

absence of barbels. These three species have barbels either one pair or two pairs. The species further differs morphologically from *D. nigrofasciatus* (Day), *D. rerio* (Hamilton) and *D. choprae* Hora in having no colour band on the body as well as on the fins.

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THE OCCURRENCE OF TREHALASE IN THE *EUBORELLIA ANNULIPES* (LUCAS) DERMAPTERA

V. VEERA RAGHAVAN AND M. MOHAMED IBRAHIMSHA

P. G. Department of Zoology, Jamal Mohamed College, Tiruchirapalli 620 020, India.

TREHALOSE, a major disaccharide in the blood of insects could not be detected in the haemolymph of New Zealand earwig *Anisolabis littorea*.¹ Paper chromatographic analysis of free sugar extracts of the whole body as well as the haemolymph and the reproductive tissues of male and female earwig *Euborellia annulipes* failed to reveal trehalose and showed glucose as the principal sugar.² However Moriarty³ reported the occurrence of trehalose in low concentrations in a British species of earwig *Forficula auricularia* and observed that there is not much of an evidence to suggest that primitive insects lacked trehalose. The present study aims at the detection of trehalase, the enzyme responsible for the cleavage of trehalose (1-0- α -D-glucopyranosyl- α -D-glucopyranoside) to glucose, which would provide evidence for the occurrence or otherwise of trehalose in earwigs.

Colonies of *E. annulipes* were raised in the laboratory from locally collected specimens. The insects were reared on soaked dog biscuits and water *ad lib*.

The insects were anaesthetised by chilling, their thorax severed and the intestine removed. The abdomen of 25 insects thus obtained were ground in an all glass homogeniser in 0.03 M ice cold citrate buffer

(pH 6.5) containing 0.01 M phenyl thiourea. The homogenate was centrifuged at 12,000 g in a Remi T-24 centrifuge for 20 min. The supernatant was extensively dialysed against extraction buffer, for over 24 hr and used as the enzyme source. The trehalase activity was determined on polyacrylamide gels⁴ using a modified trehalase specific disc gel stain method⁵ After a 90 min run the gels were incubated in 0.01 M trehalose solution for 20 min at the end of which the enzyme activity was arrested by washing the gels in distilled water and transferring them to a 0.1 M iodoacetamide solution for 8 min. After a final rinse with distilled water, the gels were immersed in freshly prepared 0.1 M tetrazolium red in 0.5 M NaOH and heated in a boiling water bath for 1.5 to 2 min with mild agitation. Red bands appear wherever aldose sugars are present.

The results were further confirmed by paper chromatography. 2% trehalose was incubated with enzyme source for 60 to 90 min at 37°C at the end of which the incubation mixture was analysed for the hydrolytic product, *viz.*, glucose by paper chromatography⁶ Incubation mixture without the substrate or enzyme source was used as controls. Glucose solution (2%) was also cochromatographed.

