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Studies on the Juvenile Hormone Extracts of the Butterfly Terias hecabe Linné

(*Lepidoptera*)

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(With 5 figures)

The corpora allata of insects produce a hormone which has been named "juvenile hormone" or "neotenin", which has been shown to influence the retention of larval characters (Wigglesworth, 1934, 1954). These glands are active in the imaginal insects also, and in the female, the hormone has been found to regulate growth of the eggs and yolk-deposition (Scharrer, 1952; Johansson, 1958). The role of the hormone in metamorphosis and reproduction has been elucidated by appropriate transplantation and ex-

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tirpation of the corpora allata in test animals. The first active extracts of the juvenile hormone were obtained by WILLIAMS (1956) as an oily substance from the abdomen of male cecropia silk moths. This finding has enabled investigators to make suitable extracts and assay the action of the juvenile hormone in insects (WIGGLESWORTH, 1958; GILBERT & SCHNEIDERMAN, 1960).

The present paper is a contribution to the study of endocrinology of Indian insects where an attempt has been made to extract the juvenile hormone of a common butterfly and to test the juvenilizing effects of the extracts on the same species.

Material and Methods

The common yellow butterfly, *Terias hecabe* LINN. (*Lepidoptera*: *Pieridae*) has been used in the investigation. Adult insects, belonging to both sexes were captured from the field on bright days, and their abdomens were cut and pulverised in a container in a small quantity of diethyl ether. Repeatedly the pulverata were extracted in quantities of ether until the supernatant was colourless. After filtration, the extract was evaporated when it left a residual, yellow, oily substance. It was dried at 60°C., for thirty minutes to remove the traces of ether. This ether-soluble material of the abdomen formed the extract (WILLIAMS, 1956).

In all experiments, the extracts were used undiluted.

1. Small droplets were injected into the body of the last larval instar or the pupa by a microneedle capillary tubing connected to an Agla micrometer syringe, each injection delivering 0.001 cc. The larger droplets (about 0.002 cc.) were blown in by mouth. The extract was injected dorsally or ventrolaterally into the first segment of the abdomen.

2. Surface of larval or pupal body was scratched gently with blunt glass rod, and a smear of the extract was applied on the surface. The scratching helped in the penetration of the hormone. In caterpillars, the application was made in the dorsum of the second abdominal segment, and in pupae, on the lateral side of the pro- and meso-thoracic segments. The smeared surface was then covered over by paraffin of low melting point. This was the adaption of the „wax test" method developed by SCHNEIDERMAN & GILBERT (1958).

3. Regeneration of thoracic legs was studied by amputation of one of the metathoracic legs of etherised last-instar caterpillars, where the wound was smeared with the extract and sealed with paraffin. In one half of the experimental lot, the amputation of both metathoracic legs was done, and only on one side the smear was applied.

The preparations of the sites of experiment were made, after fixation in Carnoy's fluid. They were dehydrated, cleared and mounted unstained in canada balsam.

Observations

1. Normal metamorphosis

The last instar caterpillar of *Terias hecabe* LINN., when fully fed, will start pupating after selecting a suitable site. The process of pupation will last for about 48 to 60 hours, beginning from the period of elaboration of silk for attachment of cremaster to complete emergence of the pupa. The pupal instar lasts for 6 to 7 days during the monsoon and post-monsoon

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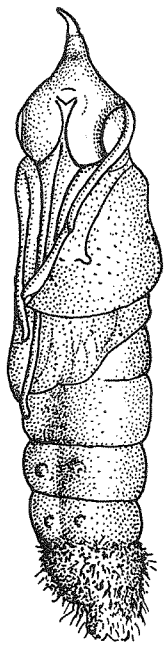
months (June to December). By January the population level of the insect falls considerably, and in summer the insects are few and the larvae are practically on the hedges. Many caterpillars die off under laboratory conditions in summer, where the temperature from February to May will be from 29 to 34 °C.

