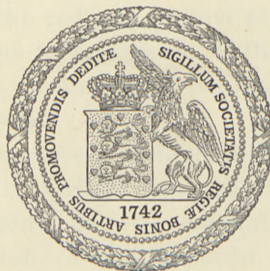


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EFFECT OF CORPUS CARDIACUM
AND OTHER INSECT ORGANS ON THE
COLOUR-CHANGE OF THE SHRIMP,
LEANDER ADSPERSUS

BY

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I. Introduction.

During later years our knowledge of colour-change in the *Crustacea* has been much furthered by the discovery of hormones controlling the movement of pigments in the chromatophores (KOLLER 1925 and later, PERKINS 1928) and by the demonstration that the sinus-gland, which in most *Decapoda* is localized in the eye-stalk, produces such hormones (HANSTRÖM 1935 and 1937, CARLSON-CARSTAM 1935 and 1936). In the majority of the *Decapoda* (with the exception of the *Brachyura*), a removal of the eye-stalks causes maximum expansion of the commonly occurring red and yellow pigments. When subsequently an extract of the eye-stalks is injected into these blinded animals, the result is a rapid and complete contraction of the pigments making the chromatophores appear as dots, while the animal as a whole acquires a transparent whitish colour. A similar effect is obtained by injection of eye-stalk extract into shrimps adapted to a dark background. These results evidently mean that the sinus-gland normally produces a hormone causing contraction of the red and yellow pigments, while absence of the hormone results in expansion of the pigments. The white pigment which occurs in many species reacts in a different and somewhat irregular way.

The *Brachyura*, however, behave differently. These crustaceans possess melanin (which is said to be absent in other decapods save *Crangon*), and a removal of the eye-stalks is followed by contraction of the melanin, whereas injection of the hormone results in expansion of this pigment. On the other hand, the yellow and red pigments, also present in many crabs, react wholly (yellow) or partly (red) as in other decapods. For details, the reader is referred to the original papers (for the

Brachyura see especially CARSTAM 1942) and to the very useful review given by HANSTRÖM in his book "Hormones in Invertebrates" (1939).

In some interesting papers (1936, 1937 a and b, 1938, 1940 a), HANSTRÖM showed that extracts of the head of various insects, when injected into blinded shrimps (*Natantia*), have a similar effect as the crustacean eye-stalk hormone, i. e. they cause a definite contraction of the red and yellow pigments, while the white chromatophores are not or only irregularly affected. In addition to this, CARLSON-CARSTAM (1935, 1936) and HANSTRÖM found that extracts of insect heads expand the black chromatophores of *Brachyura*. Thus, these extracts in their physiological effect show a striking conformity with the sinus-gland hormone.

The technique used by HANSTRÖM in these experiments was the following: The heads were crushed and extracted with seawater, the extract was boiled, filtered, and diluted to the original volume. In some cases it was found that unboiled extract had no (or only a slight) effect, while boiled extract had a considerable effect.

According to the insect species employed, the activity of the head extract showed a considerable variation. Extracts of heads of various species of *Saltatoria* had a vigorous effect, the colour of the test shrimps fading rapidly to a transparent white. The same applies to head extracts of blattids and phasmids. On the other hand, heads of some *Coleoptera*, *Hymenoptera*, and *Diptera* caused a less pronounced colour-change, and heads of certain *Lepidoptera*, adult *Odonata*, *Dermaptera*, and *Thysanura* had no effect at all (cf. HANSTRÖM 1940 a p. 10—11). In some species in which head extracts were very active, extracts of thorax and abdomen sometimes caused a weak reaction, sometimes none.

It was further proved that the insect head extract does not lose its activity when dissolved in alcohol or after being kept dry for 6 weeks; also in this respect it resembles the eye-stalk hormone (KALMUS 1938, HANSTRÖM 1940 a).

The far reaching similarity in the qualities of the two substances incited HANSTRÖM to infer that the insect head contains a hormone similar in kind to the chromatophorotropic hormone of the sinus-gland. The question of the localization of the in-

cretory organ inside the head was approached on the following line: Heads of *Dixippus* were cut transversely just behind the eyes, and extracts were made in the usual way from the anterior and the posterior parts, separately (HANSTRÖM 1938). When injected into blinded *Leander adpersus*, the extract of the hind portion caused a rapid and vigorous contraction of the chromatophores, while the anterior portion had little or no effect. Besides muscles and tracheae, the posterior half of the head contains the suboesophageal ganglion, the oesophageal (hypocerebral) ganglion, the corpora cardiaca, and the corpora allata. As the two last-mentioned organs are the only organs of the insect head which show the morphological characteristics of incretory glands, HANSTRÖM considers it likely that either the corpora allata or the corpora cardiaca are the source of the hormone in question.

In his later paper (1940 a), HANSTRÖM described some preliminary experiments with the isolated corpora cardiaca and corpora allata of blow-flies (*Calliphora*). The organs were dissected in Copenhagen by Mrs. ELLEN THOMSEN, they were dried on filter paper and sent by mail to Lund, where Professor HANSTRÖM made the extracts and injected them into blinded *Leander*. The shrimps which had received extract of the corpora cardiaca showed an almost maximum contraction of the pigments, while the corpora allata produced a less convincing reaction. Extracts of total heads (without the organs mentioned) were ineffective.

Although these experiments pointed to the corpora cardiaca as the real source of the chromatophoretropic substance, they could not be regarded as conclusive. As Professor HANSTRÖM did not wish to pursue the problem himself, it was agreed between us that the author should do so. I take the opportunity of thanking Professor HANSTRÖM.

The injection experiments were made at the »Danmarks Akvarium«, Charlottenlund. I am indebted to the governing committee and to the staff, especially to Dr. A. F. BRUUN, leader of the biological department, for providing me with working-facilities, aquaria etc.

My thanks are due to Dr. H. BLEGVAD for a material of living *Crangon vulgaris* caught in the Øresund. As a test animal,

this species was found to be inferior to *Leander adpersus*, and the few experiments made will not be included in this paper. The results were in full accordance with those obtained with *Leander*.

I have received help from several persons who provided the great number of living insects necessary for the investigation. My special thanks are due to Dr. ANKER NIELSEN, Hillerød, for collecting the specimens of *Dytiscus marginalis*.

I am very grateful to Mr. ANKER HANSEN of the Laboratory of Normal Anatomy, Royal Veterinary and Agricultural College, for taking a number of colour photographs. The ordinary photographs have been taken by the author.

The Carlsberg Foundation has supported the work with a grant.

II. Insect species used in the experiments.

The insect organs used for preparing the extracts were mainly taken from *Orthoptera* (sens. lat.), as it was convenient to use insects of a reasonable size and species which could be bred in the laboratory without too much difficulty. The following species were chosen:

Saltatoria.

Tachycines asynamorus Adel.

An apterous, long-horned grasshopper frequently found in greenhouses. The majority of the experiments has been made with organs of this species.

Gryllus domesticus L.

Phasmida.

Dixippus (Carausius) morosus Br.

Blattariae.

Blatta orientalis L.

Coleoptera.

Dytiscus marginalis L.

Diptera.

Calliphora erythrocephala Meig.

III. Remarks on the corpora cardiaca, the corpora allata, and the stomatogastric nervous system.

It is not the scope of this paper to enter into a detailed discussion of the morphology and the function of these interesting organs which, during recent years, have attracted a good deal of attention of insect physiologists and anatomists. Reviews of our present knowledge have recently been given by HANSTRÖM (1939 and 1942) and PFLUGFELDER (1941) (cf. also HANSTRÖM 1940 b). Some remarks regarding the anatomy of the organs in the species of insects treated in this work may however be useful.

DE LERMA (1937) has described the anatomy of the corpora allata and corpora cardiaca ("corpi faringei") of *Gryllotalpa*, *Blatta orientalis*, and some species of grasshoppers. His figure of *Gryllotalpa* may be regarded as a diagram of the anatomical relations of these organs prevailing in the *Saltatoria*. The elongate corpora cardiaca are situated in the head dorsally to the oesophagus. The posterior part of each corpus cardiacum is connected with the wall of the aorta, the anterior end receives a nerv from the brain (protocerebrum), while lateral nerves pass to the corpora allata. These are ovoid and lie on the sides of the oesophagus. In the median line, between the corpora cardiaca and partially united with them, we find the ganglion hypocerebrale which belongs to the stomatogastric nervous system; it receives a nerve (nervus recurrens) from the frontal ganglion.

In numerous insects, including the orders mentioned above, HANSTRÖM (1940 b and 1942) found two paired nerves which pass from the brain to the corpora cardiaca: the nervi corporis cardiaci I and II. He gave a picture of the corpora cardiaca of *Tachycines* in which he designed both nerves. The nerve pictured by DE LERMA is the n. corporis cardiaci I.

The histology of the corpora cardiaca has been described by the same authors. According to DE LERMA, they contain numerous nuclei which resemble small nerve cells, and probably they contain neuropilem, so that they still retain the nature of ganglia. On the other hand, they also possess numerous cells with vacuolized cytoplasm and acidophilous granula, which

must be secretory cells; these two types of cells are to some degree separated in different parts of the organ ("parte glandolare", "parte nervosa"). There are signs that the secretion is chiefly discharged into the aorta, and to a smaller extent through the lateral walls of the corpora cardiaca, which are bathed by the haemolymph. From these observations DE LERMA concludes that the corpora cardiaca are incretory glands.

