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STUDIES ON THE NEUROSECRETORY ACTIVITY OF THE BRAIN IN THE FRESH WATER CRUSTACEAN, *ASTACUS LEPTODACTYLUS* ESCHSCHOLZ (DECAPODA)

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The study of neuroendocrin activity in invertebrates is restricted mainly to the phylum of Arthropodes. The study of the secretory activity of the central nervous system in the group of insects and crabs is going on more or less independently, but still parallelly. In insects the process of metamorphosis, in higher-developed crabs the ability of colour change have been those physiological problems, which lead sooner or later on both fields to the recognition of the importance of the hormone secretory activity in the central nervous system. Accordingly, we are in possession of a continually-increasing amount of partial information — more or less fitting together — regarding on the one hand in insects the relation between the brain, resp. the whole retrocerebral complex and the prothorax gland, on the other hand in Decapodes the X-organ and sinus gland located in the eyestalk and next the postcomissure organ. However, the general aspect, regarding both insects and crabs, is still incomplete.

The investigations concerning the neurosecretory activity of crustaceans are dealing mainly with Decapodes and in particular with lobsters, crabs and prawns. The study of their colour change, moulting (and recently some other physiological processes) has somewhat enlightened the role and importance of the eyestalk, postcomissure organ and pericardial organ (Enami 1951, Carlisle and Dohrn 1953, Carlisle 1953, Carlisle 1959).

In insects the first step was to clear the role of the brain in the neurosecretory system, in crustaceans it is the importance of the brain which seems to be the least clear. The role of the supraoesophageal ganglion of crabs looks all the more interesting, as the important secretory centres of the eyestalk are not only in direct anatomical and probably close physiological connection with the brain through the optic nerve, but the optic lobe of the cerebral ganglion itself has also been proceeded to the eyestalk during the phylogenesis (Bliss and Welsh 1952). Besides, it can be assumed that the brain has to be in some sense the centre of further ganglions of the central nervous system the latter having their secretory activity too. The histological picture of the brain and the physiological activity of brain-extracts indicate also an important and complex role.

Histological investigations of crabs belonging to the genus *Sesarma* have shown three types of neurosecretory cells in the eyestalk (X-organ) and in some ganglions of the nervous system (ENAMI 1951). All three types of cells can be found in these crabs in the supraoesophageal ganglions too. DURAND (1956) could discern four types of secretory cells in the corresponding nerve elements of crayfish *(Orconectes virilis)* but only two of these can be found in the brain as well.

During the tests on chromatophorotropic and moult-regulating hormones physiologically active substances have been extracted first of all from the eyestalk and postcomissure organ (CARLLE 1959) but also from the brain, those substances influence the activity of chromatophores (ENAMI 1951, FINGERMAN and LOWE 1958, FINGERMAN, LOWE and SUNDARARAJ 1959) and regulate by antagonistic way the moulting (CARLISLE 1951, CARLISLE and DOHRN 1951). The reciprocal testing of insect and Crustacea hormons has shown that the specificity of certain effecting substances has so wide limits that they exceed the frontiers of species, genus and classes and render it probable that within the phylum of Arthropodes, stuffs of identical, but at least similar, chemical structure are produced in certain elements of the neurosecretory system (GABE 1953, KARLSON 1956).

Other investigations refer to pterin-like, fluorescent substances said to be connected with gen-factors in *Drosophila melanogaster* (HADORN and MITCHELL 1951) and or at least part of which are produced in certain elements of the central nervous system, resp. in the neurosecretory cells (L'HÉLIAS 1955, GERSCH and UNGER 1957, KONOK 1958). As ascertained, the production of these substances of characteristic UV-fluorescence is in relation with certain physiological processes, like moulting and metamorphosis (KONOK 1958), on the other hand these substances have a defined physiological activity, as it can be proved by tests (GERSCH and UNGER 1957, UNGER 1957). VISCONTINI and his collaborators (1955) isolated in the course of their informatory investigations from the total extract of *Astacus fluviatilis* by means of paperchromatography and then identified spectrophotometrically certain pterin derivates, which proved to be partly similar to those already known from insects (L'HÉLIAS 1955, VISCONTINI, SCHMID and HADORN 1955).

The comparative studies (as yet limited in number) point to the outlines of interesting correlations requiring further experiments. In order to approach these correlations, we have begun our investigations on the role of the supraoesophageal ganglion of crustaceans in the neurosecretory system. In our present work we wish to get the reader acquainted with a part of our investigations on *Astacus leptodactylus*.

Material and methods

Adult specimens of *Astacus leptodactylus* larger than 120 mm were used for our experiments. The animals were collected in Lake Balaton at the end of February, resp. of August. The average depth of the Lake is of 3.5 m, its water freezes once or twice in winter for a longer period (January—February). The temperature of water has an average of 26—28 C° in August.

In August the crayfishes were collected from under the shore stones, the preparation followed only later in September, until then were kept in the laboratory at a temperature of 21 C°.

In the samples in February the specimens were collected at the period when they migrated from their wintering places (deep water) to shore immediately afrer the melting of the ice. At this time the temperature of water was about 4.5 C°.

1. Paperchromatography

The supraoesophageal ganglions of both the late winter and the summer—autumn individuals were tested by means of paperchromatography. The brains were ectomised by cutting through the root of rostrum, the rostrum was removed, then the head part of the carapace was opened in circle in the direction of the thorax, up to the suture; after cutting through the dorsoventral bundle of muscles the cover was removed. After cutting through the oesophagus and the hepatopancreas and removing the intestines, we thus freed the central nervous system from the supraoesophageal ganglion together with the circumoesophageal connectives up to the suboesophageal ganglion. We cut through the nerves under a binocular microscope, ectomised the brain and put it on SCHLEICHER—SCHÜLL 2043/b paper, resp. made the chromatogram from its alcoholic extract. In the first case we carefully crushed the material on the start point with a glass wand, then imbibated it into the paper with distilled water. At the same time we prepared brains, which were homogenized in 96% ethanol in POTTER-type homogenizator. We distilled by vacuum treatment the centrifugated extract and put it on paper afterwards. It was used as a solvent a mixture consisting of buthanol — concentrate acetic acid — water (4:1:5). The chromatograms were tested in UV-light, the controls with ninhydrin. The fluorescent materials were eluated with 0.5 n NaOH then the absorption spectrum was measured with BECKMAN spectrophotometer.