2. Injection Experiments

Of the 160 caterpillars that have been injected with 0.001 to 0.002 cc., of the extract, only 9 survived. These experiments were conducted when larvae were abundant and normally active, the temperature ranging from 24 to 26 °C. All the surviving caterpillars pupated, but the pupal transformation was delayed by over 36 hours. The control larvae pupated after 60 hours while the treated ones did so only after 96 hours. The pupae were normal except two, which latter belonged to a lot where larger quantity of the hormone was injected. These were somewhat strange in that, they showed on the ventral side of the abdomen, paired prominences, which were thicker and darker, resembling imperfectly organised prolegs (Fig. 1). The skin pattern even at the site of injection was pupal, though the proleg thickenings were conspicuous.



The survival of specimens out of a total of 160, suggets a high percentage of mortality (only 5.6% surviving).

Fig. 1. Pupa of *Terias hecabe* Linn. which has received an injection of a dose of ether extract containing juvenile hormone. The injection was made on the ventrolateral side of the 1st abdominal segment. The abdomen shows the pressence of sclerotised thickenings corresponding to larval prolegs

Injection of the extract into the body of pupae was found to be lethal; none of the 110 pupae tested, survived. Injection was made into 24-hour old and 48-hour old pupae; both the categories blackened and died in the course of three days.

3. Wax Tests

The "wax test" proved to be a very satisfactory method of studying the hormonal activity of the extract.

The treated caterpillars showed no delay in moulting into the pupae. The pupal skin exhibited no special feature on the treated segment, except that in several examples the skin was rather thin and transparent. A distinctive juvenilizing effect was not observable, and the setae on the body were all of the characteristic tiny pupal type.

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No larval type of elongated seta or peg was visible in any example. One hundred caterpillars were subjected to the "wax tests" and all of them emerged as normal adults.

But the application of the extract on the thorax of the pupa yielded different results. One side alone was treated with the extract, and that only once on one pupa; the other side offered itself as the control. The hormone was applied after 24 and 48 hours of pupation.

When the butterflies emerged from such pupae, they exhibited normal, adult cuticle bearing well formed, yellow scales on the control side, while on the treated side the cuticle was black and devoid of scales (Fig. 2). The preparation of such skin revealed a general absence of sockets of scales in the region (Fig. 3); on closer examination highly aborted and tiny scales which were intermediate between scales and setae were found here (Fig. 4). The number of such intermediate scales were small, and these few ones were widely separated and their distribution resembled the few and scattered pupal setae. Both one-day-old and two-days-old pupae yielded similar results.

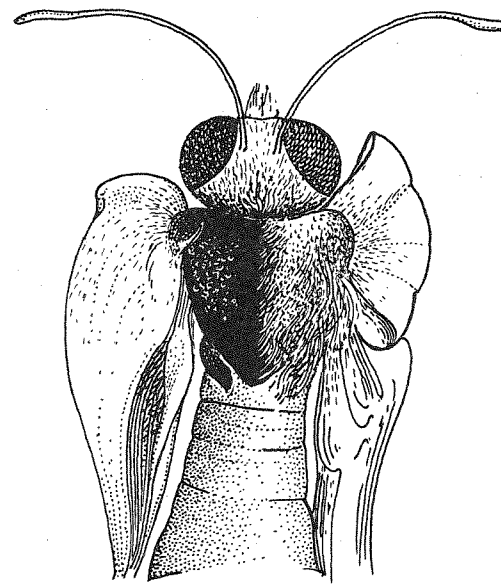

Fig. 2. Dorsal view of the adult of *Terias hecabe* LINN. which has been treated with the extract on the left side of thoracic segments, to show the absence of scales in the region

4. Regeneration of Thoracic Legs

When a metathoracic leg of a caterpillar is amputated at its base, three days before pupation, a new leg will be regenerated in full when the animal emerges out as the adult. If a smear of the hormone in the extract is laid on the wound and sealed off with paraffin, only a small stump of the leg will be present in the adult as the regenerate. Figure 5A shows such a regenerated stump on the side treated with the extract. It shows an apparently two segmented

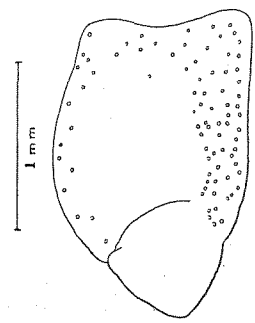

Fig. 3. Thoracic region of adult *Terias hecabe* LINN. showing the general absence of sockets of scales in the region which has been smeared with the extract and covered by paraffin, while a pupa

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but well nature, which is, however, completely undifferentiated sclerotised. From its base arises a small prominence with two small setae. This leg is remarkably similar in organisation to the typical thoracic leg of a caterpillar (Fig. 5B), which is with two segments and a claw, and bearing a ventral sense pad (?) with a pair of long setae. In some examples, regenerate was totally absent, the region exhibiting only a sclerotised patch as a scab.

