

with regularity. The X-ray pattern of hydrobiotite treated with magnesium chloride shows that the spacings of (001) reflection in parts are exactly the sum of (001) spacings for biotite (10 Å) and vermiculite (14 Å). In the X-ray pattern of hydrobiotite treated with potassium chloride the spacings of (001) reflections in parts are more regular and correspond to biotite.

Hydrobiotite may be formed from biotite or vermiculite under hydrothermal or weathering conditions.

In the Yellandlapad area vermiculite does not occur and the X-ray pattern of the analysed untreated hydrobiotite (Table I) does not reveal the presence of vermiculite by way of any regularly stratified mixed layer structure. Hydrobiotite in the present area is obviously formed from biotite and the transformation has not reached a stage in which hydrobiotite is altered to vermiculite. Field study shows that there is no hydrothermal activity and the only intrusives in the area are the quartz veins. Weathering appears to have played its part in the formation of hydrobiotite as thin section reveals the effect of leaching along the traces of cleavage planes. It is therefore surmised from the above studies, that hydrobiotite of the Pakhals of Yellandlapad area was formed from biotite by the action of dilute magnesium and possibly calcium solutions under supergene conditions as suggested by Bassett⁹ for the hydrobiotite of Libby, Montana, U.S.A. It is therefore logical to conclude that the metamorphic zone in the above-mentioned Ravlapad synclinal structure of the Pakhals in the Yellandlapad area was originally defined by andalusite and biotite, the latter now being represented by hydrobiotite.

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NATURE OF THE SEX-ATTRACTANT PHEROMONE IN A CRAB *PARATELPHUSA HYDRODROMUS* (CRUSTACEA)

RYON¹ reported that a sex-attractant pheromone released from the antennal glands along with the urine by premoult females was responsible for the males to exhibit a "searching behaviour" leading to copulation in crabs of the species *Portunus sanguinolentus*. This was based on the observation that the male crabs displayed a behavioural response to the presence of premoult female crabs, which was the same as their behaviour when they were exposed to water in which premoult females had been kept. But the female premoult crabs with sealed excretory pores failed to elicit any reaction in the opposite sex.

A similar phenomenon was incidentally noticed by us during the course of a study on the physiology of a South Indian crab *Paratelphusa hydrodromus*². We have made use of this opportunity to find out the nature of the pheromone in question about which little is known from Crustacea.

Female and male crabs numbering about 50 each were maintained separately in the laboratory in aquarium tanks as described earlier². The antennal glands from alive female crabs were fixed in 5% formaldehyde solution and aqueous Bouin's fluid and sections were prepared by paraffin embedding.

Examination of the sections under fluorescence microscope revealed the occurrence of substances showing bright yellow fluorescence in the cells lining the tube (Fig. 1) that connects the end-sac and the bladder of the formalin-fixed antennal glands of premoult, but not of intermoult or postmoult females. In Bouin-fixed antennal glands the fluorescence is absent. These observations may suggest that the fluorescent material may be of the nature of 5-hydroxytryptamine (5-HT)³. Results of histochemical tests lend support to this inference. The same material gave positive indophenol and Schmorl reactions after but not before formalin fixation. When treated with diazotized-*p*-nitroaniline the material gave a peach-red colour which turned violet on exposure to ammonia^{4,5}.

A substance yielding positive reactions similar to 5-HT is found also in the water in which premoult female crabs were kept. About 250 ml of water in which two premoult females were kept for 6 to 8 hours was centrifuged to remove the solid materials and the supernatant concentrated to 5 to 7 ml by keeping in a desiccator *in vacuo* over calcium chloride and phosphorous pentoxide. A 5 µl sample applied on Whatman

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No. 1 filter-paper gave a bright blue green fluorescence after treatment with 0.2% ninhydrin in acetone containing 10% (v/v) acetic acid, drying and heating to 100° C for 3 minutes. The control did not give this reaction which is said to be very specific for 5-HT⁶.

Bain⁸, Welsh⁹ and Maynard¹⁰ have already reported the stimulant effect of 5-HT on the heart of some decapod crustaceans, studied by them. The results reported in the present investigation may suggest that 5-HT or a substance similar to it, released from the tube of the antennal glands,