Histology

Parallelly with paperchromatographic analysis, we worked up brains histologically too.* The brain was removed by the same process and then differentiated with methylen-blue. A short alcoholic prefixing preceded the fixing for 24 hours in trichloracetic acidic BOUIN-solution, into which it was carefully moved. After the usual pretreatment, we embedded the material into paraffin and made sections in series. We stained the 5 μ sections by means of GOMORI's (1950) aldehyde-fuchsin method modificated by HALMI (1952).

Results

1. Paperchromatography

In the course of working up the material collected in August (both ways' brains put on to paper directly as well as on the chromatograms of ethanolic brain-extracts) we succeeded in discerning several substances showing distinct UV-fluorescence. The most characteristic of them was a substance fluorescenting in lilac ($R_f = 0.23$) which seemed to be identical with isoxanthopterin

* I wish to express my thanks to technical assistant Mr. KÁROLY FENYVESI for his valuable aid in preparing histological sections.

known from insect-chromatograms and also fluorescent in lilac. Parallel running has also proved the identity of the two substances.

As control, we developed the chromatograms also with ninhydrin, but there was no reaction, which means the absence of amino acids, peptids and

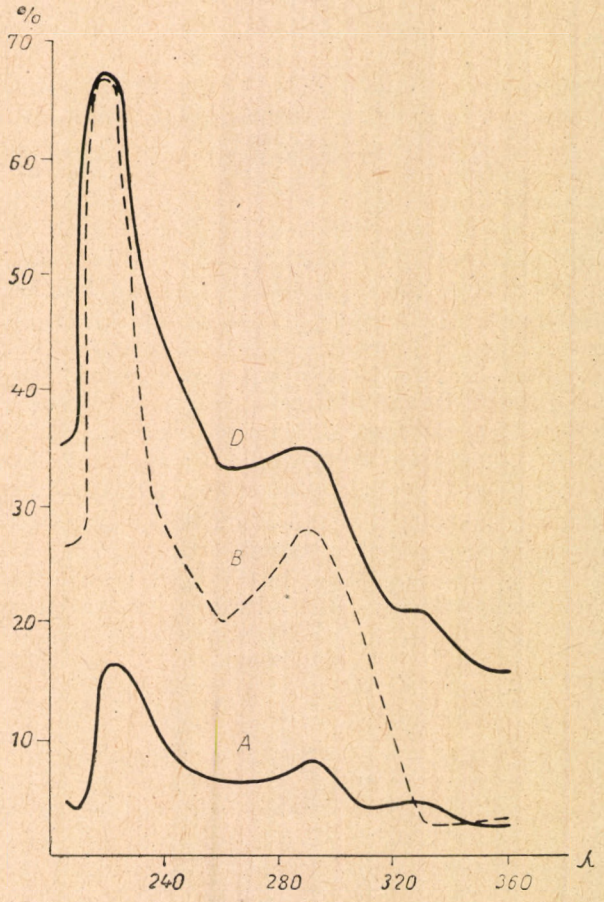

Fig. 1 Absorption spectrum of the lilac fluorescent substance (isoxanthopterin) isolated from insect and crayfish brain, in 0.5 n NaOH solution. Abscissa: wave lenghts (m μ), ordinate: absorption of light in the percentage of the total, direct quantity of light

A = *Astacus leptodactylus*, B = *Bombyx mori*, D = *Dixippus morosus*

1. ábra. Rovar és rák agyból izolált lila fluoreszcenciájú anyag (isoxanthopterin) abszorpciós spektruma 0,5 n NaOH oldatban. Abszcissza: hullámhossz (mμ), ordinata: fényabszorpció a ráeső teljes fénymennyiség %-ában.

A = *Astacus leptodactylus*, *B* = *Bombyx mori*, D = *Dixippus morosus*

proteins. We eluated the spot to identify the lilac fluorescent substance and determined its absorption spectrum. Compared to isoxanthopterin isolated from the brain and ganglions of different insects, the identity can be stated on base of absorption maximums *(Fig. 1)*. The investigations of further three

fluorescent substances (greenish-blue, yellowish-green and bluish-green) are going on, therefore we do not wish to go into details in this paper.

No substance showing UV-fluorescence could be extracted from the brain of crabs collected in late winter. Without exception all samples were negative.

2. Histology

When examining the section of crayfishes collected in August, it is very remarkable before all that —in comparison to circumstances known in insects— the quantity of cells in active phase is very high in the brain. Three different

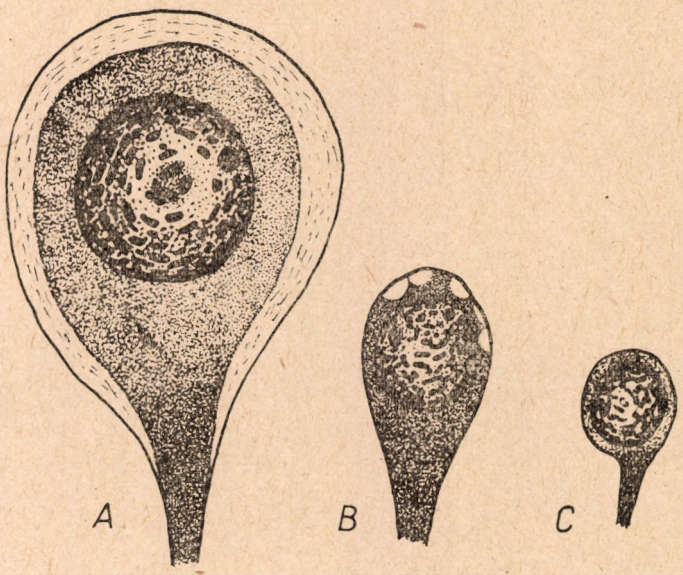

Fig. 2 Three different types of neurosecretory cells from the supraoesophageal ganglion of *Astacus leptodactylus* (natural proportions)

2. ábra. Három különböző típusú neuroszekretorikus sejt az *Astacus leptodactylus* supraoesophagealis ganglionjából (természetes arányok)

types of cells can be well-distinguished in the supraoesophageal ganglion of *Astacus leptodactylus (Fig. 2)*. In the specimens of summer and autumn, these cells were all in a very active phase, the migration of secrete is well-observable along the axon fibres.

