribution is very uniform in all ganglia studied, except for $T_1$ which lacks the dorsal group 13 (figure 2). This group usually consists of 2 neurons, but in $T_2$ it contains 4 cells. Neurons of group 12 send axons both to the left and right dorsal nerves.

Location of perikarya and the course of axonal pathways in different ganglia show a great degree of serial homology (figure 2). The arrangement follows a pattern found also in other insects, in which the function of some of the neurons has been elucidated. In *Manduca sexta,* neurons of groups 8 and 10 are presumably motoric and innervate the dorsal musculature[11]. Unpaired median neurons in the metathoracic ganglion of locusts, corresponding probably to those of group 12 in figure 2, were shown to produce octopamine and to inhibit the spontaneous contractions of the tibial extensor[12]. Cells of groups 2 or 3 apparently produce bursicon which is found within both the thoracic and abdominal transverse nerves[13,14]. No information is available on the role of other neurons shown in figure 2.

Since peptidergic neurosecretory granules, but no true cholinergic synaptic contacts, are seen in axon terminals within PTG of *Galleria*[5-7], the cells innervating the glands ought to be peptidergic. Comparison of the cobalt-filled preparations with the azan-stained sections of SG, $T_1$ and $T_2$, which were kindly provided by Prof. M. Raabe, and with the data of Delépine[15] indicated that 1 cell of the suboesophageal group 1 and both cells of group 3 in the thoracic ganglia are neurosecretory. Indeed, group 3 may be responsible for the inhibition of PTG which occurs in decapitated larvae after implantation of the thoracic but not suboesophageal or abdominal ganglia[3].

The number of perikarya localized in our study is small in comparison to the number and diversity of organs where the examined nerves terminate. This indicates that some neurons send axonal branches to several targets, and that specific influence on separate targets is achieved at the level of individual branches, similarly as suggested by Hughes[16] for the function of arthropod interneurons. This idea provides an attractive explanation of the multitude functions of insect neurohormones[17]. According to this scheme, the cells of group 3, which obviously release some of their secretion at the perisympathetic organs[14], transport peptidergic granules into PTG, where the release of granules is regulated by mechanisms pertaining to the axonal branches supplying the glands. Since the axonal transport to remote targets is characteristic of insect neurosecretory system, we suggest that regulation of neurosecretion at the axonal level is widespread in insects and enables simultaneous influence of separate targets by a single neurohormone.

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## Radioimmunoassay of plasma progesterone, testosterone, total estrogens and immunoreactive gonadotropin in the nesting and non-nesting green sea turtle, *Chelonia mydas* (L.)

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*Summary.* Plasma progesterone, testosterone, total estrogens and immunoreactive gonadotropin were measured in nesting and non-nesting sea turtles, *Chelonia mydas.* Progesterone and gonadotropin concentrations were significantly higher in nesting than in non-nesting turtles, testosterone was not significantly different in either group and total estrogens appeared to be slightly higher in the nesting group.

Despite the wealth of information on the nesting behavior and a few other aspects of its life history (see extensive bibliography in Rebel[3]) the reproductive physiology of the economically important *Chelonia mydas* is virtually unknown. Preliminary work has shown that males of this species respond to mammalian FSH, but not LH by increasing testosterone secretion as do males of the freshwater turtle, *Chrysemys picta*[4] and that it is possible to distinguish sexually immature male green turtles from females on the basis of this response to FSH[5]. This study reports changes in immunoreactive gonadotropin and plasma sex steroids in sea turtles that were sampled while ovipositing (nesting) or in the internest period between successive clutches (cruising).

Samples were collected in vacutainer tubes by D.O. at Mariculture Ltd, Grand Cayman Island (courtesy of Dr. J. Woods) from turtles maintained at Mariculture Ltd or captured in Nicaragua during the months of April, May and June, and the plasma shipped to Boston in dry ice. In a number of instances 2 or 3 samples were taken from an individual turtle prior to and post nesting. Total estrogens, testosterone and progesterone were analyzed by radioimmunoassay (RIA) as detailed in Callard et al.[6]. Plasma gonadotropin was measured by RIA using an antibody raised against chicken LH[7], which cross reacts with pituitary extracts from *Chelydra serpentina*[8], *Chrysemys picta,* and with plasma from *Chrysemys picta*[9]. Parallel inhibition curves can also be demonstrated with *Chelonia mydas* plasma (figure b). Sea turtle pituitary was not available for testing. Figure a shows values of gonadotropin, total estrogens, testosterone and progesterone in blood samples obtained while animals were nesting (ovipositing) and cruising in open water between nesting periods. Values for both gonadotropin and progesterone are significantly higher in