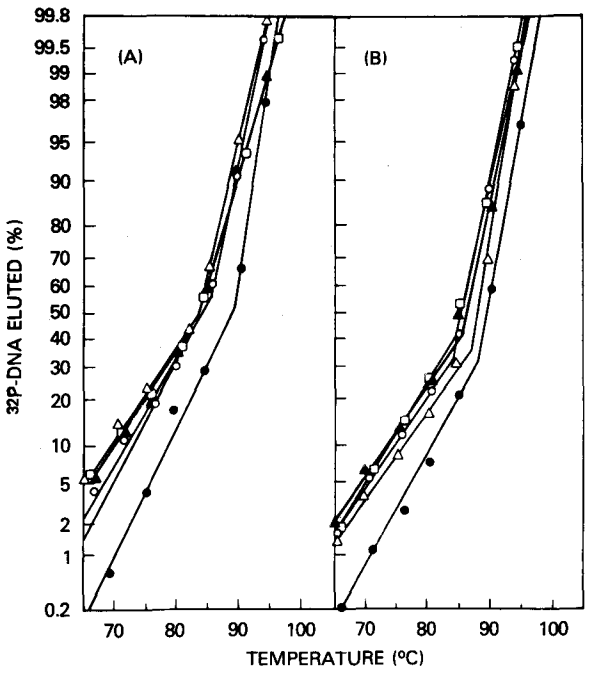

Fig. 2. Normal probability plot of thermal elution profiles of duplexes formed between reference ^{32}P-DNA and excess of unlabeled DNAs from different sources. *A* Between *N. crassa* ^{32}P-DNA and ○, *N.* sp. (Mysore); △, *N.* sp. (Lahore); ▲, *N.* sp. (Gianjor); □, *N.* sp. (Kuala Lumpur); and ●, *N. crassa* DNAs. *B* Between *N. sitophila* ^{32}P-DNA and ▲, *N.* sp. (Mysore); ○, *N.* sp. (Lahore); △, *N.* sp. (Gianjor); □, *N.* sp. (Kuala Lumpur); and ●, *N. sitophila* DNAs.

Considering the base composition and the sequence divergence data together, from the present work and from my previous work[4], it seems that *N.* sp. (Gianjor) occupies a position superimposing on or very close to that of species group *N. tetrasperma* on a phylogenetic tree. *N.* sp. (Mysore) is almost equidistantly related to both *N. sitophila* and *N. crassa* and seemingly occupies a position in the species group *N. intermedia*. The present study has been unable to assign the isolates *N.* sp. (Lahore) and *N.* sp. (Kuala Lumpur) to any particular reference species group. However, they seem to be very close to each other and relatively closer to *N. sitophila* than to *N. crassa*. Based on crossing behavior, however, all the strains of *N.* sp. (Lahore) were tentatively assigned to the species group *N. crassa*[10]. Assignments of the isolates to the known species groups, in this work, is treated as only tentative and certainly not final. But results in this study conform largely to the established strain assignments[10] of the isolates. It is believed that the data presented here will be helpful to other workers interested in *Neurospora* genetics and evolutionary biology.

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Neurosecretory control of Corpora allata activity in cockroach, *Periplaneta americana* L.

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Summary. The brain neurosecretory material is involved in the control of Corpora allata activity during post-embryonic development. A high concentration of neurosecretory material within the Corpora allata restrains the activity of the gland.

It is well known that the neurosecretory cells (NSC) of the protocerebrum, prothoracic gland (PTG) and Corpora allata (CA) govern the development of insects. However, while the control mechanism of brain NSC and PTG are understood that of CA is not quite clear as yet. Most of the references about the control of CA are concerned about their activity in relation to reproduction. Engelmann[2] held that the activity of CA during ovarian development in *Leucophaea maderae* is regulated purely by nervous stimuli from the brain and suboesophageal ganglion. But Highnam[3] in *Schistocerca gregaria* established that the median NSC of pars intercerebralis liberate a substance which is involved in the activation of the CA of adult females. The median NSC have been found to control protein metabolism which may allow an activation of CA. Strong[4] in *Schistocerca paranensis* suggested that lateral NSC activate the CA. Scharrer[5–7] demonstrated experimentally that the inhibition of CA is brought about by the protocerebral NSC. She also suggested the involvement of neurosecretory components in the regulation of cyclical activity of CA[8]. Khan and Fraser[9] gave histological evidence that the neurosecretory material restrains the activity of CA. The aim of the present study was to examine in more detail the correlation between the neurosecretion and the activity of CA, and to investigate further the role of neurosecretion in the control of CA activity during development of *Periplaneta americana*.

