development of the potato tuber moth, P. operculella are reported in this paper.

T. terrestris plants were collected from the field, shade-dried, powdered and continuously extracted with solvent petroleum ether (40-60°C) in Soxhlet extractor for 48 h and the solvent was completely evaporated<sup>6</sup>. A specified quantity of the extract was dissolved in acetone to get the desired concentrations of 0.1, 0.25, 0.5, 0.75, 1.0 and 1.25% on weight basis. Acetone 1% served as the control.

The eggs, larvae and pupae were subjected to the treatment. A specified quantity of eggs, larvae and pupae (tables 1 and 2) were placed on Whatman No. 1 filter paper in petri dishes (5 cm dia) and sprayed with hand atomizer. The petri dishes were air-dried for 15 min to prevent the development of fungus. Treated larvae were provided with pieces of potato with a few eye buds as a source of food.

Table 1 Effect of T. terrestris extract on egg hatchability of P. operculella

Conc. (%)	% of egg hatch ± S.D	
0.10	82 ± 3.12	
0.25	$68 \pm 1.02$	
0.50	$52 \pm 1.01$	
0.75	$40 \pm 2.52$	
1.00	$25 \pm 1.13$	
1.25	$10 \pm 0.91$	
Control	$98 \pm 0.81$	

Each value represents averages of four replicates and each consisting of 25 eggs.

**Table 2** Effect of T. terrestris extract 0.5% on larval mortality and adult emergence of P. operculella

Stages	Mortality (%)	Adult emergence (%)
I Instar		
Control	$4 \pm 1.92$	$96 \pm 1.82$
Treated	$40 \pm 3.32$	$60 \pm 1.42$
II Instar		
Control	$3 \pm 2.41$	$97 \pm 0.82$
Treated	$38 \pm 1.02$	$62 \pm 1.21$
III Instar		
Control	$2 \pm 2.01$	$98 \pm 1.01$
Treated	$35 \pm 0.91$	$65 \pm 1.12$
IV Instar		
Control	$3 \pm 3.71$	$97 \pm 2.01$
Treated	$36 \pm 2.41$	$64 \pm 1.13$
Pupae		
Control	0	$100 \pm 0.81$
Treated	$40 \pm 1.38$	$60 \pm 3.51$

Each value represents four replicates and each consisting of 20 individuals.

The egg hatchability (table 1) decreased with increase in concentration of the extract. Egg hatchability of 98% was recorded in control. The hatchability of the treatment ranged from 10 to 82%. Similar studies were carried out by Rajendran and Gopalan<sup>7</sup>, who reported that the eggs of *D. cingulatus* did not hatch when treated with the plant extract, *Polyscias quilfoylei* at higher doses.

I to IV instar larvae (35-40%) and pupae (40%) failed to become adults when they were treated with the 0.5% plant extract (table 2). Extracts of T. terrestris recorded higher juvenilizing effect than P. hysterophorus at 500  $\mu$ g when tested on the penultimate instar of D. cingulatus<sup>5</sup>.