HANSTRÖM (1940 b, 1942) confirmed DE LERMA's observations and accepted his idea as to the function. Especially in *Tachycines*, he found the tissue of the corpora cardiaca virtually flooded with fuchsinophile drops, "deren Vorhandensein die Hypothese einer inkretorischen Tätigkeit der betreffenden Organe kräftig unterstützt. Diese Hypothese muss jedoch natürlich auf experimentellem Wege bestätigt werden."

In *Orthoptera* and blattids the corpora allata are paired organs. They consist of a great number of uniform cells with oval nuclei rich in chromatin; cell-boundaries are not visible. As is well known, the corpora allata are proved to be incretory organs which especially influence the metamorphosis and the ovarian development of insects (WIGGLESWORTH, WEED-PFEIFFER, PFLUGFELDER, BOUNHIOL, ELLEN THOMSEN, and others, vide E. THOMSEN 1942).

Own observations.

Tachycines asynamorus.

In several respects, the organs of *Tachycines* resemble those of *Grylotalpa* as described by DE LERMA, but in some features the two species differ.

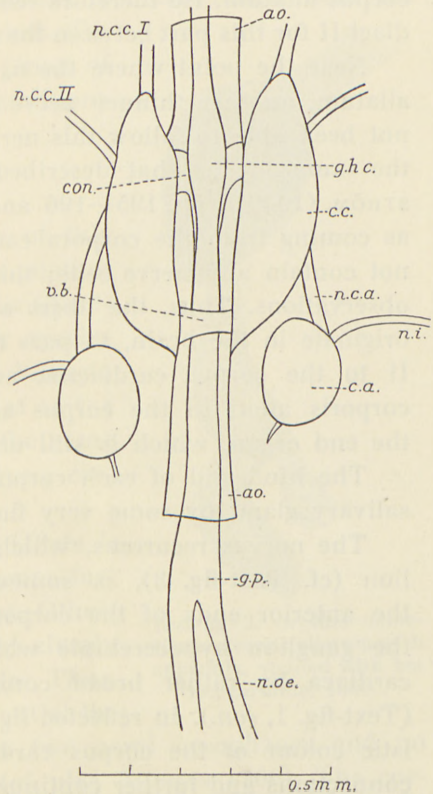
The corpora cardiaca (Text-fig. 1 and Plate I, fig. 1) are elongate organs measuring about 0.4—0.5 mm in the adult. They are situated behind the brain and are fastened to the dorsal side of the oesophagus by fine connective tissue and tracheae. Their caudal ends are fixed to the fat body by some very fine threads. The corpora cardiaca are intimately connected with the dorsal vessel, they may be described as thickened parts of the lateral walls of the aorta; in most of their length, the two corpora are separate, held together dorsally and ventrally by the ordinary thin wall of the aorta, but the posterior ends are united by a ventral bridge (Text-fig. 1, *v.b.*).

In dissections the corpora cardiaca are mostly conspicuous due to their bluish white colour. A similar colour is generally displayed by the corpora cardiaca of all other insect species I have dissected, and it is remarkable that, according to a personal communication by Professor HANSTRÖM, the sinus gland of *Decapoda* has the same bluish white hue.

The anterior end of each corpus cardiacum receives a well developed nerve from the brain; it enters the corpus cardiacum ventrally near the ganglion hypocerebrale (HANSTRÖM 1940 b, fig. 188). While, no doubt, this nerve is the nervus corporis cardiaci I, another thinner nerve, which is connected with the corpus cardiacum a little further behind (also ventrally), is probably the n. corporis cardiaci II.

The corpora allata are nearly spherical. Anteriorly each of them receives the nervus corporis allati, a rather stout nerve coming from the ventral side of the anterior part of the corpus cardiacum. It looks as if the nerve fibers really originate from the n. corporis cardiaci II and only pass through the ventral part of the corpus cardiacum for a short distance.

In this connection, it should be remembered that PFLUGFELDER (1937) describes the n. corporis allati of *Dixippus* as coming directly from the brain, and only receiving a few nerve fibers from the corpus cardiacum (Text-fig. 3). HANSTRÖM (1940 b), however,



Text-fig. 1. *Tachycinus asynamorus*. ao. aorta; c.a. corpus allatum; c.c., corpus cardiacum; con., connection between c. cardiacum and ganglion hypocerebrale (g.h.c.); g.p. posterior ganglion; n.c.a., nervus corporis allati; n.c.c.I, nervus corporis cardiaci I; n.c.c.II, nervus corporis cardiaci II; n.i. nerve from c. allatum; n.oe., nervi oesophagei; v.b., ventral bridge. — Dorsal view. 70×. Semi-diagrammatic.

holds that this nerve has a similar course as in other insects, entering the corpus cardiacum, where some of its nerve fibers branch between the cells, while others possibly pass directly to the corpus allatum. He therefore retains the name of n. corporis cardiaci II for this part between the brain and the corpus cardiacum.

Near the point where the n. corporis allati enters the corpus allatum, another thinner nerve (Text-fig. 1, *n.i.*) issues. I have not been able to follow this nerve to its end, but it is probably the same one as that described by NABERT (1913) and HANSTRÖM (1940 b, pp. 195—196 and 226). These authors regard it as coming from the corpora cardiaca, as the corpora allata do not contain any nerve cells; this is in accordance with my own observations. Thus, the fibers of this unnamed nerve seem to originate in the brain, to pass through the n. corporis cardiaci II to the corpus cardiacum and, from there, through the n. corporis allati to the corpus allatum before they continue to the end organ, which is still unknown.

The hind end of each corpus allatum is connected with the salivary gland by some very fine and short threads.

The nervus recurrens, which comes from the frontal ganglion (cf. Text-fig. 3), is somewhat swollen at the height of the anterior ends of the corpora cardiaca. This thickening is the ganglion hypocerebrale which is joined with the corpora cardiaca by rather broad connections ventrally to the aorta (Text-fig. 1, *con.*). In reflected light, it is seen that the characteristic colour of the corpus cardiacum is also shown by these connections and further continues as a fine layer down the sides of the nervus recurrens. It seems possible that this colour indicates the occurrence of glandular cells.

In *Gryllotalpa* (DE LERMA), *Rhaphidophora* (ANDER 1939), and several species of *Saltatoria* investigated by BORDAS (1900), the nervus recurrens branches out at the ganglion hypocerebrale into two nerves which are sometimes called the nervi oesophagei. In *Tachycines*, however, the nerve continues undivided in a caudal direction, forming another ganglion somewhat behind the corpora allata, and then it branches into the nervi oesophagei. BORDAS found a similar course of the nervus recurrens in two species of *Mantidae* and three species of *Blattidae*. He

terms the posterior ganglion the "ganglion stomacal", while HOFER (1886) in *Blatta orientalis* describes it as "hinteres dreieckiges Ganglion des nervus recurrens" (cf. his fig. 1, Plate 1). In this species the nervus recurrens is much longer, and the ganglion is situated further behind. *Tachycines* seems to be the only genus of the *Saltatoria* in which such conditions hitherto have been observed.

Gryllus domesticus.

The most interesting feature of this species is shown by the corpus allatum which has a remarkable appendix (Text-fig. 2), not found in any other species investigated and apparently hitherto undescribed. It is not transparent as the corpus allatum proper, but of the same bluish white colour as the corpus cardiacum.

The nervus corporis allati (Plate I, fig. 2) leaves the corpus cardiacum further behind than in *Tachycines*. Otherwise, the relations of the organs closely resemble those found in *Gryllotalpa*. Thus, the nervus recurrens branches out at the ganglion hypocerebrale into the two nervi oesophagei, and no additional ganglion is found (cf. also BORDAS 1900). The corpora cardiaca unite posteriorly.

Dixippus (Carausius) morosus.

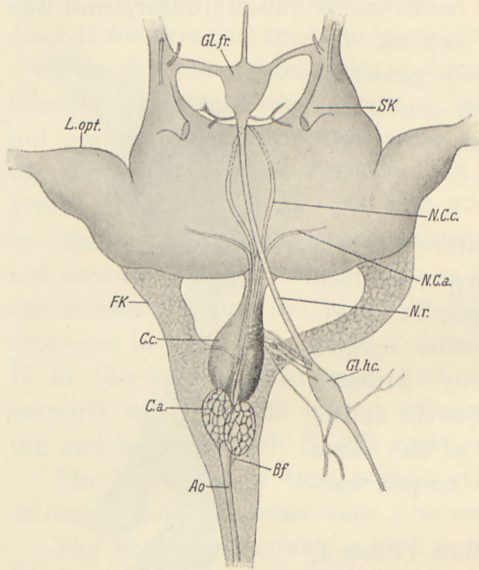
The stomatogastric nervous system and the corpora cardiaca and allata have been studied by several authors. The most detailed description has been given by PFLUGFELDER (1937) (cf. Text-fig. 3). I have nothing to add to his record, only it should be emphasized that the very close connection between the corpus cardiacum and allatum makes it almost impossible to separate these organs without injuring one or the other, most often the corpus cardiacum (cf. also Plate II,



Text-fig. 2. *Gryllus domesticus*. Corpus allatum with appendix, stained with borax-carmin. 100X.

fig. 3). This means that secretion may flow out into the 1% NaCl in which the insect is immersed for dissection or that even small bits of tissue adhere to the corpus allatum. This may

involve impurity of the extract of the corpus allatum and even of other organs.