Cells type A

Very large cells with a diameter varying between 60—120 (!) μ *(Figs. 2* and *3)*. Their plasm is homogeneous. In a more advanced secretory phase, vacuoles are sometimes formed on the edges of the plasm and secretory drops can be observed on the surface of the vacuoles. At the release of the secretory product, the stainable content is more or less regularly concentrated from the nuclear membrane inwards to the nucleus. Consequently, on the inner side of the nuclear membrane, a thimble-like chromophobe area is being formed. The

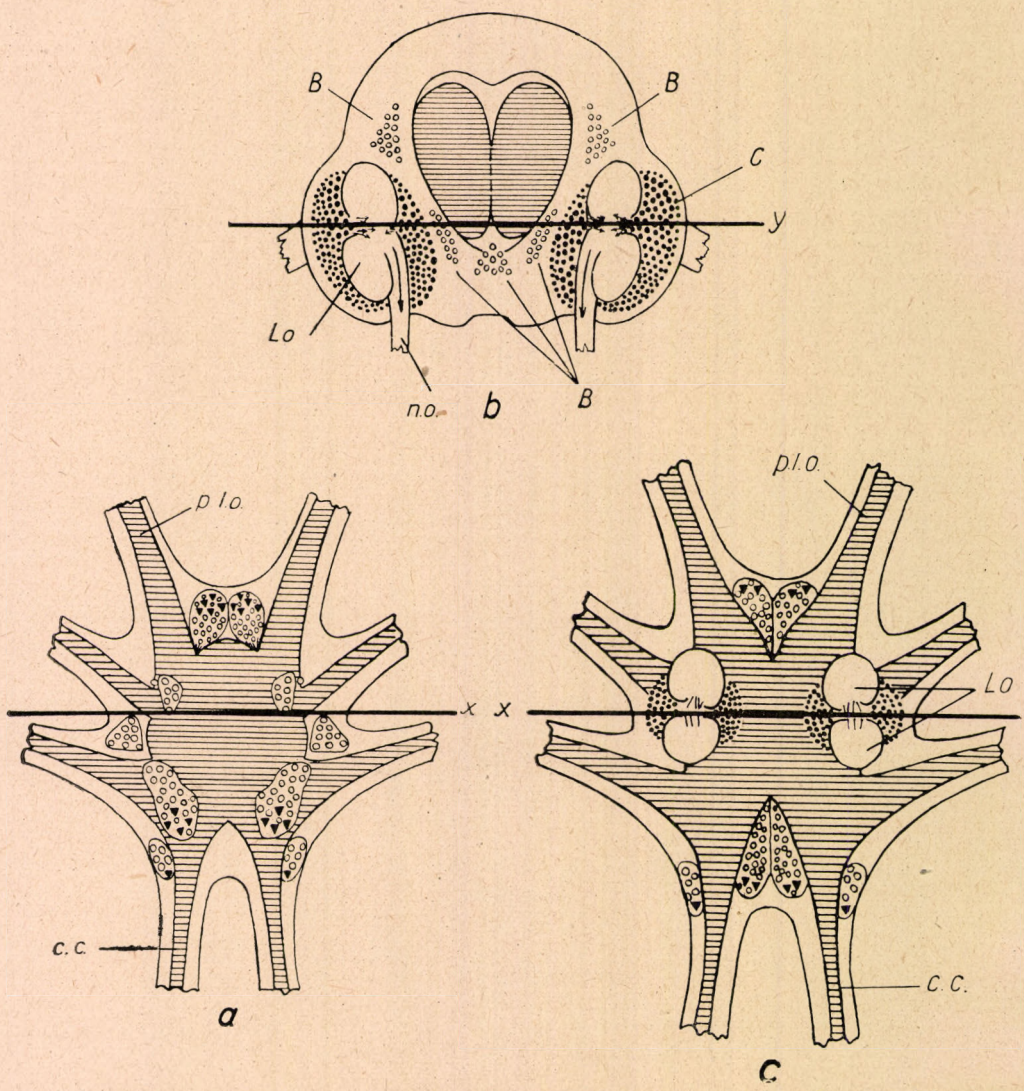

Fig. 7 Arrangement of neurosecretory cells in the brain of *Astacus leptodactylus*. *Fig. A :* upper sight of brain, *Fig. B :* transverse section of brain, *Fig. C :* picture of the brain's section in the plane marked in *Fig. B :* (B = B-type neurosecretory cells, C = C-type neurosecretory cells, C. c. = circumoesophageal connective, Lo olfactory lobe, n. o. = olfactory nerve, p. l. o. = peduncle lobi optici, × = straight line, Fig. B shows a section made in a plane vertical to it; *y* = straight line shows that vertical plane in which *Fig.* C figures the brain. ▼ = sign of A-type secretory cells, o = B-type secretory cells, ● = C-type secretory cells). Sizes of *a* and *c* are equal

7. ábra. A neuroszekretorikus sejtek elrendeződése az *Astacus leptodactylus* agyában. *a ábra :* az agy felülnézetben, *b ábra :* az agy kereszmetszetben, *c ábra :* az agy képe a *b ábrán* jelzett síkban készült metszetben. (B = B-típusú szekréciós sejtek, C = C-típusú szekréciós sejtek, C.c. = circumoesophageális connectivum, Lo = lobus olfactorius, n.o. = nervus olfactorius, p. l. o. = pedunculus lobi optici, x = egyenes, melyre merőleges síkban készült keresztmetszetet mutat be a *b ábra :* y = az egyenes jelzi azt a merőleges síkot, melyben a *c ábra* mutatja be az agyat. v = A-típusú szekréciós sejtek jelzése, 0 = B-típusú szekréciós sejtek, 0 = C-típusú szekréciós sejtek) *a* és *c* méretei azonosak.

size of the nucleus is varying between 28—38 μ. Generally a peripherically located nucleolus can be observed in the nucleus. These giant cells appear usually in a relatively limited number (14—16 pcs) associated to B-type cells on the frontal and caudal ends of the brain *(Fig. 7.)*

Cells type B

These cells are well-comparable partly to ENAMI's α-cells (1951), partly to DURAND Type 3 secretory cells. The measures of the cell body vary between 19 and 25 μ. Their plasm is homogeneously dispersed, full of fine granules. The aggregation of the granules is also observable but less frequently. The smaller vacuoles are formed on the inner side of the cell membrane and occasionally get into contact *(Fig. 6)* The release of the secretory substance from the cell can be well-noticed *(Fig. 4)*. The measure of the nucleus is 16—19 μ. Two nucleoli are well-observable in the nucleus, especially during the later secretory phase. These cells are to be found on a rather well-limited territory *(Fig. 7)*, forming a bundle, in rather large quantity (frontally about 80—100, caudally 50—60 and laterally also about 50—60). Naturally, the number of cells is only approximate, but, considering the measures of cells, attention was paid not to count a cell several times.