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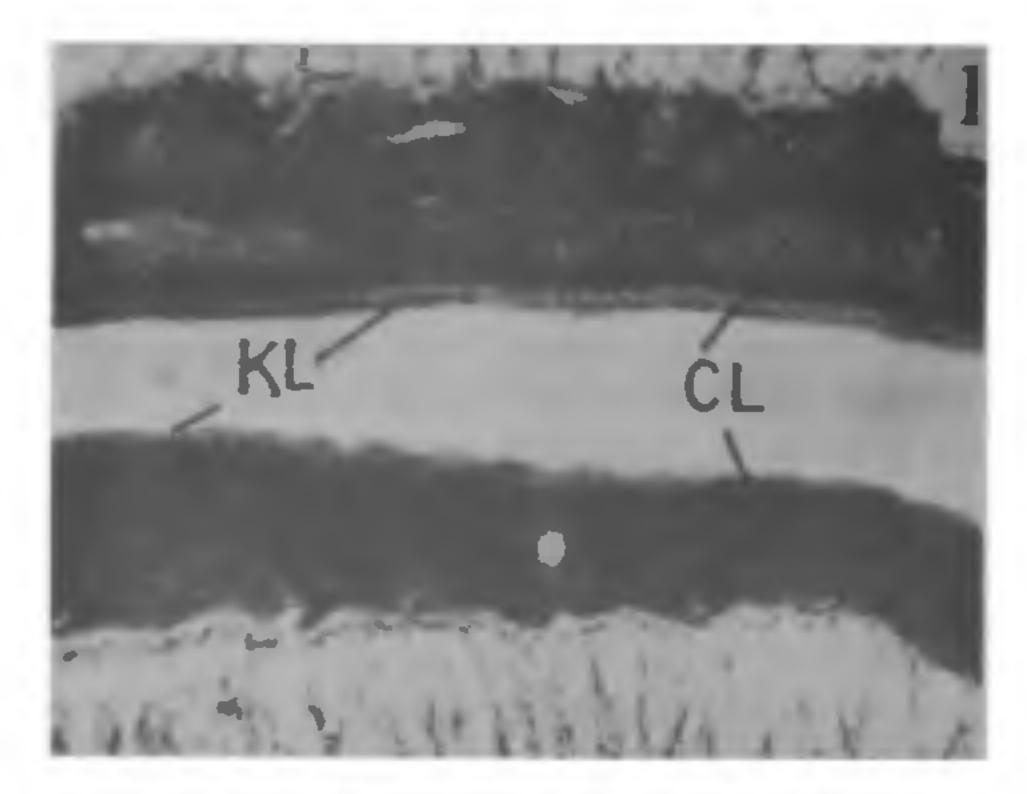
- 1. Slama, K. and Williams, C. M., Biol. Bull., 1966, 130, 235.
- 2. Stall, G. B., Med. Rigiksfac Landbouw Wentens chappen G. ent, 1967, 32, 393.
- 3. Prabhu, V. K. K., John, M. and Ambika, B., Curr. Sci., 1973, 42, 725.
- 4. Gopakumar, B., Ambika, B. and Prabhu, V. K. K., Entomon, 1977, 2, 259.
- 5. Gunasekaran, K. and Chelliah, S., In: Proc. national seminar on behavioural physiology and appropriate management of crop pests, TNAU, Coimbatore, June, 1985.
- 6. Jacobson, M., Redfern, R. E. and Mills, Jr., Lloydia, 1975, 38, 455.
- 7. Rajendran, B. and Gopalan, M., In: Proc. national seminar on behavioural physiology and appropriate management of crop pests, TNAU, Coimbatore, June, 1985.

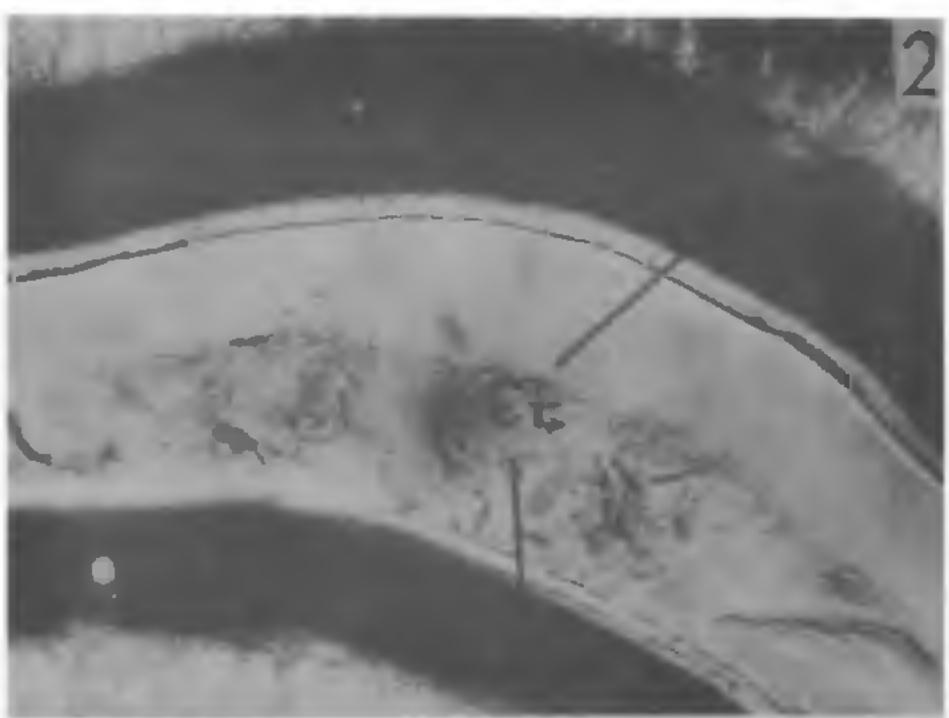
# OCCURRENCE OF KERATIN AND COLLAGEN IN THE INNER LINING OF THE OESOPHAGUS IN THE CRAB, CHARYBDIS ANNULATA (FABRICIUS)