Text-fig. 3. *Dixippus morosus*. Brain and stomatogastric nervous system. Ventral view. Ao, aorta; Bf, fibrils; C.a., corpus allatum; FK, fat-body; GL.fr., ganglion frontale; GL.hc., ganglion hypocerebrale; L.opt., optic lobe; N.C.a. nervus corporis allati (= n. corporis cardiaci II after HANSTRÖM); N.C.c., nervus corporis cardiaci I; N.r., nervus recurrens; SK, oesophageal connective. (After PFLUGFELDER).

Blatta orientalis.

The corpora allata and cardiaca etc. of this species have been described by earlier authors (especially HOFER 1887, POLICE 1910, BRETSCHNEIDER 1914, and DE LERMA 1937), while BORDAS (1900), NABERT (1913), and HANSTRÖM (1940 b) have treated *Blattella germanica* and other species. The investigators disagree on certain points and, therefore, I think it necessary to give a short description of my own observations which are based mainly on dissections

and partly on sections of the organs.

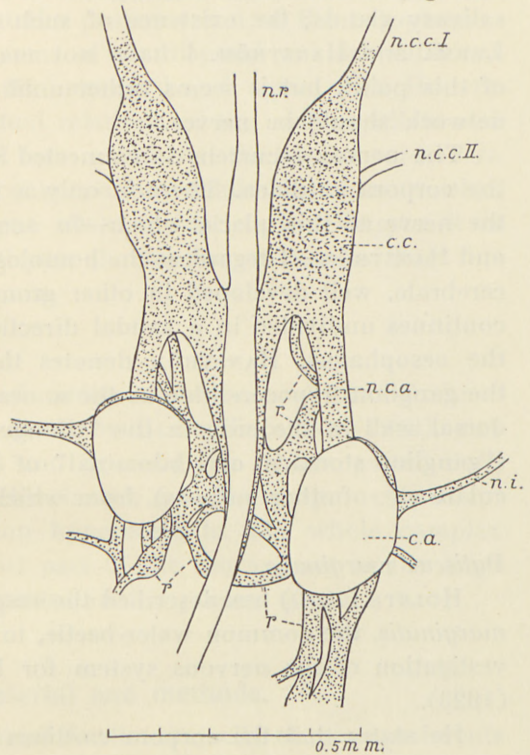
In dissected preparations the corpora cardiaca tend to diverge anteriorly (Text-fig. 4); this is probably due to a tearing of the thin wall of the aorta, as in sections they are found to adhere closely and to form the ventral wall of the aorta (Plate II, fig. 4, cf. also HANSTRÖM 1940 b, fig. 221). Posteriorly, they disjoin from the wall of the aorta which lies dorsally to them. Each corpus cardiacum receives two nerves; the foremost and coarsest is the n. corporis cardiaci I, while the other, which enters the corpus cardiacum further back, probably represents the n. corporis cardiaci II. Posteriorly, the corpora cardiaca are united with the nervus recurrens, continuing as narrow layers on each side

of this nerve almost as far as the hind end of the corpora allata.

From each corpus cardiacum comes a lateral band-like branch which goes to the corpus allatum and which must be regarded as the nervus corporis allati. It is remarkable that in fresh preparations this broad band has the same bluish white colour as the corpus cardiacum; it also contains many nuclei resembling those of the corpus cardiacum, so that its nervous character is not very obvious. It is likely, however, that it does contain nerve fibers which proceed through the corpus allatum to the fine nerve going out from this organ (Text-fig. 4, *n.i.*).

The corpora allata are dorsoventrally flattened, disc-like organs, in sections tending to show a narrow lumen which may be an artefact. They are connected with the corpora cardiaca not only by the nervus corporis allati already referred to, but also by an irregular network of tissue between each corpus allatum and the caudal continuation of the corpus cardiacum on the same side (Text-fig. 4, *r*).

The configuration of this network shows great individual variation. There is also a connection between the networks of the two sides, usually as a ventral bridge below the nervus recurrens. Similar, rather irregular threads connect the corpora allata with the salivary glands which lie just behind them.



Text-fig. 4. *Blatta orientalis*. *n.r.*, nervus recurrens; *r.*, network between corpora allata and corpora cardiaca. Other letters as in Text-fig. 1. Dotted: organs of bluish colour. Dorsal view. 70×. Semi-diagrammatic.

The above description in most points agrees with the old account by HOFER (1886), while DE LERMA does not picture any network between the corpora allata. HOFER assumed that the corpora allata were ganglia which give off nerves to the salivary glands; the existence of such nerves is denied by DE LERMA and HANSTRÖM. I have not made any close inspection of this point, but it seems rather unlikely to me that the whole network should be nerves.

The nervus recurrens is connected with the caudal ends of the corpora cardiaca. There is only a very slight thickening of the nerve at this place, which—in accordance with DE LERMA and HANSTRÖM—I regard as the homologon of the ganglion hypocerebrale, well developed in other groups. The nervus recurrens continues undivided in a caudal direction on the dorsal side of the oesophagus, HANSTRÖM denotes the posterior part behind the ganglion hypocerebrale as the n. oesophageus. It ends on the dorsal wall of the crop in the “triangular ganglion” of HOFER (“ganglion stomacal ou abdominal” of BORDAS, ganglion ventriculare etc. of other authors), from which two branches originate.

Dytiscus marginalis.

HOLSTE (1910) has described the respective organs in *Dytiscus marginalis*, the common water-beetle, in connection with his investigation of the nervous system for KORSCHULTZ'S monograph (1923).

He states that the corpora cardiaca (“Ganglien des Rückengefäßes”) are not very distinctly circumscribed (“ihre Form ist wenig scharf umrissen”); they do not resemble typical ganglia, but are more band- or pad-like in shape. They are intimately connected with the wall of the dorsal vessel and receive paired short nerves from the protocerebrum. They are said to innervate the dilatator muscles of the oesophagus through a very fine nerve on the median side posteriorly, to be connected with the nervus recurrens through a fine nerve, and to send out a lateral stouter nerve, which goes below the optic nerve and fuses with a branch of the maxillary nerve.

The corpora allata (“Tracheenganglien”) are described as elongate-spherical bodies lying close to the oesophagus. From HOLSTE'S fig. VIII it is seen that they are intimately connected

with the corpora cardiaca, almost as in *Dixippus*, without any intervening nerve.

The nervus recurrens is said to be devoid of a ganglion hypocerebrale, it continues as a strong unpaired nerve ending with a ganglion ventriculare at the posterior end of the oesophagus.

The number of specimens at my disposal was not sufficient for a detailed study of the organs, but I agree with HOLSTE as to the shape and mutual relations of the corpora allata and cardiaca. In my journal I have noted that the corpus cardiacum has an irregular, flattened shape, almost reminding of an amoeba; it adheres with the corpus allatum. Just as in *Dixippus* it is next to impossible to extirpate the corpus allatum without injuring the corpus cardiacum.

Calliphora erythrocephala.

The corpus allatum and cardiacum of the adult blow-fly have recently been studied in detail by ELLEN THOMSEN (1942), and I refer to the description and figures given in her paper. Both organs are unpaired in this insect, and the corpus cardiacum is fused with the ganglion hypocerebrale. The whole complex is situated in the foremost part of the thorax, not in the head as in the other species treated in the present paper.

IV. Material and methods.

The decapod species used for the majority of the experiments was the common edible shrimp *Leander adspersus* Rathke. The shrimps were bought at intervals on the fish-market of Copenhagen and taken to the aquarium. There was a rather high initial mortality, but after some days, the survivors had settled in the tank and—at least in the autumn and the winter—seemed to thrive well, they ate profusely and lived for weeks or even months. In some periods—especially during the warmer months and in the coldest periods of winter—it was impossible to procure living shrimps, and this is the cause of the limited number of animals in some of my experiments.

According to KOLLER (1936), *Crangon* has four clearly different pigments (melanin, white, yellow, and red), whereas *Leander adspersus* is said to show a remarkable chromatic variation in

the appearance of its pigments, which are described as white, yellowish white, chrome yellow, orange, rust coloured, red, brownish red, blackish brown, and blue. These are not all separate pigments, but with some reservation KOLLER combines them as follows: *a.* chrome yellow—rust coloured; *b.* red—blackish brown; *c.* white—yellowish white. He considers the first named colour of each group as representing only a dilute form of the second, and he even suggests the possibility that all colours of groups *a* and *b* represent one pigment only, while *c* is distinct from this. The diffuse blue pigment is different from all the others.

HANSTRÖM (1937) describes the occurrence of yellow and red pigments, besides the white and the blue. After extirpation of the eye-stalks, the yellow and the red pigments are maximally expanded, and the adaptation to the background disappears; the white pigment reacts to light as before the operation.

Similar results were obtained by CARSTAM (1942). He describes the red ("reddish brown") pigment as varying in appearance according to the degree of expansion; when maximally contracted, it looks almost black. The yellow pigment mostly occurs in the same chromatophores as the red one, sparsely in monochromatic yellow cells. The colour of the shrimps in the first stage of expansion is predominantly yellow, later the red pigment starts moving and then follows the same chromorhizae as the yellow. The same author also stresses the difference in the appearance of blinded shrimps and specimens adapted to a dark background. The former are reddish brown, all pigments are maximally expanded, the red forming a thin layer and thus appearing lighter red. The latter are darker, blackish brown, the yellow pigment is maximally expanded, while the reddish brown is only $\frac{3}{4}$ expanded and therefore appears darker. Experiments on the relation of time to degree of expansion lead CARSTAM to conclude that the yellow and the "brown" (red) pigments are controlled by two separate contracting hormones.