Discussion

That ether-extracts of abdomen of adults of *Terias hecabe* LINN., contain quantities of the juvenile hormone, is very well illustrated by the results

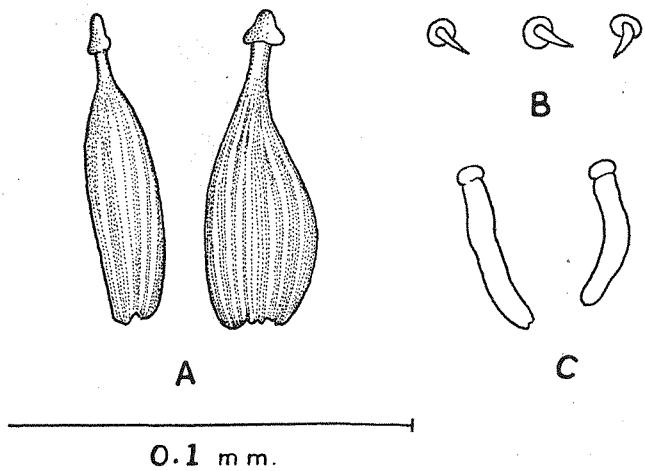

Fig. 4. Structure of typical imagnial scales (A); typical pupal setae (B); and the intermediate type (C) obtained as a result of wax test, taken from region shown in Fig. 2

obtained in the present investigation. The existence of this hormonal principle in etherethanol extracts of bodies of adult *Periplaneta*, larval *Pieris* and larval *Rhodnius* have been reported by WIGGLESWORTH (1958). Injection experiments have induced a high rate of mortality in caterpillars of *Terias* and in pupae they have been completely lethal. A high degree of similar mortality has been recorded by WIGGLESWORTH (1958), though SCHNEIDERMAN & GILBERT (1959) have pointed out that diluted extracts have been very well tolerated. However, the fact that some caterpillars of *Terias* have pupated, is suggestive of the fact that in some cases at least, undiluted extracts will be tolerated. The normal pupation, and the emergence of the normal butterflies indicate that the juvenile hormone content of the extract has not succeeded in exercising juvenilizing effects except in the production of a localised change in two examples. This tends to support the view that the last instar larva can inactivate the juvenile hormone (WIGGLESWORTH, 1952; GILBERT & SCHNEIDERMAN, 1960). However,

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a marked sensitivity of the region bearing the prolegs to higher titre of juvenile hormone has been reported by PIEPHO (1950) who obtained pupae with larval prolegs and imagines with skin bearing underlying proleg structures, by supplying several corpora allata to an wound site on the ventro-