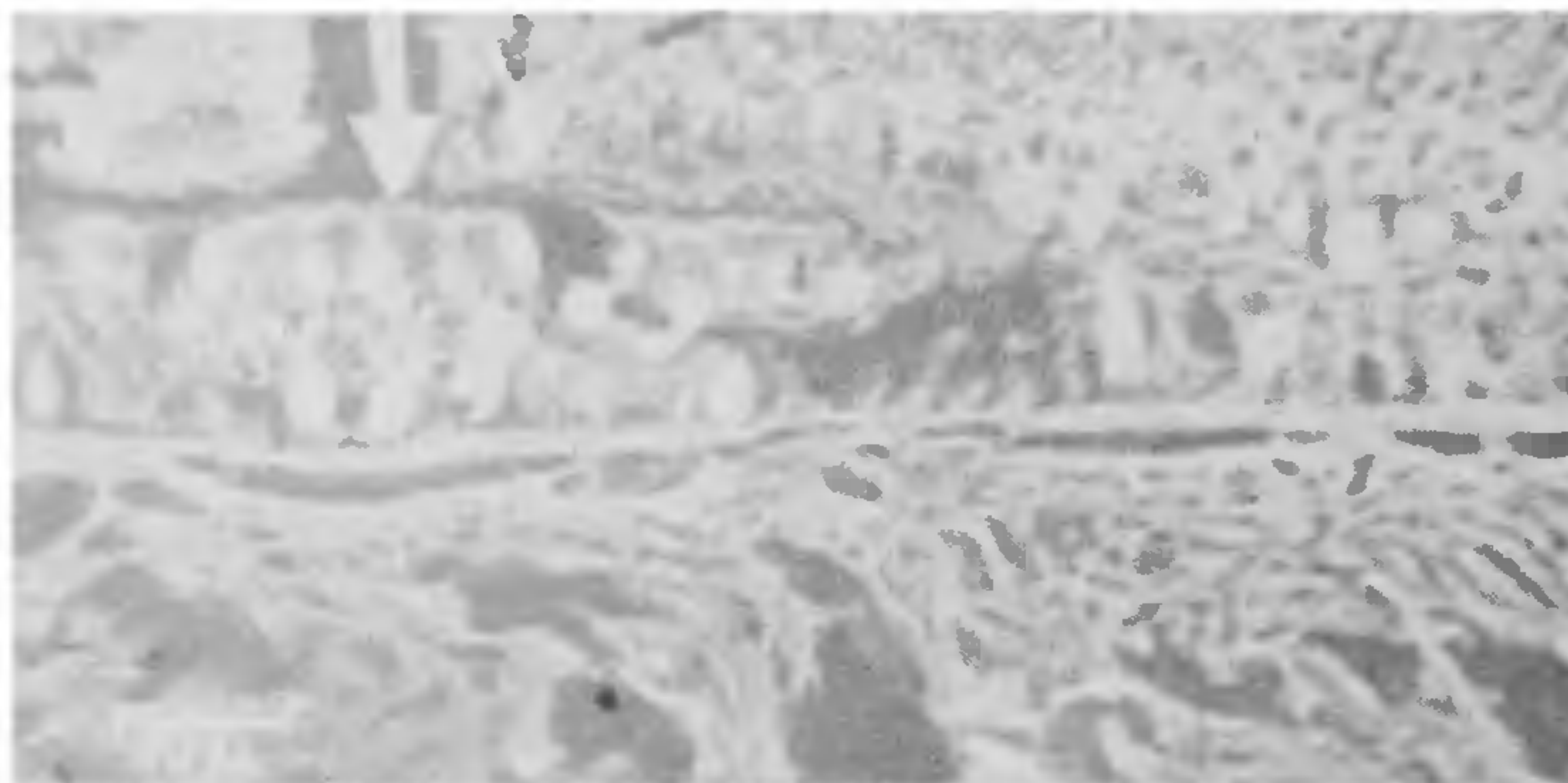


FIG. 1. Longitudinal section through the tubule of the antennal gland of *Paratelphusa hydrodromus*, viewed under fluorescence microscope. The arrow indicates the fluorescent substances.

These observations may indicate that a substance similar to 5-HT is present in the cells of the tube of the antennal glands in the premoult female crabs and this substance is released into the medium. If this is the sex-attractant pheromone, one would expect that on its removal from the water, the male crabs should not exhibit the search behaviour.

A sample of water in which a few premoult female crabs were kept, was poured into two troughs A and B. The 5-HT-like substance from the water in trough-A was removed following the procedure of Bertler *et al.*⁷ and the water in trough-B was left to serve as control. Two male crabs were released in both the troughs. The one in the trough-B showed searching behaviour, while the other remained normal. The latter exhibited searching behaviour on transferring to the trough-B.

Experiments using authentic sample of 5-HT confirmed the observations reported above. A 100 ml sample of 5-HT solution (10^{-4}) was added to 4 litres of water and the same poured in 2 troughs x and y. 5-HT from trough-x was removed by Bertler *et al.*'s method⁷ and the other trough was left as it was. A few male crabs were released in both the troughs and their behaviour observed. The males in trough-y reacted instantaneously exhibiting searching behaviour, while no such display was shown by those in trough-x. The latter when released in trough-y reacted as the former.

serves as the sex-attractant pheromone in the crabs studied.

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