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STUDIES ON THE NEUROENDOCRINE ACTIVITY IN THE CEN RAL NERVOUS SYSTEM OF NEWLY HATCHED CRAYFISHES (*ASTACUS LEPTODACTYLUS* ESCHZ.) RELATED TO THE LIGHT ADAPTATION

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As has been established by previous studies performed on *Astacus* (KONOK 1961, 1962) a very significant role may be attributed generally to the neuroendocrine integration system in the reflex processes responsible for the adaptation of the organism to its environment, especially in the case of invertebrates. As it is evidenced by former investigations and the findings of several authors (WATERMAN 1961), this holds true especially in connection with light, one of the most important ecological factors.

The time in which neuroendocrine activity begins in the course of ontogeny, the modes of its changes, and its role are interesting and important problems worth to be investigated from the point of view of both ecology and physiology.

The investigations reported in this paper were designed to examine that phase of ontogeny in which the independent life of the individual animals starts. Hatched from eggs the young crayfishes are living still for a while under the protection of the female (clutching at the pleopods, and leaving them only occasionally), nevertheless, they are already exposed directly to various environmental effects, as light for instance, which induce active reflexes in them. All these considerations necessarily support the assumption that there may exist in the neuroendocrine system a certain degree of activity, which is primarily responsible for the existence and co-ordination of processes of adaptive character.

Material and methods

108 young animals (*Astacus leptodactylus* ESCHZ.) hatched in aquarium originating from one female were used in these investigations. The one day old youngs were separated from the pleopods of the female. One group of them was placed in an illuminated (1000 Lux) basin, another group in a darkened. After 48 hours the two groups were interchanged. Samples were taken for control from the two groups at the end of this 48 hours long exposure to constant light conditions. After the interchange of animals, samples were taken from the two groups (from both basins) at every thirty minute during a period of two hours.

The position of pigments in the chromatophores of all prepared animals was defined at first, directly under microscope. The observation did not involve any special difficulty in the case of these translucent animals, as compared with the more adult specimens possessing a shell strongly incrustated (KONOK 1961). After registering the state of the chomatophores, the whole animals was fixed in Bouin solution (instead of acetic acid trichloracetic acid was used). Then the material was embedded into celloidin-paraffin and sections were made in series. The sections were stained by aldehyde-fuchsin method (GOMORY, 1950) modified by HALMI (1952).*

Results

Chromatophores

Chromatophores characteristic of the genus *Astacus* containing red and white carotenoids as well as blue (astaxanthin) pigments are already present in the otherwise translucent integument of young crayfishes. In comparison to adult individuals the number of xanthophores is considerably less than that of erythrophores. Erythrophores resp. the red as well as the blue pigment are found in relatively large number in the integument. An adaptive movement of pigments depending upon changes in light conditions is observable similar to that found in case of adult animals. The arrangement of pigments produced in response to illumination is characterized by the dispersion of red white pigments and the simultaneous concentration of astaxanthin pigments. On the effect of darkness the reverse process takes place (KONOK 1961).

These changes occur unambiguously and simultaneously in every chromatophore located on the whole surface of the body, within 100—120 minutes subsequent to changes taking place in light conditions *(Fig. 1)*.

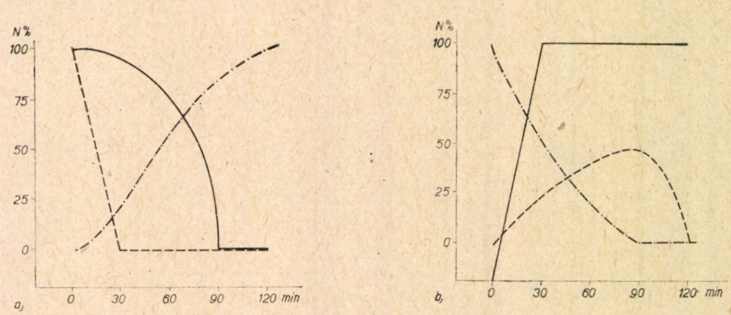

Fig. 1 Adaptive movement of pigments in the chromatophores in light (A) and dark (B) adapted conditions. Ordinate: the expansion of pigments in terms of percentage of maximum expansion (approximative values), abscisse: time. ———— = red, ------ = white, — · — · — · — = blue pigments

1 ábra. Pigmentek adaptációs mozgása a kromatofórokban fényhez (A) és sötétséghez (B) való adaptáció esetében. Ordináta: a pigmentek expanziója a maximális szétterültség %-ában (megközelítő értékek), abszcissza: az adaptáció időbeni lefolyása. ———— = piros, ------- = fehér, — · — · — · — = kék pigmentek

* I should like to express here my thanks to technical assistants Mrs. Brigitta Szabó and Miss Etel Szabó for their valuable assistancy in the microtechnical work.