Cells type C

These are identical in every respect with the γ-cells described by ENAMI (1951) and with the DURAND (1956) Type 4 secretory cells *(Fig. 2)*. They occur in great number, about 600—700 are noticeable on defined territories of the brain, located bilaterally on the surface of the olfactory lobe *(Fig. 7)*. These cells are characterized by a great nucleus (13—15 μ) related to the plasm, the diameter of the cell body being only 18—20 μ. The plasm is fairly stainable and homogeneously dispersed granules can be noticed in it. The transport of secrete in the bundle of axon of the grape-like cell colony is well observable *(Fig. 5)*.

Some sections show clearly the sharply-outlined characteristic localization of B and C type cells just as well as the pathway of the bundle of axons, owing to the fact that the transported secretory substance is itself well-stainable. The passage of the secrete can be followed from the group of secretory cells partly inside the ganglion, partly through the nerves entering the ganglion *(Fig. 8)*.

The histological picture of the supraoesophageal ganglion from the material collected at the end of February shows that neurosecretory activity begun in these animals about this time, after the winter rest period. In fact, in some animals we found in inactive phase every cell of all three types *(Figs. 9, 10, 11)*. In other animals collected at the same time, the beginning of the neurosecretory activity can be recognized. From this point of wiew, the B-type neurosecretory cells occupy the first place, because in some specimens they could be demonstrated in a rather advanced secretory phase, what is more, their product appeared in the axon too.

In the A-type giant cells the secretory activity can only be observed in the beginning phase, the quantity of secrete being small. In the inactive phase the nucleus of cells, type A and B is stainable light-pink and homogeneously, only the nucleolus or nucleoli light up in vivid purpur. The plasm of these inactive cells is colourless *(Figs. 9 and 10)*.

In contrary to the above two cell types, no February animal showed the slightest sign of activity in C-type cells. Although these signs are not very remarkable in the summer period either, still in these animals — compared to those of August — the measures of the C-type cells, which are in the average the same, are smaller (cell body: 15—18 μ, nucleus: 12—13 μ; on the other hand, their plasm which in summer is hardly stainable is completely colourless similarly to those of the bundle of axon deriving from them which show no traces of secrete either *(Fig. 11)*.

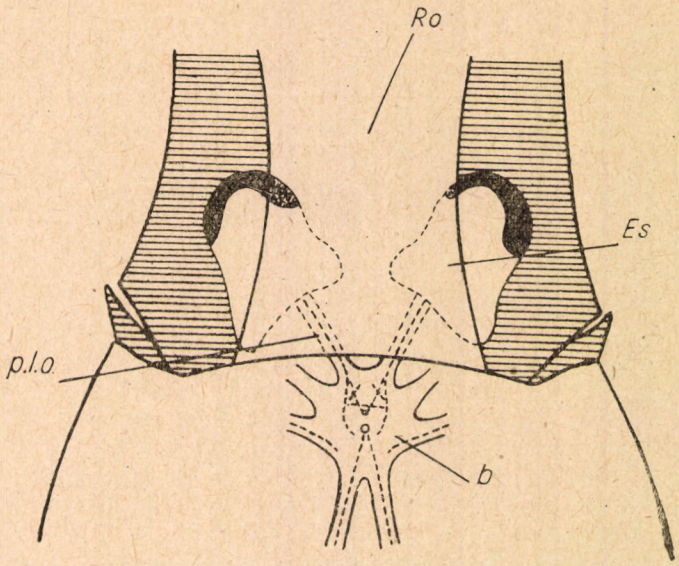

Fig. 8 The site of eyestalks and brain after removal of the carapace. Dotted lines show the path of secrete transport (b = brain, e. s. = eyestalk, p. l. o. = peduncle lobi optici, ro = rostrum).

8. ábra. A szemnyelek és az agy helyzete a carapax eltávolítása után. Az agyban a szaggatott vonalak jelzik a szekrétumtranszport útját. (b = agy, e.s. = szemnyél, p. l. o. = pedunculus lobi optici, ro = rostrum)

There was no difference in any type of cells between the secretory activity of males and females.

In the series of sections, in several sections, simmetrically located, two capillaries of 40 μ diameter could be noticed in a lenght of about 0.9 mm which extended into the circumoesophageal connective *(Fig.12)*. The stainable neurosecrete was well-observable in the small capillars. Naturally, the direction of migration of the secretory material could not be defined, but considering the foregoing, it seems very probable that the material flowing into the capillars, originates in the B-type neurosecretory cells.

Discussion

Although we used to distinguish certain types of cell of the supraoesophageal ganglion of *Astacus leptodactylus* by the letters of the A, B, C, they are not to be confounded, and can only partly be considered identical with those types

of neurosecretory cells which MATSUMOTO (1954) and PARAMESWARAN (1955) presented from the thoracic ganglion of *Eriocheir japonicus*, resp. *Paratelphusa hydrodromus* which they noted similarly.

The A-type cells reported in the present paper, though not identical, may yet in respect of some morphological characteristics — like the measure of cells and the small number of cells — be brought in connection with the giant B-type cells of ENAMI (1951) and with MATSUMOTO's A-type cells (1954), further with the Type 2 neurosecretory cells of DURAND (1956). Though ENAMI found this type of cell in the brain, in DURAND's opinion, these cells occur exclusively in the eyestalk (X-organ) and can never be found in the supraoesophageal ganglion. MATSUMOTO treats this type of cell only from the point of view of the thorax ganglion.

These A-type cells could be checked without any doubt in the winter and summer specimens of *Astacus leptodactylus*. They are to be found only in small quantity, 14—16 pcs altogether and especially on the frontal part of the brain *(Fig. 7)*. Their measure surpasses sometimes those given by ENAMI (1951) and DURAND (1956) even that of MATSUMOTO (1954), as we found in several cases cell diameters above 100 μ.

