

Det Kongelige Danske Videnskabernes Selskab

Biologiske Skrifter, bind 6, nr. 5

Dan. Biol. Skr. 6, no. 5 (1951)

WEISMANN'S RING
AND RELATED ORGANS IN
LARVAE OF DIPTERA

BY

MATHIAS THOMSEN



København

i kommission hos Ejnar Munksgaard

1951

DET KONGELIGE DANSKE VIDENSKABERNES SELSKAB udgiver følgende publikationsrækker:

L'Académie Royale des Sciences et des Lettres de Danemark publie les séries suivantes:

	Bibliografisk forkortelse <i>Abréviation bibliographique</i>
Oversigt over selskabets virksomhed (8°) <i>(Annuaire)</i>	Dan. Vid. Selsk. Overs.
Historisk-filologiske Meddelelser (8°) Historisk-filologiske Skrifter (4°) <i>(Histoire et Philologie)</i>	Dan. Hist. Filol. Medd. Dan. Hist. Filol. Skr.
Arkæologisk-kunsthistoriske Meddelelser (8°) Arkæologisk-kunsthistoriske Skrifter (4°) <i>(Archéologie et Histoire de l'Art)</i>	Dan. Arkæol. Kunsthist. Medd. Dan. Arkæol. Kunsthist. Skr.
Filosofiske Meddelelser (8°) <i>(Philosophie)</i>	Dan. Filos. Medd.
Matematisk-fysiske Meddelelser (8°) <i>(Mathématiques et Physique)</i>	Dan. Mat. Fys. Medd.
Biologiske Meddelelser (8°) Biologiske Skrifter (4°) <i>(Biologie)</i>	Dan. Biol. Medd. Dan. Biol. Skr.

Selskabets sekretariat og postadresse: Ny vestergade 23, København V.
Selskabets kommissionær: EJNAR MUNKSGAARD's forlag, Nørregade 6,
København K.

*L'adresse postale du secrétariat de l'Académie est:
Ny vestergade 23, Copenhague V, Danemark.*

*Les publications sont en vente chez le commissionnaire:
EJNAR MUNKSGAARD, éditeur, Nørregade 6, Copenhague K, Danemark.*

Det Kongelige Danske Videnskabernes Selskab

Biologiske Skrifter, bind **6**, nr. 5

Dan. Biol. Skr. **6**, no. 5 (1951)

WEISMANN'S RING
AND RELATED ORGANS IN
LARVAE OF DIPTERA

BY

MATHIAS THOMSEN



København

i kommission hos Ejnar Munksgaard

1951

CONTENTS

	Page
Introduction	3
Material and Technique	5
<i>Calliphora erythrocephala</i> Meig.	6
<i>Tabanus</i> sp.	13
<i>Thereva</i> sp.	19
<i>Ptychoptera</i> sp.	20
Discussion	23
1. Comparative Morphology	23
2. Cytological Observations	26
Summary	29
Literature	30

Introduction.

In the year 1864 AUGUST WEISMANN published his classical work on the development of the *Diptera*. In this he described and pictured a remarkable ring-shaped organ which surrounds the aorta and is traversed by tracheae. He considered it to be of a mechanical function serving as support of the aorta, and he stated that it degenerated at metamorphosis. During the later part of the 19th century "the ring of Weismann" was observed in the larvae of several higher *Diptera* and mentioned by various authors, as KÜNCKEL D'HERCULAIS (1875) (*Volucella*), LOWNE (1890—95) (*Calliphora*), and PANTEL (1898) (*Thrixion*). Further HEWITT (1914) described it in *Musca domestica*, KEILIN (1917) in *Phaonia* (*Muscidae*) and THOMPSON (1921) in *Miltogramma* (*Tachinidae*). Some of these investigators (notably LOWNE and KEILIN) described new facts, especially relating to the innervation of the organ, but its function remained obscure.

In the 30'es the role of hormones in the development of insects became evident, and some investigators turned their attention to the enigmatic ring of Weismann as a possible hormone-producing organ. In 1935 FRAENKEL by ligaturing experiments showed that pupation in the blow-fly (*Calliphora*) was obviously due to a substance produced in the anterior part of the larva, and BODENSTEIN (1938) working on *Drosophila* arrived at the same result.

Prompted by FRAENKEL's experimental work BURTT (1937) undertook a reinvestigation of Weismann's ring in the larva of *Calliphora*, suspecting this organ of being the source of FRAENKEL's »pupation hormone«. He gave a good description of the anatomy of the organ and concluded that Weismann's ring represented the highly modified corpora allata of other insects. At the same time he described the corpora allata and allied organs in the larvae of *Chironomus* and *Tipula*. In a later paper (1938) BURTT showed that *Calliphora* larvae experimentally deprived of Weismann's ring did not pupate, but continued to feed, and he concluded that the ring did actually secrete the "pupation hormone" of FRAENKEL.

At the same time E. HADORN (1937 a and b), working on *Drosophila* mutations, found that the mutant "lethal giant", in which development is arrested just before or immediately after formation of the puparium, may be induced to normal puparium formation by implantation of a small organ situated in front of the brain and evidently homologous with the Weismann's ring of *Calliphora*. HADORN called this organ the ring-gland and suggested that it might be the homologue of the corpora allata of

other insects. In a later paper SCHARRER and HADORN (1938) showed that the ring-gland of *Drosophila* contained two types of cells, and they tentatively interpreted the larger cells as representing the corpora allata and the smaller ones as the corpora cardiaca. NYST (1941) suggested that the whole ring-gland might correspond to the c. cardiaca.

The question of the homology of the ring-gland continued to be discussed during the following years. E. THOMSEN (1940) discovered the unpaired corpus allatum of the adult *Calliphora*, *Lucilia*, and *Musca* and stated that the likewise unpaired c. cardiacum is fused with the ganglion hypocerebrale of the stomatogastric nervous system. Confirming unpublished results of DRUMMOND, she later (1941) showed that the adult c. allatum is represented in the Weismann's ring of the *Calliphora* pupa by an anterior group of small cells, and she ventured the hypothesis that the larger cells forming the lateral parts of the ring might be homologous with the so-called "pericardial gland" found by PFLUGFELDER (1938) in *Phasmidae*. The "pericardial gland" lies close to the aorta and the c. allata-cardiaca and, like the large lateral cells of the ring-gland, degenerates in the adult insect.

In the 40's M. VOGT published some important papers on the histology of the ring-gland of *Drosophila* and *Calliphora* (1941, 1942, and 1943) in connection with her experimental work. Her studies showed that the ring-gland comprises three types of cells: The cells of the c. allatum ("Zentralteil"), the c. cardiaca (fused in *Calliphora*) and the main cells ("Hauptzellen") which surround and cover the two other groups. The homology of the main cells was not considered.

DAY (1943) described the anatomy of the ring in the larva of *Lucilia sericata* and the changes of the organ during metamorphosis. He found the c. allatum, but concluded that "the large ring-gland cells" (VOGT's "main cells") represented the c. cardiaca, which according to his description were reduced in the adult fly, but did not disappear completely.

POULSON (1945) made a study of the embryology of the ring-gland of *Drosophila*, in the hope of solving the difficult question of the homologies. He likewise came to the result that "the large ring-gland cells" were the larval c. cardiaca, but added that the small cells found in the ventral part of the gland comprise cells of the hypocerebral ganglion together with imaginal c. cardiacum cells which replace the larval c. cardiacum when this degenerates.

In the same year (1945) BODENSTEIN published a description of the corpora allata of various *Culicidae*. The paired c. allata of the mature larvae were covered by a layer of larger cells of unknown nature, attached anteriorly to a transverse trachea.

Recently some French authors have made valuable contributions to our knowledge of the endocrine organs of the *Diptera*.

POSSOMPÈS (1946) published an interesting paper on the endocrine glands of the *Chironomus* larva, correcting earlier descriptions by BURTT (1937) and ZEE and PAI (1944). This was followed by an account of the larva of *Tipula* (POSSOMPÈS 1947), which had been briefly described previously by BURTT (1937). These papers will be mentioned again below.

ARVY and GABE (1947) undertook a renewed study of the endocrine glands of the *Chironomus* larva, applying refined cytological and histochemical methods. They gave excellent descriptions of the chondriome and Golgi bodies, but like POSSOMPÈS they did not identify the *c. cardiacum* of this larva.

Finally CAZAL (1948) published his comprehensive memoir on the endocrine retro-cerebral glands of the whole insect class. In the chapter on the *Diptera* the following forms were especially treated: *Tipula*, *Culex*, *Tabanus*, and *Eristalis*, while some others were more briefly mentioned. In some cases only imagines had been investigated, but with regard to *Culex* and *Eristalis* the organs of the larva were described. I shall return to this important paper on the following pages.

CAZAL has changed the now generally accepted term *corpus cardiacum* to *c. paracardiacum*. Though it must be admitted that this name is more precise, I have hesitated in following him on account of the already existing terminological confusion relating to this organ, which has been described under 6—8 different names.

The great diversity in the morphology of the *corpus cardiacum-allatum* system of the few *Diptera* already studied, the conflicting results of different investigators, and the great physiological interest attached to these organs made a broad comparative study comprising representatives of as many families as possible seem a promising task. However, the appearance of the papers by POSSOMPÈS and CAZAL prompted the author to give up this plan, which would no doubt have taken a long time to complete, and so four genera only are treated in this paper. They represent, however, the three main branches of the *Diptera*, and the study throws some light on the difficult question of the homology of the very specialized complex organ found in the higher *Diptera*. The cytology of the organs has been studied in some detail.

Material and Technique.

The larvae of *Calliphora* used were reared on dog meat at a constant temperature of 20° C.

The larvae of *Tabanus* and *Ptychoptera* were collected and sent me (together with larvae of *Chironomus* and *Eristalis*) from the Laboratory of Freshwater Biology at Hillerød. I am very grateful to Professor Kai Berg and Dr. Anker Nielsen for their unfailing helpfulness, without which this investigation could hardly have been carried through. My thanks are also due to Dr. Helge Volsøe, who has collected most of the *Thereva* larvae, and to Dr. I. Agrell of Lund, who sent me larvae of a *Tipula*, which were used for comparison. I am also greatly indebted to Professor Pierre Cazal (Montpellier), Dr. L. Arvy (Paris), and Professor Bertil Hanström (Lund) for providing me with some dyes and other chemicals. Mr. N. Haarløv (M. Sc.), Mr. E. Rasmussen (M. Sc.), and Miss Else Mohr have rendered valuable technical assistance.

Most of the larvae were dissected under a binocular. In some cases the organs were drawn in the living state or after fixation with alcohol, but generally the dissected specimens were fixed for later sectioning. A number of fixing fluids were tried: the

fluids of Kahle, San Felice, "Alcoholic Bouin" (after Duboscq-Brasil), Hollande's modification of Bouin's fluid, Helly, Altmann, Flemming, and Champy.

Alcoholic Bouin followed by Masson's trichrome stain, as recommended by CAZAL (1948), proved an excellent method for a general study of the anatomy, and in some cases for the secretory cells. The author tried both the original Masson stain (iron haematoxylin¹, Ponceau de xyloidine, aniline blue), and Foot's modification, in which aniline blue is replaced by light green, and a mixture of Ponceau and acid fuchsine takes the place of the pure Ponceau (FOOT 1933).

Fixation in Flemming's fluid (formula of BAKER 1945b) and staining with Heidenhain's iron haematoxylin gave fine preparations of nuclei, mitochondria and in some cases beautiful secretory granules. Fixation in Champy's fluid (24 hours), and after-treatment with 1 % osmic acid for several days in an incubator at about 35° C. is highly recommended by CAZAL as a good method for the Golgi bodies of various cells and especially for the chromophile cells of the *c. cardiacum*. If the cytoplasmic inclusions are sufficiently blackened by the osmium, the sections are mounted unstained, otherwise they are stained in the usual way with iron haematoxylin. The author has likewise obtained excellent results with this method, but in some cases (the neurosecretory brain cells of *Tabanus*) the secretory granules were not visible after this treatment, though very conspicuous after Flemming's and some other fluids.

The author also tried the special modification of Altmann's method for mitochondria introduced by GABE (1947), but without success, perhaps due to an inferior quality of the available carmine.

Sections were cut at 5 or 10 μ . The thick and tough cuticle of some of the larvae made the cutting difficult, but the use of butylic alcohol for dehydration, as recommended by some investigators, proved a considerable advantage. Pieces were taken through the usual stages of alcohol till 96 %, then through two changes of butylic alcohol and then directly into melted paraffin wax (two changes).

The microphotographs were taken with a Zeiss microscope "Lumipan" and the small Zeiss "Phoku" camera. The drawings were made by Mrs. B. Strubberg and the author.

The Carlsberg Foundation has supported the work with a grant.

Calliphora erythrocephala Meig.

Though the papers of BURTT (1937) and VOGT (1943) have contributed much to our knowledge of the ring-gland in this species, some points still remained doubtful or contradictory. A renewed study, therefore, seemed desirable. The following description applies to the third stage larva after it has ceased to feed, just prior to the formation of the puparium.

¹ It was found preferable to perform the Heidenhain method at room temperature (mordanting for 1/4—1 hour and staining for a similar period) instead of at a higher temperature as usually recommended. In this way there is less risk that the sections loosen from the slide.

Anatomy. The shape of Weismann's ring is seen from plate I, fig. 1. The organ shows some individual variation, and the shape also varies according to its more or less stretched state, but the main features remain the same. The ring-gland consists of two elongate lateral parts, which unite posteriorly below the aorta. Anteriorly they diverge, but are connected by a transverse part of varying length, often somewhat bent or angular. From the centre of the transverse part a tongue-like process extends forwards, recognizable as part of the glandular tissue by the conspicuous round cell nuclei (fig. 1, wra); this prolongation has not been mentioned by previous investigators, though in fact it is always present. Furthermore the glandular process continues anteriorly in a band-like structure (cb) which goes to the pharynx. This is the "cephalo-pharyngeal band" of LOWNE (1890—95), originally described by WEISMANN (1864). The whole organ is rarely or never circular, but forms a sort of frame more nearly resembling a triangle or a diamond through which the aorta passes. The terms "ring" or ring-gland are, however, convenient. As observed by BURTT, the aorta is attached to the ring-gland dorsally and ventrally, but is free laterally. Two conspicuous tracheae, branches of the main longitudinal trunks, pass through the lateral parts of the ring and are united by a transverse commissure imbedded in the anterior transverse part of the ring. When the tracheae have left the ring-gland they enter the mediad sides of the hemispheres of the brain.

In the late third stage larva Weismann's ring is situated just above the brain and the optic disc (the rudiment of the imaginal eye), but in fixed preparations it may sometimes be displaced to a more posterior position. The length is 400—500 μ .

While the gross anatomy hitherto recorded can easily be observed in dissected specimens, either fresh or after fixation and staining with borax carmine, the innervation demands a study of sections.

As found by PFLUGFELDER (1937) and HANSTRÖM (1938, 1940) and later confirmed by CAZAL (1948), the corpus cardiacum-allatum system of insects is generally innervated by two pairs of nerves from the brain, the nervi corporis cardiaci I and II of HANSTRÖM (n. paracardiaci interni and externi of CAZAL). In the *Diptera*, however, there is generally only one pair of nerves, as the internal and the external nerve of each side have fused, and CAZAL seems to hold that this character is valid for all *Diptera*. He does not mention the fact that BURTT (1937) described two pairs of nerves going from the brain to Weismann's ring in the *Calliphora* larva. VOGT's paper (1943) contains no information as to the innervation of the ring-gland. DAY (1943), in his paper on the larva of *Lucilia*, mentions one pair of nerves, which he identifies with the n. corporis cardiaci I of HANSTRÖM. On the other hand he states that the ring-gland "also receives nerve fibers from the hypocerebral ganglion, which is situated on the recurrent nerve". As these conflicting opinions seem to throw some doubt on the statement of BURTT, I have given particular attention to the matter. According to BURTT, the n. recurrens, coming from the pharynx, passes between the hemispheres and the oesophagus to a ganglion on the proventriculus, just behind the brain; the recurrent nerve gives off a dorsal branch which joins the hindmost

part of the ring and goes to a ganglion on the floor of the aorta. This ganglion, which he identifies with the "median ganglion" of LOWNE, further receives two nerves on each side from the cerebral commissure. I can confirm his description.

BURTT's semidiagrammatic figure (his fig. 5) illustrates the nervous connections in a way which is very nearly correct. His picture shows that one of the n. c. cardiaci is thinner than the other; I have also observed the difference, and can add that it is the n. c. cardiaci internus (I), which is the thinner nerve. The external nerves emerge from the median sides of the hemispheres, the internal pair a little further forwards, from the posterior side of the pars intercerebralis.

The question of the homology of the "median ganglion" of LOWNE and BURTT is important to our understanding of the morphology of the ring-gland. BURTT himself considered the "median ganglion" as the fused "oesophageal ganglia", i. e. what we now call the corpora cardiaca (CAZAL: c. paracardiaca); though his material was, perhaps, hardly sufficient to permit such a conclusion, he was probably right. VOGT, after a closer study of the histology of the ring, likewise interpreted the "median ganglion" as representing the corpora cardiaca. She did not mention the possible existence of a hypocerebral ganglion in the larva, though the ventral part of the gland complex of the adult fly according to E. THOMSEN (1941 og 1942) (corroborated by HANSTRÖM) comprises the joined c. cardiacum and ganglion hypocerebrale. DAY (1943) is not quite clear in his statements, for although he speaks of the hypocerebral ganglion as situated on the recurrent nerve (cf. the quotation above), he also says (p. 3) that the hypocerebral ganglion actually forms the ventral part of the ring. CAZAL regards this part (i. e. the "median ganglion" of BURTT) as the fused corpora cardiaca, but he differs from previous investigators in regarding the ganglionic cells found in this part in the pupa and the imago as representing the ganglion ventriculare, while he thinks that the ganglion hypocerebrale has disappeared both in the larval and later stages. Quite a different view was taken by POULSON (1945), who regards the lateral parts of the ring as the corpora cardiaca.

I think, however, that the cytological characteristics prove beyond doubt that the "median ganglion" of the larva—as held by BURTT, VOGT, and CAZAL—does actually represent the fused corpora cardiaca (cf. below). But a close study of my sections has convinced me that the hypocerebral ganglion has not disappeared, but is present in the *Calliphora* larva as a more or less pronounced swelling of the recurrent nerve at (or just behind) the point where it gives off a branch to the ring-gland. This thickened portion contains an agglomeration of nerve cells, and it can hardly be interpreted in any other way (plate III, fig. 9gh). Behind the hypocerebral ganglion the n. recurrens continues to the proventriculus, where it ends in a small, but distinct ganglion ventriculare. In the pupa the recurrent nerve is much shortened, and the hypocerebral ganglion is incorporated in the ring-gland.

It should be added that the n. recurrens can be followed in the sections along the dorsal side of the oesophagus far in front of the brain, finally ending in a small ganglion, the frontal ganglion, above the pharynx.

Cytology. By far the largest part of the ring is formed by the cells which VOGT calls "Hauptzellen" (main cells) and which I shall provisionally term the R-cells. These are seen in several figures of this paper (see especially plate I, fig. 2—3 and plate II, fig. 5—7). They are the largest cells of the gland, being 30—50 μ in diameter.