Eyestalk

In the ocelli of young crayfishes the movement of proximal retinal pigments in response to illumination occurs in the same way as has been previously reported in the case of adult specimens (Konok 1962), namely in darkadapted state the pigments are located below the basal membrane, in response to light, however, they migrate above it. Under reversed conditions there is a movement of pigments in the opposite direction. In comparison to adult animals some differences exist in the time required for the adaptation of proximal retinal pigments, namely in case of young specimens the light-adapted state sets in sooner (within 20—40 minutes), whereas the adaptation to darkness needs a longer period (90—120 min).

Similarly there are differences between young and adult specimens in the anatomical properties of the eyestalk, for instance in the direction of the position of the single medullae, further in the morphologically well defined

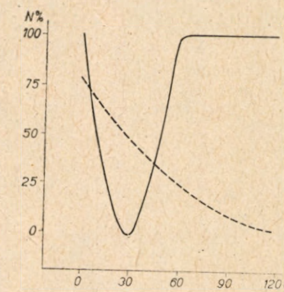

Fig. 3. Quantity changes in granular cells in terms of percentage of maximal value. ———— = adaptation to darkness, -------- = adaptation to light

3. ábra Granulociták mennyiségének változása a maximális érték %-ában. ———— = sötétséghez való adaptáció, -------- = fényhez való adaptáció

appearance of the so called „sensory pore" (rudimentary eye-papilla) in the eyestalks of young animals *(Fig. 2)*. In the case of young crayfishes the common axis of medullae is at 50—60° angles to the axis of eyestalk, whereas in adult animals the direction of the two axes is nearly parallel.

The sinus gland is well developed, and axon terminals in large number are located dispersely between the medulla externa and medulla terminalis on the inner sides of eyestalks. The axon terminals constituting the sinus gland are coloured lilac-red in both dark- and light-treated animals, if they are kept at constant conditions for 48 hours. Nevertheless, after 30—60 minutes subsequent to the change in light conditions this colouring changes blueish-lilac.

In the eyestalk of permanently illuminated animals the absolute quantity of granular cells is high. Upon darkening their number falls almost to zero during the first 30 minutes, and subsequently rises again suddenly to a high value. Conversely, in animals kept in darkness, the amount of granular cells decreases to minimum from a good average value within about 100 minutes in response to illumination *(Fig. 3)*.

In comparison to more adult specimens the medulla terminalis X organ is even more undeveloped in young crayfishes, and consists of one group of secretory cells located on the external, lateral surface of the medulla terminalis. Not only the absolute quantity of secretory cells is lower, but even giant

A type cells are absent (KONOK 1960). The X organ does not display secretory activity. The existence of sensory pore X organ (KNOWLES and CARLISLE 1956) is not demonstrable.

Central nervous system

The central nervous system of young specimens of *Astacus leptodactylus* still shows anatomical and micromorphological differences in comparison to adult animals. These differences are obviously characteristic of an earlier stage of development.

The fusion of originally six pair ganglia, constituting the subesophageal ganglion is still not complete, resp. the connectives between the other five pair

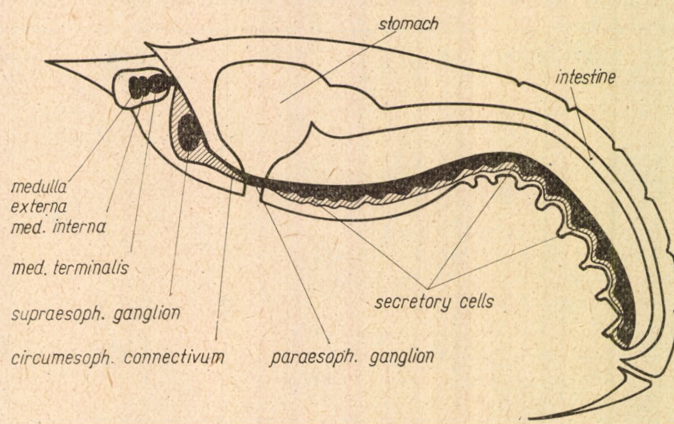

Fig. 5. The central nervous system of three days old *Astacus leptodactylus* (lateral view). Secretory cells are marked by stripes

5. ábra. 3 napos *Astacus leptodactylus* központi idegrendszere (oldalnézet). A csíkozott rész szekréciós sejtállomány

of ganglion in the cephalothorax and the six pair ganglia located in the single segments of abdomen are still so short that there is an almost direct contact between the adjoining ganglia. Nevertheless, the single ganglia are well developed but, due to the insufficient length of the connectives, they are accumulated in such an extent that the single ganglia are covered by a continuous, common ,,envelope'' of secretory cells *(Fig. 4)*. The secretory cells are located peripherally on the ventral side of nerve cord *(Fig. 5)*.