Considering both the morphological character and the anatomical localization *(Figs. 2* and *7)* of the B-type neurosecretory cells, these can be identical in all probability with ENAMI's (1951) α-cells, as well as with DURAND's (1956) Type 3 neurosecretory cells. Similarly, the C-type cells, regarding their morphological data, may be considered identical with the γ-cells proved by ENAMI (1951) in the supraoesophageal ganglion of certain *Sesarma* species, further with MATSUMOTO's (1954) D-type cells in the thorax ganglion of *Eriocheir japonicus* and with DURAND's (1956) Type 4 neurosecretory cells from the brain of *Orconectes virilis*. In relation to the described cells in other crabs mentioned above, the difference appears only in their anatomical sites *(Fig. 7)* and in the fact that in the active period, the plasm of cells can perceptibly be stained, and in the bundle of axon deriving from the cells the migration of secretory material can be well-observed.

Regarding MATSUMOTO's (1953) C-type cells, DURAND's (1956) supposition seems to be very probable that these cells described in the thorax ganglion are more corresponding to ENAMI's (1951) α-cells, than to β-cells, and this way they can be better made conform to DURAND's Type 3 cells and to B-type cells of *Astacus leptodactylus*.

Studying the diagram *(Fig. 7)* showing the situs of the different cell types, attention is called on the systematic distribution of each kind of cell. The neurosecretory cells mostly periferically seated and are grouped along the nerves which enter, resp. leave the brain. The C-type cells can be found completely isolated on the interior and exterior surface of the olfactory lobes, in grape-bunchlike groups. Their number is the greatest among all types of secretory cells, about 6—700 take place bilaterally on the surface of the olfactory lobes. The bundle of axons leaving the groups of cell are entering from inside and outside into the olfactory lobe. The secretory material is well-stainable in the axons, in the two lobes and in the fibres of the olfactory nerve during the period of high secretory activity in the summer—autumn exemplares *(Fig. 7)*.

The A- and B-type secretory cells appear partly together, but never get mingled. The largest group of B-type cells is seated on the frontal part of the

brain, on the territory which corresponds to the pars intercerebralis of insects. Beside these cells grouped simmetrically in two bunches, the A-type cells appear peripherically towards the eyestalks. About 6—8 giant cells can be checked beside about 80—100 B-type secretory cells *(Fig. 7)*.

The other large group of B-type cells is also seated peripherically and simmetrically with the longitudinal axis of the brain, in the triangle formed by the entering circumoesophageal connectives at the caudal end of the brain *(Fig. 7)*. Beside about 50—60 B-type cells — similarly to the frontal side — there appear some more 4—6 A-type cells. The root of the hindermost part of nerves leaving the brain is surrounded by a ring of B-type cells, associated to some giant cells. The grouping of B-type cells are to be found on the external side at the origin of the two proceeding connectives *(Fig. 7)*.

BLISS and WELSH (1952) observed in the crab *Gecarcinus lateralis* an axon-net like arrangement which could be distinctly recognised in the brain of *Astacus* too. Particular interest should be paid to the two little capillars proceeding from the circumoesophageal connective and containing stainable secretory substances they are probably even collective giant axons *(Fig. 12)*.

The axon-fibre bundles transporting secretory material, entering bilaterally and parallelly into the two peduncles lobi optici could be well-distinguished. Although the path of axons transporting secrete could not be quite cleared in the supraoesophageal ganglion of *Astacus*, in this case the scheme of BLISS and WELSH (1952) seems to be insufficient. The transversal path of axon bundles transporting secrete could also be observed, e.g. between the two olfactory lobes *(Fig. 8)*.

Examining the neurosecretory centres of the brain from the physiological point of wiew, it has to be taken into consideration that these neurosecretory cells may apparently be responsible — amongst others — for the production of hormones playing role in moulting and on the other hand for those of chromatophorotropic characteristics. CARLISLE and DOHRN (1953), further CARLISLE (1953) in the course of their investigations on extracts obtained from the brain of *Lismata seticaudata* arrived at the conclusion that the production of a moult-accelerating factor may be assumed in the supraoesophageal ganglion. This hormon is presumably the antagonist of the moult-inhibiting factor described by DURAND (1956) in the case of *Orconectes virilis*, in the Type 2 neurosecretory cells of the X-organ of eyestalk. If a connection can be established between DURAND's Type 2 neurosecretory cells and the A-type cells of *Astacus*, this moult-accelerating factor may be produced in the brain too.

At the same time we have to suppose on base of ENAMI's (1951) investigations on *Sesarma* species the presence of two factors of chromatophorotropic character roted by him as S-and N-hormone, as well as the presence of certain principle acting on chromatophors, according to FINGERMAN and his collaborators (1958, 1959), both in the cerebral ganglion.

The comparison of the histological picture of the secretory conditions of winter and summer as well as the summing up of the results of the parallelly performed paperchromatographic analysis practically permits to say only as much about the physiology of *Astacus* that during the wintering-period every neurosecretory activity stops. Neurosecretory activity will then gradually begin at the end of February. First the B-type cells begin their activity, then the A-type giant cells and at last the C-type secretory cells. The factor fluorescent in lilac, identified as isoxanthoperin, may be brought probably into con-

nection with the C-type cells, owing to the fact that in contrary to the samples of August, they do not yet appear in the material of February. That is the only type of cell which showed no activity in any specimen of February. It follows from the above that the isoxanthopterin ought to be produced in the C-type cells of the supraoesophageal ganglion, on the surface of the olfactory lobes. Relative to its physiological role and importance, we may partly refer to ENAMI's (1951) investigations, which bring into contact the origin of the chromatophorotropic factor signed N-hormone with the γ-cells (C-type neurosecretory cells of *Astacus)*. On the other hand, CARLISLE's and DOHRN's (1953) experiments are of interest, because the absorption spectrum of a moult-accelerating factor isolated from the brain and made by BECKMAN-type spectrophotometer in the UV-spectrum shows close resemblance to the isoxanthopterin isolated from the brain of *Astacus*. At the same time, we have to take into consideration the investigations of L'HÉLIAS (1955), GERSCH and UNGER (1957) resp.UNGER (1957), which pretend that this same factor (isoxanthopterin) acts as chromatophorotropic hormone in insects. On base of the above-said, it does not seem impossible that the isoxanthopterin may be identified with one or the other of the chromatophorotropic hormones tested by ENAMI or FINGERMAN.

These hypotheses are not yet sufficiently well-founded. The role and correlation of these hormones has to be cleared up by further experiments. In the interest of the possibility of a higher rank synthesis, the investigations have to be converged to the recognition of the basical correlations between structure and function. With other words, harmony has to be created between histological and physiological data and at the same time, these experiments have to attain biochemical level. Doubtlessly, there are some efforts being made, but it is absolutely necessary to complete the data in our possession.