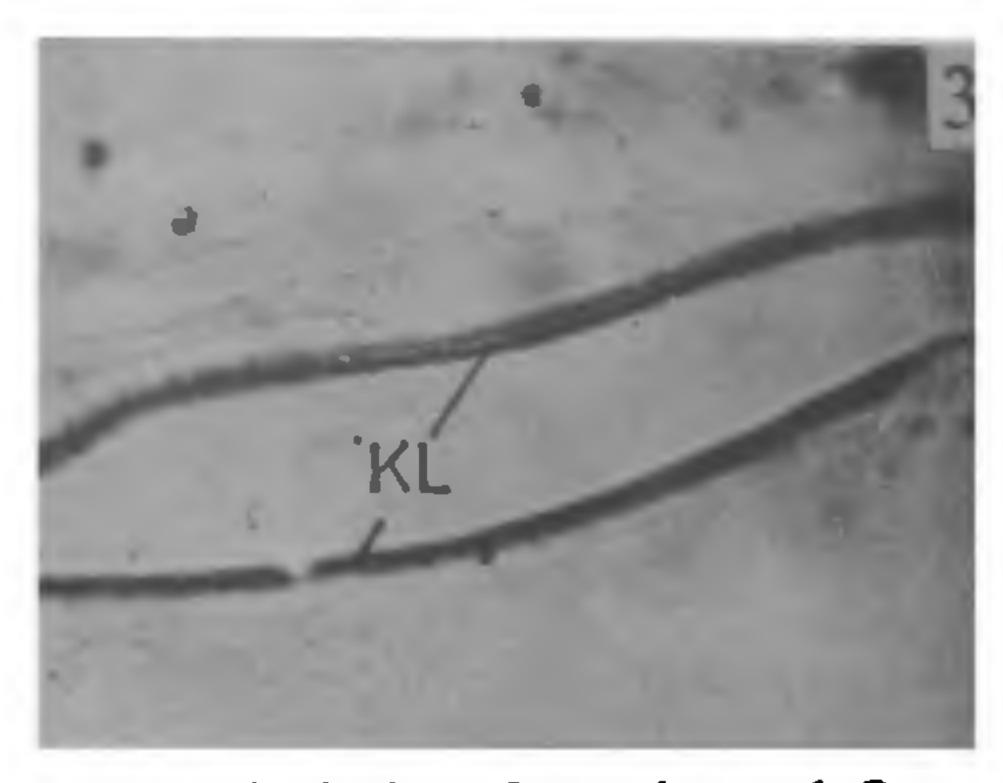
## K. SHYAMASUNDARI, B. TRINADHA BABU and K. HANUMANTHA RAO

Department of Zoology, Andhra University, Visakhapatnam 530 003, India.

CRUSTACEAN oesophagus is lined by a cuticular layer to withstand against pressure and abrasion







Figures 1-3. Section of oesophagus. 1. Oesophageal lining showing inner collagen and outer keratin layers—Azan; 2. Oesophageal lining showing inner collagen layer—Aniline blue, and 3. Oesophageal lining showing outer keratin layer—Rhodamine B. [CL, Collagen layer; KL, Keratin layer].

caused by the intake of food material. The foregut and hindgut are distinguished from the midgut by their ectodermal origin and chitinous lining<sup>1</sup>. However, Pike2 observed that the oesophageal lining consists of two separate layers, an outer cuticular layer and an inner chitinous layer. Barker and Gibson<sup>3</sup> also observed these two layers in the oesophagus of Homarus gammarus and termed them as epicuticle and endocuticle. Shyamasundari and Hanumantha Rao4 were the first to describe the cuticular lining of the stomodaeum and proctodaeum as composed of elastin and collagen in amphipods. Very recently, Erribabu et also made similar observations on the cuticular lining of the oesophagus in the crabs. In the present investigation on Charybdis annulata the lining of the oesophagus is observed cytochemically to contain an outer keratin layer and an inner collagenous layer.

The oesophageal lumen of C. annulata is lined by an uncalcified two-layered cuticle of about 25–30  $\mu$ m thick (figure 1). The cuticular lining is followed by the basement membrane and columnar epithelial layer.

The chemical nature of the inner and outer layers of the cuticular lining of the oesophagus is observed by conducting a number of cytochemical tests (table 1).

When the sections of oesophagus were treated with periodic acid/Schiff (PAS) technique the inner layer showed strong positive nature and it was resistant to PAS/saliva digestion, while the outer layer was negative to this test revealing the diffe-

Table 1 Cytochemical tests applied to the cuticular lining of the oesophagus

	Results	
Cytochemical test applied	Inner layer	Outer layer
Heidenhain's Azan	Blue	Red
Mallory's triple stain	Blue	Orange to red
Aniline blue	Deep blue	Yellow
Periodic acid/Schiff (PAS)	++	_
PAS/saliva	++	_
Orcein/Van Gieson	Red	Brown
Aldehyde fuchsin	_	++
Luxol fast blue G in methanol	++	-
Rhodamine B	_	+++
Potassium permanganate/ alcian blue	-	++

<sup>+++,</sup> Intensely positive; ++, Strongly positive; +, Moderately positive; -, Negative.

rence in chemical composition. The inner layer stained blue with Azan (figure 1), Mallory's triple stain and aniline blue stain (figure 2). It gave a strong positive reaction with Luxol fast blue G in methanol and failed to react with aldehyde fuchsin. These results suggest that collagen may be involved in the composition of inner layer. The outer layer stained red with Azan (figure 1) and orange to red with Mallory's triple stain. This layer is intensely positive to aldehyde fuchsin (AF), Rhodamine B (figure 3) and potassium permanganate/alcian blue thus confirming the presence of keratin type of protein.