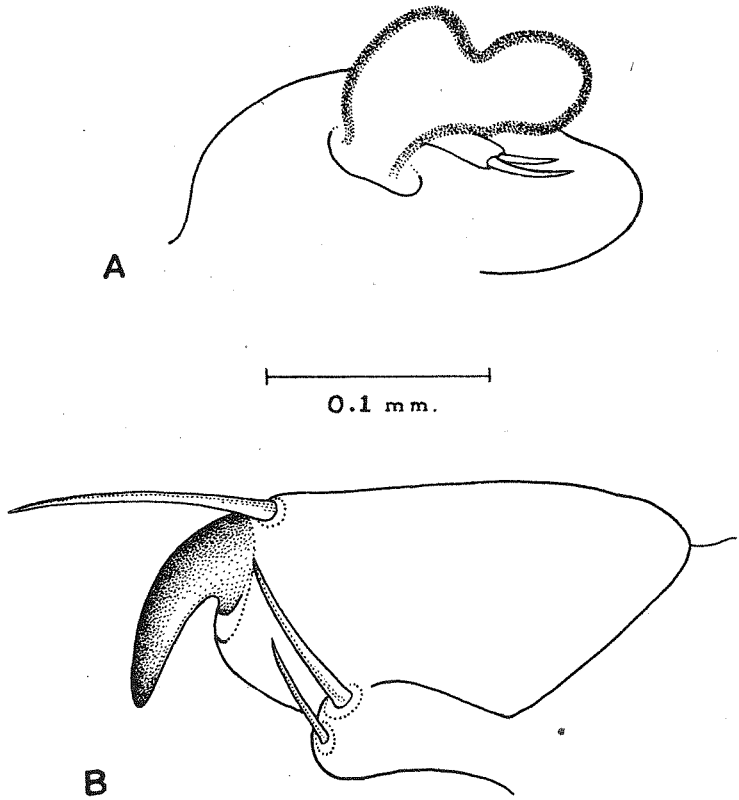

Fig. 5. A. Regenerate of metathoracic leg from adult *Terias hecabe* LINN. under the influence of the juvenile hormone extract. It is faintly bilobed and bears the typical larval sensory pad
B. Normal metathoracic leg of a caterpillar of *Terias hecabe* LINN. The sensory pad and the two segmented nature are well marked

lateral side of the last instar caterpillar of *Galleria*. The indications of prolegs on pupae of *Terias* reported in this paper, are also illustrative of this sensitivity to increased dosage; this would tend to suggest that the inactivation of the juvenile hormone presumed earlier could only be partially complete.

Wax tests applied to the early pupa form the most valuable methods of studying the juvenilizing effects of the ether-extracts of adult abdomen. Structures intermediate between imaginal scales and larval setae were

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laid out on the skin, in a pattern conforming to that of pupae, on regions of skin of adults, on the treated sides. Similar structures of an intermediate nature have been recorded on transplanted skin of Lepidoptera by NAYAR (1954) where the pupal stadium was made to skip over. The juvenilizing effects of the corpora allata have been recorded in transplants in other insects also (in *Iphita* by NAYAR, 1956), and in studies using extracts similar observations have been recorded by WIGGLESWORTH (1958) and GILBERT & SCHNEIDERMAN (1960).

Application of full strength extract of juvenile hormone to tip of abdomen of *Rhodnius* resulted in the failure of differentiation of valvulae of the genitalia and retention of the sclerotised ring around anus (WIGGLESWORTH, 1958). This shows that differentiation of appendages also is influenced by the juvenile hormone. PFLUGFELDER (1939) has shown that in *Dixippus*, the presence of corpora allata is needed for the normal progress of regeneration. But BODENSTEIN (1955) finds that the prothoracic gland hormone is the one related to normal regenerative processes. The moulting hormone (produced by the prothoracic gland) is present in the last instar larva and the pupa to produce the pupal and imaginal moults respectively in *Terias*, but the presence of extra juvenile hormone from the extracts, has induced the production of a stump, resembling a larval thoracic leg, thus exhibiting a distinctive juvenilizing effect. This would also be indicative of a possibility that the inactivation of the juvenile hormone which has been presumed to occur in the injection experiments, may not be so effective in this instance, because of the topical relationship between the blastema and the small quantity of the juvenile hormone supplied in the smear made on the wound.

Acknowledgment

The author is indebted to the post-graduate students of the Department of Zoology, University College, Trivandrum, for 1961/62, for help in the collection of butterflies and extraction of the juvenile hormone.

Summary

1. Diethyl ether-extracts of whole abdomen of adults of *Terias hecabe* LINN., (*Lepidoptera*: *Pieridae*) were made to test the juvenile hormone-effects of the same.

2. Experiments were done on caterpillars and pupae of *Terias*, involving injection and topical application of the extracts.

3. A high rate of mortality occurred when injections were given. All pupae died, while about 5.6% of the caterpillars alone pupated, when injections were made. In two exceptional cases, indications of prolegs were noticeable on the abdomen of the pupae as tanned swellings.

4. Topical application on skin of caterpillar, followed by sealing with paraffin ("wax tests") showed no effect on the insects and the larvae moulted into normal pupae and adults. But topical application on pupae produced on the adult skin an absence of true scales on the treated area. Instead the patches showed intermediates between scales and setae arranged sparsely like pupal setae.