Summary

On base of histological investigations and paperchromatographic analysis of the supraoesophageal ganglion of the fresh water crab *Astacus leptodactylus* ESCHSCHOLZ, the following could be concluded:

1. Three different types of neurosecretory cells (A, B, C) are located in majority peripherically and grouped around the roots of nerves originating in the brain, in definite number and arrangement.

2. Compared to the investigations of ENAMI, MATSUMOTO and DURAND, except the A-type giant cells, the other two types of neurosecretory cells are identifiable with ENAMI's α-, resp. with DURAND Type 3 secretory cells, on the other hand with ENAMI γ- and DURAND Type 4 neurosecretory cells resp. with MATSUMOTO's D-cells.

3. The A-type giant cells can be compared in certain relation to ENAMI's giant B-cells, resp. the DURAND Type 2 neurosecretory cells.

4. The C-type cells are to be found in great quantity (about 6—800 pcs laterally located only on the surface of the olfactory lobes). These axons proceed into the olfactory lobes.

5. Great neurosecretory activity can be observed in all three types of cells in the cerebral ganglions of summer and autumn samples.. On the contrary, in the winter, end February samples the C-type cells are in a completely

inactive state. In the other two types of cell secretory activity is just beginning about this time.

6. Inside the brain, the transport of neurosecrete in the axon bundles can be well-analysed. We observed the migration of the secretory material through the peduncle lobi optici. We could further observe two little canals (supposed to be giant axons) proceeding from the circumoesophageal ganglion to the brain, in which the transport of neurosecrete was demonstrable.

7. During the summer—autumm active secretory period, among others a lilac fluorescent compound could be isolated from the brain by means of paper chromatography. On base of its R_f value and its absorption spectrum measured in the UV-spectrum and other characteristics, it proved to be identical with the isoxanthopterin isolated from the brain of insects.

8. To clear the role the isoxanthopterin is supposed to have, we made comparisons partly with the conditions found in insects, partly in connection with the neurohormones of chromatophorotropic character which are produced in the brain of crabs and which control moulting.

9. Isoxanthopterin could not be demonstrated in the brain of winter specimens. Owing to this fact, the point of its production can only be brought into correlation with C-type cells which are at this time inactive.

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VIZSGÁLATOK A KECSKERÁK, *ASTACUS LEPTODACTYLUS ESCHSCHOLZ* (DECAPODA) AGYÁNAK NEUROSZEKRETORIKUS TEVÉKENYSÉGÉVEL KAPCSOLATBAN

Konok István

Összefoglalás

A Balatonból augusztus végén és február végén gyűjtött rákok supraoesophageális ganglionjában vizsgáltuk a szekréciós aktivitás hisztológiai képét. A hisztológiai vizsgálatokkal párhuzamosan papírkromatográfiás úton is analizáltuk az állatok cerebrális ganglionjait.

Az *Astacus leptodactylus* agyában háromféle (A, B, C) szekréciós sejttípust tudtunk megkülönböztetni. Ezeknek a sejteknek csak egy része (B, C) egyeztethető össze más szerzőknek édesvízi- (Enami 1951) és tengeri tarisznyarákok (Matsumoto 1954) illetve egy amerikai folyami rák (Durand 1956) szemnyeléből és különböző idegelemeiből kimutatott szekréciós sejtjeivel.

Az egyes sejtféleségek meghatározott eloszlásban, többnyire szőlőfürt-szerű csoportokban helyezkednek el az agy felszínén, illetőleg a C-típusú sejtek a lobus olfactorius felületén. Az A- és B-típusú sejtek csoportjai az agyból kiinduló idegek, továbbá a pedunculus lobi optici és a circumoesophagealis connectivum tövénél helyezkednek el.

Az A-típusú óriás-sejtek 60—120 μ átmérőjűek, magvuk 28—38 μ nagyságú. Mindig B-típusú sejtek csoportjának szegélyén figyelhetők meg. Összesen mindössze 14—16 található belőlük az agyban. A B-típusú szekréciós sejtek jóval nagyobb számban (mintegy 200 darab) több, élesen elhatárolt csokorszerű csoportban jelennek meg, szimmetrikus elrendeződésben. A B-típusú sejtek nagysága 19—25 μ között váltakozik, sejtmagvuk mérete 16—19 μ. A C-típusú, viszonylag nagy magvú (13—15 μ) sejtek (sejtméret 18—20 μ) szőlőfürt-szerű tömege (6—800 darab) speciálisan a lobus olfactorius falára tapad laterálisan. Az egyes sejtféleségekre jellemző továbbá a nucleolusok száma és a vacuolarizáció folyamata.

A nyár—őszi példányok agyában igen aktív neuroszekretórikus tevékenységet figyeltünk meg mindhárom sejttípus esetében. Az egyes sejtcsoportokból kiinduló axonnyalábokban jól megfigyelhető a szekrétum áramlása. Követhető a szekréciós anyag vándorlása egyrészt a cerebrális ganglionon belül, másrészt pedig az agyból való kililletve beáramlása a pedunculus lobi optici-n, a circumoesophagealis connectívumokon és az egyes idegpárokon keresztül.

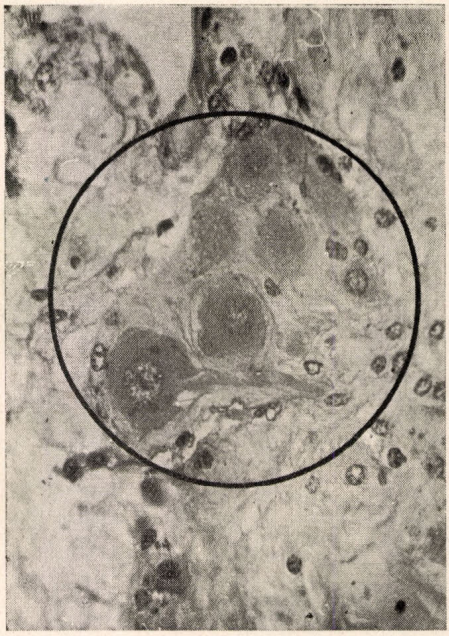
A februárvégi gyűjtésből származó anyagban, egyes példányok esetében még egyetlen sejttípusban sem volt megfigyelhető szekréciós tevékenység. Más példányokban viszont az A- és B-típusú sejtekben már kimutatható a szekréciós aktivitás megindulása. C-típusú sejtekben viszont ebben az időszakban még egy állatban sem észleltük a szekréciós tevékenység bármiféle jelét is. Úgy látszik tehát, hogy a téli nyugalmi periódus után elsőként a B-típusú, majd az A-típusú óriás-sejtek kezdik meg hatóanyagtermelésüket, végül pedig a C-sejtek aktiválódnak.