It could be concluded that the lining of the inner wall of the oesophagus in *C. annulata* is made up of an outer keratin and an inner collagen layers.

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- 1. Reddy, A. R., Proc. Indian Acad. Sci., 1937, B6, 170.
- 2. Pike, R. B., Galathea, L.M.B.C. Memoir, 1947, p. 34.
- 3. Barker, P. L. and Gibson, R., J. Exp. Mar. Biol. Ecol., 1977, 26, 297.
- 4. Shyamasundari, K. and Hanumantha Rao, K., Curr. Sci., 1973, 42, 134.
- 5. Erribabu, D., Shyamasundari, K. and Hanumantha Rao, K., Curr. Sci., 1979, 48, 224.
- 6. Erribabu, D., Shyamasundari, K. and Hanumantha Rao, K., J. Exp. Mar. Biol. Ecol., 1982, 58, 175.

## PROLACTIN-DEPENDENT ACTIVATION OF MAMMALIAN SPERM METABOLISM

Y. DHANANJAYA REDDY, J. NAGESWARA RAO, M. PRASAD and S. GOVINDAPPA Department of Zoology, Sri Venkateswara University, Tirupati 517 502, India.

PROLACTIN enhances the male sertility in many animal species<sup>1, 2</sup>. Administration of OPRL stimulates testicular growth and spermatogenesis in mice and rats<sup>3, 4</sup>. Bromocriptine, an ergot alkaloid, inhibits prolactin secretion and release by its direct action on pituitary acidophils<sup>5</sup>. Bromocriptine administration decreased prolactin levels in the serum of male albino rats<sup>6</sup>. Since information on the sperm

metabolism and PRL concentration is meagre, an attempt was made to understand the effect of prolactin on sperm metabolism.

Adult male Wistar strain albino rats,  $70\pm2$  days old and  $160\pm5$  g body weight were administered with  $20\,\mu\mathrm{g}$  of bromocriptine mesylate (Sandoz Pharmaceuticals, Switzerland) per rat per day for 15 days as suggested earlier. The second group of six animals received subcutaneous injections of OPRL (NIH, Bethesda, USA) at a dose of  $1\,\mu\mathrm{g}$  per g body weight per day for 5 days as described earlier. Injections of vehicle (physiological saline) were given to the third group of animals (controls). The animals were maintained at laboratory conditions and fed a standard rat diet obtained from the Hindustan Lever Ltd., Bombay and water was supplied ad libitum. All the animals were sacrificed at 24 h after last injection.

Both control and experimental albino rats were sacrificed by cervical dislocation. The cauda epididymis was cut out and taken into the medium containing physiological saline. The tissue was minced and teased gently for the release of spermatozoa as suggested by Chinoy and Sanjeevan<sup>9</sup>. The sperm samples with motile sperm were taken for the analysis.

The testes were isolated with minimum mechanical stress. The adhering blood was blotted and used for biochemical analysis. The sperm motility was observed under the microscope and comparison was drawn between control and experimental groups of animals.

The cholesterol content<sup>10</sup>, levels of ATPase<sup>11</sup>, sorbitol dehydrogenase<sup>12</sup>, glucose 6-phosphate dehydrogenase<sup>13</sup>, acid phosphatase<sup>14</sup>, alkaline phosphatase<sup>14</sup>,  $17\beta$ -hydroxy steroid dehydrogenase<sup>15</sup>, and  $3\beta$ -hydroxy steroid dehydrogenase<sup>15</sup> were estimated by the methods described earlier.

Prolactin levels have been modulated in adult male albino rats through the administration of exogenous prolactin and bromocriptine. The rats administered with exogenous PRL represented hyperprolactinemic conditions and those administered with bromocriptine recorded hypoprolactinemic conditions. This observation was in close consonance with the reports of earlier investigators  $^{5, 16-19}$ . The testicular cholesterol content decreased conspicuously in PRL-administered rats (table 1) indicating its active mobilization probably into androgenesis. The activity levels of  $3\beta$  and  $17\beta$ -hydroxy steroid dehydrogenases (HSD) were significantly elevated in the tissue indicating improved androgenesis in the testis. Since