The spheroid or ovoid nucleus is also large (diam. 20—25 μ) and contains a conspicuous nucleolus and very large chromosomes. After fixation in Flemming's fluid and staining in iron haematoxylin the chromosomes are especially clear and show a spiral structure resembling a mitotic telophase. As mitoses were never observed in my preparations, we have here a case of nuclei in the resting stage, containing distinct chromosomes of a telophasic appearance. BURTT (1937) and VOGT (1943) have observed that these cells undergo a remarkable growth during the last larval stage, both the nuclei and the cell-bodies increasing several times in size. DAY (1943) states that the same cells in the *Lucilia* larva appear to be in the "polytene" condition. But the typical polytene chromosomes of the salivary glands present a different structure: they are much thicker and have distinct transverse bands, so the chromosomes of the ring-gland cells are not likely to belong to that type. As only one nucleolus is visible in the nucleus (rarely two), the R-cells may be diploid. However, in well-fixed preparations (Flemming's fluid) the nucleolus sometimes seems to be a composite body, which may indicate that the nuclei are polyploid, perhaps due to some sort of endomitosis.

The cytoplasm of the large R-cells is rather clear, though densely packed with short, filiform mitochondria, which stain with Heidenhain's and other stains. After fixation in Champy's or Flemming's fluids another element is also visible, viz. almost spherular granules of varying size. They are most distinct after prolonged treatment with osmic acid, but do not look like typical Golgi bodies (they are never ring-shaped). Sometimes a few vacuoles are seen in the cells, generally lying in the periphery away from the nucleus; in such cases the granules are most numerous around the vacuoles (plate I, fig. 3v). These observations seem to indicate that the granules are secretory, and in fact they resemble the granules observed by CAZAL in the corresponding cells of the *Eristalis* larva after Champy's osmic-iodine technique (CAZAL 1948, p. 186, fig. 165). On the other hand granules of almost the same size and shape are found in many other cells of the *Calliphora* larva after osmification, so that the identification is open to doubt. The reticular appearance of the cytoplasm mentioned by CAZAL and illustrated in his fig. 165 in my opinion is due to the mitochondria.

The R-cells generally form two epithelium-like layers, between which some elongate or even spindle-like cells form a sort of core (plate I, fig. 2). Nerve fibres are sometimes observed between the cells. The homology will be discussed later.

The epithelium lining the tracheae which pass through the ring-gland consists of smaller cells staining intensely with haematoxylin.

The corpus allatum of the larva was identified and studied by VOGT (1943), who originally designated it as "Zentralteil". It is situated in the centre of the anterior transverse part of the ring, where it forms a somewhat flattened ventral layer (plate I,

fig. 4ca). The number of cells is about 25—27. Especially in specimens fixed in OsO_4 (Flemming or Champy) it is seen that the cells have a peculiar shape, which to my knowledge has never been observed in the *c. allata* of other insects. They are elongate, almost spindle-shaped, with their axes corresponding to the longitudinal axis of the body. Further the cells have an almost fibrillar structure, which seems to be due to a more or less parallel orientation of the mitochondria. The spindle-shape is not quite so distinct after fixation in alcoholic Bouin or S. Felice, and therefore was not observed by VOGT, who used the latter fixative. The cytoplasm contains osmiophile granules of varying size resembling those of the R-cells, but partly larger (plate II, fig. 6). They are visible after fixation in both Champy's and Flemming's fluids and do not show a lighter internum as do typical Golgi bodies. They are sometimes blackened so intensely by the osmic acid that the whole *c. allatum* appears very dark (plate II, fig. 7). The nuclei are much smaller than those of the R-cells, spheroid or sometimes roundly elongate in shape. It seems that the *c. allatum* is especially connected with the central, spindle-shaped R-cells, which, as mentioned above, are situated between the superficial layers of the large R-cells. Nerve fibres could not be identified.

As already mentioned the ring-gland has an anterior projection. This is mainly composed of the R-cells. From this part "the cephalo-pharyngeal band" stretches forward. The cells of this organ form one or two layers. They are larger than the R-cells, elongate, and after fixation in S. Felice show a fibrillar structure and sometimes numerous vacuoles; the cell borders are indistinct. After osmification black granules are seen in the cytoplasm (plate I, fig. 4). The nuclei resemble those of the R-cells, but are longer. It may be added that the posterior end of the band is wedged in between the anterior part of the ring-gland and the aorta (plate II, fig. 5cb). Probably it is this part of the band which is seen in VOGT's fig. 8 and regarded by her as "Pericardialzellen", while DAY (1943) in his fig. 4 designates them as "Durchlasszellen". The real function of this structure is not quite clear. It is presumably neither nervous nor glandular, and perhaps only serves as a mechanical support of the ring as held by LOWNE (1890—95). In some larvae just before formation of the puparium the cells seem to be degenerating. The disappearance of the band during the pupal stage makes possible the backward displacement of the ring-gland recorded by DRUMMOND, E. THOMSEN (1942), and DAY (1943).

As mentioned above, the posterior part of the ring, where the two lateral parts meet below the aorta, forms the so-called "median ganglion", which is of special interest for our morphological interpretation of the whole ring-gland. BURTT (1937) and VOGT (1943) have pointed out that this part contains fuchsinophile nerve cells, which they considered to represent the fused corpora cardiaca. CAZAL (1948) supported this view. In the larva of *Eristalis tenax* he found that the corresponding cells are intensely blackened by prolonged osmification in a similar way as the *c. cardiacum* cells of various other insects. They therefore belong to his "chromophile type", while he states that "les cellules chromophobes sont peu abondantes".

In my sections of the ring-gland of the *Calliphora* larva these chromophile cells

are plainly seen. They are stained red by acid fuchsine and blackened by osmic acid. They lie somewhat scattered in the "median ganglion", but are most numerous in its central part (plate III, fig. 8 and 9cc). However, a few cells showing the same qualities are found in the lateral parts of the ring (as also seen by BURTT and VOGT) and even in the swollen part of the recurrent nerve, which I regard as the ganglion hypocerebrale (plate III, fig. 9gh). In the *Calliphora* larva the chromophile cells have rather short prolongations so that in sections they look polygonal or triangular. I have not been able to find any secretory granules in them, but they contain numerous mitochondria of the usual shape (short curved threads).

There is still another sort of cells in the same region, which deserve more attention than they have hitherto obtained. They were briefly mentioned by DAY (1943), who seems to regard them as nerve cells. They are definitely smaller than the chromophile cells, and their cytoplasm is almost untinged after osmification, though slightly stained by iron haematoxylin. They form clusters and are more numerous than the chromophile cells. They very much resemble the nerve cells in the nervus recurrens, and for some time I considered them to represent the ganglion hypocerebrale. It is, however, more likely that these cells represent the chromophobe cells which CAZAL (1948) has described from the c. cardium of various insects; they agree in every respect with his description. I have not been able to decide whether these cells have axons of their own, so that it cannot be said whether they should be regarded as nerve cells, glia cells, or something else.

To complete the description of the endocrine system of the *Calliphora* larva, it would be desirable to localize the brain cells from which the nerves innervating the ring-gland arise. According to HANSTRÖM and CAZAL these cells in other insects form four groups: (1) the two median groups, situated in the pars intercerebralis of the protocerebrum, the axons of which form the internal nerves (nervi corporis cardiaci I of HANSTRÖM, nervi paracardiaci interni of CAZAL), and (2) the two lateral groups, likewise situated in the protocerebrum, giving rise to the n. c. c. II or n. paracardiaci externi. In some insects, notably *Hymenoptera*, *Lepidoptera*, and *Diptera* (cp. especially HANSTRÖM 1938, 1940, and 1941, and several papers by B. and E. SCHARRER, where further literature is cited), these cells show definite signs of being neurosecretory cells. This has been confirmed by CAZAL (1948), who found a "sécrétion chromophile" to be most distinct in adults of *Phasmidae*, *Panorpa*, *Tipula*, and *Tabanus*. On the other hand CAZAL states that in some insects the corresponding cells are very similar to ordinary nerve cells, and in other cases they also resemble nerve cells, but are distinguished by their larger size and abundant cytoplasm.

In the case of the *Calliphora* larva I have been able to find what are probably the median groups, while the lateral groups are not distinct. The identification of these "median groups" is, however, only based on their localization and some cytological characteristics, as it has not been possible to follow with certainty the nerve fibres from these brain cells to the ring-gland. The cells form two rather conspicuous groups lying at the anterior surface of the protocerebrum near the median line (plate IV,

fig. 10). The number is difficult to decide, but it may be about 12—14. The cell body is large and rounded or pear-shaped. There is one thick axon issuing from each cell; in some cases this axon resembles an almost empty tube in which a fine fibrilla is sometimes discernible (plate IV, fig. 12B). The axons form a definite bundle with medioposterior direction. The cells stain red with Ponceau and acid fuchsine and are distinctly darker than the surrounding brain cells when stained with iron haematoxylin, this being due to the great number of short filiform mitochondria which fill the cytoplasm. After fixation in Champy's fluid and prolonged osmification they show distinct dictyosomes (Golgi bodies) of the typical disc- or ring-shape (plate IV, fig. 11 and 12A), occurring chiefly between the nucleus and the axon. There is a striking resemblance between these cells and the mother cells of the n. c. *cardiaci interni* of *Hydrous piceus* drawn by CAZAL in his fig. 101 and likewise osmium-impregnated. The Golgi bodies sometimes show a mulberry-like appearance similar to that described by THOMAS (1948). In certain cases a vacuole occurred between the nucleus and the axon. Only in a single larva did some of these cells contain bigger bodies blackened by the osmic acid. It could be observed that these bodies consisted of small granules, which looked like mitochondria, and it seemed that they also contained some dictyosomes.

It may be mentioned here that mitoses were sometimes observed in the ordinary brain cells, especially in early third stage larvae.

It may be appropriate to describe also the so-called "garland", even if nothing is known as to a secretory function of this organ. The garland, originally detected by WEISMANN in the *Calliphora* larva and later found in several other larvae of *Diptera* (KOWALEVSKY 1887, KEILIN 1917, et alia), forms a V-shaped string of cells stretching dorsally from side to side with its two ends adhering to the salivary glands and its central and posterior part situated above the oesophagus and below the aorta. The string consists of two or more (3—4) rows of loosely connected cells, mostly fusiform or ovoid, and most of them binucleate. The cytoplasm presents an interesting picture, especially after staining with Foot's modification of Masson's trichrome stain (plate V, fig. 14). The peripheral layer of the cells appears faintly green and contains a great number of fine threads, mostly showing an orientation perpendicular to the surface. The identification of these threads as mitochondria leaves little doubt. The interior is filled with spherular bodies of somewhat varying size and likewise tinged green; in the centre of the cell they are less numerous, this part often appearing much lighter than the rest. Between these bodies a third element occurs: fine strings or rather rows of minute granules, which sometimes tend to adhere to net-like structures; these strings stain intensely red like the nuclei. In spite of their different staining ability it seems most likely that they are also of a mitochondrial nature.

In material fixed in Champy's fluid and treated with OsO_4 the spherules show a brownish colour while the peripheral and central mitochondria are faintly grey and hardly different inter se. Between these, especially in the periphery of the cell,

smaller granules occur which look darker than the spherules. Whether they are simply earlier stages of these or they also comprise some Golgi elements could not be decided. No typical dictyosomes were observed.

The physiological role of the garland cells is not known with certainty, but they are generally classified with the pericardial cells, to which they show a close affinity, as "nephrocytes" (KOWALEVSKY 1887 og 1889, KEILIN 1917), or more recently as micro-phagocytes (see WIGGLESWORTH 1939). Kowalevsky's well-known experiments showing that certain coloured substances are stored in these cells prove their phagocytic powers. But I cannot refrain from saying that the spherular inclusions of the cells in *Calliphora* look very much like secretory granules. However this may be, it is of interest to point out the striking resemblance between these cells and the so-called "glandes post-cérébrales antérieures" described by POSSOMPÈS (1946) in the larva of *Chironomus* and reinvestigated with finer cytological methods by ARVY and GABE (1947). Obviously these "glands" are in fact two giant "garland cells" (cp. the figures in the last-named paper with my fig. 14). It should be mentioned that ARVY and GABE regard the "anterior post-cerebral glands" as being in "intense secretory activity".

The pericardial cells of the *Calliphora* larva contain similar mitochondria and spherules as the garland cells, though the difference between the peripheral and central mitochondria is hardly observable. In the young third stage larva, in which I have studied them, most of the cells have only one nucleus, but some binucleate cells can be found (plate V, fig. 13). It is, however, obvious that the garland cells should be regarded as specialized pericardial cells, a fact which does not necessarily exclude a secretory function besides the generally acknowledged phagocytic activity.¹

Tabanus sp.

One might expect that the highly specialized development of the ring-gland in the *Cyclorrhapha* would be easier to understand when compared with the corresponding organs in more primitive *Diptera*. Therefore a study was made of the ring-gland of the *Tabanus* larva, as a representative of the *Diptera brachycera*. The endocrine glands of the adult *Tabanus* have been described by CAZAL (1948), but those of the larva were unknown. The species was obtained in the autumn from a ditch in Hillerød. The larva hibernates in the last stage and evidently pupates in spring, but unfortunately no pupae and very few larvae could be found after hibernation, therefore no adults were obtained, and the species could not be determined. The larvae studied were in the last larval stage, preparing for hibernation. If offered food (small living blow-fly larvae) most of them attacked the prey and sucked it out, but some *Tabanus* larvae refused to feed and had evidently already entered into the hibernating stage. These larvae could be kept in the laboratory for long periods without food.

Anatomy. On a first view the ring-gland resembles that of the blow-fly larva,

¹ YAO (1950), in a paper on cell phosphatase in *Drosophila*, likewise suggests that the pericardial cells are secretory.

but some important differences are soon observed. The organ is situated just behind the brain, the anterior part actually lying in the cleft between the two hemispheres (plate V, fig. 15 and 16). The shape is almost that of a triangle with the apex pointing backwards. The posterior part is plainly demarcated from the rest and forms a rounded body which is either longer than broad and pear-shaped or heart-shaped, or reversely broader than long. This portion is the corpus allatum, which is about 100—150 μ long, while the length of the whole ring-gland is 300—400 μ .

Compared with the conditions in the *Calliphora* larva, the *c. allatum* of *Tabanus* is remarkable by its large size and by forming the posterior part of the ring-gland. Further it should be noted that the whole ring-gland lies on the dorsal side of the aorta, which consequently does not traverse the opening of the "ring" as in *Calliphora*, though it is visible through the opening when looked at from above (plate V, fig. 15). The rest of the ring-gland forms a horseshoe-like body, the anterior and central part of which may be more or less marked off as a rounded prominent pad. From a tracheal arch in front of the brain a number of median branches, generally four, pass backwards between the hemispheres, the external one on each side penetrates into the hemisphere, while the central tracheae (2 or 3) pass below the anterior part of the ring-gland and probably send fine branches into this organ and the aorta.

In the dissected larva it is easy to see the nervus recurrens passing along the dorsal side of the oesophagus and ending on the proventriculus. It proved impossible to find the hypocerebral ganglion, and no nervous connection between the *n. recurrens* and the ring-gland could be detected. In transverse sections the recurrent nerve has been followed forwards to a small ganglion in the ventral wall of the aorta in front of the brain and the optic disc, and it is possible that this ganglion should be interpreted as the hypocerebral ganglion. According to CAZAL the adult *Tabanus* has a typical hypocerebral ganglion just behind the brain. The frontal ganglion is found in the pharyngeal mass in a similar situation as in the *Calliphora* larva.

In the *Tabanus* larva there is only one pair of *nervi corporis cardiaci*. As in most *Diptera* a fusion of the external and internal nerve on each side has occurred. These nerves will be described below.

Cytology. The big corpus allatum is made up of a great number of small cells (plate VI, fig. 17). The shape of these is highly variable and seems to be influenced by the method of fixation; generally the cells are polygonal, often elongate or even spindle-shaped, sometimes resembling the *c. allatum* cells of the *Calliphora* larva. The nuclei are spheroid and contain a distinct nucleolus and small and bigger chromatin bodies. After fixation in Flemming the cytoplasm is seen to be filled with rod-shaped mitochondria, which seem to end with a thicker knob or granule. CAZAL (1948) has given similar pictures of the *c. allatum* in the *Eristalis* larva (cp. his fig. 164, p. 186), and he regards these granules as secretory droplets and assumes that the mitochondria are involved in the secretion. My observations do not warrant a similar conclusion, as I have not been able to follow the gradual development of larger drops described by CAZAL.

Prolonged post-osmification of Champy-fixed material results in rather a striking appearance of the *c. allatum* (plate VI, fig. 19). The central cells (plate VII, fig. 2) contain small cup- or ring-shaped, blackened granules which look like Golgi bodies. In the peripheral cells (plate VII, fig. 22) these granules are mostly replaced by larger spheroid bodies with a thin black surface layer and a light interior; besides these are found irregular lumps of osmium-blackened material without a distinct border, and, between the other structures, faintly coloured mitochondria. One can hardly evade the idea that the secretory material originates from the Golgi bodies, probably from their internum, which seems to undergo a process of liquefaction during which the body swells to a thin-walled spherule and finally flows out into the cytoplasm as a diffuse substance. This conception is, of course, quite hypothetical, but not inconsistent with modern views (see f. ex. BAKER 1945 and 1950, THOMAS 1948, GRESSON 1949).

Only in one case a typical *nervus corporis allati* was observed, in all other larvae examined the *c. allatum* and the rest of the ring-gland were in intimate contact, and the nerve fibres to the *c. allatum* did not appear as a nerve in the anatomical sense.

The bulk of the ring-gland is formed by the R-cells (main cells) which resemble those of *Calliphora* (plate VI, fig. 18*wr*). They are of a much larger size than the cells of the *c. allatum*. On the surface they are arranged in epithelium-like layers, but in the interior of the gland they are rather irregular in shape. The nuclei are large and pale, with one or two big nucleoli and scattered, small, winding, thread-like chromosomes. Filiform mitochondria are abundant in the cytoplasm. After osmification for 6—8 days a varying number of granules of small size appear, whether these should be regarded as Golgi bodies or as secretory granules is difficult to decide.

In sections stained after Masson-Foot the surface of the ring-gland is stained a vigorous red, but branched bluish strands are seen running between the cells; they are connected with the outer surface and may perhaps be a supporting structure. The cuticle (except the epicuticle) is stained greenish blue in the same sections.

The antero-ventral portion of the ring-gland has two conical projections which are not visible on plate V, fig. 15 and 16, where they are concealed by the hemispheres and the dorsal parts of the ring-gland. They are easily found in sagittal or horizontal sections (plate VII, fig. 20). They become narrower and continue in the *n. c. cardiaci* coming from the brain; there is a gradual transition from the ring-gland to the nerve, as the nerve fibres are covered with a layer of cells resembling those of the gland.

The *n. corporis cardiaci* and the transitory zone (*trz*) are remarkable by a conspicuous content of granules, which show a vivid red colour after staining with Masson's trichrome stain or with acid fuchsine, and are also intensely stained by haematoxylin and blackened by osmium. In Champy-fixed material it is seen that the coloured substance consists of small densely heaped granules or minute rods which show a remarkable resemblance to mitochondria. The surrounding cytoplasm is yellow. This may perhaps mean that a diffuse secretion is formed by the mitochon-

dria. No Golgi bodies were observed in the cells. These granules (secretory material?) are always most conspicuous in the transitory zone, while they disappear in the anterior half of the nerve. In the ring-gland proper the granules can sometimes be observed along the nerve fibres which enter the gland, but here it is difficult to distinguish them from the usual mitochondria.