Material and methods. The different instar nymphs were reared at 25 °C temperature. The heads of the nymphs of known ages were fixed in aqueous Bonins fluid and em-

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bedded in paraffin wax. Serial sections were cut at 6 µm and stained with paraldehyde fuchsin and chrome alum haematoxylin phloxin stains.

Results and discussion. The 1st instar nymphs underwent moulting 12 days after hatching. The brain neurosecretory material (stained purple with Paraldehyde fuchsin and blue black with Chrome alum haematoxylin phloxin stains) is rare in the CA of 1st instar nymphs for the first 6 days but increase in concentration from the 7th to the 12th day, and the stainable material was also observed in transit in nervi corporis allati I (NCA I) during these days. The CA undergo reduction in size in 7 to 12 days old nymphs as the concentration of neurosecretory material increases within them. The CA of 12 days old nymphs have abundant neurosecretory material in the axons of NCA I residing in intercellular spaces within the gland. The CA of 12 days old nymphs (about to moult) are definitely smaller in size than those of early nymphs (figures 1 and 2) and possess low cytoplasmic volume and aggregated nuclei. This histological picture presented by the CA of 12 days old nymphs indicates their inactive state. Thus the accumulation of relatively large amount of neurosecretory material in the CA inhibits the activity of the glands before moulting. The CA of freshly moulted 2nd instar nymphs still contain an appreciable amount of neurosecretory material and the glands are smaller in size. But a few days after moulting, the CA dispense with almost all neurosecretory material. The CA now increase in size and possess more cytoplasm and less concentrated nuclei. The CA have now resumed their activity and appear to be actively engaged in producing juvenile hormone. They continue to show signs of secretory activity till the 6th or 7th day. During this period an appreciable amount of neurosecretory material has accumulated in the Corpora cardiaca (this is also true for 1st stage nymphs)

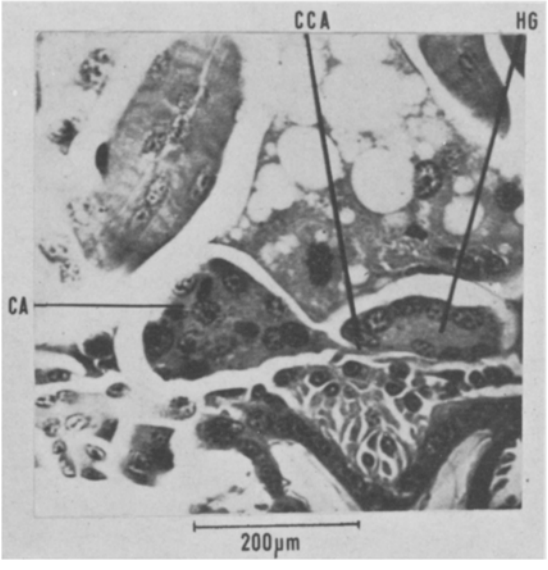

Fig. 1. Photomicrograph of a section through the Corpora allata (CA) of an 8-day-old 1st instar nymph of *P. americana* showing allatal commissure (CCA) and hypocerebral ganglion (HG). Paraldehyde fuchsin, Groat's haematoxylin and indigo carmine stain.