My investigations were not centered upon a study of the nature of the pigment; but my observations on a great number of living *Leander adspersus* mainly tend to confirm the view of CARSTAM, especially as to the difference in the reaction of the yellow and red pigments in normal and blinded animals. In a

few points, however, my opinion differs from his. First, I find the red pigment actually red, not "reddish brown" or even "brown". Secondly, I was at one time inclined to believe that besides the red, yellow, and white pigments—and the diffuse blue substance—a real black pigment existed in this species. It is true that the red pigment, when highly contracted, appears as an almost black lump in the center of the chromatophore, but there are also chromatophores which appear partly black in the expanded phase. Such blackish chromatophores are present on the ventral side of the eye-stalks; they are of large size and the pigment has a peculiar bluish black colour. By re-examination I found that the dilute pigment in the finer branches of the cells looks really blue, so that it may be a case of intracellular occurrence of the usual blue pigment. As to the blackish looking cells which often occur along the median dorsal line of the carapace and the abdomen (Plate III, fig. 5) these probably contain condensed red pigment.

As stated by CARSTAM, most of the chromatophores contain red and yellow pigments. A smaller number contains red and white pigments; it is remarkable that the white chromorhizae lie nearer to the cuticle than the others.

Of the newly caught shrimps, the bigger ones are often rather dark, brownish, the yellow pigment is highly, the red one only partly expanded. The smaller specimens are generally of a lighter, yellow colour. Some specimens are greyish or greenish grey; in these, the diffuse blue pigment occurs as circular areas round the chromatophores in which the red pigment is wholly contracted, while the yellow one is more or less expanded.

The blue pigment is often, but not always, observed when the red pigment contracts after injection of certain extracts (cf. below). It appears first on the antennae, especially on the inner edge of the two basal joints of the first antenna and on the inner border of the exopodite of the second antenna, and at the same time on the sides of the rostrum, which are parallel to the basal joints of the antennae. The uropods may begin to show the blue colour simultaneously, and in such individuals the rest of the body soon follows. Much individual variation occurs, in some specimens only parts of the antennae and the

mouth-parts turn faintly bluish, and in many shrimps no trace of blue colour is observed; in well-defined cases it is clearly seen that the blue pigment is formed as dots around the rapidly contracting red chromatophores. These observations are in accordance with the view of KEEBLE and GAMBLE that the blue substance is formed by the red pigment during contraction.

The shrimps were kept in a large tank with light quartz sand on the bottom, the temperature of the water being about 10° C. They showed a rapid adaptation to the light bottom, becoming conspicuously paler than they were at their arrival (Plate III, fig. 5). In preparation for the injection, the eyes of a number of shrimps (e. g. 100) were cut off by means of fine scissors; each eye was taken separately, generally on two consecutive days. In spite of this, the mortality following the amputation was considerable, often about 40 per cent. Possibly, the losses might have been reduced by more elaborate methods, but in view of saving time the simple technique also used by previous investigators was maintained. The individuals which survived the first one or two days after the last operation often lived for weeks in the tank.

On the day after the removal of the second eye, the shrimps had generally adopted a more or less marked red colour (Plate III, fig. 6). However, a considerable individual variation existed, as some specimens were only yellowish, others (the majority) lighter or darker red. In the latter, the red chromorhizae form a very delicate network all over the surface making the animal appear brilliantly red (Plate IV, fig. 8); as far as possible only such individuals were used for the injections.

The insects were dissected in the Zoological Laboratory of the Royal Veterinary and Agricultural College. The organs were placed on filter paper, labelled, dried for one or two days in an incubator at 60° C., and kept in a desiccator for later use. Mostly, organs from five individuals were placed on each paper (e. g. 5 brains or 10 corpora allata); in some cases, it was necessary to use a smaller number, rarely a greater one.

On the day fixed for the injection, the central part of the filter paper was cut out, and 1 cm³ sea-water (from the aquarium) was added. Under the binocular the filter paper was divided into small bits and fibers and was left for about one

hour to extract. Then, the fluid with its content was transferred to a small test tube and was heated in a water bath to 100°C . After cooling it was filtered and was now ready for use. Generally, the fluid appeared quite clear and colourless, only extracts of whole heads, thoraces, and abdomina were more or less yellow or brown even after repeated filtration.

In most cases, the extracts were made a few hours before the injections took place, but, a few times, an extract was kept in a frigidaire till the next day, which did not involve any discernible reduction of the activity, while storing at room temperature resulted in a destruction of the active substance.

Before injection, the extract was cooled by keeping the filled syringe in cold water for a few minutes. The shrimps only stand the injection if the temperature of the fluid is about the same (not higher) as that of their own body, but when this is the case, they generally recover very rapidly and may live for a long time afterwards. Each individual received 0.1 cm^3 of the extract which was injected into the dorsal part of one of the first abdominal segments. The number of shrimps used for each experiment was generally 4—6, as the changes could not be followed exactly when a greater number was used. The effect of the treatment was controlled by regular inspection every 5 minutes; the general colour-tone and the shape of the chromatophores, as seen under the binocular, were noted. The observations were mostly confined to the red and "blackish" chromatophores which are easily observed. Already 2 minutes after injection, the most active extracts had caused a distinct fading of the colour, and after 10 minutes the shrimps were pale and transparent, simultaneously the pigments showed a maximum or submaximum contraction.

It is difficult to measure the effect in a really exact way. After some practice, however, I found it possible to characterize the momentary degree of contraction by such descriptive terms which allow us to compare the results of single experiments. For further simplification, each experiment was given a "mark" expressed as from one to three + or as \div , (+) indicating a smaller effect than +. The whole scale thus comprises 7 degrees of effect, *viz.* +++, ++(+), ++, +(), +, (+), and \div .

It should be added that the lower plus degrees differ from

the higher ones not only in the stage of contraction reached but also in the slower reaction. (+) means a just discernible contraction of the pigment; + designates a definite, but faint contraction, the shrimp generally showing a yellowish colour, and so on.

In the following, a description of an experiment from my journal is given.

Experiment P 23. *Tachycines* 14. ^{21/11} 1941.

Strength: 10 corpora cardiaca in 100 cm³ sea-water.

16 h⁰⁵. 0.1 cm³ of the extract is injected into each of 4 blinded (red) *Leander adspersus*.

16 h⁰⁷. Colour distinctly fading.—Red pigment showing beginning contraction (cf. Plate IV, fig. 9).

16 h¹⁰. The biggest individual, originally dark red, is now greyish; its red pigment is distinctly contracted (as Plate IV, fig. 10). Three smaller specimens (originally somewhat lighter red) are now pale (whitish), their pigments are even more contracted, blue colour is beginning to appear.

16 h¹⁵. Biggest individual pale (whitish); its pigment highly contracted (as Plate V, fig. 11). The three others are quite transparent; pigment showing submaximal contraction.

16 h²⁰. Biggest individual quite transparent; pigment submaximally contracted (as Plate V, fig. 12). Three others as before.

No further change.

+++

V. Experiments.

The results of the experiments are summarized in tables 1—6.

1. *Tachycines asynamorus*.

As I had a rather abundant supply of this grasshopper, most of the experiments were made with organs of this species (cf. Table 1).

Extracts of entire heads (5 heads to 1 cm³ sea-water) injected into blinded (red) *Leander adspersus* (0.1 cm³ per individual) caused a rapid contraction of the red pigment, reaching

Table 1.
Tachycines asynamorus.

Organ	Strength (number of organs/cm ³)	Number of shrimps inj.	Results	
Head (total)	5/1	10	+++	..
Thorax	5/2	6	+(+)	..
—	-	2	+	..
—	-	2	?	..
—	-	6	..	÷
Abdomen	5/2	6	+	..
—	-	5	(+)	..
—	-	4	?	..
—	-	3	..	÷
Brain	5/1	23	..	÷
—	4/1	3	..	÷
Suboesophageal ganglion	5/1	24	..	÷
Optic lobe	10/1	16	..	÷
—	9/1	6	..	÷
—	8/1	4	..	÷
Frontal ganglion	5/1	22	..	÷
—	-	2	?	..
Oesophageal connectives	10/1	7	..	÷
Corpus allatum	19/1	3	..	÷
—	10/1	29	..	÷
—	-	2	(+)	..
—	-	5	+	..
Corpus cardiacum ...	10/1	33	+++	..
— ...	10/10	4	+++	..
— ...	10/100	18	+++	..
— ...	10/1000	4	+++	..
— ...	-	6	++	..
— ...	-	8	+(+)	..
— ...	10/10,000	4	++	..
— ...	-	11	+	..
— ...	-	9	..	÷
— ...	10/100,000	4	+	..
— ...	-	2	(+)	..

Table 1 (continued).
Tachycines asynamorus.

Organ	Strength (number of organs/cm ³)	Number of shrimps inj.	Results	
Corpus cardiacum				
÷ G. hypocerebrale	10/1	9	+++	..
— ...	10/100	9	+++	..
— ...	-	2	++(+)	..
— ...	10/1000	2	+(+)	..
— ...	10/10,000	2	..	÷
Head ÷ C. card. ÷ C. allat. ÷ G. hypocerebr. ÷ Suboes. ggl.	5/1	2	++	..
—	-	2	+(+)	..
—	-	2	(+)	..
—	5/10	2	(+)	..
Ganglion hypocerebrale	5/1	7	+(+)	..

its maximum in about 10 minutes. This is in agreement with the results obtained by HANSTRÖM (1940 a) with head extracts of 7 other species of grasshoppers belonging to as many genera.