In the ring-gland the nerve fibres can be followed to two groups of central cells, one left and one right (plate VI, fig. 18cc). There is no doubt that these groups represent the corpora cardiaca, which in the *Tabanus* larva are not fused as in *Calliphora*. Each cell-group (plate VII, fig. 20) comprises at least two types of cells: (1) Rather large cells with abundant cytoplasm and of simple rounded shape, sometimes pear-shaped or elongate. They differ from the R-cells by their more numerous mitochondria, which are filiform, but a little shorter and thicker than those of the R-cells; there are a few, not very typical Golgi bodies. (2) Smaller cells with little cytoplasm and with smaller nuclei than (1). Their nuclei contain several small chromatin granules, but no distinct nucleoli like the first-mentioned cells. They seem to occur along the nerve fibres and along the periphery of the groups of larger cells.

It is obvious that the larger cells (1) correspond to the chromophile cells of Cazal, but it should be noted that they do not show such pseudopodia-like ramifications as found by CAZAL in certain insects. The homology of the small cells is more difficult to decide, but they show an obvious resemblance to the small cells of the c. cardiacum of the *Calliphora* larva, and it is likely that they should be interpreted in the same way, i. e. as the chromophobe cells of Cazal. On the other hand this name does not, of course, explain the function or origin of the cells. Probably they are either glia cells or nerve cells.

In the adult *Tabanus* there is likewise only one pair of nerves to the c. cardiaca (CAZAL 1948); each nerve is formed by the union of two intracerebral roots originating from two groups of brain cells, recognisable by their special affinity to certain stains. They are stated to resemble the c. cardiacum in this respect. The external root of each nerve comes from a lateral cell-group consisting of 3—4 cells, the internal one from a median group consisting—as it seems—of several cells lying in the pars intercerebralis.

In the brain of the larva similar cell-groups are observed; they are really much more distinct than in the *Calliphora* larva. The cells in question appear red after Masson's trichrome stain and are intensely coloured by haematoxylin in other cases, especially after fixation in Flemming's or Helly's fluids (plate VIII, fig. 24). As in the adult each hemisphere contains two groups of cells, a ventral one comprising only 2—4 cells, and a dorsal one made up of a greater number, about 30 cells. It is difficult to count the latter group exactly, as dubious cells are often observed which may be transitory stages.

In one case only, I succeeded in following the internal root of the nerve in its whole course to the mother cells. Just inside the hemisphere the nerve branches into two roots, and the internal (anterior) of these proceeds forward with a paramedian

course crossing the transverse nerve fibres of the pars intercerebralis (plate X, fig. 32). On the anterior side of this the nerve fibres bend in a curve into the other hemisphere and continue in a dorsal direction on the front of the brain for about 20μ . Then again they bend somewhat inwards and backwards to end in a group of about 30 intensely red nerve cells. These must be the "median" (internal) group, though in the *Tabanus* larva it is scarcely more median than the other group of few cells. They are both situated on the antero-median side of the hemisphere, the small group more

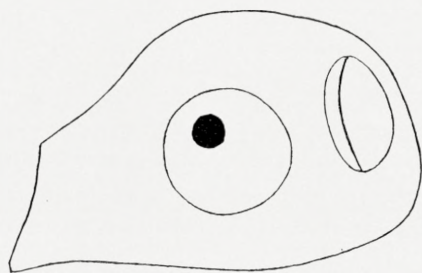


Fig. 1.

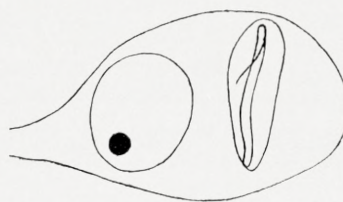


Fig. 2.

Neurosecretory brain cells with »thread vacuoles« (*Tabanus*).

ventrally than the numerous group. Neither of the groups is strictly situated in the pars intercerebralis, but near this part.

The cells of both groups show signs of a secretory activity, and I do not hesitate in designating them as neurosecretory cells. Cells fixed in Flemming's fluid and stained with iron haematoxylin are especially conclusive. The cytoplasm of the dorsal (internal) group of numerous cells is intensely blackened and appears to be filled with small filiform mitochondria and roundish granules of different sizes, sometimes also occurring in the axons (plate IX, fig. 27). Many cells (all?) show a large vacuole with a fine thread or thin rod lying in the wall of the vacuole. The exact course of the thread is very difficult to observe, but it seems to be an irregular spiral which makes one or several turns in the wall of the vacuole (plate VII, fig. 23). Similar "thread vacuoles" are seen after other fixatives and stains (text-fig. 1—2). They are not confined to the neurosecretory cells, but may also be observed in other large nerve cells of the brain. I have not found any reference in the literature to similar observations by other investigators. The threads may of course be dismissed as artifacts, but their rather regular occurrence after different fixation and staining does not make this supposition very likely. I have not seen them in any other species which I have investigated.

In Flemming-fixed sections the ventral (external) group of neurosecretory cells show a striking appearance. The cytoplasmic granules are very distinct, their size and shape variable, the larger ones showing a darker rim and a somewhat lighter interior. An especially beautiful cell, found in a sagittal section, is shown in plate VIII, fig. 24—26. This cell is larger than the surrounding brain cells and has a very thick axon which is literally stuffed with granules of varying size, between which lighter coloured

mitochondria are discernible. A few similar cases have been observed, but more frequently the axons are cut transversely. It is hardly possible to interpret such pictures in any other way than as cells in a state of intense secretion. For the following reasons the granules cannot be Golgi bodies (but may be Golgi products): (1) Golgi bodies do not ordinarily appear by the technique applied to these preparations, (2) Golgi bodies are not seen in other nerve cells of the same sections, and (3) the granules do not resemble the Golgi bodies shown by other methods (cp. below).

This conclusion receives further support from the appearance of the two cell-groups after other technical treatment.

In Bouin-fixed larvae stained with Masson's trichrome stain the few cells of the ventral (external) group contain numerous granules of a luminous red colour, obviously identical with the granules seen in the Flemming-fixed cells just mentioned (plate IX, fig. 28). In some cases, however, the granules are bluish (from the haematoxylin), but surrounded by a diffuse red colour. The same cells contain small filiform inclusions, stained by the haematoxylin, no doubt mitochondria. They tend to be arranged in a regular way, sometimes forming a definite collection with a more or less concentric arrangement of the single mitochondria. Also vacuoles, with or without threads, can be seen.

After the same treatment the cells of the dorsal (internal) groups present a similar appearance (plate IX, fig. 30). They are pear-shaped with the pointed ends directed against the median plane and continuing in the axons. This end of the cell is reddish, either due to its content of red granules or to a diffuse red colour. Mitochondria occur in the whole cell, but are especially numerous in the blunt end (opposite the axon), this part therefore appearing bluish. Vacuoles with threads also occur. Near the cells the axons are thick, but eventually they attenuate to fine nerve fibres. Now and then luminous red granules occur in small groups along these nerve fibres (cp. p. 17).

In the Masson-stained sections it is not infrequent outside the two conspicuous groups to observe single cells with an appearance suggesting a secretory activity. One is almost led to assume that secretion may occur to some extent in many brain cells, so that the so-called neurosecretory cells may only be specialized cases with a more pronounced secretory function.

Sections fixed in S. Felice's fluid, which is generally considered a good fixative for chromosomes, but not for cytoplasm, show rather a striking difference in the appearance of the two groups of neurosecretory cells. The few cells of the ventral group (plate IX, fig. 29) contain a great number of conspicuous granules, which stain with haematoxylin and show a darker external layer and a lighter coloured interior, just like those found in Flemming-fixed cells. The granules can also be seen in the thick proximal part of the axon. The mitochondria are but faintly coloured. In the cells of the dorsal group, however, such granules are not visible, but the mitochondria are more distinct and form similar collections as seen in the Bouin-fixed material. These cells show also one or a few vacuoles with or without threads. The

cause of this difference is unknown, but it may be connected with the phase of secretion.

In accordance with the extensive experience of CAZAL it was expected that Champy-fixation followed by prolonged osmification would make the neurosecretory cells especially distinct. This did not prove to be the case in my material. On the contrary it was very difficult in such sections to localize these cells as they did not differ much from other large brain cells. Only by means of the previously gained knowledge of their position in the brain it was possible to identify the dorsal (internal) groups with certainty. These cells (plate X, fig. 31) show the pear-shape described above. They contain distinct osmium-blackened elements varying in size from minute granules to bigger bodies, mostly consisting of smaller granules grouped as a chaplet. The largest of the granules forming the chaplet show a light internum and a black externum. In some cases it seems that the ring is double, made up of two parallel chaplets. The granules and rings are most numerous in the narrow part of the cell which continues in the axon. These inclusions must be Golgi bodies, probably in different stages of development. The same cells contain a few vacuoles. Also other cells of the brain and the ventral ganglionic mass contain similar Golgi bodies.

The author is well aware of the difficulty of drawing definite conclusions on physiological processes from cytological data, which are highly dependent on the technique applied, but with all reservation the following working hypothesis is suggested: In the neurosecretory brain cells the first stage of the secretory cycle is probably represented by the small osmiophile granules. These grow into typical Golgi bodies (dictyosomes) in a way similar to that described by THOMAS (1948). The Golgi bodies are involved in the formation of the secretory droplets seen especially in the Flemming-fixed sections (cp. "Discussion" p. 27).

Thereva sp.

I have been able to examine some larvae of the genus *Thereva* found in the soil in two localities in Denmark. As *Th. nobilitata* Fabr. and *Th. annulata* Fabr. are the commonest species in this country (LUNDBECK 1908), it is probable that the larvae belong to one or both of these species. The family *Therevidae* like the *Tabanidae* are included in the *Diptera brachycera*.

The extremely thick and tough cuticle makes the sectioning rather difficult, so only a few complete series were obtained. The observations stated below, though not exhaustive, may still be of some interest.

Dissection of the larvae showed that the brain is situated in the mesothorax with the imaginal discs of the eyes lying in front. Two tracheae, branches of the main longitudinal trunks, pass just above the eye-discs and enter the hemispheres on their medial sides (plate XI, fig. 33).

The endocrine organs are seen immediately behind the brain in close contact

with the aorta. The arrangement differs from that found in the larvae of *Tabanus* and also from that of the *Calliphora* larva. The corpus allatum is a conspicuous crescent- or kidney-shaped body lying on the dorsal side of the aorta. The breadth is about 100 μ , the length 50 μ . Its anterior ends are connected with a still larger organ (about 150 μ long and 200 μ broad), the lateral parts of which are visible on both sides of the aorta when seen from above; these lateral parts are connected ventrally below the aorta. The comparatively large cells of the organ are plainly seen under the binocular. These cells—as will be shown below—correspond to the R-cells found in *Tabanus* and *Calliphora*; thus it can be stated that the *Thereva* larva possesses a real ring-gland, which—unlike the case in *Tabanus*—surrounds the aorta.

The big c. allatum (plate XI, fig. 34) consists of a great number of small cells with comparatively large spheroid or ovoid nuclei. It is surrounded by a fine membrane. After treatment with Champy and post-osmification thread-like mitochondria are seen in the cytoplasm, but no Golgi bodies were observed. After Bouin-fixation numerous vacuoles appear in the cells.

The much larger cells of the lateral lobes and ventral part of the ring-gland are rather variable in shape. The large nuclei are spheroid or elongate. The cytoplasm has a reticular appearance, obviously due to numerous, thin, filiform mitochondria, seen in osmium-treated sections. The whole appearance so much resembles the R-cells of *Calliphora* and *Tabanus*, that there cannot be any doubt as to the identity with these. Among the described R-cells a few others are found, which differ from those in the smaller size of the nuclei and the darker cytoplasm after osmification. In other series similar cells are stained red by Ponceau. The resemblance with the c. cardiacum cells of *Tabanus* is obvious, (plate XI, fig. 35 cc).

There is only one pair of nerves going from the medial sides of the brain to the ventro-median part of the ring-gland. Two or three large pear-shaped cells which stain intensely red with Ponceau lie on the anterior side of each hemisphere, very close to the median plane (plate XI, fig. 36). It is probable that these cells represent the internal groups of neurosecretory cells, but the nerve-fibres going to the ring-gland could not be traced through the brain. Other red-staining cells situated further back on the medial surface of the hemisphere, may represent the external groups, but the evidence is insufficient.

There is a single recurrent nerve passing along the dorsal side of the oesophagus and terminating in a small ganglion ventriculare at the anterior end of the proventriculus. No hypocerebral ganglion could be found.

Ptychoptera sp.

This species may serve as a representative of the primitive suborder of the *Diptera nematocera*. The well-known larva with its retractile "tail" is common in freshwater rich in decomposing organic substances.

Anatomy. In spite of the considerable size of the whole larva, dissection of the endocrine glands and adjacent organs proved rather difficult. Dissection from the ventral side gave the best results. It is useful to stretch the oesophagus and the tracheae, which are normally S-shaped, with needles to study the *situs viscerum*. The larva may then be fixed with alcohol or other fluids in the stretched condition, or the oesophagus and the salivary glands are cut loose and tilted forwards to expose the endocrine organs which are situated dorsally to the oesophagus just behind the well-developed (eucephalous) head-capsule. In plate XII, fig. 37 the distance between the head and the corpora allata is exaggerated owing to the stretching mentioned above. In the *Ptychoptera* larva the brain and the suboesophageal ganglion are included in the head, while in the larvae of *Calliphora*, *Tabanus*, and *Thereva* they are situated in the thorax. It may further be mentioned that there is a remarkable group of "garland cells", which laterally adhere to the anterior ends of two elongate parts of the fat-body and, stretching inwards, are fixed to each side of the oesophagus (plate XIII, fig. 38 *gu*).

Most conspicuous among the endocrine organs are the corpora allata, two oviform or spindle-shaped organs lying close to the aorta, to which they are connected at their posterior ends (plate XII, fig. 37). The pointed apex of the *c. allatum* continues in a fine trachea. Measurements of the *c. allatum* in a fresh condition showed a length of 150–180 μ , in fixed specimens 80–120 μ . The two organs of the same individual may differ in size. By dissection it is hardly possible to ascertain what are the corpora cardiaca, but, situated at the posterior end of the *c. allatum* and between this and the aorta, one observes a number of large, roundish cells (fig. 37 *cc*), which further study has proved to represent the *c. cardiacum*. Each of these cell-groups receives a nerve, which can be seen along the side of the aorta; this is the *nervus corporis cardiaci*, remarkable by its relative thickness. Another interesting organ connected with the *c. allatum* is a thin transparent tissue of cellular structure (*gp*), which extends as a broad band from the anterior transverse trachea to the middle part of each salivary gland to which it is fixed. It is seen (plate XII, fig. 37) that the above-mentioned small trachea, a fine branch from the transverse trachea going to the *c. allatum*, occupies part of the inner margin of the band. Further the anterior end of the *c. allatum* normally seems to be connected with the band. It is obvious that this thin tissue corresponds to the so-called "glandes pérित्रachéennes" described by POSSOMPÈS (1946) in the larva of *Chironomus*.

The *n. c. cardiaci* coming from the medial sides of the hemispheres are connected with a well-developed elongate ganglion hypocerebrale, which receives the *nervus recurrens* from the anterior part of the head and gives off the two long *n. oesophagei* running on each side of the oesophagus, partly fixed to its wall, and ending in the proventriculus.

Cytology. The corpora allata consist of irregular cells with roundish nuclei, each containing one nucleolus. In Flemming-fixed material the chromatin tends to form disc-like bodies adhering to the inner side of the nuclear membrane. It some-

times looks as if they protruded on the external surface of the membrane, but this may be due to shrinking of the nucleus during fixation. After the same treatment the cytoplasm is seen to contain vacuoles and filiform mitochondria; some mitochondria are unusually long. Evidence of secretion is slight, though in Masson-stained sections indistinct red droplets, possibly secretory, may be found in the cells. After Champy's fluid and post-osmification for 6 days the cytoplasm contained numerous very small, spheroid, osmium-blackened granules, besides vacuoles (plate XIV, fig. 43). Whether these granules are Golgi bodies or secretory granules is difficult to decide, they do not show the typical shape of dictyosomes. The *c. allatum* is covered with a single layer of very thin cells with some flattened nuclei.

As already mentioned the *corpus cardiacum* is made up of a small number of fairly large cells lying behind the *c. allatum* and between this gland and the aorta, intimately connected with both organs. The thin sheath covering the *corpus allatum* continues as a still finer membrane around the *c. cardiacum* cells but part of it covers the surface of the *c. allatum* between this and the *c. cardiacum*. These membranes are stained blue by Masson's trichrome stain.

The large *c. cardiacum* cells lying at the posterior end of the *c. allatum* continue as a layer on the wall of the aorta. The number of cells varies; 5—10 have been counted. In some cases a few similar cells (2—5) occur at the anterior end, but they may be lacking. On the mediad side there is a gradual transition between the *c. cardiacum* and the *nervus corporis cardiaci*, which is covered with cells resembling those of the gland itself.

In preparations fixed in Flemming's or Champy's fluids the *c. cardiacum* cells are dark, containing irregular dark masses, probably consisting of a secretory material (plate XIV, fig. 41, 43cc). The same substance is found in the cells along the nerve, but disappears at some distance from the brain. In most cases the cells show a simple, rounded shape, but in some sections of material fixed in Champy's fluid and post-osmificated they present a very interesting picture (plate XIV, fig. 42). From the anterior end of the cell a rather thick and well-defined process projects forward in the direction of the *n. c. cardiaci*. Near the cell most of these processes show a distinct swelling, which is blackened by the osmium like the content of the cell itself. Sometimes it looks as if lumps of the same material were lying between the cells, though it cannot be excluded that in reality these lumps belong to similar cytoplasmic processes. It cannot be safely decided whether the processes continue into the nerve (*n. c. cardiaci*), though this is probably the case. Sometimes it looks as if there were two nuclei in one cell, a large one and a smaller one, but it is more likely that the smaller nuclei belong to chromophobe cells scattered among the chromophiles.

How should these observations be interpreted? The processes very much resemble axons, and the whole appearance of the cells reminds one of the neurosecretory brain cells (cp. *Tabanus*, plate VIII, fig. 25). As the cells of the *c. cardiacum*—in the general opinion of the authors—are secretory nerve cells, this interpretation can hardly be objected to from a morphological point of view. On the other hand it looks

as if secretory material produced in the cells of the *c. cardiacum* migrated through the axons in the direction of the brain, while it is otherwise assumed that material originating in the neurosecretory brain cells proceeds through or along their axons to the *c. cardiacum*, as stated especially by B. and E. SCHARRER. Perhaps the explanation is that the secretory material from the neurosecretory cells, both those situated in the brain and those of the *c. cardiacum*, diffuses from the axons into the blood at some distance from the cells. The close topographical relation between the *c. cardiacum* and the *c. allatum* may suggest that the latter organ may be specially dependent on the hormone from the *c. cardiacum* (cp. JOLY 1945).