A nyári, aktív szekréciós periódus idején, papírkromatográfiás úton izolálni lehetett az agyakból — többek között — egy lila színben fluoreszkáló vegyületet is. Ez az anyag R_f-értéke, fluoreszcencia-színe, UV-tartományban mért abszorpciós spektruma és más jellemzői alapján azonosítható volt rovarok agyából izolált isoxanthopterinnel.

Vizsgálatainkat a rovarokra vonatkozó irodalmi adatokkal (L'HÉLIAS 1955, GERSCH és UNGER 1957, UNGER 1957, KONOK 1958), a rovarokkal és rákokkal foglalkozó összehasonlító vizsgálatokkal (GABE 1953, KARLSON 1956), továbbá a rákok kromatofór- és vedlésszabályozó hormonjaival kapcsolatos fiziológiai ismeretekkel összevetve, az isoxanthopterin valószínűleg azonosítható lesz majd további vizsgálatokban az egyik kromatofórokra ható trop-hormonnal, illetve az egyik vedlés-szabályozásban szerepet játszó hatóanyaggal.

Az isoxanthopterint a téli példányok cerebralis ganglionjában nem sikerült megtalálni. Ez a tény egyrészt ugyancsak a fenti meggondolásokra utal, másrészt pedig azt teszi valószínűvé, hogy az ezidőben egyedül inaktív C-típusú sejtekben termelődik.

Plate 1 — 1. tábla



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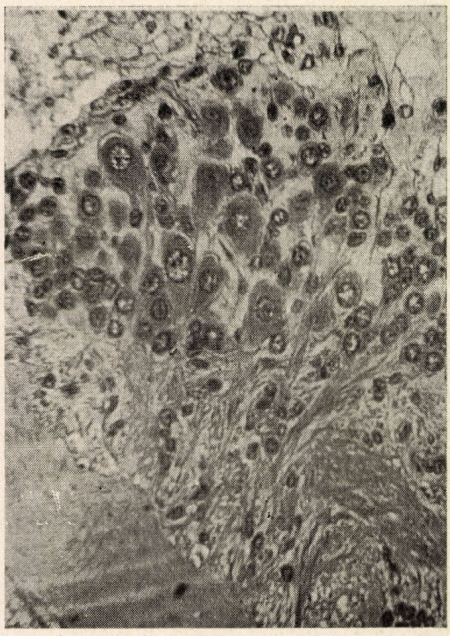

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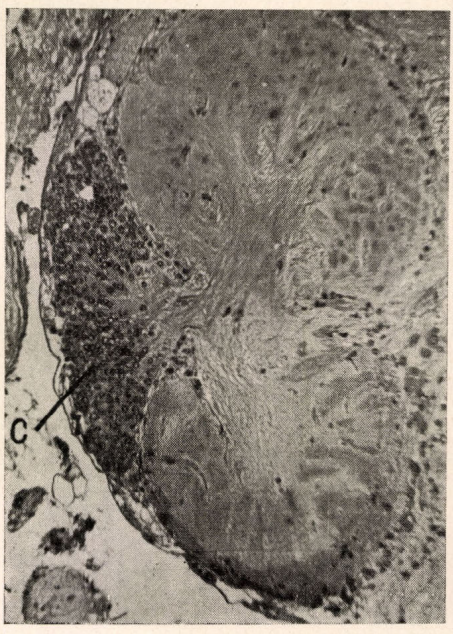

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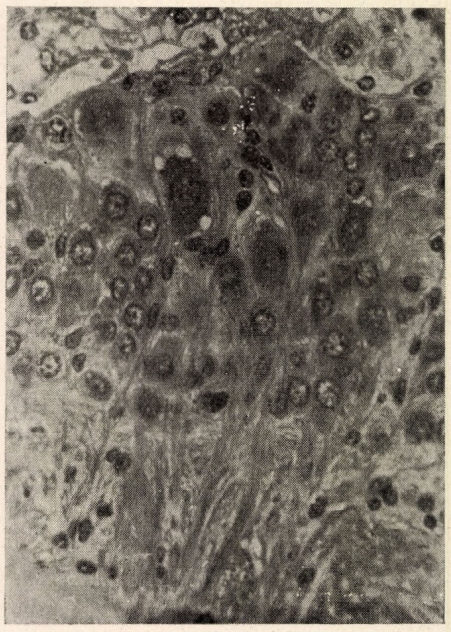

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Fig. 3. Group of active A-type giant neurosecretory cells of the brain of summer—autumn *(Astacus)* specimen. Fixed in trichloracetic acidic BOUIN, stained with HALMI's aldehyde-fuchsin method. (200 ×)

3. ábra. Aktív A-típusú óriás neuroszekréciós sejtek csoportja nyár-őszi példány *(Astacus)* agyában. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (200×)

Fig. 4. Active B-type neurosecretory cells from August-exemplars *(Astacus)* from the frontal brain part (pars intercerebralis). The axons originating in these can well be seen. Fixed in trichloracetic acidic BOUIN, stained with HALMI's aldehyde-fuchsin method. (250×)

4. ábra. Aktív B-típusú szekréciós sejtek augusztusi állat (Astacus) agyának frontális területéről (pars intercerebralis). Jól látszik az axonok lefutása. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (250×)

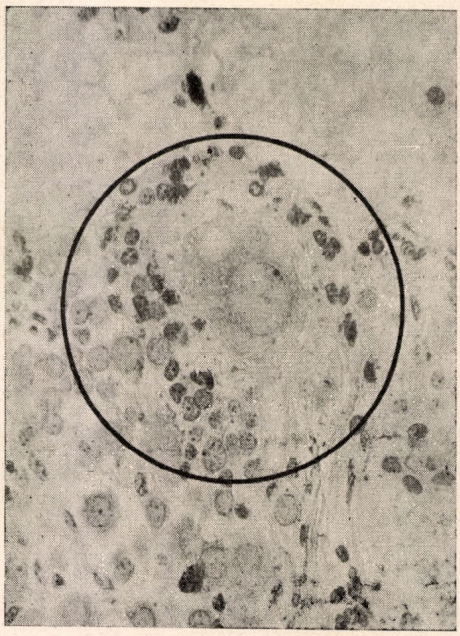
Fig. 5. Active C-type neurosecretory cells from the brain of summer—autumn *Astacus* The proceeding of axons into the olfactory lobe can be distinctly remarked. Fixed in trichloracetic acidic BOUIN, stained with HALMI's aldehyde fuchsin method. (70×)