Extracts of thorax and abdomen¹ gave varying results, in some cases the effect being definitely positive, in other cases doubtful or even negative. The most probable explanation is that the active substance is carried with the blood from the head to other regions of the body, where it is found in varying concentration.

Extracts were then made of all organs of the head which might be imagined as possible sources of the chromatophoretropic substance.

Extracts of the brain, the suboesophageal ganglion, the optic lobe, and the frontal ganglion, when injected into red shrimps, showed no effect on the chromatophores. Only 2 out of 24 shrimps tested with extract of the frontal ganglion were dubious. As BROWN and EDERSTROM (1940) state

¹ It was necessary to use more fluid to extract these voluminous parts of the body (2 cm³ for every 5 individuals).

that the oesophageal connectives of *Crangon* produce a hormone which influences the colour-change of this shrimp causing an expansion of the black pigment in the telson and uropods, I have in a few experiments tried to apply an extract of the oesophageal connectives of *Tachycines*; injected into blinded *Leander adpersus* this substance was without any effect.

It was of special interest to test extracts of the corpus allatum, as this organ is known to be an incretory gland of great importance in the developmental physiology of insects, producing hormones which control the metamorphosis and the ovarian development. So, the corpus allatum might a priori be suspected as the possible cause of the chromatophoretropic effect of the head. However, 32 out of 39 test animals did not react at all to the injection of this extract, while 7 individuals showed a weak contraction of the red pigment. It is obvious, therefore, that the substance causing the very conspicuous effect following injection of extracts of entire heads cannot have its origin in the corpus allatum.

The results of all experiments hitherto mentioned contrast sharply with those obtained with the corpus cardiacum. An extract made of 10 corpora cardiaca (i. e. organs from 5 individuals) in 1 cm³ sea-water was injected into blinded shrimps, each specimen as usual receiving 0.1 cm³, corresponding to 1 organ. This extract was tested on 33 red shrimps which all showed a very rapid and complete contraction of the pigment, accompanied by a prompt colour-change from red to a transparent white (Plate III, fig. 7). Already 2 minutes after the injection, a definite fading was noticeable, and after about 10 minutes the colour-change was complete, the red, yellow, and "black" chromatophores being in a state of maximum contraction. At this stage, most of the chromatophores, when regarded with a weak objective, appeared as rounded dots, but a higher magnification revealed that many of them, especially the larger ones, actually were irregular, verrucous lumps (Plate V, fig. 13). During contraction some of the larger chromatophores, especially the large cells along the median line of the carapace, passed through a stage in which the pigment was condensed in the proximal parts of the chromorhizae, while nothing could be seen in the center of the cell; these stages presented a picture somewhat resembling the

metaphase of a mitosis (Plate V, fig. 12). In some specimens the diffuse blue pigment appeared to a varying extent (cf. p. 17). The changes following injection of this extract corresponded completely to those produced by extracts of the whole head.

It should, however, be noted that in these experiments no special care was taken to separate the corpora cardiaca from the ganglion hypocerebrale which is intimately connected with them (Text-fig. 1). The corpora cardiaca are plainly glandular, in dissections they are seen to be actually distended by fluid, while the ganglion does not show any histological or other signs of secretion; so it is very unlikely that the ganglion has anything to do with the effect on the crustacean chromatophores. Later, I succeeded in severing the ganglion from the corpora cardiaca, and it could be proved that the extract of the corpora cardiaca had still a very strong effect. In a single experiment with an extract of the isolated ganglion hypocerebrale (5 organs per 1 cm³) a weak effect was found, but in accordance with other experiences (cf. below) I cannot consider this a proof of a secretion of the ganglion; I am inclined to regard it as due to admixture of a small quantity of the contents of the corpus cardiacum.

In some other experiments the suboesophageal ganglion, the corpora allata, the corpora cardiaca, and the ganglion hypocerebrale were removed, and an extract was made of the rest of the head. This extract had a varying but definitely positive effect. The organs remaining in the head were the brain, the optic lobes, and the frontal ganglion, which, when tried separately, had no effect on the chromatophores; apart from these, the head contained the oesophagus, muscles, tracheae, fat cells etc. which are not likely to be concerned with the effect. It is probable, therefore, that the response to this extract is due to secretion of the corpus cardiacum present in the blood or absorbed in the tissues of the head. Obviously, if this extract of "the rest of the head" would not show any effect on the chromatophores, the explanation given above (p. 22) of the positive reaction obtained with extracts of thorax and abdomen could hardly hold.

The above conclusion is supported by the fact that the extract of the corpora cardiaca remains active even after extreme

dilution. In Table 1, several experiments (4) are combined and arranged according to a decreasing concentration. The difference in the results of the single experiments may either be due to a variation—individual or temporary—in the production of the active substance by the corpora cardiaca, or to differences in the threshold value of the test specimens, or to technical circumstances.

Dilution to 1:100 of the original strength, i. e. 10 organs in 100 cm³ water, showed no appreciable decrease in the effect. In some cases, even 1:1000 gave the same result, in other experiments, however, this strength showed a somewhat reduced activity. After dilution to 1:10,000 of the basic extract the fluid was in most cases still active, but on 9 test specimens no change could be observed. Even after diluting to 1:100,000 of the original strength there was in some cases a faint reaction, while in other cases no reaction could be observed.

The real dilution is, however, not correctly expressed by these figures, since already the basic extract represents a considerable dilution of the active substance. The size of a corpus cardiacum is about 0.25 mm × 0.37 mm × 0.12 mm (measured on sections); the volume is then at the most 0.01 mm³. If the whole volume is considered to be an active secretion, the basic extract of 10 organs per 1 cm³ represents a dilution of 1:10,000. This means that the figures should be multiplied by 10,000, so that the secretion of the corpus cardiacum should be active after a dilution of 1:100 millions or even 1:1000 millions. The sensitivity of the chromatophores to the active substance of the corpus cardiacum would thus be extremely high. In this connection it should be mentioned that the crustacean eye-stalk hormone is stated to be active after a dilution of at least 1:500,000 (quoted from HANSTRÖM 1939, p. 107). KROGH and REHBERG (*vide* KROGH 1930, p. 185), in perfusion experiments with frogs, found that 1 part pituitrine to 50,000—1 million parts perfusion fluid was still able to maintain the tonus of the capillaries.

2. *Dixippus morosus*.

The stick-insect *Dixippus morosus* has been the object of several investigations by PFLUGFELDER, who produced a considerable amount of evidence regarding the function of the cor-

Table 2.
Dixippus morosus.

Organ	Strength (number of organs/cm ²)	Number of shrimps inj.	Results	
Head (total).....	5/1	10	+++	..
Thorax.....	5/2	10	++	..
Abdomen.....	5/2	10	+	..
Brain.....	6/1	8	..	÷
—	5/1	4	..	÷
—	-	9	++(+)	..
—	4/1	6	+	..
Suboesophageal ganglion	5/1	12	..	÷
—	4/1	6	?	..
Optic lobe.....	12/1	7	..	÷
—	9/1	6	+	..
—	8/1	12	..	÷
Frontal ganglion	5/1	10	+	..
—	-	10	..	÷
Corpus allatum.....	10/1	14	+	..
—	-	6	++	..
—	10/10	4	+	..
—	10/100	4	..	÷
Corpus cardiacum...	10/1	11	+++	..
—	10/2	3	+++	..
—	10/4	3	+++	..
—	10/8	4	+++	..
—	10/16	3	+++	..
—	10/64	3	+++	..
—	10/640	3	++	..
—	10/6,400	3	+	..

pora allata. The physiology of the corpora cardiaca is completely unknown.

As already found by HANSTRÖM, extracts of the whole head of this species cause a rapid and complete contraction of the red pigment of blinded *Leander adpersus* (Table 2). A somewhat

weaker effect is produced by extract of the thorax, and a still weaker one by the abdomen.

Extracts of the isolated organs of the head gave somewhat surprising results. As in the case of *Tachycines*, extracts of the corpora cardiaca were very active and in no way inferior to the entire head. When diluted, the extract retained its activity down to a dilution of 1:6,400 of the basic extract. However, at least in some experiments, extracts of the other organs also gave positive results, i. e. they caused a more or less pronounced contraction of the pigment. Thus, brain extract was negative in 12 cases, but in 15 cases it caused a distinct contraction. The optic lobe and the frontal ganglion showed a similar effect, while extract of the suboesophageal ganglion had no or at the most a very doubtful effect. On the other hand, extract of the corpus allatum always caused contraction of the chromatophores, though the effect disappeared when the fluid was diluted to 1:100 of the original strength.

These observations claim an explanation. One might infer that the above-mentioned organs really produce a substance similar in effect to the hormone of the corpus cardiacum. In the case of the corpus allatum this might be true, but the very irregular results obtained with the four other organs hardly warrant such a conclusion. It seems much more likely that the effect is due to admixture of some secretion of the corpus cardiacum. As already mentioned above (cf. p. 11 and Plate II, fig. 4), it is hardly possible to remove the corpus allatum from the corpus cardiacum without injuring the latter, and this involves that the secretion flows out among the organs still present in the head under dissection, i. e. the brain, the optic lobe, and the frontal ganglion, so that these organs + the corpus allatum may happen to contain traces of the active hormone, even if they are washed in sea-water before being dried. Of the organs in question, only the suboesophageal ganglion is removed before the corpus allatum and the corpus cardiacum are dissected, and the fact that the extract of this ganglion has practically no effect seems to support the hypothesis given above. It is further in agreement with the experience that the extract of the corpus cardiacum retains its effect even when highly diluted.