The cells of the "peritracheal gland" (plate XIII, fig. 40) generally form single layers. They contain numerous unorientated, filiform mitochondria; no Golgi bodies were observed. Flemming-fixed material shows vacuoles with a few rounded, greyish globules, probably fat droplets. The nuclei contain threadlike chromosomes and one or more nucleoli; they very much resemble the nuclei in the R-cells of the *Calliphora* larva (plate I, fig. 3). ARVY and GABE (1947), in their beautiful paper on the *Chironomus* larva, state, against POSSOMPÈS (1946), that the cells of this organ are intensely basophilic. This is also the case in the *Ptychoptera* larva. ARVY and GABE have found that this basophilia is due to a rich content of ribonucleic acid.

I have not been able to identify with certainty the brain-cells from which the axons forming the *n. c. cardiaci* originate.

Discussion.

1. Comparative Morphology.

It is evident from the preceding description that the ring-gland of the *Calliphora* larva represents a complex and highly specialized system comprising the unpaired corpus allatum and the likewise unpaired *c. cardiacum*, both totally enveloped by a third component, the R-cells, which form the major part of the ring. The diagram text-fig. 3 (p. 24) shows this composition. The author is in accordance with BURTT and VOGT as to the interpretation of "the fuchsiphile ganglionic cells" of the posterior part of the ring as *c. cardiacum* cells. These cells are clearly identical with the chromophile cells described by CAZAL in many insects, but the same region also contains several smaller cells, which evidently represent the chromophobe cells of CAZAL.

The ring-gland of the *Tabanus* larva mainly differs from that of *Calliphora* in the following points: (1) the much larger *c. allatum* is not enveloped by the R-cells, but forms a separate, though closely connected posterior portion of the ring; (2) the *c. cardiacum* is represented by two cell groups imbedded in the antero-lateral parts of the ring; (3) the aorta does not pass through the ring; (4) the ring-gland is not traversed by the tracheae. Thus the *Tabanus* larva in the morphology of the ring-gland represents a somewhat more primitive stage.

The ring-gland of the *Thereva* larva resembles that of *Tabanus* in possessing

a similar separate and large *c. allatum* with a posterior position, but agrees with *Calliphora* in the fact that the ring surrounds the aorta.

The three genera mentioned thus agree in an important character: the *c. cardiaea* are completely covered by the R-cells which form the bulk of the complex organ. In the two representatives of the *Brachycera* the large *c. allatum* forms a separate

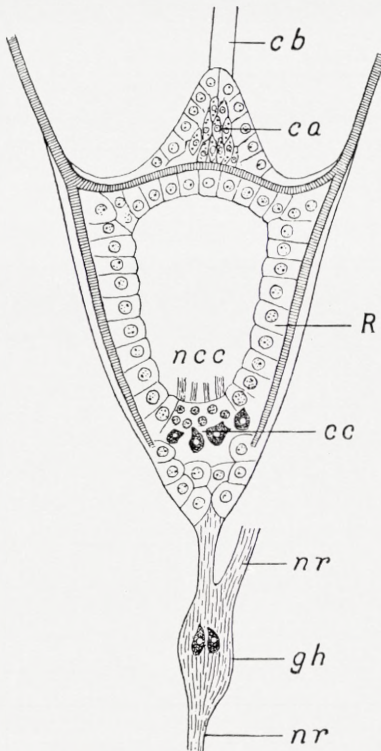


Fig. 3.

Diagram of Weismann's ring in the larva of *Calliphora*. *ca* *c. allatum*, *cb* cephalo-pharyngeal band, *cc* *c. cardiaea*, *gh* ganglion hypocerebrale, *ncc* nervi corporis cardiaci, *nr* nervus recurrens, *R* R-cells (traversed by trachea).

posterior part of the complex, but in the cyclorrhaphous *Calliphora* this organ is much smaller, situated anteriorly, and enveloped by the R-cells, so that the three constituents are virtually fused. As this is also the case in the larvae of *Lucilia* (DAY 1943), *Drosophila* (VOGT 1941, 1942), and *Eristalis* (CAZAL 1948), it is possible that this character is valid for the whole group of the *Cyclorrhapha*.

In the only representative of the *Nematocera* here studied, the larva of *Ptychoptera*, there is no ring-gland, but separate paired corpora allata and *c. cardiaea*; the *c. allata* are of considerable size, while the *c. cardiaea* are reduced to a few cells forming somewhat irregular groups. Thus, as might be expected, this genus shows a much more primitive and typical arrangement of the endocrine glands than the larvae of the higher *Diptera*. The *Ptychoptera* larva, however, shows another character of great interest, as it possesses a peculiar tissue resembling that found by POSSOMPÈS in the *Chironomus* larva and termed by him the "glande pérित्रachéenne". Its position and cytological appearance makes it very probable that it is the homologue of the R-cells. The hypothesis is supported by the relation of this tissue to the tracheae: The trachea along the anterior margin of the "glande pérित्रachéenne" is evidently the same as the trachea traversing the ring-gland of the *Calliphora* larva. POSSOMPÈS, who was the first to suggest the homology, pointed out that the "glandes pérित्रachéennes" like the

large lateral cells of Weismann's ring atrophy at metamorphosis. Finally the appearance of the peritracheal gland in the larva and pupa of *Culex* (BODENSTEIN 1945, CAZAL 1948), gives what must be considered as a proof of the homology. In this genus the peritracheal gland at the same time surrounds the trachea and forms an external cover around each *c. allatum*. The *c. cardiaea* are separate (CAZAL fig. 138). Thus *Culex* shows an interesting transition from the more primitive pattern of *Chironomus* and *Ptychoptera* on the one hand to the specialized cases of the higher *Diptera* on the other. "Peritracheal glands" also occur in *Psychoda*

and *Phlebotomus* (CAZAL), but so far have not been found in the *Tipula* larva (POSSOMPÈS 1947).

It is tempting to look for similar "organs" in other insect classes, and as already mentioned in the introduction E. THOMSEN (1941) postulated that the R-cells (large lateral cells) of the *Calliphora* larva might be the homologue of the "pericardial gland" of *Phyllium* and *Dixippus* described by PFLUGFELDER (1938, 1940). This organ has a similar situation close to the *c. allata-cardiaca* and the aorta and, like the R-cells, attains its highest development in the last larval stage and degenerates shortly after the final moult. The cytological picture differs in so far as the nuclei of the pericardial gland cells are lobate and polymorphous.

This hypothesis was opposed by POULSON (1945), who maintained that the large ring-gland cells originate from the stomodaeum, i. e. from the ectoderm, while the "pericardial gland" of the *Phasmidae* according to PFLUGFELDER is derived from the mesoderm. This conclusion, perhaps, after all is not quite decisive, considering the great difficulty of tracing such minute cell groups at early embryonic stages.

POSSOMPÈS (1946) supported the hypothesis of E. THOMSEN. So did CAZAL (1948), but he did not distinguish between "pericardial gland" and "pericardial cells". PFLUGFELDER (1938) emphasized the difference between these groups of cells in *Phyllium*: the cells of the "pericardial gland" have granular cytoplasm and lobate nuclei, while the usual pericardial cells show a fibrillar structure of the cytoplasm and simple rounded nuclei. The rather unfortunate name of "pericardial gland" is, of course, apt to lead to confusion.

It should, however, be emphasized that E. THOMSEN (*loc. cit.*) did not suggest a homology of the lateral ring-gland cells with true pericardial cells, but only with the "pericardial gland" of *Phasmidae*. In fact a comparison between the R-cells of the *Calliphora* larva and pericardial cells of the same species shows a pronounced difference (fig. 3 and fig. 13). The pericardial cells are filled with distinct mitochondria and contain a number of spherular bodies (just like the garland cells), while in the R-cells the mitochondria are much less stainable and granules are few and often invisible; also the nuclei look different.

While the homology between the "pericardial gland" of phasmids and the R-cells is at least very probable, so far nothing definite is known as to the occurrence of similar cells in other orders of insects. The "ventral glands" found in several groups of lower insects (PFLUGFELDER 1938, 1940 and 1947) have been homologized with the intersegmental organs of larval *Odonata*, with the prothoracic glands of *Lepidoptera*, *Hymenoptera*, and the blattid *Leucophaea*, and with some doubt also with the "parenchymatous tracheal organs" of *Nepa* (B. SCHARRER 1948). No doubt the glandular tissues of insects deserve a closer study, and it may be expected that hitherto undescribed organs may be found which will help to bridge the gaps still existing in our knowledge.

One more point ought to be mentioned: the striking difference in the size of the *c. allatum* of the investigated larvae, that of *Calliphora* being much smaller than

those of the other forms. It is tempting to correlate this fact with differences in the life-history and especially with the fact that while *Calliphora* has a rapid and continuous development with many annual generations, all the others have but one generation a year and hibernate as full-grown larvae. Only such full-grown larvae were studied. The large size of the *c. allatum* (as also its cytological appearance) evidently shows that this organ is extremely active, probably producing juvenile hormone which may play a role in preventing metamorphosis.

2. Cytological Observations.

Mitochondria¹ have been observed in all or most of the cells studied. They occur mostly as short curved threads or rods which are coloured by haematoxylin in Heidenhain's iron haematoxylin method. They are most distinct after osmium fixation.

Golgi bodies are very distinct in some of the cells of the nervous system after impregnation with osmic acid. In the most convincing cases these elements show the typical ring-shape of invertebrate "dictyosomes". Such pictures should probably be regarded as representing bodies or drops consisting of a chromophile external layer of a lipoid nature and a chromophobe internum (see BAKER 1945, THOMAS 1947 and 1948). In some cases also chaplet-like rings consisting of smaller granules were observed (plate X, fig. 31).

Neurosecretory brain cells. The Golgi bodies were especially clear in those brain cells of the *Calliphora* larva from which the axons going to the ring-gland (the internal paracardiac nerves of CAZAL) probably originate. It is almost certain that these cells correspond to the "median group" of neurosecretory cells found in several other insects. In the adult *Calliphora* neurosecretory brain cells are conspicuous in the living animal by their bluish colour (E. THOMSEN, 1948). A microscopical study of these cells will appear later. It is surprising, however, that in the larva it was almost impossible to find secretory material in the corresponding cells; both early and late third stage larvae have been studied, and only in one larva somewhat doubtful signs of secretion were observed. Whether the abundant and well-developed Golgi bodies suggest a preparatory stage to secretion remains to be proved.

In the *Tabanus* larva the signs of neurosecretion in the brain are very convincing. Such cells as the one pictured on plate VIII contain a great number of dark bodies of varying size in the cell-body and in the thick proximal part of the axon. The smallest are minute roundish granules, the larger ones irregularly shaped lumps which are undoubtedly secretory products. The small granules show some resemblance to Golgi bodies, but these are generally invisible after Flemming-fixation, and the gradual transition in size seems to show that the small granules are younger stages of the large bodies.

It is also important that identical granules were observed after fixation with

¹ In this paper the term mitochondria comprises both filiform and granular elements of the chondriome.

Helly's, S. Felice's, and Bouin's fluids. They are often stained bluish black by haematoxylin, but after Masson's trichrome stain they sometimes stain a bright red colour. The secretory nature of these granules seems undeniable. On the other hand it is somewhat surprising that they are not visible in the sections after fixation with Champy's fluid and prolonged osmification; it will be remembered that CAZAL found this method of special value for the demonstration of secretory granules in the nerve cells and incretory organs of insects. However, when this method is used the neurosecretory brain cells of the *Tabanus* larva show unusually large and beautiful Golgi elements (fig. 31). Even if modern investigators claim that the pictures of Golgi bodies obtained by osmium impregnation are to some degree artifacts, the Golgi elements do exist in the cell as natural cytoplasmic inclusions. It seems very likely therefore to assume that they are involved in the formation of the secretory granules in a similar way as that suggested by THOMAS (1948). The "mulberry" stages described by this author resemble some of the Golgi elements seen in my sections. THOMAS also holds that the Golgi products observed by him are "clearly identical" with the neurosecretory granules of B. and E. SCHARRER, but he shows some reluctance in accepting the hypothesis of neurosecretion and instead suggests the somewhat revolting idea that the material produced within the Golgi system is absorbed by the nucleus. Even if my own observations are quite insufficient to decide this question, I am much inclined to support the hypothesis of neurosecretion. In this connection it should be mentioned that in some larvae of *Diptera* (especially *Tipula*) the cells in question show the same characteristic bluish colour as the *c. cardiaca* of many insects and the neurosecretory cells of the adult blow-fly.

Another problem is how the secretory products are extruded from the cells. B. and E. SCHARRER, who have described neurosecretory brain cells in a number of vertebrates and invertebrates, assume that the granules or droplets actually move along the axon to the innervated organ (in insects to the *c. cardiacum*). It is of course very difficult to prove this from a study of sections, though in some insects the stained granules have been observed following the axons in their whole intercerebral route (cp. also p. 23). Future studies may perhaps solve this intricate problem.

Cytology of the *c. cardiacum*. The *c. cardiacum* in the larvae investigated is reduced to rather few cells, which in the *Ptychoptera* larva adhere to the *c. allatum*, while in those of *Tabanus*, *Thereva*, and *Calliphora* they are imbedded in the ring and surrounded by the R-cells ("the peritracheal gland")¹. The cells of the *c. cardiacum* generally appear intensely red after staining with Ponceau or acid fuchsine and are blackened by osmium after fixation in Champy's fluid and post-osmification. The black colour after the latter method is mainly caused by numerous minute granules which are densely heaped in the cells; this is especially clear in the *Tabanus* larvae. These granules look very much like mitochondria. CAZAL (1943, p. 148) similarly states that the abundant mitochondria of the chromophile cells contribute materially

¹ In the larva of *Chironomus* the *c. cardiacum* is probably represented by the cells which POSSOMPÈS in his preliminary paper (1946) has described as "cellules ganglionnaires du nerf stomacal".

to the chromophily of these cells, but are not its only cause: the cytoplasm between the granules is also chromophile.

As the red colour obtained by the first-mentioned methods (Ponceau, acid fuchsine) stains the cells uniformly, it seems possible that the secretion consists of a diffuse substance, surrounding the mitochondria. A few Golgi bodies have been observed in the cells. The cytological picture presented by the *c. cardiacum* cells is thus very different from that of the neurosecretory cells at least in the *Tabanus* larva. This fact is of some importance with regard to the interesting hypothesis of B. and E. SCHARRER that the neurosecretory cells and the *c. cardiacum* form one physiological system comparable to the hypothalamus-neurohypophysis of vertebrates.

The cells of the *c. cardiacum* of *Calliphora* and *Tabanus* are of a simple shape. Also in the larva of *Ptychoptera* the cells mostly appear simple and rounded, but in a few cases of Champy-fixed sections they showed cytoplasmic processes resembling axons (cp. p. 22). After fixation in Champy's or Flemming's fluids the *c. cardiacum* of *Ptychoptera* either appears uniformly black or contains irregular dark lumps, probably a secretory material; the same substance occurs in the nervus *c. cardiaci*. This substance, strangely enough, was not stained red by Ponceau.

The morphology and function of the chromophobe cells of the *c. cardiacum* are imperfectly known. Their incapability of staining makes a study very unpromising.

Cytology of *c. allatum*. The *c. allatum* of the *Calliphora* larva with its elongate cells with longitudinally orientated mitochondria is the most specialized one hitherto described. The numerous osmiophile granules are either Golgi bodies or secretory products.

In the other larvae the *c. allatum* resembles the usual type ("type parvocellulaire" of CAZAL), though the shape of the cells may vary considerably. In the *Ptychoptera* larva osmium-impregnation results in the appearance of numerous small granules resembling those of the *Calliphora* larva. In the *Tabanus* larva, however, prolonged osmification produces an intense blackening of the organ. The peripheral cells contain spherular granules of different size, generally with a black rim and a light interior, and further irregular dark masses without a distinct border (plate VII, fig. 22). The centrally situated cells have smaller and darker granules, some compact and others showing a light interior. An obvious interpretation of this picture would be that the last named granules are Golgi bodies which form secretory products in their interior. They eventually appear as droplets which grow to a certain size and then burst, flowing out into the cytoplasm. The process has advanced to its final stage in the periphery of the *c. allatum*, from which the secretion is probably released into the haemolymph.

This account of the secretion of the *c. allatum* differs much from that of CAZAL, who derives the secretory products from the mitochondria and finds that the stage of secretion is always more advanced in the centre than in the periphery. His statement does not only apply to the lower orders of the *Pterygota*, but also to the larva of *Eristalis* (CAZAL, fig. 164). How this discrepancy should be explained is difficult

to say. In the case of the *Tabanus* larva, however, I find it impossible to fit my observations into CAZAL's theory. It is thus seen that the secretion in the c. allatum of the *Tabanus* larva, like that of the neurosecretory brain cells, is probably produced by the Golgi bodies, while the secretion of the c. cardiacum does not show any clear relation to the Golgi bodies, but seems somehow to be connected with the mitochondria—as also held by CAZAL.

It is likely that the granules observed in the R-cells (plate I, fig. 3) are also of a secretory nature, but this is not quite clear.

It should be added that there are so many experimental results proving the secretory or incretory function of the c. allata, c. cardiaca, and large lateral ring-gland cells that a negative histological find cannot disprove this theory, but should probably be referred to a temporary pause in the secretory cycle.

Summary.

1. The present paper contains a detailed description of the anatomy and cytology of the ring-gland (Weismann's ring) of the third stage larva of *Calliphora erythrocephala*. The ring is a complex organ comprising the c. allatum and the c. cardiacum enveloped by the cells of the third component: the large lateral cells (R-cells).

2. With the view of elucidating the homology of the different parts of the ring-gland, especially the R-cells, the ring-gland of some other larvae of *Diptera*, belonging to more primitive groups, have been studied. These were the larvae of *Tabanus* sp. and *Thereva* sp. (*Diptera brachycera*) and the larva of *Ptychoptera* sp. (*Diptera nematocera*).

3. The larvae of *Tabanus* and *Thereva* have ring-glands resembling those of the *Cyclorrhapha* inasmuch as the c. cardiacum is enveloped by the R-cells which form the main part of the ring. The c. allatum, however, is much bigger, more demarcated and forms a separate posterior part of the complex organ.

4. In the larva of *Ptychoptera* a more primitive state is found, resembling that of other orders of insects. There are paired c. allata and c. cardiaca. The former are two well-defined pluricellular organs, while the latter consist of a few, large cells adhering to the c. allata. The R-cells are represented by two cellular bands or sheets connected with a transverse trachea and the c. allata. Similar tissues were described by POSSOMPÈS (1946) under the name of "glande péritrachéenne" in the larva of *Chironomus*.

5. The hypothesis of E. THOMSEN (1941) that the lateral ring-gland cells (R-cells) may be homologous with the "pericardial gland" of the *Phasmidae* described by PFLUGFELDER is supported. The "pericardial gland" should be strictly distinguished from the ordinary pericardial cells.

6. The "garland cells" of WEISMANN in the larva of *Calliphora* are large pericardial cells. Similar cells are found in the larva of *Ptychoptera*.

The so-called "glandes post-cérébrales antérieures" (POSSOMPÈS 1946, ARVY

and GABE 1947) of the *Chironomus* larva, previously misinterpreted as corpora allata by some authors and as *c. cardiaca* by others, are in reality two especially large garland cells. In their detailed cytology they agree completely with the garland and pericardial cells.

7. The brain cells from which the axons innervating the *c. cardiacum* originate have been especially studied in the larva of *Tabanus*. These cells are undoubtedly neurosecretory cells which show the cytological picture of intense secretion. In a few cases the secretion could be followed along the axons. The secretory products probably arise from the Golgi bodies of the cells. In the larva of *Calliphora* the corresponding brain cells did not as a rule show any definite signs of secretion.