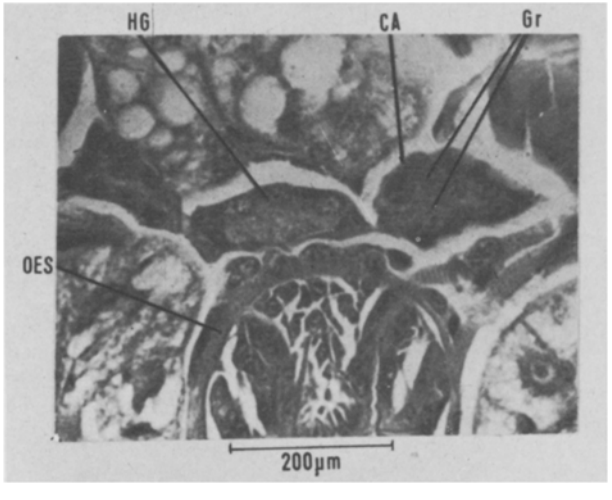

Fig. 2. Photomicrograph of the frontal section of the Corpora allata of a 12-day-old 1st instar nymph of *P. americana* showing abundant PF-positive neurosecretory granules. Note the small size of Corpora allata and crowded condition of nuclei. Oes, Oesophagus; HG, hypocerebral ganglion; CA, Corpora allata; Gr, Neurosecretory granules.

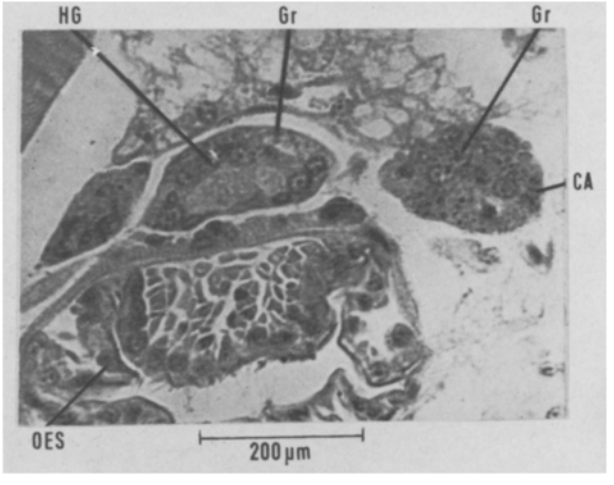

Fig. 3. Photomicrograph of the frontal section of the Corpora allata of a 7-day-old 2nd instar nymph of *P. americana* showing abundant PF-positive neurosecretory granules. Letterings same as in figure 2.

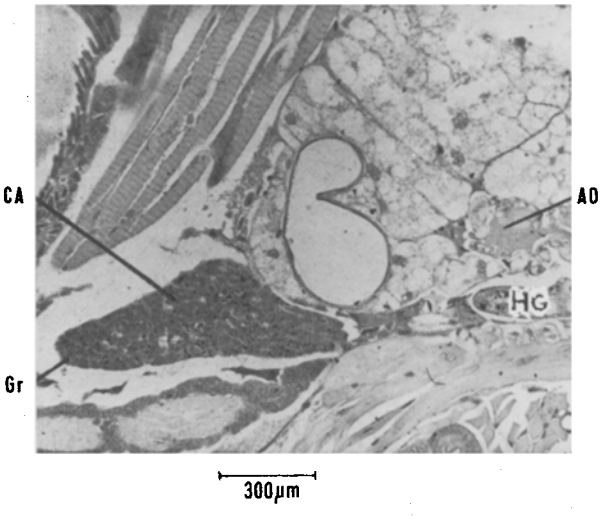

Fig. 4. Photomicrograph of the frontal section of the Corpus allatum of an adult female *P. americana* killed 1 h after the final moult showing abundant neurosecretory granules (Gr). HG, hypocerebral ganglion; Ao, Aorta. Note the small size of the gland and crowded nuclei.

from where an overflow of material begins to CA via NCA I from the 7th day onwards. Consequently the neurosecretory material becomes again more abundant in the CA on the 7th day. This overflow of neurosecretory material renders the glands inactive (figure 3). The moulting of 2nd stage nymphs occurs from 12th to 14th day. The CA of 2nd instar nymphs ready to moult, and also those of freshly moulted 3rd stage nymphs, present histological characteristics of an inactive gland and contain abundant neurosecretory material. This pattern is repeated in subsequent nymphal instars too.