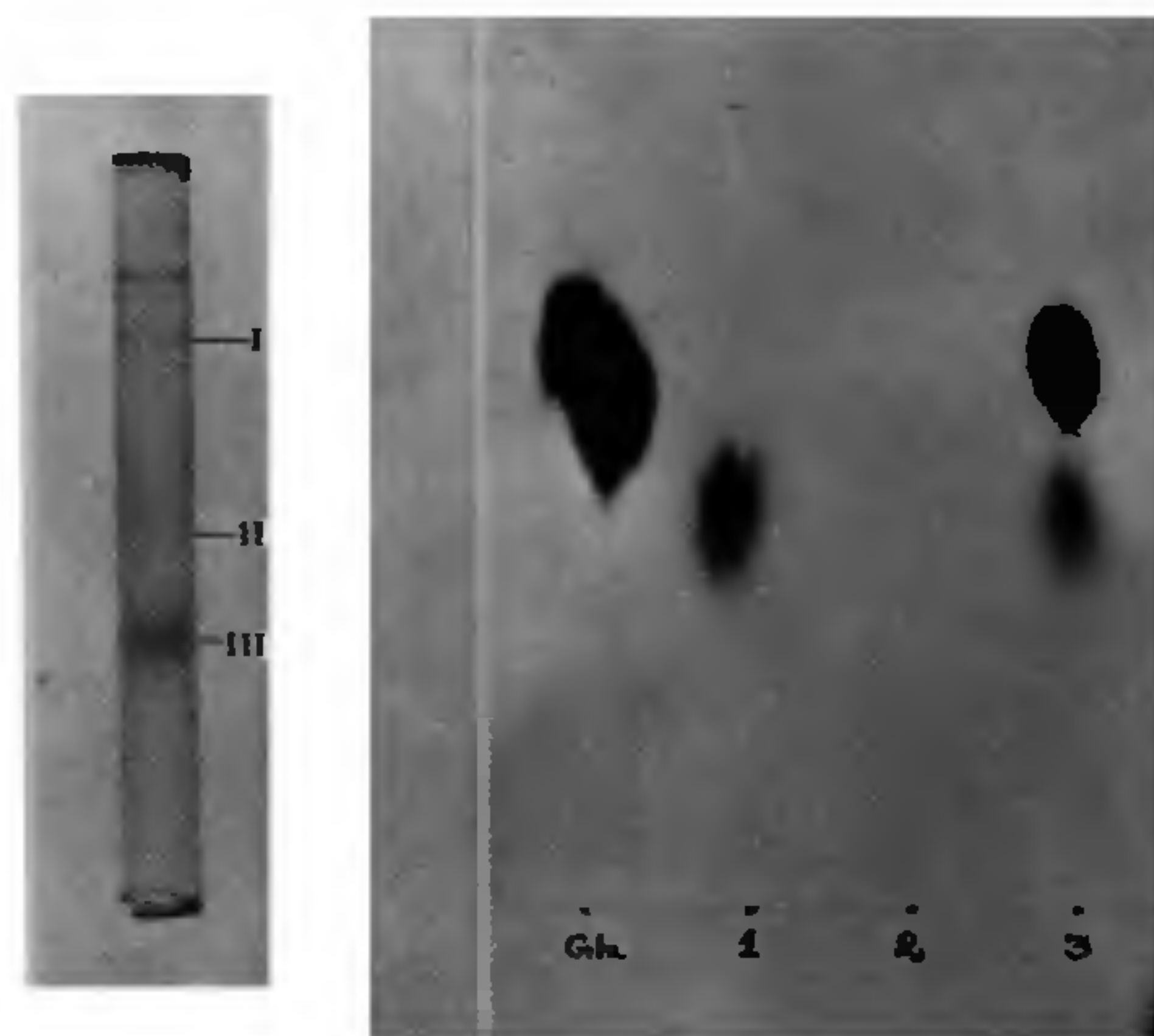


Figure 1 Electropherogram of the crude abdominal extract of *E. annulipes*. Gel stained specifically for trehalase. I, II and III indicate the multiple molecular forms of trehalase.

Figure 2 Paper chromatogram showing the formation of glucose ($\mu\gamma$ per spot) from trehalose incubated with the crude abdominal extract of *E. annulipes* at 37°C in citrate buffer. (Glu) Glucose. 1. Control without extract. 2. control without trehalose, and 3. experimental.

Trehalase activity could be detected in three regions on the gels (figure 1). The first fraction (I) is a slow moving one occurring as a discrete band and the last, fast moving fraction (III) occurs as a broad diffuse band. In between, there is a second fraction (II) having relatively a weak reaction with tetrazolium red. *In vitro* incubation of trehalose with the enzyme source revealed glucose as the hydrolytic product and undigested trehalose in the paper chromatogram (figure 2).

The detection of trehalase from the abdominal extracts of *E. annulipes* sets at right the controversy regarding the occurrence or otherwise of trehalose in earwigs. It is quite possible that the disaccharide occurs at very low concentrations in the haemolymph of the insect. Moriarty³ reported a concentration range of only 0.1 μg to 1.1 μg for female and 0.5 to 1.5 μg for males per μl of haemolymph of *F. auricularia* although trehalose concentration in the blood of most insects examined fall within the range of 5 to 50 μg per μl ⁷. Also the detection of trehalase renders such hypotheses, as that trehalose as a blood sugar appeared later than the origin of class insecta or that the earwigs could have lost the disaccharide secondarily from the system², untenable.

More interestingly, the trehalase of *E. annulipes* is electrophoretically heterogeneous, which essentially means that the insect has multiple molecular forms of the same enzyme. Isozymes of trehalase have been reported in *Phormia regina*⁸, isozyme A being restricted to midgut and blood and isozyme B restricted to head, muscles and rectal papillae. The two isozymes are electrophoretically and kinetically heterogeneous. Also earlier work⁹ has shown considerable difference between intestinal and muscle trehalases of insects. Further work is underway to partially purify the isozymes of the earwig and study their tissue specificities and kinetic properties.

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EFFECT OF α -METHYL DOPA ADMINISTRATION ON THE PHEROMONAL BLOCK TO IMPLANTATION IN MICE

S. C. SAHU AND C. J. DOMINIC

Department of Zoology, Banaras Hindu University, Varanasi 221 005, India.

THERE is a large body of evidence which suggests that the neuroendocrine cause of the male-induced implantation failure (the Bruce effect) in newly inseminated mice is the decreased hypophysial prolactin release leading to the failure of corpus luteum development¹⁻³. The Bruce effect can be prevented by treatment with pimozide, an antagonist of dopamine receptors⁴ or chlorpromazine, an inhibitor of dopamine activities⁵. These studies suggest the involvement of the dopaminergic neurons in the mediation of the pheromonal stimulus leading to the Bruce effect. In the present report, the ability of α -methyl dopa (1,3-(3,4-dihydroxyphenyl)-2-methylalanine), which stimulates hypophysial prolactin release through suppression of dopamine synthesis, to prevent the Bruce effect was evaluated.

All females and the stud males employed in the study belonged to the Parkes (P) strain and the alien males used for inducing implantation failure to the wild strain. The females were approximately 3 month old virgins. They were mated with P males and on finding the vaginal plug (day 0 post coitum)^{1,6} were separated from the stud males and housed individually in cages, 40×15×10 cm. Twenty four hours later (day 1 post coitum) they were subjected to one of the following treatments. *Group I*: Individual exposure to a confined alien male for 3 days (from 1000 hr on day 1 to 1000 hr on day 4 post coitum) and administration of α -methyl dopa, 6 mg/female/day, on days 1 to 5 post coitum. *Group II*: Individual exposure to a confined alien male for 3 days as in Group I and administration of normal saline, 0.1 ml/female/day, on days 1 to 5 post coitum. *Group III*: Left undis-