5. ábra Aktív C-típusú szekréciós sejtek a lobus olfactorius felületén nyár-őszi peldány *(Astacus)* agyában. Az axonok behúzódása a lobus olfactoriusba jól megfigyelhető. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (70×)

Fig. 6. Vacuoles in B-type neurosecretory active cells, from the frontal part of the brain *(Astacus)*. Fixed in trichloracetic acidic BOUIN, stained with aldehyde-fuchsin according to HALMI's method. (270×)

6. ábra. Vacuolumok B-típusú aktív szekréciós sejtekben az agy frontális területéről *(Astacus)*. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (270×)

Plate 2 — 2. tábla



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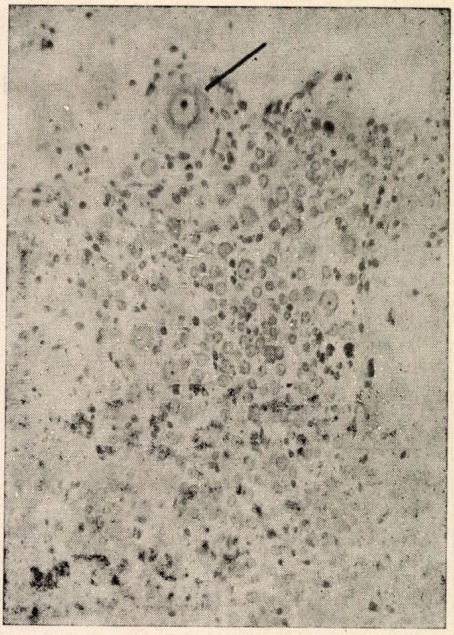

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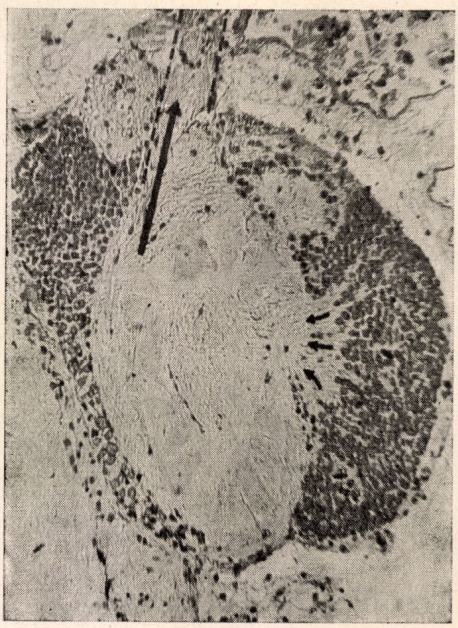

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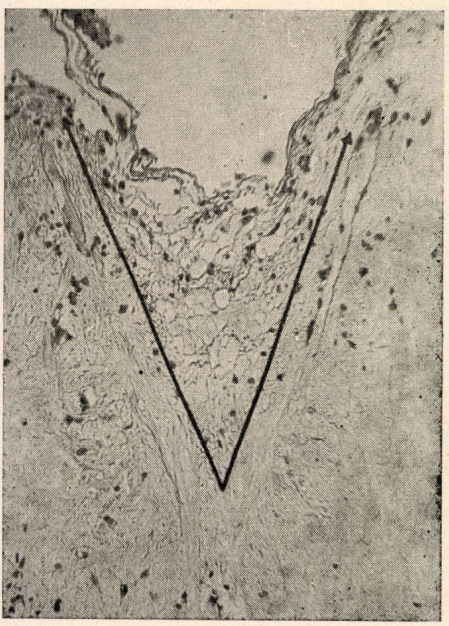

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Fig. 9. A-type secretory cells of the frontal part of the brain from end February material *(Astacus)*. The lightly stainable homogeneous nucleus with a nucleolus, the nonstainable plasm and the proceeding axon can well be seen. Fixed in trichloracetic acidic BOUIN, stained with aldehyde-fuchsin according to HALMI's method. (250×)

9. ábra. A-típusú neuroszekretorikus sejt februárban gyűjtött *Astacus* agyának frontális területén. Jól megfigyelhető a halványan színeződő homogén mag egy nucleolusszal, a nem festődő plazma és a kiinduló axon. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (250×)

Fig. 10. B-type secretory cells from February material *(Astacus)*. The great quantity of cells as well as the plasm and nucleus characteristic for the inactive phase can be distinctly noticed. There is an A-type cell on the edge of the picture. Fixed in trichloracetic acidic BOUIN, stained with aldehyde-fuchsin according to HALMI's method. (130×)

10. ábra. B-típusú szekréciós sejtek februári anyagból *(Astacus)*. Jól látszik a sejtek nagy száma, továbbá az inaktív fázisra jellemző mag és plazma. A kép szélén egy A-típusú sejt látható. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehydfuchsin festés (130×)

Fig. 11. Inactive secretory cells on the surface of the olfactory lobe (February *Astacus)*. The arrow shows the direction of axons. The bundle of fibres completed with the dotted line is the olfactory nerve. Transverse section. Fixed in trichloracetic acidic BOUIN, stained with aldehyde-fuchsin according to HALMI'sme thod. (70×)

11. ábra. Inaktív C-típusú szekréciós sejtek a lobus olfactorius felületén (februári *Astacus)*. A nyíl mutatja az axonok irányát. A szaggatott vonallal kiegészített rostköteg a nervus olfactorius. Keresztmetszet. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (70×)

Fig. 12. Collective giant axons conducting into the circumoesophageal connectives in the brain (February *Astacus)*. Fixed in trichloracetic acidic BOUIN, stained with aldehyde-fuchsin according to HALMI's method. (70×)

12. ábra. A circumoesophagealis connectivumokba átvezető kollektív óriásaxonok februári példány *(Astacus)* agyában. Triklórecetsavas BOUIN fixálás és HALMI-féle aldehyd-fuchsin festés (70×)