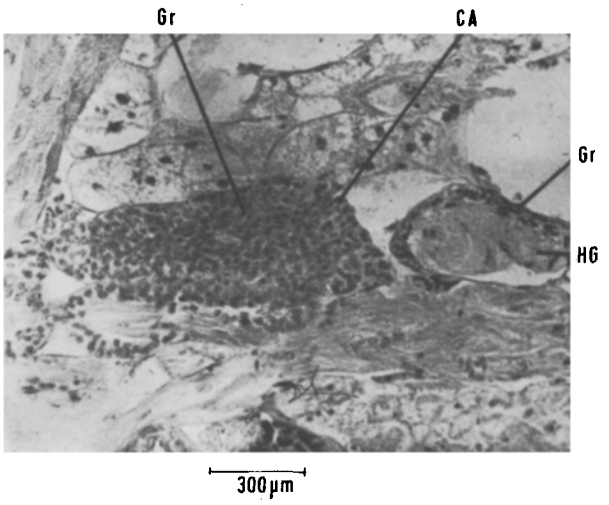

Fig. 5. Photomicrograph of the frontal section of the Corpora allata of an adult female killed 4 h after the final moult showing sparse neurosecretory granules (Gr). HG, hypocerebral ganglion. Note the scattered nuclei and relatively large size of the gland.

The CA of freshly moulted adult females (1 h after final moult) are loaded with neurosecretory material and are much smaller than those of 4 h old females. The CA of 1 h old females have a low volume of cytoplasm and highly crowded nuclei and can therefore be regarded as inactive (figure 4). But only 4 h after the final moult, the CA of adult females show a notable increase in size, have more cytoplasm and less crowded nuclei, and contain only a few neurosecretory granules (figure 5). It seems that, as soon as most of the neurosecretory material has gone out from the CA, the restraint or inhibition is over and the CA resume their activity.

However, the present histological findings can be interpreted in another way also. It may well be that the small amount of brain hormone reaching the CA perhaps stimulates the glands for a certain time; then, as the time for moulting approaches, the activity of the CA which has to be restrained is checked by the overflow and consequent presence of abundant neurosecretory material in these glands. The other possibility is that the presence of small amount of brain hormone does not stimulate the glands but just allows them to do a certain degree of their function. The secretory cells of the CA are allowed to produce a certain amount of juvenile hormone under the supervision of brain NSC (which may not be working completely independently of the CA) for a certain period after which the overflow and high concentration of brain neurosecretory material restrains their activity. In any case, the involvement of brain neurosecretory material in the control of the activity of CA seems to be fairly certain.

A fungitoxic principle from the leaves of *Lawsonia inermis* Lam.

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Summary. During antifungal screening of higher plants, the leaves of *Lawsonia inermis* were found to exhibit strong fungitoxicity. On chemical investigation, the antifungal factor was found to be 2-hydroxy-1,4-naphthoquinone (Lawsone). The minimum effective dose against test organism was found to be 1000 ppm. Lawsone was found to exhibit fungicidal activity, wide fungitoxic spectrum and nonphytotoxicity.

Plants are known to contain various antimicrobial substances[2,3]. Although the antifungal activity of extracts of higher plants is well demonstrated[4–7], the characterization of the fungitoxic principle has received little attention. The leaves of *Lawsonia inermis* Lam. were found to exhibit strong toxicity during antifungal screening of higher plants. The present communication deals with the isolation, identification and fungitoxic properties of the active principle.

Experimental procedures and results. 20 g of leaves as well as stem, root and bark were extracted separately with methanol and screened for antifungal activity by a modified paper disc method[8] against the test organism *Helminthosporium oryzae* Breda de Haan. Of various parts tested, only the leaves of *Lawsonia inermis* exhibited fungitoxicity. For the isolation of the active principle, 500 g of fresh leaves were extracted with methanol several times until the last eluate became colourless. The solvent from this extract was evaporated under reduced pressure. The residue was dissolved in distilled water and extracted with n-butanol and chloroform separately. The solvents from the n-butanol and the chloroform fractions and water from the aqueous fraction were removed by evaporation under reduced

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