8. In the *Tabanus* larva the secretion in the *c. allatum* cells also seems to be intimately related with the Golgi bodies, while the *c. cardiacum* presents a very different cytological picture, the secretion apparently arising round the mitochondria.

Zoological Laboratory, Royal Veterinary and Agricultural College, Copenhagen.

Literature.

- ARVY, L. and GABE, M. (1947): Contribution à l'étude cytologique et histologique de la larve de *Chironomus plumosus* L. *Revue Canad. Biol.* 6.
- BAKER, J. R. (1945a): The structure and chemical composition of the Golgi element. *Quart. Jl. Micr. Sci.* 85.
- (1945b): *Cytological Technique*, 2nd ed. London.
- BODENSTEIN, D. (1938): Untersuchungen zum Metamorphoseproblem I. Kombinierte Schnürrungs- und Transplantationsexperimente an *Drosophila*. *Roux' Arch.* 137.
- (1945): The corpora allata of mosquitoes. *Connecticut Exp. Sta. Bull.* 488.
- BURTT, E. T. (1937): On the corpora allata of dipterous insects. *Proc. R. Soc. London B.* 124.
- (1938): On the corpora allata of dipterous insects. II. *Proc. R. Soc. London B.* 126.
- CAZAL, P. (1948): Les glandes endocrines rétro-cérébrales des Insectes (étude morphologique). *Bull. biol. France et Belg. Suppl.* 32.
- DAY, M. F. (1943): The homologies of the ring-gland of *Diptera brachycera*. *Ann. Ent. Soc. Amer.* 36.
- DE ROBERTIS, E. D. P., NOWINSKI, W. W. and SAEZ, F. (1948): *General Cytology*. Philadelphia and London.
- FOOT, N. C. (1933): The Masson trichrome staining methods in routine laboratory use. *Stain Technology* 8.
- FRAENKEL, G. (1935): A hormone causing pupation in the blowfly *Calliphora erythrocephala*. *Proc. R. Soc. London. B.* 118.
- GABE, M. (1947): Sur l'emploi du picrate de Vert de méthyle pour la différenciation de la coloration d'Altmann. *Bull. Histol. Appliq.* No. 1.

- GRESSION, R. A. R. (1948): Essentials of General Cytology. Edinburgh.
- HADORN, E. (1937a): An accelerating effect of normal "ring-glands" on puparium-formation in lethal larvae of *Drosophila melanogaster*. Proc. nation. Acad. Sci. Washington 23.
- (1937b): Hormonale Kontrolle der Pupariumbildung bei Fliegen. Naturw. Berlin 25.
- HANSTRÖM, B. (1938): Zwei Probleme betreffs der hormonalen Lokalisation im Insektenkopf. Kungl. Fysiogr. Sällsk. Handl. Lund, N. F. 49.
- (1939): Hormones in Invertebrates. Oxford.
- (1940): Inkretorische Organe, Sinnesorgane und Nervensystem des Kopfes einiger niederer Insektenordnungen. Kungl. Svenska Vetensk. Handl. 18.
- (1941): Einige Parallelen im Bau und in der Herkunft der inkretorischen Organe der Arthropoden und der Vertebraten. Kungl. Fysiogr. Sällsk. Handl. Lund, N. F. 37.
- HEWITT, C. G. (1914): The House-fly. Cambridge.
- JOLY, P. (1945): La fonction ovarienne et son contrôle humoral chez les Dytiscides. Arch. Zool. exp. 84.
- KEILIN, D. (1917): Recherches sur les Anthomyides à larves carnivores. Parasitology 9.
- KOWALEVSKY, A. (1887): Zum Verhalten des Rückgefäßes und des guirlandenförmigen Zellenstrangs der Musciden während der Metamorphose. Biol. Cbl. 6.
- (1889): Ein Beitrag zur Kenntnis der Exkretionsorgane. Biol. Cbl. 9.
- KÜNCKEL d'HERCULAIS, J. (1875): Recherches sur l'organisation et le développement des Volumes etc. Paris.
- LOWNE, B. T. (1890—95): The Anatomy, Physiology, Morphology and Development of the Blow-fly. I—II. London.
- LUNDBECK, W. (1908): Diptera danica. II. Copenhagen and London.
- NYST, R. H. (1941): Contribution à l'étude de l'hormone nymphogène. Ann. Soc. Roy. Zool. Belg. 72.
- PANTEL, J. (1898): Le *Thrixion halidayanum* Rond. Essai monographique sur les caractères extérieurs, la biologie et l'anatomie d'une larve parasite du groupe des Tachinaires. La Cellule 15.
- PFLUGFELDER, O. (1937): Bau, Entwicklung und Funktion der Corpora allata und cardiaca von *Dixippus morosus* Br. Z. wiss. Zool. 149.
- (1938): Weitere experimentelle Untersuchungen über die Funktion der Corpora allata von *Dixippus morosus* Br. Z. wiss. Zool. 151.
- (1940): Wechselwirkungen von Drüsen innerer Sekretion bei *Dixippus morosus* Br. Z. wiss. Zool. 152.
- (1947): Über die Ventraldrüsen und einige andere inkretorische Organe des Insektenkopfes. Biol. Zbl. 66.
- POSSOMPÈS, B. (1946): Les glandes endocrines postcérébrales des Diptères. I. Étude chez la larve de *Chironomus plumosus* L. Bull. Soc. Zool. France 71.
- (1947): II. Étude sommaire des corpora allata et des corpora cardiaca chez la larve de *Tipula* sp. Bull. Soc. Zool. France 72.
- POULSON, D. F. (1945): On the origin and nature of the ring-gland (Weismann's ring) of the higher *Diptera*. Trans. Connecticut Acad. 36.
- SCHARRER, B. (1941): Neurosecretion. II. Neurosecretory cells in the central nervous system of cockroaches. J. Comp. Neur. 5.
- (1948): The prothoracic glands of *Leucophaea maderae* (Orthoptera). Biol. Bull. 95.
- (1949): Hormones in insects. In: Hormones, chemistry, physiology, and clinical applications, edited by G. Pincus and K. V. Thimann. Vol. I. New York.
- SCHARRER, B. and HADORN, E. (1938): The structure of the ring-gland (Corpus allatum) in normal and lethal larvae of *Drosophila melanogaster*. Proc. nation. Ac. Sci. Washington 24.

- SCHARRER, B. and SCHARRER, E. (1949): Neurosecretion IV. A comparison between the inter-cerebralis-cardiacum-allatum system of the insects and the hypothalamo-hypophyseal system of the vertebrates. Biol. Bull. 87.
- SCHARRER, E. and SCHARRER, B. (1945): Neurosecretion. Physiol. Rev. 25.
- THOMAS, O. L. (1947): The cytology of the neurones of *Helix aspersa*. Quart. Jl. Micr. Sci. 88.
- (1948): A study of the spheroid system of sympathetic neurones with special reference to the problem of neurosecretion. Quart. Jl. Micr. Sci. 89.
- THOMPSON, W. R. (1921): Recherches sur les Diptères parasites. I. Les larves des *Sarcophagidae*. Bull. Biol. Fr. et Belg. 54.
- THOMSEN, E. (1940): Relation between corpus allatum and ovaries in adult flies (*Muscidae*). Nature (London) 145.
- (1941): Ringdrüse und Corpus allatum bei Musciden. Naturw. Berlin. 29.
- (1942): An experimental and anatomical study of the corpus allatum in the blow-fly *Calliphora erythrocephala* Meig. Vid. Medd. Dansk Naturh. Foren. 106.
- (1948): Effect of removal of neurosecretory cells in the brain of adult *Calliphora erythrocephala* Meig. Nature (London) 161.
- VOGT, M. (1941): Anatomie der pupalen *Drosophila*-Ringdrüse und ihre mutmassliche Bedeutung als imaginales Metamorphosezentrum. Biol. Zbl. 61.
- (1942): Ein drittes Organ in der larvalen Ringdrüse von *Drosophila*. Naturwiss. Berlin. 30.
- (1943): Zur Kenntnis des larvalen und pupalen Corpus allatum von *Calliphora*. Biol. Zbl. 63.
- WEISMANN, A. (1864): Die Entwicklung der Dipteren. II. Die nachembryonale Entwicklung der Musciden. Z. wiss. Zool. 14.
- WIGGLESWORTH, V. B. (1939): The Principles of Insect Physiology. London.
- YAO, T. (1950): The localization of alkaline phosphatase during the postembryonic development of *Drosophila melanogaster*. Quart. Jl. Micr. Sci. 91.
- ZEE, H. C. and PAI, S. (1944): Corpus allatum and corpus cardiacum in *Chironomus* sp. Amer. Naturalist 78.

The following papers were only received during the printing and could not be considered in the text:

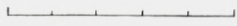
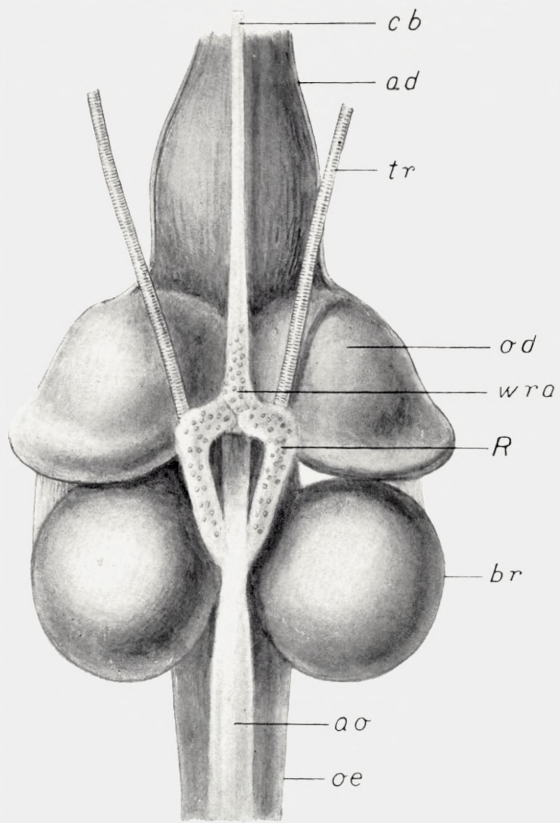
- PFLUGFELDER, O. (1949): Die Funktion der Pericardialdrüsen der Insekten. Sonderdruck aus »Verhandl. d. deutsch. Zoologen in Mainz 1949.«
- POSSOMPÈS, B. (1948): Les corpora cardiaca de la larve de *Chironomus plumosus* L. Bull. Soc. Zool. France 73.
- (1948): Les glandes endocrines post-cérébrales des Diptères III. Étude chez la larve de *Tabanus* sp. Ibidem.
-

PLATES

PLATE I

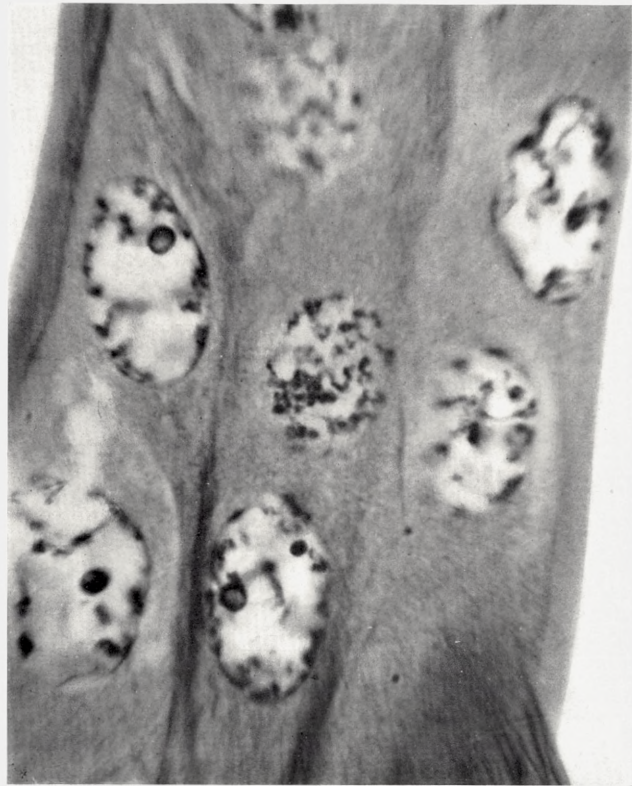
Calliphora erythrocephala.

- Fig. 1. Weismann's ring of late third stage larva in natural position, dorsal view. *ad* imaginal disc of antenna; *ao* aorta; *br* brain; *cb* cephalo-pharyngeal band; *od* optic disc (rudiment of imaginal eye); *oe* oesophagus; *R* lateral part of Weismann's ring; *tr* trachea traversing the ring-gland; *wra* anterior prolongation of ring-gland.
- Fig. 2. Section of ring-gland with R-cells. Note the spiralized chromosomes. — Flemming; Heidenhain. Photo. 900 ×.
- Fig. 3. Horizontal section of R-cells. *v* vacuoles surrounded by granules. — Flemming; Heidenhain. 800 ×.
- Fig. 4. Horizontal section of anterior part of ring-gland. *ca* corpus allatum with osmiophile granules; *cb* cephalo-pharyngeal band. The large pale nuclei belong to the R-cells. — Champy, osmic acid (8 days); unstained. Photo. 320 ×.

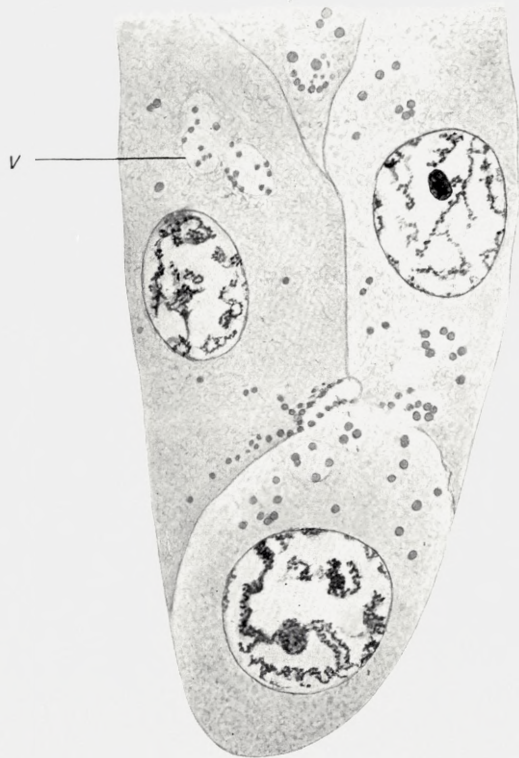


0.5 mm.

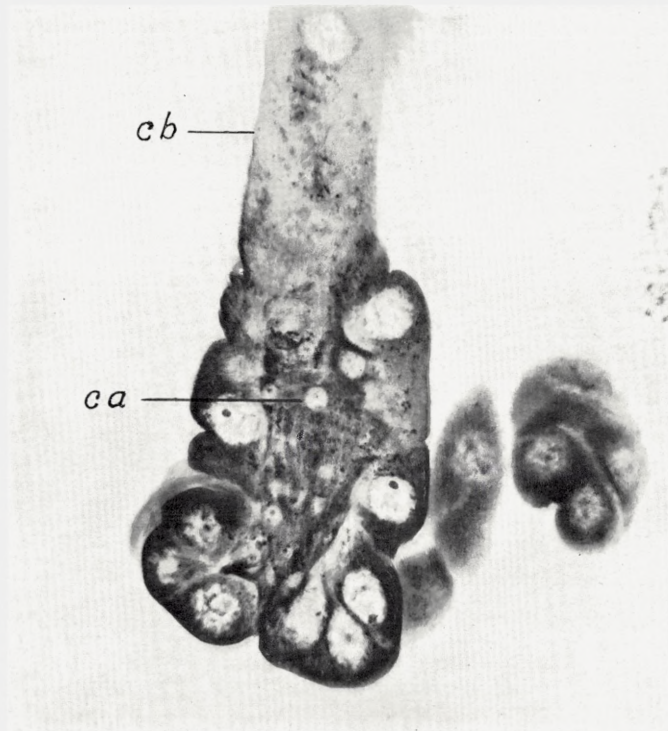
1



2



3

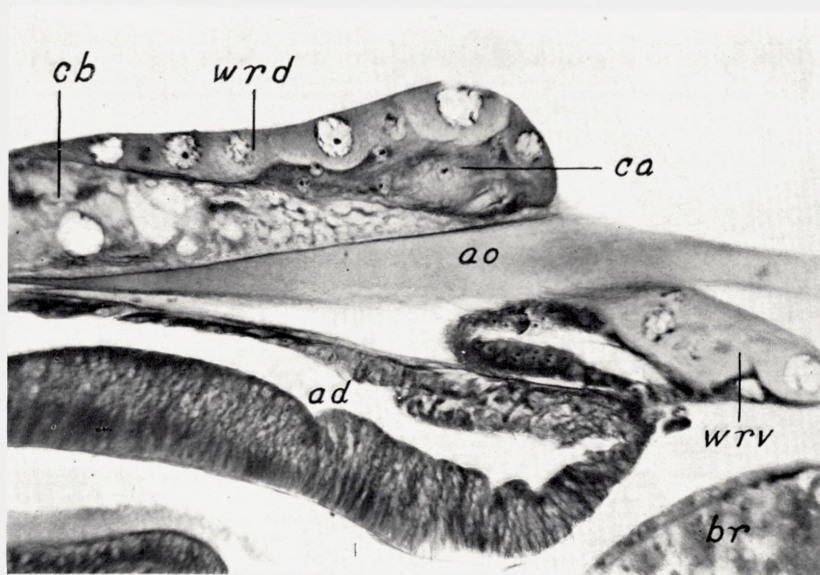


4

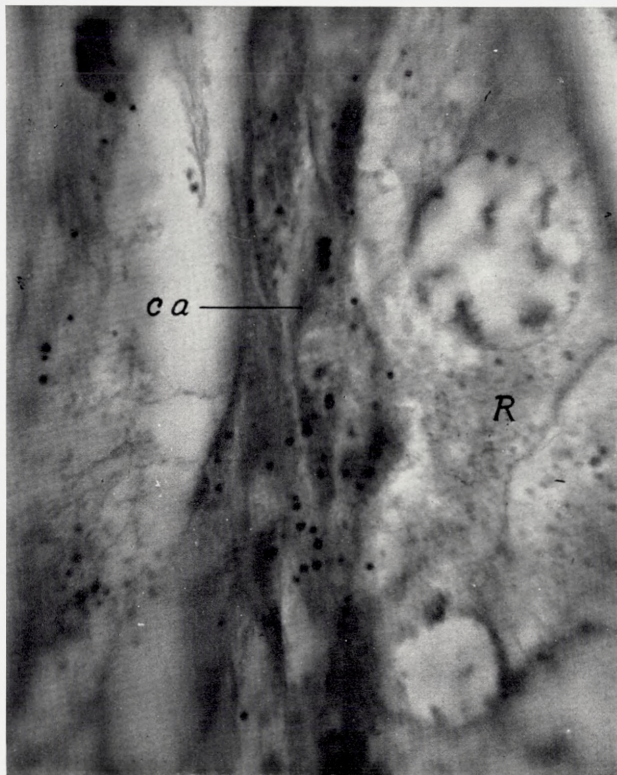
PLATE II

Calliphora erythrocephala.