3. *Blatta orientalis*.

The experiments with the cockroach organs (Table 3) give a simpler and clearer picture than did the preceding ones. The extracts of the brain, the suboesophageal ganglion, and the optic lobe had no effect on the chromatophores, while extract of the

Table 3.
Blatta orientalis.

Organ	Strength (number of organs/cm ³)	Number of shrimps inj.	Results	
Head (total).....	5/1	10	+++	..
Brain	5/1	8	..	÷
Suboesophageal ganglion.....	7/1	4	..	÷
—	5/1	10	..	÷
Optic lobe.....	6/1	4	..	÷
Corpus allatum.....	16/1	4	..	÷
—	10/1	4	..	÷
—	-	6	+	..
Corpus cardiacum...	16/1	5	+++	..
— ...	16/2	3	+++	..
— ...	16/10	3	+(+)	..
— ...	10/1	6	+++	..
— ...	-	4	++(+)	..
— ...	10/2	2	+(+)	..
— ...	10/10	4	(+)	..

corpus allatum was negative in 8 cases, and in 6 others caused a slight contraction of the pigment.

The extract of the corpus cardiacum induced a rapid and complete discoloration of the injected shrimps. This only applies to the basic strength, as a dilution to 10/10 already reduces the effect to a just observable reaction. Thus, the hormone of *Blatta* is either much less effective than that of *Dixippus* and *Tachycines*, or it is produced in a smaller quantity.

4. *Gryllus domesticus*.

Extracts of the corpus cardiacum of the cricket showed a surprisingly small effect on the pigment of *Leander* (Table 4); in one experiment even no change of the chromatophores or

Table 4.
Gryllus domesticus.

Organ	Strength (number of organs/cm ³)	Number of shrimps inj.	Results	
Head (total).....	5/1	10	(+)	..
Brain.....	5/1	6	..	÷
Suboesophageal ganglion	5/1	4	..	÷
Corpus allatum + ap- pendix	20/1	6	+	..
—	10/1	7	..	÷
Corpus allatum ÷ ap- pendix	10/1	4	..	÷
Appendix.....	10/1	4	..	÷
Corpus cardiacum...	20/1	6	+	..
— ...	10/1	5	+	..
— ...	10/1	4	..	÷

of the colour was obtained, this result being unique among the species examined. It should be noticed that the extract of entire heads had also a small effect only.

5. *Dytiscus marginalis*.

The few experiments performed with this representative of the *Coleoptera* (Table 5) led to results which are apparently contradictory to those hitherto regarded, since the extract of the corpus allatum had almost the same effect on the chromatophores as the corpus cardiacum. This might indicate that the corpus allatum of this beetle contains a pigment-activating hormone; however, this is not the only possible explanation, as

Table 5.
Dytiscus marginalis.

Organ	Strength (number of organs/cm ³)	Number of shrimps inj.	Results	
Brain.....	5/1	2	..	÷
—	-	4	?	..
—	-	2	(+)	..
—	3/1	6	(+)	..
Suboesophageal ganglion.....	5/1	13	..	÷
Corpus allatum.....	10/1	8	+++	..
—	-	1	++	..
—	10/10	2	++	..
—	10/100	4	+	..
—	10/1000	2	..	÷
Corpus cardiacum...	10/1	16	+++	..
— ...	10/100	2	++	..
— ...	10/1000	3	+	..
— ...	10/10,000	2	..	÷

in this species—just as in *Dixippus*—the two organs are so intimately connected that I have not been able to remove the corpus allatum without injuring the corpus cardiacum. The positive result of the corpus allatum may therefore be due to an admixture of the hormone of the corpus cardiacum.

The extract of the corpus allatum retained its activity after dilution to 1:100 of the original strength, while the extract of the corpus cardiacum was still active when diluted to 1:1000; however, these facts are only based on a single experiment, as only few beetles were available.

6. *Calliphora erythrocephala*.

Finally, I have made some experiments with organs of blowflies. I owe thanks to Dr. ELLEN THOMSEN for dissecting the flies and excising the organs. In *Calliphora* the corpus allatum and the corpus cardiacum are unpaired and extremely small; in mature females the corpus allatum measures only about $80 \times 100 \times 80 \mu$ (E. THOMSEN 1942). For this reason it was deemed

Table 6.
Calliphora erythrocephala.

Organ	Strength (number of organs/cm ³)	Number of shrimps inj.	Results	
Corpus allatum.....	20/1	20	..	÷
—	-	4	?	..
Corpus cardiacum...	20/1	23	++	..
—	-	2	+	..
Head.....	20/1	8	+(+)	..
—	-	15	+	..

necessary to use 20 organs (corresponding to 20 flies) to 1 cm³ of sea-water, each shrimp as usual receiving 0.1 cm³ of the extract, but even then the concentration is weaker than that used as basic strength in the other experiments.

Table 6 shows that the extract of the corpus allatum injected into 20 shrimps had no effect at all, while 4 cases were dubious. On the other hand, the extract of the corpus cardiacum as usual caused a rapid colour-change; the shrimps became pale, and the pigment contracted, though not quite to the maximum degree shown in Plate V, fig. 13.

These results agree well with the general trend of the previous experiments. On the other hand it seems surprising that entire heads also gave positive results, in spite of the fact that they do not contain the corpus cardiacum. In *Calliphora* and other flies, both this organ and the corpus allatum lie further back, in the foremost part of the thorax (E. THOMSEN 1942). I have not attempted to investigate whether any single organ really present in the head (e. g. the brain) causes a similar effect, but the experiments with *Tachycines* previously reviewed make it probable that the positive reaction after injection of extract of the entire head is caused by the hormone of the corpus cardiacum present in the blood and tissues of the head.

These results differ somewhat from those obtained by HANSTRÖM (1940 a) with the same species. In his experiments, extract of the corpus cardiacum (14/1) injected into blinded *Leander adpersus* brought about a conspicuous reaction "bis

zur beinahe Maximalkontraktion der Mehrzahl der Chromatophoren". Extract of the corpus allatum (same strength) in 3 injected specimens induced a weaker positive response, while the reaction of 2 others was doubtful. Finally, extract of entire heads (14/1) in 5 treated shrimps had no effect on the chromatophores.

VI. Discussion.

The experiments recorded in this paper prove that the colour-change which is seen in *Leander adspersus* after injection of extracts from entire heads of certain insects is primarily induced by a substance present in the corpus cardiacum. In five species of insects, extracts of the isolated corpora cardiaca caused a contraction of the red, yellow, and "black" pigments, which, both as to the rate of the process and to the final stage reached, did not differ from that obtained by injection of extracts of the entire head. In four of the species, the effect must be described as a very conspicuous and rapid one, leading to a maximum contraction of the red pigment in the course of about 10 minutes. Only in *Gryllus*, the contraction stopped at a rather early stage, but it is very significant that in this species also the extract of the entire head had only a small effect, so that in all species there is full accordance between the corpus cardiacum and the head as a whole with regard to their pigment-activating effect.

Also the sixth species studied, *Calliphora erythrocephala*, agreed with the others, extract of the corpus cardiacum causing a corresponding contraction of the pigment of *Leander*. In this species only, the effect of the corpus cardiacum cannot be compared with that of the whole head, since the corpus cardiacum is not situated in the head, but in the thorax. As it was found that head extract of *Calliphora* nevertheless induced contraction of the pigment, some experiments were performed with extracts of *Tachycines* heads, from which the corpora cardiaca and the corpora allata had been removed (p. 24). These extracts also gave a positive effect, though much weaker than that produced by extracts of the corpora cardiaca of the same species. The only possible explanation seems to be that secretion of the corpus cardiacum, present in the blood and tissues of the head, is the

cause of the positive reaction of the head without the corpora cardiaca and allata, both in the case of *Tachycines* and *Calliphora*. If this holds, it is possible to explain the weak positive response sometimes obtained with extracts of the thorax and the abdomen of *Tachycines* in a similar way.

The experiments with *Tachycines* gave an unequivocal result: extracts of various other organs of the head did not produce any change of the chromatophores; only in a few cases did extract of the corpora allata cause a slight contraction. Hence, there can be no doubt that in this species the pigment-activating effect of the entire head is due solely to the corpora cardiaca.

In *Blatta*, the results are equally clear, as the corpora cardiaca in all experiments had a positive effect, while other organs (with the exception of a single experiment) were negative. Almost the same applies to *Gryllus*, but with *Dixippus* and *Dytiscus* the results were more ambiguous, as not only extracts of the corpora cardiaca but also those of other organs, notably the brain and the corpora allata, sometimes induced a positive response of the chromatophores. As to the brain, the results were irregular, sometimes negative, in other experiments positive but varying in degree; so this case can most likely be explained in the same way as the results with "the rest of the head" of *Tachycines* (cf. above). Extracts of the corpora allata, however, in all experiments with *Dixippus* and *Dytiscus* gave a positive result (down to a certain dilution). It is possible, therefore, that these organs really produce (or contain) a substance similar in its effect to that produced by the corpora cardiaca, but this conclusion is not cogent. It should be remembered that, among the types investigated, only in *Dixippus* and *Dytiscus* it was impossible to sever the corpora cardiaca from the corpora allata without injuring the former or both organs. Presumably, the extirpated corpora allata were more or less smeared with the secretion of the corpora cardiaca, or even tiny bits of the latter in some cases adhered to the corpora allata.