5. Application of extracts on amputated surface of metathoracic legs of caterpillars, resulted in the production of stump-like regenerates in the adult butterflies. These

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regenerates were bilobed, with a seta-bearing ventral organ, resembling a larval thoracic leg.

6. The findings have been discussed in the light of recent literature on juvenile hormone and its physiology.

Zusammenfassung

1. Es wurden Äther-Extrakte ganzer Abdomina von adulten *Terias hecabe* L. (*Lepidoptera*: *Pieridae*) gemacht, um deren Juvenilhormonwirkung zu prüfen.

2. Die Versuche wurden mit Raupen und Puppen von *Terias* bei Injektion und örtlicher Anwendung der Extrakte durchgeführt.

3. Bei Injektionen trat hohe Mortalität auf. Alle Puppen starben, während nur 5,6% der Raupen zur Verpuppung kamen. In zwei Ausnahmefällen wurden Andeutungen von Beinen am Abdomen der Puppen als gelbliche Anschwellungen bemerkt.

4. Örtliche Anwendung auf der Haut von Raupen mit Wachsabschluß (wax tests) zeigte keine Wirkung; die Raupen entwickelten sich zu normalen Puppen und Imagines. Aber örtliche Anwendung bei Puppen bewirkte auf der Haut der Imagines das Fehlen echter Schuppen an der behandelten Stelle. Stattdessen zeigten diese Stellen Zwischenformen zwischen Schuppen und Borsten, die wie Puppenborsten zerstreut angeordnet waren.

5. Bei Anwendung von Extrakten auf der Wundfläche amputierter Metathorakalbeine von Raupen erfolgte bei den adulten Tieren die Bildung eines stummelförmigen Regenerates, das zweilappig und mit einem borstentragenden Ventralorgan an ein larvales Thorakalbein erinnert.

6. Die Ergebnisse werden unter Berücksichtigung der neueren Literatur über Juvenilhormone und ihre Physiologie diskutiert.

Резюме

1. Из целых абдоменов взрослых *Terias hecabe* L. (*Lepidoptera* : *Pieridae*) изготовлялись эфирные вытяжки, для испытания действия ювенильных гормонов.

2. Опыты проводились на гусеницах и куколках *Terias* в условиях ин'екции и местного применения вытяжек.

3. При ин'екциях получилась большая смертность. Все куколки погибли, в то время, как лишь 5,6% гусениц окукливались. В двух исключительных случаях отмечались на абдомене куколок следы ног в виде желтоватых опуханий.

4. Местное применение вытяжек на коже гусениц с wax tests не оказало действия; гусеницы продолжали свое развитие и превращались в нормальные куколки и imagines. Однако, последствием местного применения было отсутствие чешуек на месте применения. Вместо этого на таких местах показались промежуточные формы между чешуйками и щетинками, расположенными разбросанно, как у куколок.

5. При применении экстрактов на раневой площади ампутированных гусеничных ног метаторакса у взрослых насекомых образовался культяобразный регенерат, который являясь двудольным и обладая брюшным органом с щетинками, напоминал личиночную грудную ногу.

6. С учетом новейшей литературы о ювенильных гормонах и их физиологии обсуждаются полученные результаты.

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Beiträge zur Kenntnis der Adoretini der aethiopischen Region

(*Coleoptera*: *Lamellicornia*, *Melolonthidae*, *Rutelinae*)

II. Teil [1])

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(Mit 9 Textfiguren)

1. Ist *Adoretus oedipus* Fairmaire ein *Trigonochilus*?

Der von Fairmaire (1903) vom Mt. d'Ambre in Madagascar beschriebene *Adoretus oedipus* wurde von Ohaus (1912) in seiner „Revision der *Adoretini*" in das Genus *Trigonochilus* Brenske (1896) gestellt. Die Art soll im Bau der Oberlippe, der Borstenpunkte auf der Halsschildscheibe und in der starken Verschiedenheit der äußeren Klauen an den Hinterfüßen mit *Trigonochilus* übereinstimmen. Ohaus war sich aber über die Stellung dieser

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