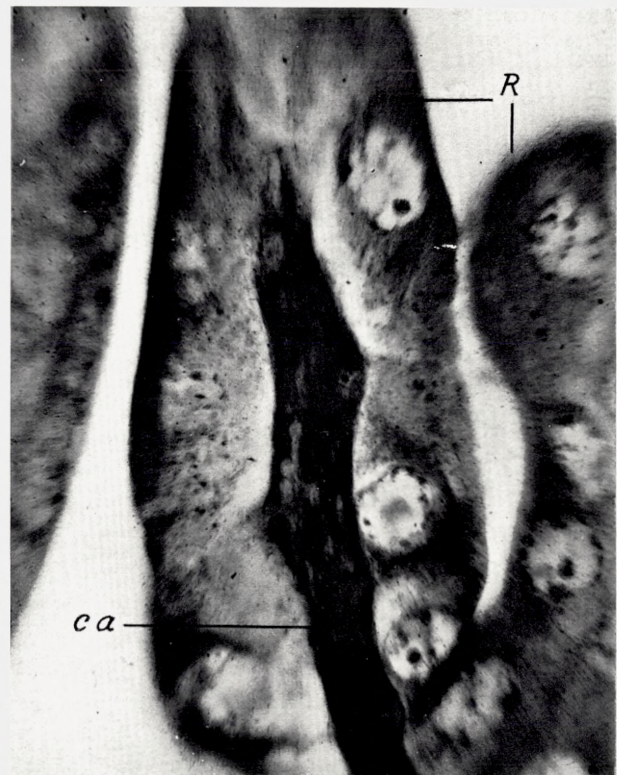
- Fig. 5. Sagittal section through ring-gland, etc. *ad* antennal disc; *ao* aorta; *br* brain; *ca* c. allatum; *cb* cephalopharyngeal band; *wrd* antero-dorsal part of ring-gland (R-cells); *wrv* ventral part of ring-gland. — S. Felice; Heidenhain, acid fuchsine. Photo. 200 ×.
- Fig. 6. Horizontal section of corpus allatum. *ca* c. allatum, spindle-shaped cells with blackish granules; *R* R-cells with large spheroid nuclei. — Champy, osmic-acid (9 days); unstained. Photo. 900 ×.
- Fig. 7. Similar section from another larva. Letters as in fig. 6. Note the intense blackening of the c. allatum. — Champy, osmic acid (6 days); Heidenhain. Photo. 550 ×.



5



6



7

PLATE III

Calliphora erythrocephala.

- Fig. 8. Horizontal section of posterior part of Weismann's ring (formerly called "median ganglion"), *cc* *c. cardiacum*, the line points to a chromophile cell, to the left of which a group of chromophobe cells is visible; *nec* nervus corporis cardiaci externus; *nr* nervus recurrens; *nrv* branch of this to Weismann's ring (the connection is found in the neighbouring section); *R* R-cells enveloping *c. cardiacum*. — Champy, osmic acid (4 days); Heidenhain. 300 ×.
- Fig. 9. Similar section from another larva. *cc* chromophile cells of *c. cardiacum* imbedded in R-cells; *gh* ganglion hypocerebrale with two chromophile cells; *nr* nervus recurrens. — Flemming, osmic acid (7 days); unstained. Photo. 400 ×.

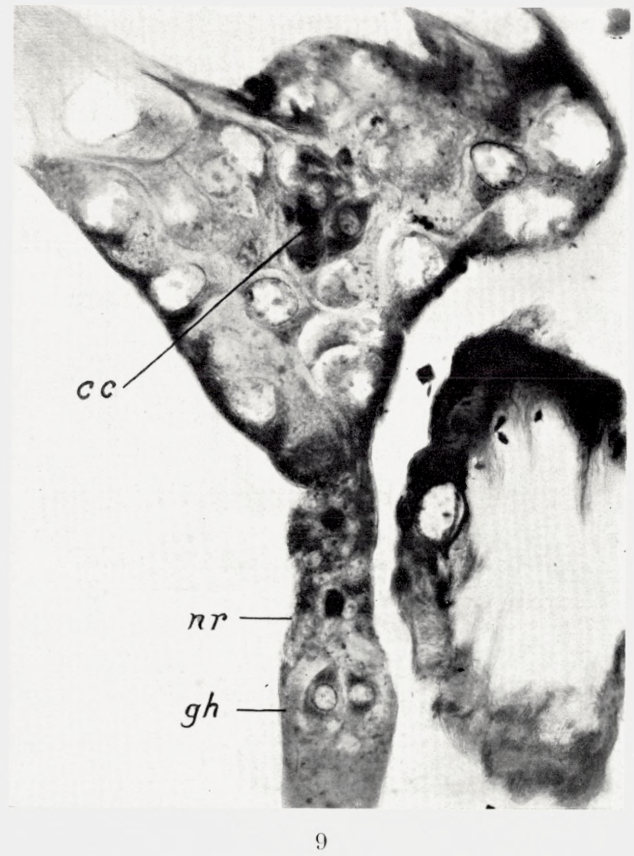
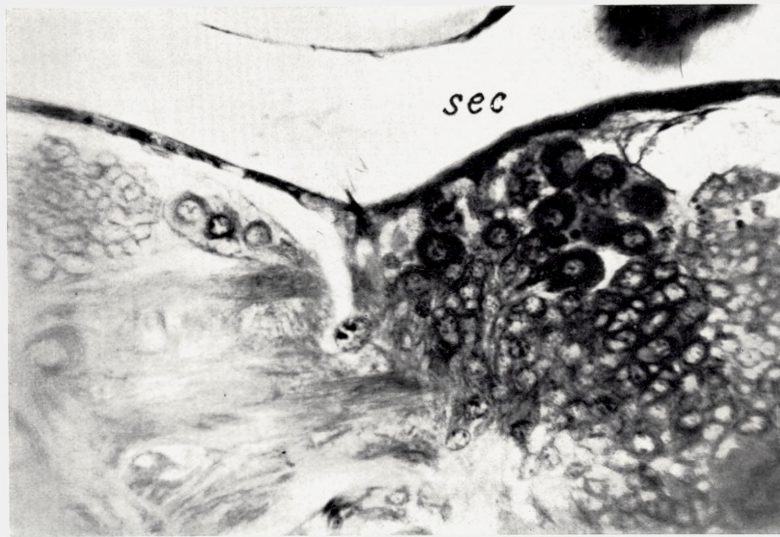


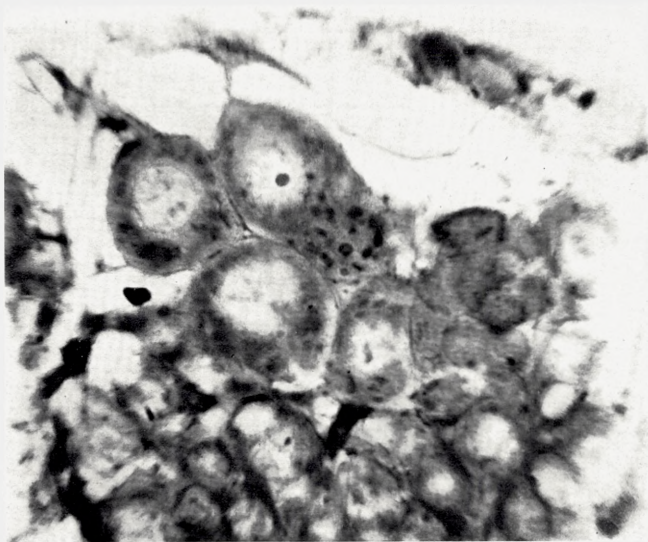
PLATE IV

Calliphora erythrocephala.

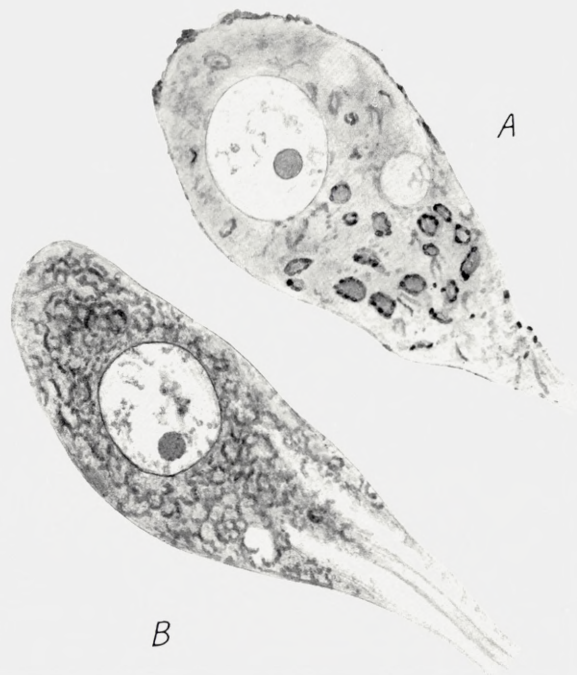
- Fig. 10. Horizontal section through antero-median part of brain (pars intercerebralis). *sec* neurosecretory cells of right hemisphere. — Flemming; Heidenhain. Photo. 320 \times .
- Fig. 11. Neurosecretory brain cells with Golgi bodies from the pars intercerebralis of another larva. — Champy, osmic acid (7 days); Heidenhain. Photo. 900 \times .
- Fig. 12. Neurosecretory brain cells. *A* one of the cells from fig. 11 drawn at a higher magnification showing Golgi bodies; *B* cells from brain fixed in Flemming's fluid and stained with Heidenhain's iron haematoxylin showing mitochondria and tube-like axon. 1650 \times .



10



11



12

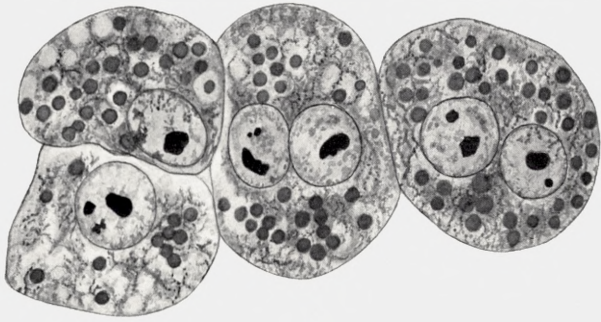
PLATE V

Calliphora erythrocephala.

- Fig. 13. Pericardial cells of early third stage larva. Two binucleate cells and two mononucleate twin-cells, probably originated from division of one of the former type. Cytoplasm with spheroid granules and thread-like mitochondria. — Alcoholic Bouin; Masson-Foot. 1275 \times .
- Fig. 14. Garland cell of older third stage larva. Granules and mitochondria (cp. text). — Alcoholic Bouin; Masson-Foot. 1275 \times .

Tabanus sp.

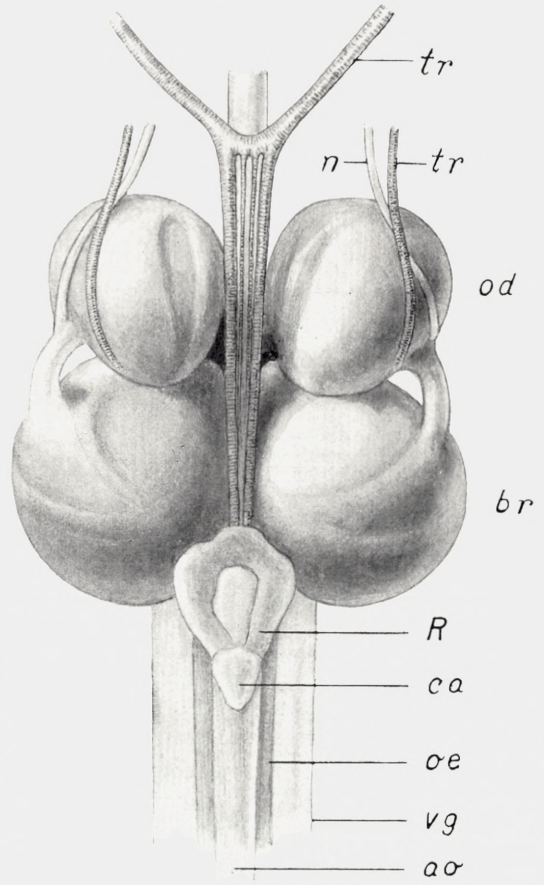
- Fig. 15. Ring-gland and brain, etc., of last stage larva, dorsal view. *ao* aorta; *br* brain; *ca* c. allatum; *n* nerve; *od* optic disc; *oe* oesophagus; *R* lateral part of ring-gland; *tr* tracheae; *vg* fused ventral ganglia.
- Fig. 16. Similar preparation from left side. *pv* proventriculus; other letters as in fig. 15.



13

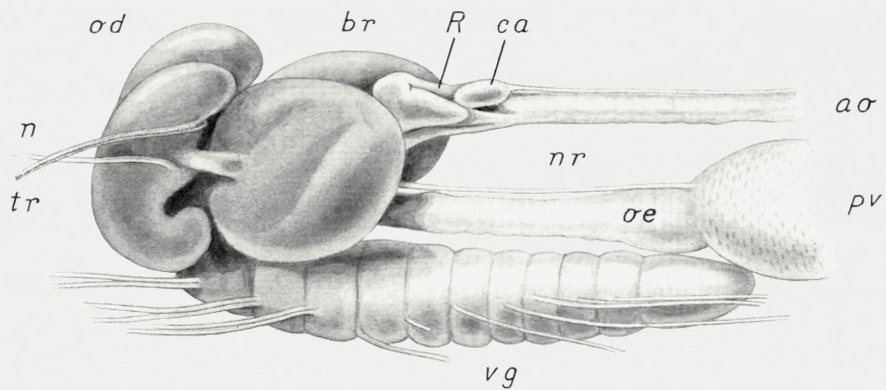


14



0.5 mm.

15



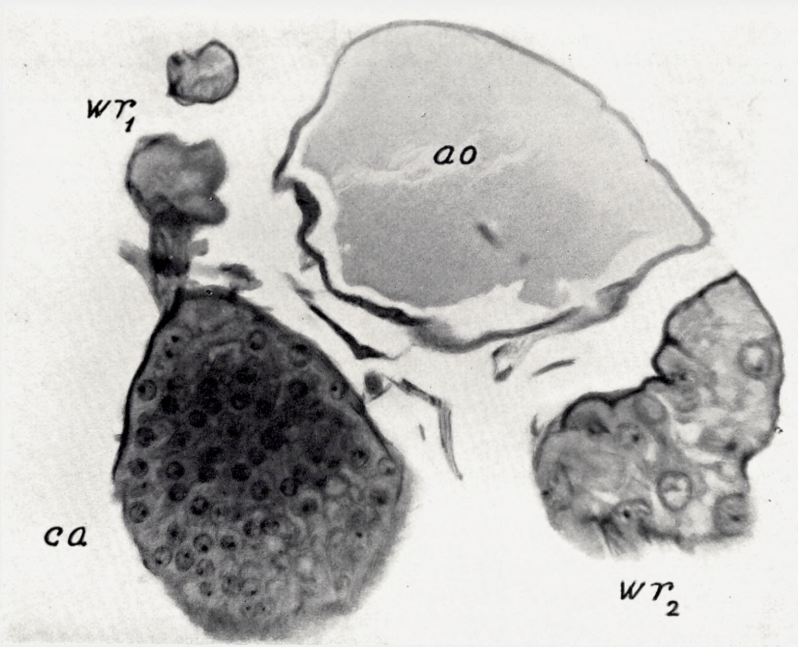
0.5 mm.

16

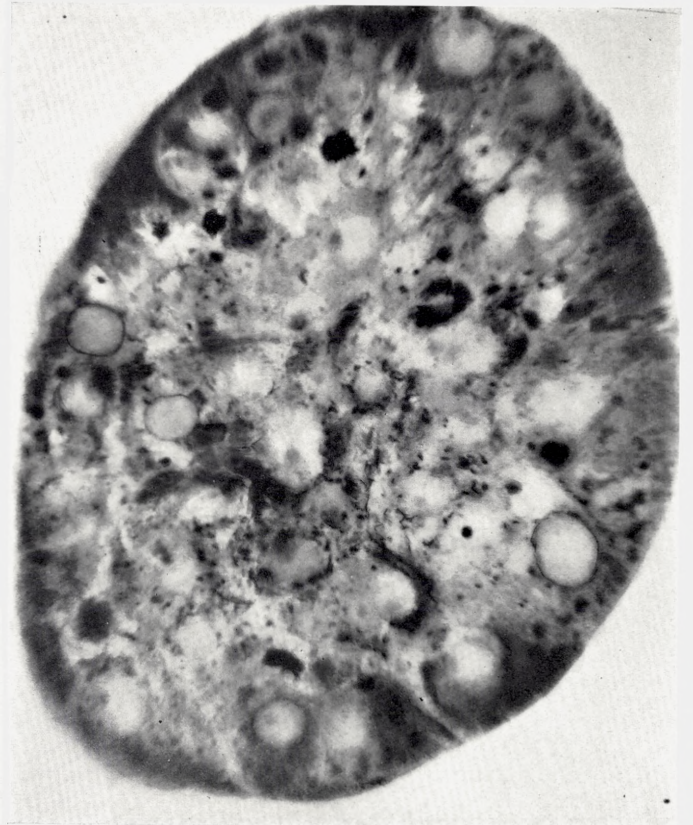
PLATE VI

Tabanus sp.

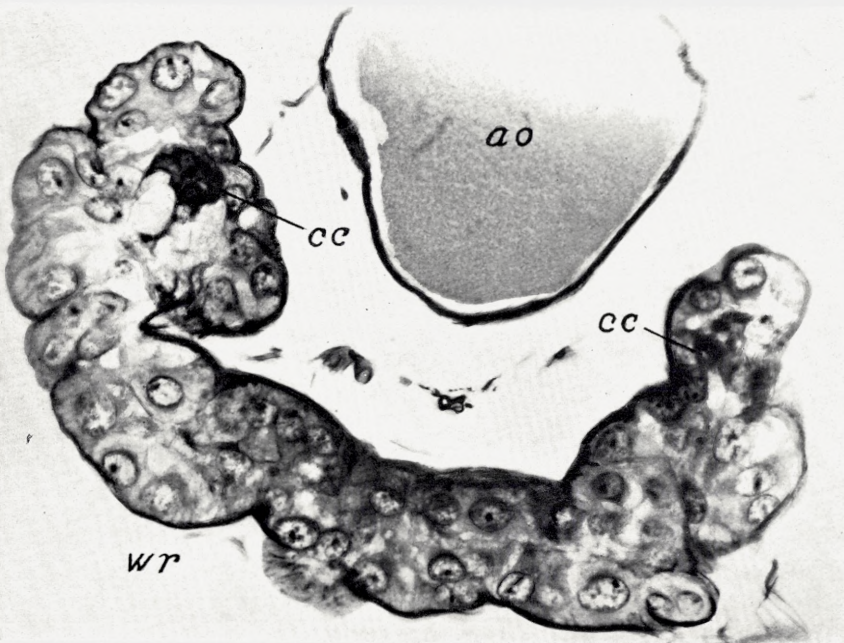
- Fig. 17. Section of ring-gland showing posterior part of the organ. *ao* aorta; *ca* *c. allatum*; *wr₁* and *wr₂* left and right lateral parts of ring-gland superficially cut. — Alcoholic Bouin; Masson-Foot. 400 ×.
- Fig. 18. Another section from the same series, at a more ventral level. *ao* aorta; *cc* the two groups of *c. cardiacum* cells imbedded in the R-cells; *wr* ring-gland (R-cells). — Same technique. 400 ×.
- Fig. 19. Section through *c. allatum* with Golgi bodies in the central cells and secretory granules in the external ones (cf. plate VII, fig. 21—22); nuclei pale. — Champy; osmic acid (10 days); unstained. Photo. 900 ×.