The probability that such admixture may suffice to involve a positive effect of the whole extract is supported by the experiments with diluted extracts of the corpus cardiacum, which show that even highly diluted fluids may retain their positive effect. In the case of *Dixippus*, it is significant that extract of

the corpus allatum loses its pigment-concentrating power already at a dilution of 10/100 (10 organs in 100 cm³), while the corpus cardiacum is active even at a dilution of 10/6,400. These facts involve that the content of active substance in the basic extract of the corpus cardiacum must be at least 600 times as great as in that of the corpus allatum. This is in good agreement with the hypothesis given above, which, after all, I consider the most likely one. In corresponding experiments with *Dytiscus* the difference is not so pronounced.—In the case of *Calliphora* the extract of the corpus allatum was mainly negative, while the corpus cardiacum had always a positive effect.

Thus, all evidence so far available points to the corpus cardiacum as the source of the chromatophoretropic substance of the insect head. Regarding the nature of this substance, the following facts are at hand: It is resistant to boiling, soluble in water and—as shown by other investigators—in alcohol. It does not lose its activity after being kept dry for a long period; I have found pronounced effect of preparations which had been kept in a desiccator for 15 months. In the case of some species (as *Tachycines asynamorus*) the substance retains its activity even after extreme dilution. The activity is not specific, but extends to members of another systematic class. Finally, it may be noted that the corpus cardiacum shows the anatomical characteristics of a ductless gland, the secretion of which, according to DE LERMA, is discharged into the blood. These facts apparently justify to regard the active substance of the corpus cardiacum as a hormone. HANSTRÖM reached the same conclusion with regard to the active principle of the entire head.

As the hormone of the corpus cardiacum, when injected into blinded shrimps, influences the chromatophores in exactly the same way as the hormone of the sinus-gland, it seems likely that the two substances are chemically related, though they need not be identical.

Several authors (HANSTRÖM, E. THOMSEN, and others) have stressed the anatomical and physiological resemblance existing between the corpora allata of insects and the hypophysis of vertebrates. HANSTRÖM (1941) extended the analogy to cover also the corpora cardiaca which he compared with the neurohypophysis (pars posterior), while the corpora allata were com-

pared with the adenohipophysis (pars anterior); this comparison was based on the ontogeny and the anatomical relation of the two organs. As also emphasized by HANSTRÖM, the analogy should not be carried too far, but it is useful as far as the better known conditions of the vertebrates can guide us in the study of the invertebrates. In this respect, the experiments recorded in the present paper may be of some interest, the results showing a certain analogy to conditions in the vertebrates. The corpus cardiacum in insects produces a hormone which, at the time being, is only known through its action on the chromatophores of crustaceans. The hypophysis of the lower vertebrates forms one or more hormones which influence the movement of pigments in the chromatophores. In mammals, however, the pars intermedia of the hypophysis produces a hormone ("intermedin") which, when injected into frogs or fishes, induces quite similar changes of the chromatophores, while its rôle in the physiology of mammals is unknown. The analogy is not complete, since the pars intermedia is regarded as belonging to the adenohipophysis, whereas the corpus cardiacum—according to HANSTRÖM's interpretation—should be compared with the neurohipophysis. However that may be, the resemblance is rather striking.

It is evident that the part played by the corpus cardiacum in the physiology of insects themselves has not been elucidated by this investigation. It might be imagined that the colour-change of insects in some way should be regulated by the corpora cardiaca, though available evidence is not much in favour of this view. Even if this could be proved, the corpora cardiaca must have other additional functions, as they occur and are well-developed in all insects (except some apterygotes), while only a few insects are able to change their colour.

Summary.

1. It has previously been shown by HANSTRÖM that extracts of the head of various insects, when injected into blinded shrimps, cause a contraction of the pigment similar to that produced by the sinus-gland hormone. In the present paper a closer analysis of this phenomenon has been attempted.

2. The following species were used: *Tachycines asynamorus* Adel., *Gryllus domesticus* L., *Dixippus (Carausius) morosus* Br., *Blatta orientalis* L., *Dytiscus marginalis* L., and *Calliphora erythrocephala* Meig. Most of the investigations were made on the first named species.

3. The corpora allata, corpora cardiaca, and the stomatogastric nervous system of *Tachycines* and *Blatta* are described in some detail.

4. Extracts were made of several organs of the head, comprising the corpora cardiaca and allata. They were tested by injection into shrimps (*Leander adspersus*) which through amputation of the eye-stalks had been deprived of their own pigment-contracting hormone. Such individuals are red, owing to the maximum expansion of the predominant red pigment.

5. In all experiments, extracts of the corpora cardiaca induced a contraction of the pigment equivalent to that caused by the entire head. In the case of *Tachycines* even extremely dilute extracts still produced an observable effect, in the other species the activity disappeared at somewhat higher concentrations. The order was: *Tachycines*, *Dixippus*, *Dytiscus*, *Blatta*, *Calliphora*, *Gryllus*.

6. The positive results sometimes obtained with extracts of the brain, "the rest of the head", i. e. the head without corpora cardiaca and allata, the thorax, and the abdomen are most likely caused by secretion of the corpus cardiacum present in the blood and tissues. The positive effect of the corpora allata in *Dixippus* and *Dytiscus* is considered due to admixture of substance of the corpus cardiacum, owing to the difficulty of completely separating the two organs in these species.

7. The conclusion is drawn that the chromatophoretropic effect of extracts of entire insect heads is most probably solely due to a substance of the corpus cardiacum. This substance is regarded as a hormone.

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Addendum.

In a review by B. SCHARRER (*Physiol. Rev.* 21,3, 1941) I have recently found a brief reference to a paper by BROWN and MEGLITSCH (*Biol. Bull.* 79, 1940), at present inaccessible to me, in which the authors state an effect of corpora cardiaca, cerebral and frontal ganglia of insects on the chromatophores of crustaceans.

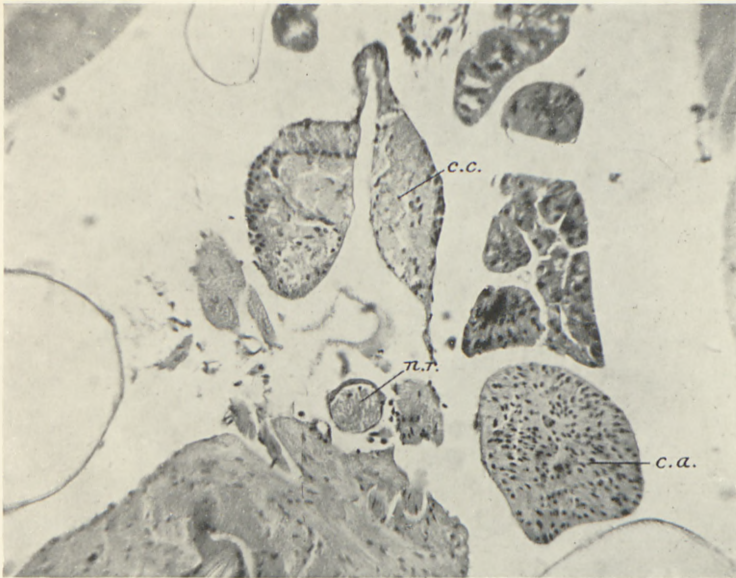


Fig. 1. *Tachycines asynamorus*. Transverse section of head. *c.a.*, corpus allatum; *c.c.*, corpus cardiacum; *n.r.*, nervus recurrens. 105 \times .

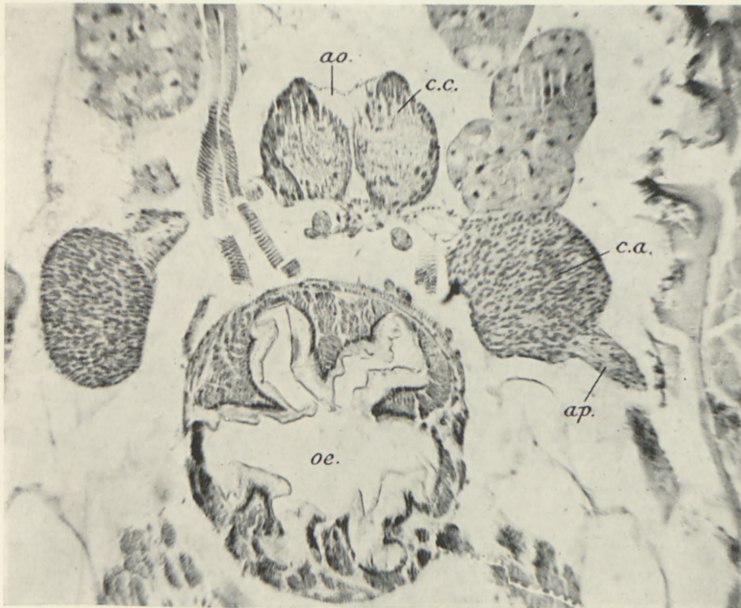


Fig. 2. *Gryllus domesticus*. Transverse section of head. *ao.*, aorta; *c.a.*, corpus allatum; on the right side the appendix (*ap.*) is partly visible; *c.c.*, corpus cardiacum; *oe.*, oesophagus. 105 \times .