17



19

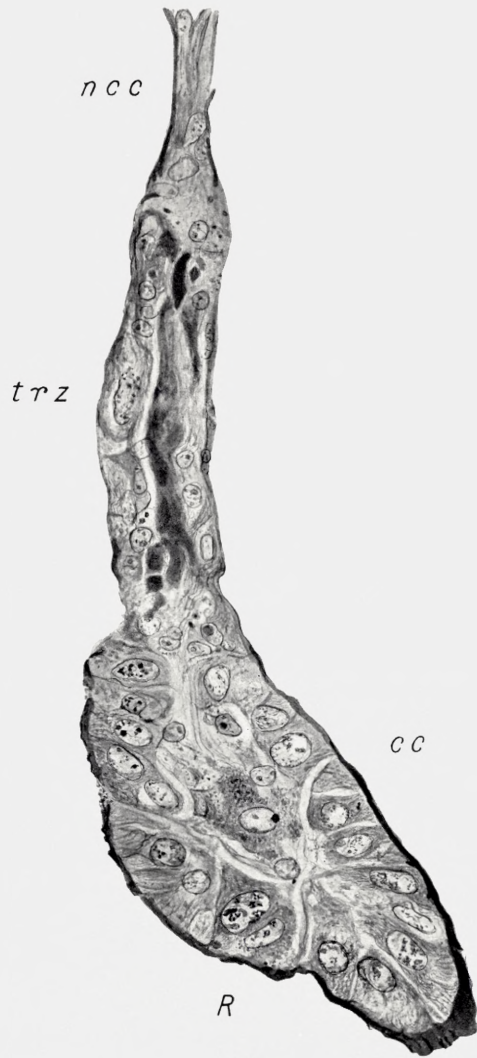


18

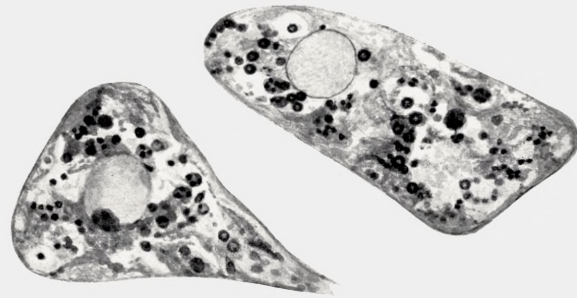
PLATE VII

Tabanus sp.

- Fig. 20. Longitudinal section through one of the anterior lobes of the ring-gland. *cc* *c. cardiacum* (in centre, surrounded by R-cells); *ncc* *nervus corporis cardiaci*; *R* R-cells; *trz* transitory zone with black granules. — Flemming; Heidenhain. 250 ×.
- Fig. 21. Two cells from the central part of the *c. allatum* with Golgi bodies. — Champy, osmic acid (6 days); Heidenhain. 1760 ×.
- Fig. 22. External cells of the same *c. allatum*. *d* secretory droplets, *s* diffuse secretory material. 1760 ×.
- Fig. 23. Neurosecretory brain cell from dorsal group (cp. plate VIII, fig. 24). *v* vacuole with coiled thread in the wall. Flemming; Heidenhain. 2000 ×.



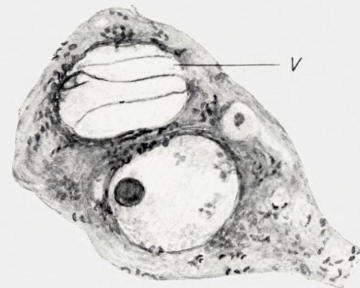
20



21



22

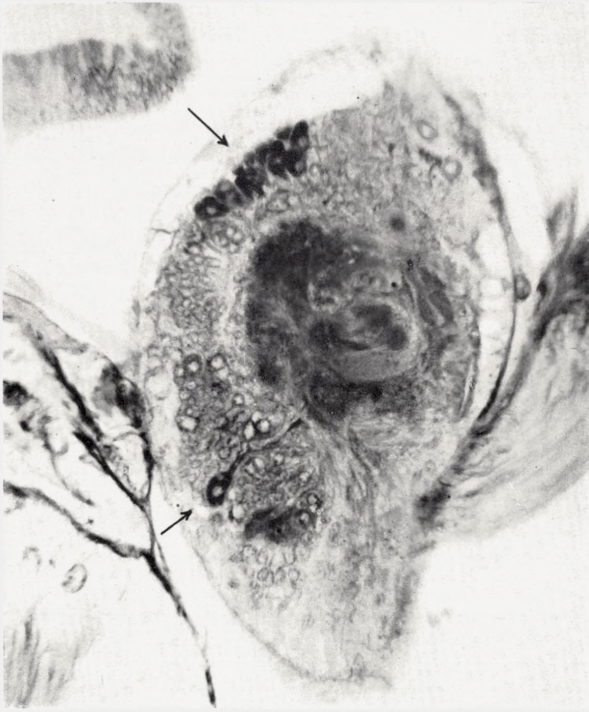


23

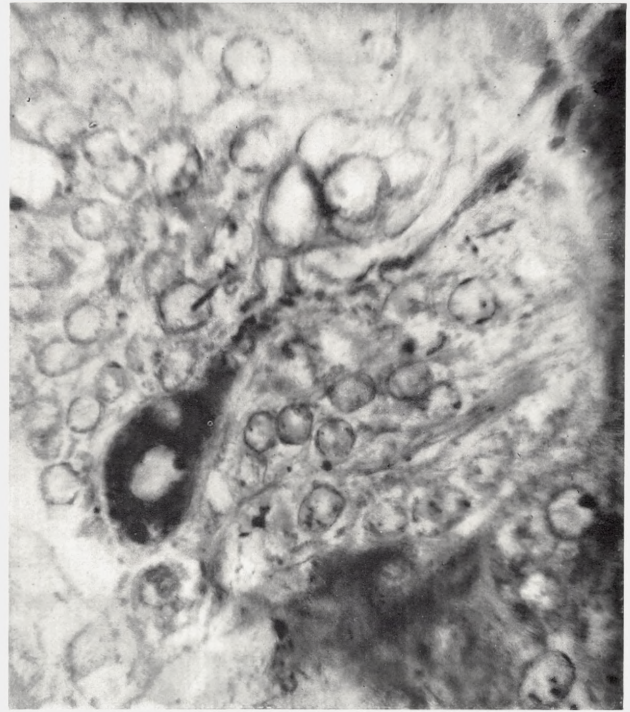
PLATE VIII

Tabanus sp.

- Fig 24. Sagittal section of hemisphere near median plane (front of brain to the left). Arrows indicate the dorsal (internal) and ventral (external) neurosecretory cells. — Flemming; Heidenhain. Photo. 200 \times .
Fig. 25. The ventral neurosecretory cell from fig. 24 at a higher magnification. — Photo. 900 \times .
Fig. 26. Drawing of the same cell at a still higher magnification. Secretory granules in cell and axon, vacuoles in cell body. Note also the "thread-vacuoles" in three of the brain cells next to the neurosecretory cell. — Flemming; Heidenhain. 1500 \times .



24



25

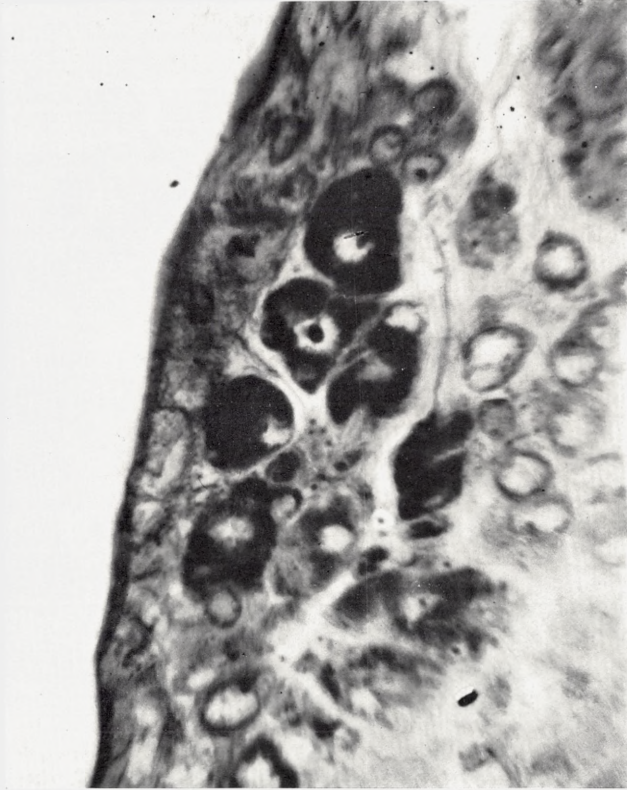


26

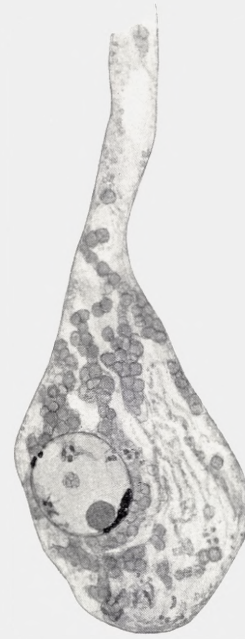
PLATE IX

Tabanus sp.

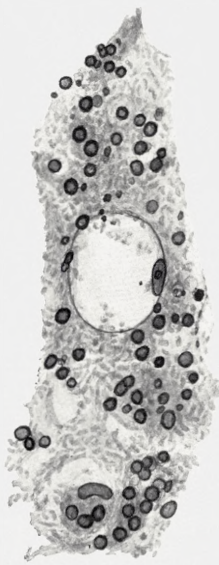
- Fig. 27. Part of anterior surface of hemisphere with dorsal group of neurosecretory cells (blackish). — Flemming; Heidenhain. Photo. 900 \times .
- Fig. 28. Neurosecretory cell from ventral (external) group. Secretory granules stained red. — Alcoholic Bouin; Masson. 1650 \times .
- Fig. 29. Neurosecretory cell from ventral (external) group. Secretory granules stained blue. — S. Felice; Masson. 2400 \times .
- Fig. 30. Neurosecretory cells from dorsal (internal) group. *mi* dense collection of mitochondria; *rg* part of cell with diffuse red colour. — Alcoholic Bouin; Masson. 1650 \times .



27



28



29



30

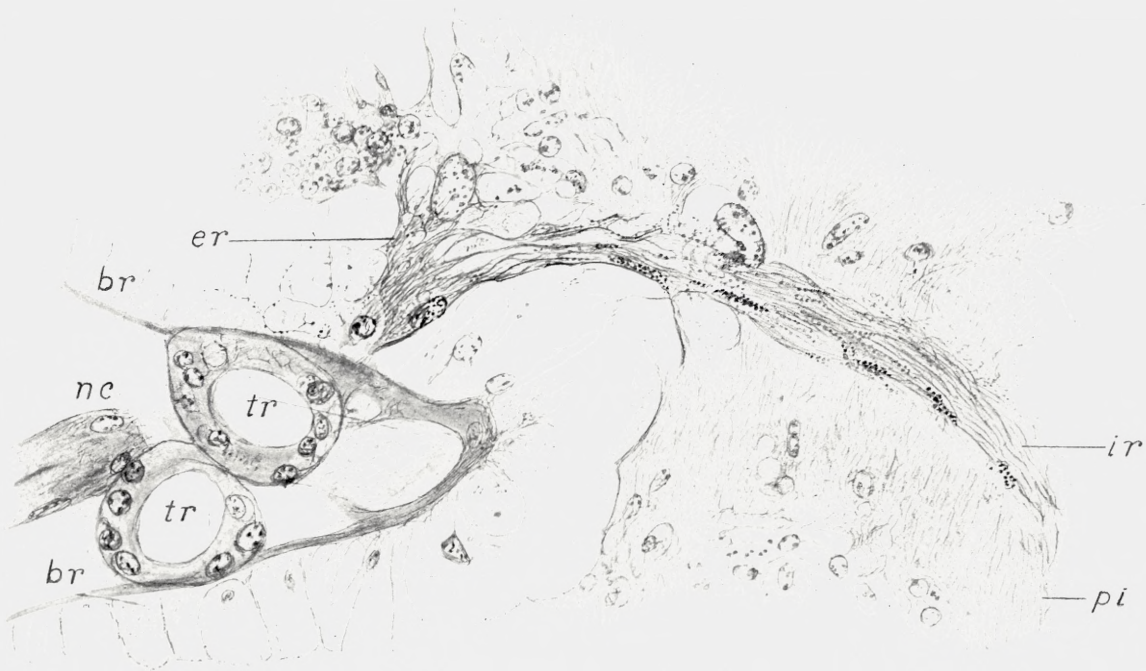
PLATE X

Tabanus sp.

- Fig. 31. Neurosecretory cells from dorsal (internal) group with granular and chaplet-like Golgi bodies. — Champy, osmic acid (10 days); unstained. 1650 ×.
- Fig. 32. Horizontal section through the pars intercerebralis of the brain. *br* posterior surface of the two hemispheres; *er* external root of nervus corporis cardiaci (only partly visible); *ir* internal root of the same nerve with several collections of granules (stained red in the section); *nc* part of nervus corporis cardiaci behind the brain (in a neighbouring section connected with the roots); *pi* commissural nerve fibres in pars intercerebralis; *tr* tracheae. — Alcoholic Bouin; Masson. 500 ×.



31

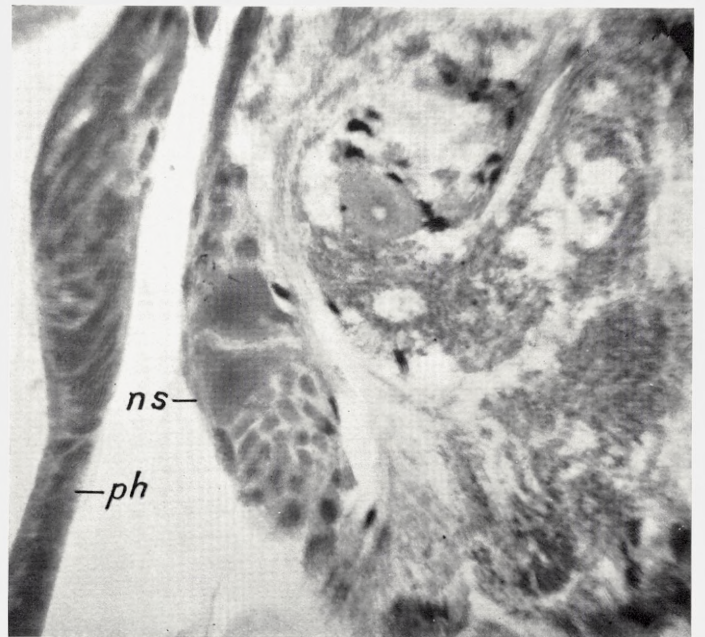
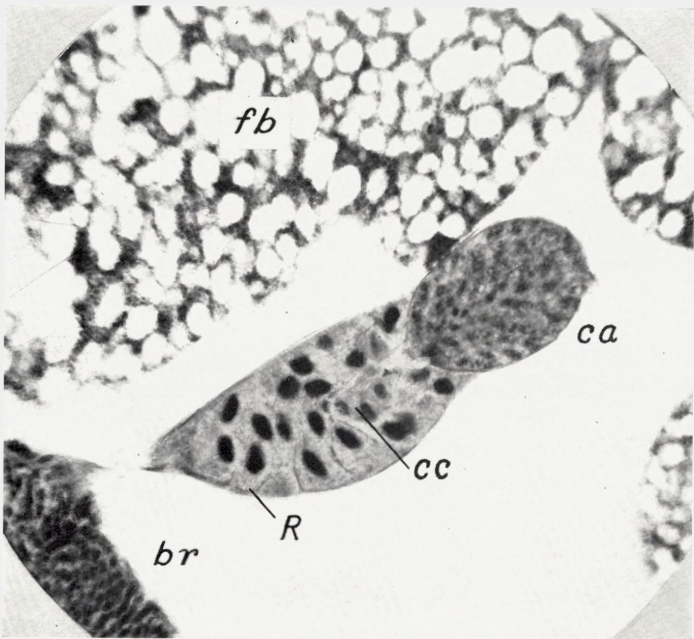
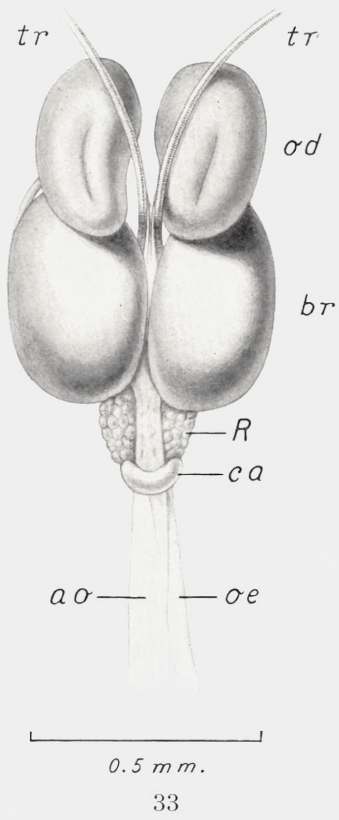


32

PLATE XI

Thereva sp.

- Fig. 33. Ring-gland and brain etc. of last stage larva, dorsal view. *ao* aorta; *br* brain; *ca* c. allatum; *od* optic disc; *oe* oesophagus; *tr* tracheae.
- Fig. 34. Horizontal section through ring-gland and adjacent organs. *ao* aorta; *br* brain; *ca* c. allatum; *fb* fat-body; *R* R-cells in lateral parts of ring-gland. — Alcoholic Bouin; Masson-Foot. 300 ×.
- Fig. 35. Sagittal section through lateral part of ring-gland. *br* brain; *ca* c. allatum; *cc* c. cardiacum; *fb* fat-body; *R* R-cells. — Alcoholic Bouin; Masson-Foot. 300 ×.
- Fig. 36. Sagittal, paramedian section through foremost portion of left hemisphere with two neurosecretory cells (*ns*) lying at the surface; *ph* posterior wall of optic disc. — Alcoholic Bouin; Masson-Foot. 400 ×.



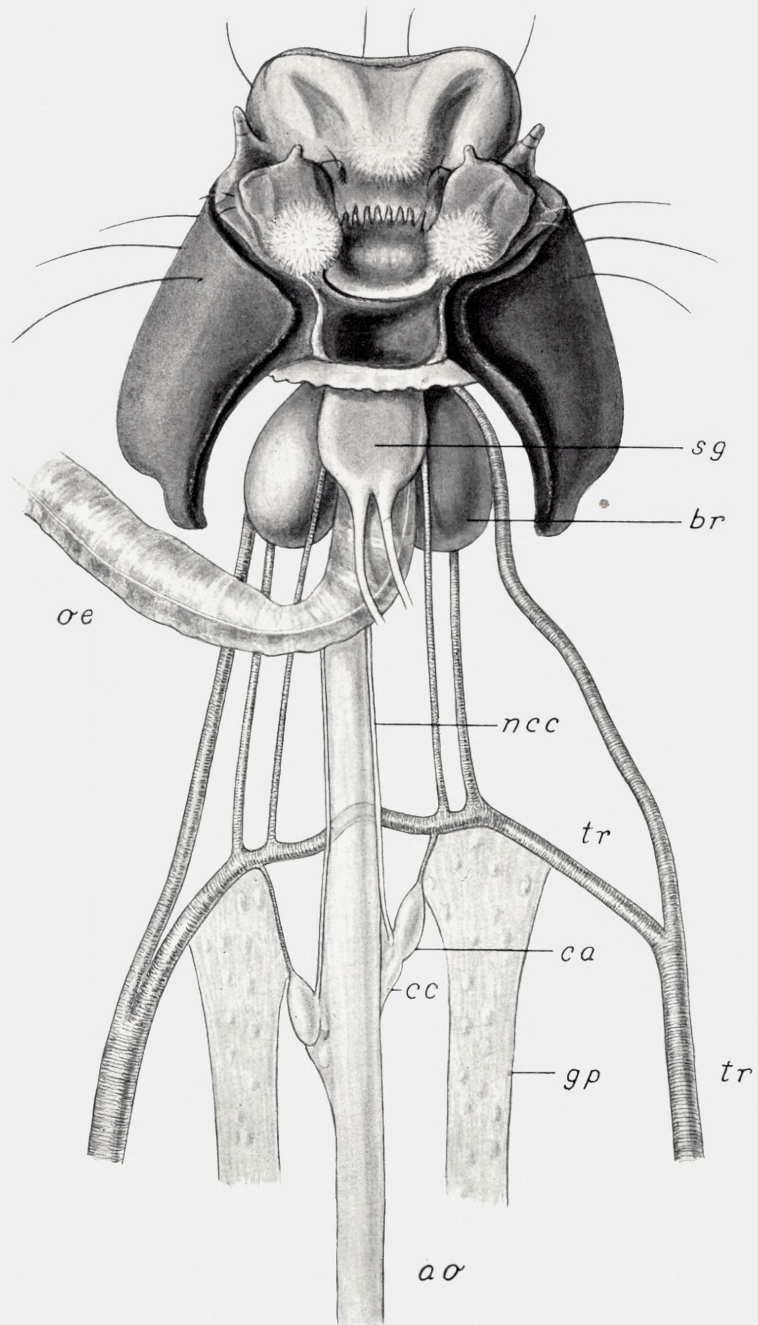
35

36

PLATE XII

Ptychoptera sp.

Fig. 37. Ventral view of head capsule and adhering organs. *ao* aorta; *br* brain (the line points to the left hemisphere); *ca* c. allatum; *cc* c. cardiacum; *gp* "peritracheal gland" (R-cells); *ncc* nervus corporis cardiaci; *oe* oesophagus (artificially bent); *sg* suboesophageal ganglion; *tr* tracheae.

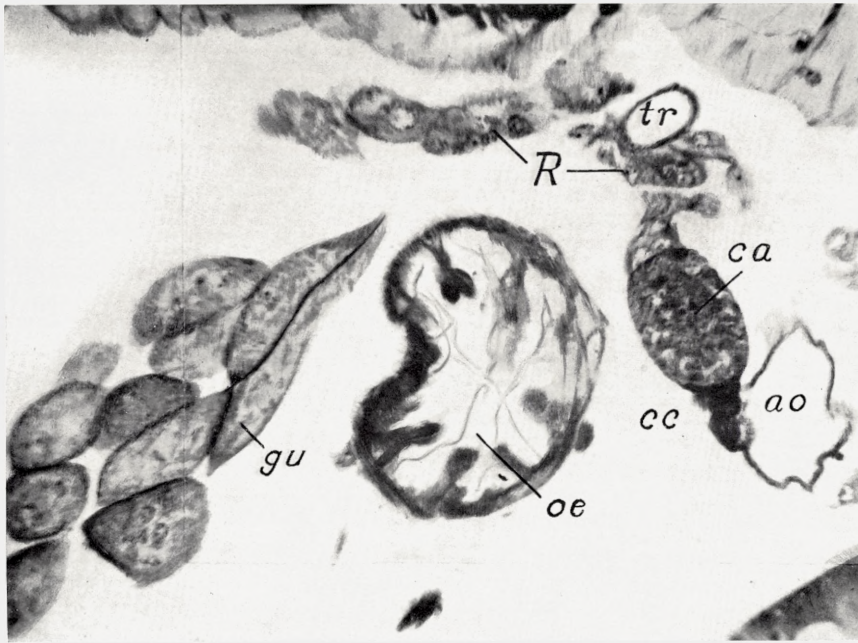


0.5 mm.

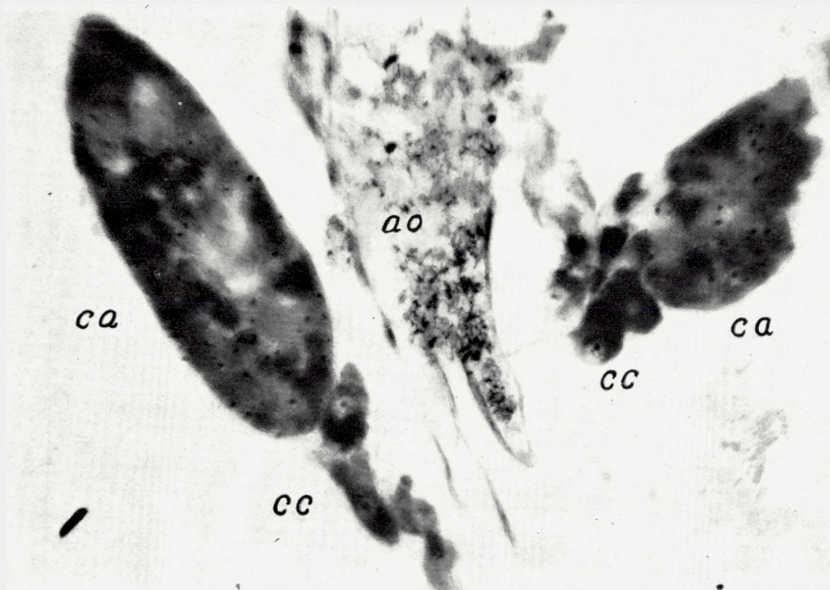
PLATE XIII

Ptychoptera sp.

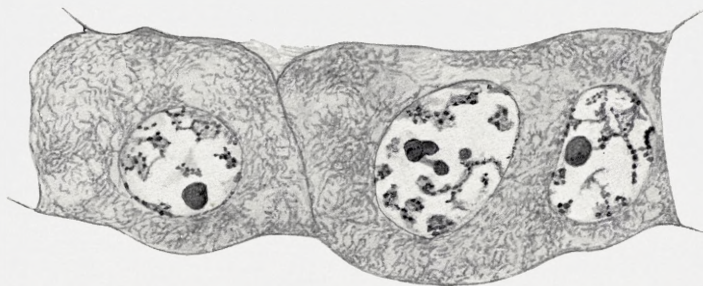
- Fig. 38. Oblique section through endocrine glands and surrounding organs. *ao* aorta; *ca* c. allatum; *cc* c. cardiacum; *gu* garland cells; *oe* oesophagus; *R* R-cells of "peritracheal gland"; *tr* trachea. — Fleming; Heidenhain. 200 ×.
- Fig. 39. Horizontal section through endocrine glands. Letters as in fig. 38. — Champy; Heidenhain. 500 ×.
- Fig. 40. R-cells of "peritracheal gland". Note spiralized chromosomes and mitochondria in the cytoplasm (cp. plate I, fig. 3). 1200 ×.



38



39

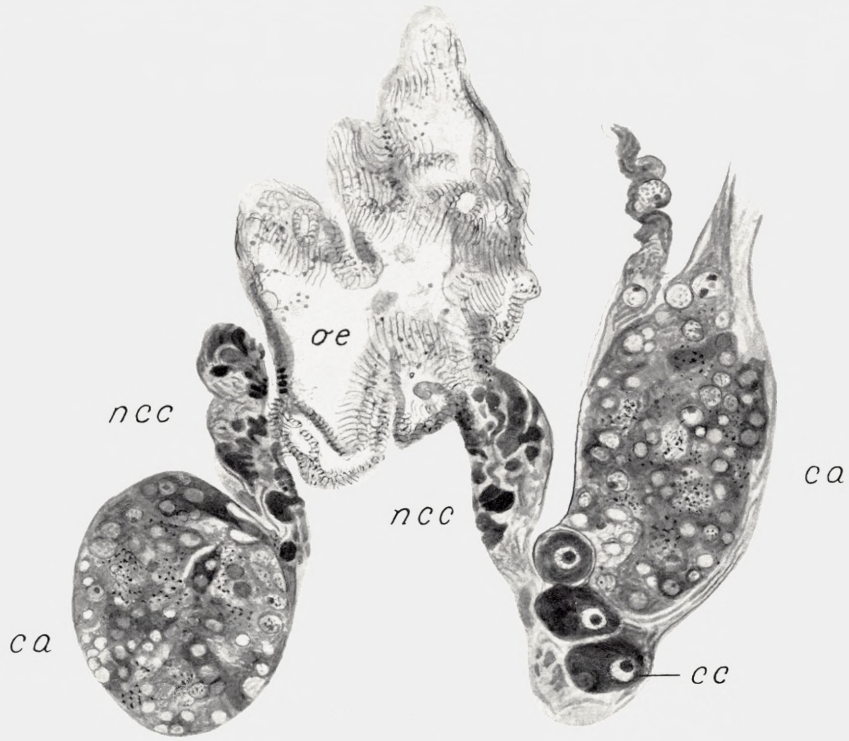


40

PLATE XIV

Ptychoptera sp.

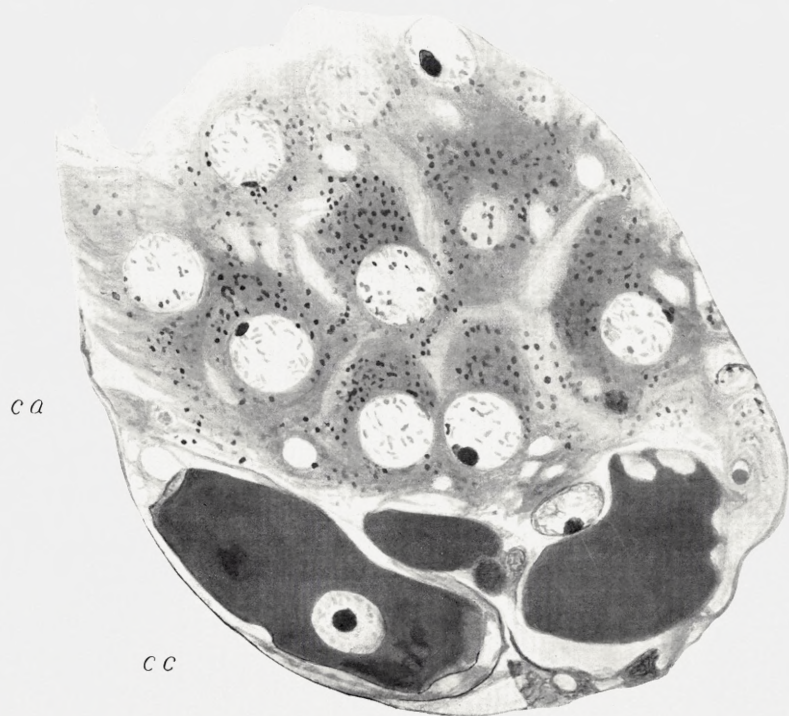
- Fig. 41. Nearly horizontal section through endocrine glands. *ca* *c. allatum*; *cc* *c. cardiacum*; *ncc* *nervus corporis cardiaci*; *oe* *oesophagus*. Osmiophile granules in *c. allata*, intense blackening of *c. cardiacum* and adjacent part of cardiac nerve. — Champy, osmic acid (6 days); unstained. 450 ×.
- Fig. 42. Cells of the *c. cardiacum* (*cc*) with axon-like processes (*cp.* text); *ca.* part of *c. allatum*. — Champy, osmic acid (6 days); Heidenhain. 1000 ×.
- Fig. 43. Section through *c. allatum* (*ca*) with osmiophile granules, and *c. cardiacum* (*cc*); from another larva. — Same technique. 2000 ×.



41



42



43

Det Kongelige Danske Videnskabernes Selskab

Biologiske Skrifter

Dan. Biol. Skr.

Bind 2 (kr. 61.00)

kr. ø.

1. BÖCHER, TYGE W.: Beiträge zur Pflanzengeographie und Ökologie dänischer Vegetation. I. Über die Flechtenheiden und Dünen der Insel Läsö. 1941 4.00
2. SØRENSEN, THORVALD: Untersuchungen über die Therophytengesellschaften auf den isländischen Lehmf lächen (*»Flags«*). 1942 3.00
3. ORLA-JENSEN, S.: The Lactic Acid Bacteria. Die echten Milchsäurebakterien. Ergänzungsband. 1943 18.00
4. WESTERGAARD, M.: Cyto-Taxonomical Studies on *Calamagrostis Epigeios* (L.) Roth, *Ammophila Arenaria* (L.) Link, and their Hybrids (*Ammophila Baltica* (Flügge) Link). 1943 8.00
5. NIELSEN, K. BRÜNNICH: The Asteroids of the Senonian and Danian Deposits of Denmark. *Opus posthumum* edited by TH. MORTENSEN and ALFRED ROSENKRANTZ. 1943 9.50
6. ROSENINGE, L. KOLDERUP, and LUND, SØREN: The Marine Algæ of Denmark. Contributions to their Natural History. Vol. II. Phæophyceæ. II. Corynophlaeaceæ, Chordariaceæ, Acrothrichaceæ, Spermatochnaceæ, Sporochneæ, Desmarestiaceæ, Arthrocladiaceæ. With supplementary Comments on Elachistaceæ. 1943 6.00
7. BÖCHER, TYGE W.: Studies on the Plant Geography of the North-Atlantic Heath Formation. II. Danish Dwarf Shrub Communities in Relation to those of Northern Europe. 1943 12.50

Bind 3 (kr. 60.50)

1. OLSEN, SIGURD: Danish Charophyta. Chorological, Ecological and Biological Investigations. 1944 22.00
2. JESSEN, KNUD, and HELBÆK, HANS: Cereals in Great Britain and Ireland in Prehistoric and Early Historic Times. 1944 8.50
3. GRAM, K., JØRGENSEN, C. A., og KØIE, M.: De jyske Egekrat og deres Flora. 1944 26.00
4. ORLA-JENSEN, S., OLSEN, ERIK, and GEILL, TORBEN: Senility and Intestinal Flora. A Reexamination of Metchnikoff's Hypothesis. 1945 4.00

Bind 4 (kr. 72.00)

1. BÖCHER, TYGE W.: Beiträge zur Pflanzengeographie und Ökologie dänischer Vegetation. II. Über die Waldsaum- und Graskrautgesellschaften trockener und halbtrockener Böden der Insel Seeland mit besonderer Berücksichtigung der Strandabhänge und Strandebenen. 1945 20.00
2. SØRENSEN, THORVALD, and GUÐJÓNSSON, GUÐNI: Spontaneous Chromosome-Aberants in Apomictic Taraxaca. Morphological and Cyto-Genetical Investigations. 1946 10.00
3. BÖCHER, TYGE W., CHRISTENSEN, TYGE, and CHRISTIANSEN, M. SKYTTE: Slope and Dune Vegetation of North Jutland. I. Himmerland. 1946 12.00
4. BÖCHER, TYGE W.: *Dichothrix gelatinosa* sp. n. Its Structure and Resting Organs. 1946 2.00

	kr. ø.
5. ROSENVINGE, L. KOLDERUP, and LUND, SØREN: The Marine Algæ of Denmark. Contributions to their Natural History. Vol. II. Phæophyceæ. III. Encoeliaceæ, Myriotrichiaceæ, Giraudiaceæ, Striariaceæ, Dictyosiphonaceæ, Chordaceæ, and Laminariaceæ. 1947	14.00
6. RAVN, J. P. J.: Om Nyker-Omraadets Kridtfafler. 1946.....	4.00
7. KNISELY, MELVIN H., BLOCH, EDWARD H., and WARNER, LOUISE: Selective Phagocytosis. I. Microscopic Observations concerning the Regulation of the Blood Flow through the Liver and other Organs and the Mechanism and Rate of Phagocytic Removal of Particles from the Blood. 1948.....	10.00

Bind 5 (kr. 82.50)

1. NIELSEN, ANKER: Postembryonic Development and Biology of the Hydroptilidæ. A Contribution to the Phylogeny of the Caddis Flies and to the Question of the Origin of the Case-Building Instinct. 1948	30.00
2. JØRGENSEN, ERIK G.: Diatom Communities in Some Danish Lakes and Ponds. 1948.....	18.00
3. LEMCHE, HENNING: Northern and Arctic Tectibranch Gastropods. I. The Larval Shells. II. A Revision of the Cephalaspid Species. 1948	18.00
4. SØRENSEN, THORVALD: A Method of Establishing Groups of Equal Amplitude in Plant Sociology Based on Similarity of Species Content and its Application to Analyses of the Vegetation on Danish Commons. 1948.....	7.50
5. NILSSON, TAGE: On the Application of the Scanian Post-Glacial Zone System to Danish Pollen-Diagrams. 1948.....	9.00

Bind 6

(uafsluttet / en cours de publication)

1. ORLA-JENSEN, S., ORLA-JENSEN, ANNA D., and SNOG-KJÆR, AGNETE: Biological Researches on the Silage Process. 1948.....	2.00
2. LUND, SØREN: The Marine Algæ of Denmark. Contributions to their Natural History. Vol. II. Phæophyceæ. IV. Sphacelariaceæ, Cutleriaceæ, and Dictyotaceæ. 1950.	10.00
3. TUXEN, S. L.: Über den Lebenszyklus und die postembryonale Entwicklung zweier dänischer Protürengattungen. 1949	10.00
4. PAULSEN, OVE: Observations on Dinoflagellates. Edited by Jul. Grøntved. 1949.	9.00
5. THOMSEN, MATHIAS: Weismann's Ring and Related Organs in Larvae of Diptera. 1951.	15.00
6. NIELSEN, ANKER: Contributions to the Metamorphosis and Biology of the Genus <i>Atrichopogon</i> Kieffer (Diptera, Ceratopogonidæ). With Remarks on the Evolution and Taxonomy of the Genus. 1951	16.00
7. BÖCHER, TYGE W.: Cytological and Embryological Studies in the Amphipod <i>Arabis Holboellii</i> Complex. 1951.....	9.00

Bind 7

(uafsluttet / en cours de publication)

1. NYGAARD, GUNNAR: Hydrobiological Studies on some Danish Ponds and Lakes. Part II: The Quotient Hypothesis and some new or little known Phytoplankton Organisms. 1949	40.00
2. CHRISTENSEN, PAUL J. HOLST: Studien über die postembryonale Entwicklung bei <i>Cochlidion Limacodes</i> Hufn. (Fam. <i>Cochlididae</i> , <i>Lepidoptera</i>). 1950	10.00