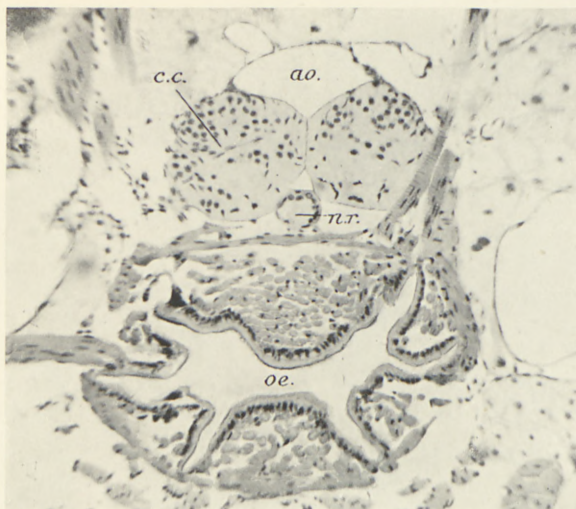


Fig. 3. *Blattella orientalis*. Transverse section of head. Letters as in preceding figures. 105 \times .

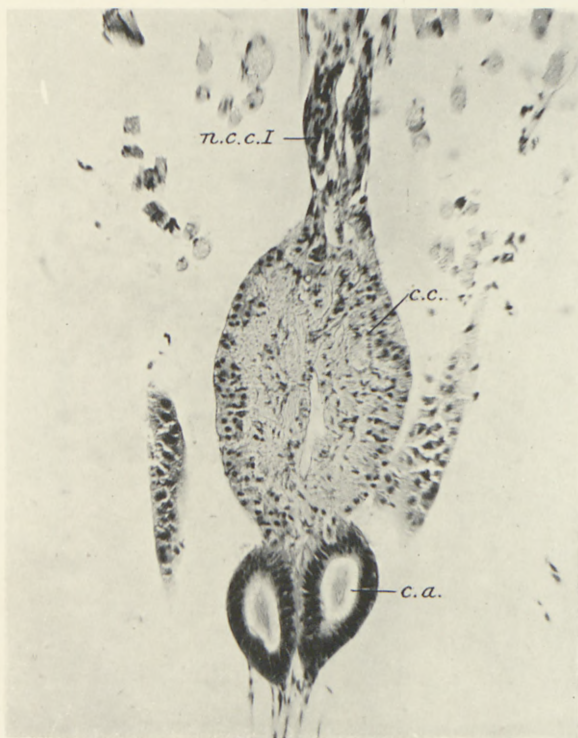


Fig. 4. *Dixippus morosus*. Horizontal section of head. *n.c.c.I.*, nervus corporis cardiaci I. Note the intimate connection between the corpora cardiaca (*c.c.*) and the *c. allata* (*c.a.*) 105 \times .



Fig. 5. *Leander adspersus*. Living specimen from light sand bottom. $1\frac{1}{2}\times$.



Fig. 6. Maximal expansion of pigment after amputation of eye-stalks; living specimen. $1\frac{1}{2}\times$.

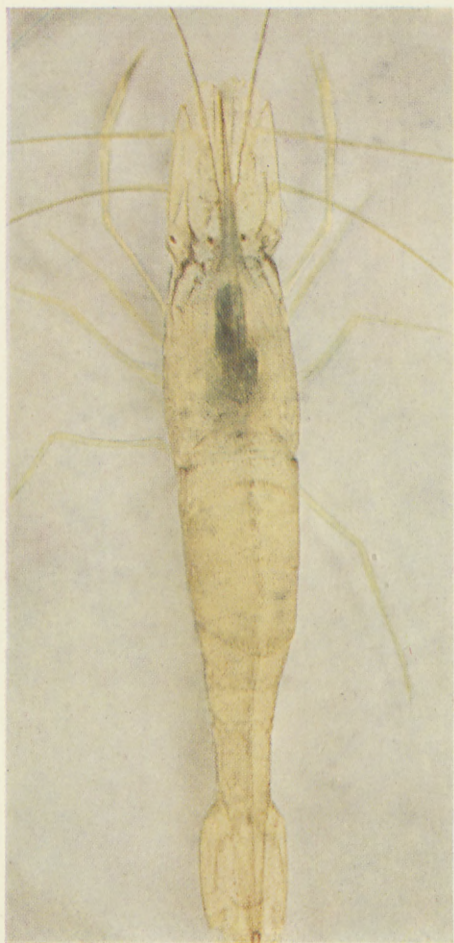


Fig. 7. Effect of injection of corpus cardiacum extract: complete contraction of pigment; living specimen. $1\frac{1}{2}\times$.

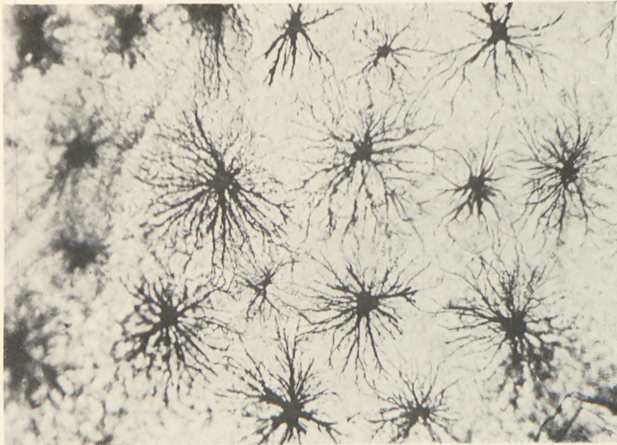


Fig. 8. *Leander adpersus*. Maximally expanded red chromatophores after amputation of eye-stalks. 75 \times .

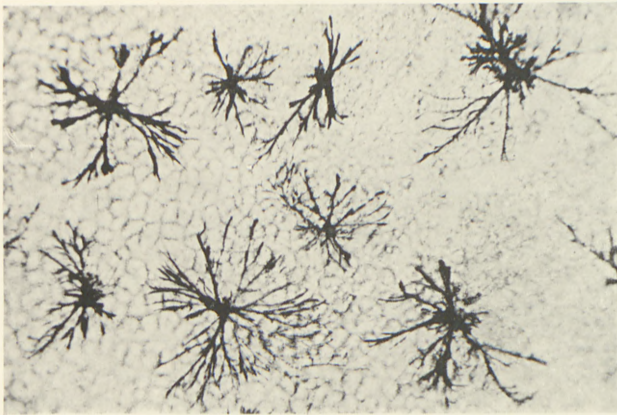


Fig. 9. Beginning contraction of red chromatophores. 75 \times .

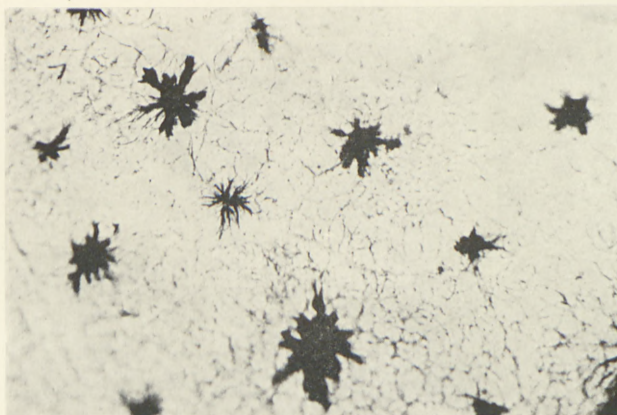


Fig. 10. Red chromatophores halfway contracted: early stage after injection of extract of corpus cardiacum of *Tachycines*. 75 \times .



Fig. 11. Proceeding contraction of red pigment; extract of corpus cardiacum of *Tachycines*. 75 \times .

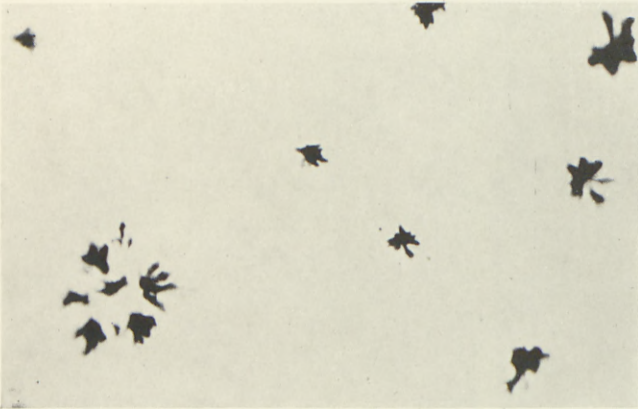


Fig. 12. Chromatophores showing submaximal contraction of red pigment. Left: large chromatophore, in which the pigment is arranged in a figure resembling a mitotic metaphase. Extract of corpus cardiacum of *Tachycines*. 75 \times .

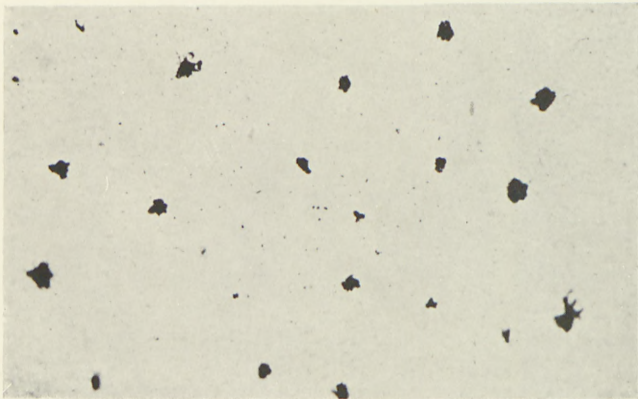


Fig. 13. Maximum contraction of red pigment: final stage after injection of extract of corpus cardiacum of *Tachycines*. 75 \times .