Paraesophageal ganglia are located laterally on both sides of the esophagus on each of the two connectives combining the supraesophageal and subesophageal ganglia. Not only motoric neurons but also a great number of secretory celles *(Fig. 6)* are present in these paraesophageal ganglia, in opposition to similar organs reported in other groups of crustaceans (CARLISLE and KNOWLES 1959).

A large number of secretory cells is to be found in the central nervous system even if their absolute number is considered. The cell types marked as A, B and C by KONOK (1960) are all identifiable. Morphological signs of very strong secretory activity are found in both A and B cells. Separated units of secretory cell groups are observable in the ventral nerve cord. The transport of secretory substances in the axons is also observable. No unambiguous relationships were demonstrable either in this case between the functional phases of cells and the single phases of the adaptation to illumination (KONOK 1962).

Discussion

The young specimens of *Astacus leptodactylus* newly hatched from eggs already respond normally to various light conditions, although they still live under the protection of the female, clutching at the pleopods under its abdomen, and leaving their abode chiefly only in night hours, in the daytime, however, only under the cover of stones and other objects. Even carotenoid pigments characteristic of adult animals are already present in the integument in large amounts. The number of xanthophores in the integument is still small, that of the erythrophores, however, is great. The pigmentmosaic patterns of adaptive character produced in this phase of development are obviously due to the increased light-sensitivity of the crayfishes having still completely translucent unincrustated integument.

The central nervous system is still in an early phase of development. The neuroendocrine activity—obviously related not only to the regulation of colour adaptation—is also still incomplete. Whereas, namely, there are secretory neurons of increased activity to be found in great number in the whole central nervous system, the medulla terminalis X organ in the eyestalk is not yet completely developed, and shows morphological signs of inactivity. This observation directly leads to the conclusion that no importance should be attributed to X organ in the activation of chromatophores.

The morphological picture supports the supposition that the paraesophageal ganglia represent a new source of secretory substances. This observation necessitates further studies not only in the case of *Astacus leptodactylus*. Namely, on basis of these considerations, another function and importance should be attributed to these organs as contrasted with some paraesophageal ganglia described earlier in some other crustanceans (CARLISLE and KNOWLES 1959) since they do not contain exclusively motoric neurons but also secretory ones.

Summary

Evaluating macro- and micromorphologically the experiments concerning the light adaptation in newly hatched crayfishes the followings were established as regards their neuroendocrine activity.

1. There are already all types of chromatophores resp. all kinds of pigments with the exception of xanthophores present in great quantity in the integument. The number of xanthophores is still small both in absolute and in relative sense.

2. The light-adaptive arrangement of pigments takes place in a way similar to that observed in adult animals. The accommodating migration of the proximal retinal pigments of ocelli equals also to that observed in adults.

3. In comparison with adult animals there are morphological differences regarding the direction of the position of single medullae in the eye-stalk.

The number of secretory neurons present in the medulla terminalis X organ is still low, there are no A type cells present, and no activity is observable.

4. Changes in the relative quantity of granular cells in relation to the single phases of adaptation are also observable.

5. The central nervous system is not yet completely developed morphologically. Indicative of that is the incomplete fusion of the six pair ganglia forming the subesophageal ganglion, further the fact that the other ganglion pairs of the nerve cord are still in direct contact with each other—due to their size, resp. to the shortness of their connectives.

6. A continuous common secretory cell ,,envelope'' covers ventrally not only the fused ganglia but also those connected with each other.

7. One paraesophageal ganglion is present on each connective running along on both sides of the esophagus. The basic difference between these ganglia and similar organs described by other authors in other groups of crustaceans is that they do not contain only motoric neurons but a considerable part of the small sized ganglia is constituted of secretory neurons.

8. The secretory cells present in large number in the brain and in the ganglia of the nerve cord are in the phase of a strong activity. The existence of such a strong neuroendocrine activity is suggested also by observations made on both the hormone transport and the morphological picture of sinus gland.

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A NEUROENDOKRÍN AKTIVITÁS VIZSGÁLATA A FÉNYADAPTÁCIÓVAL KAPCSOLATBAN, FRISSEN KELT KECSKERÁK (*ASTACUS LEPTODACTYLUS* ESCHSCHOLZ) IVADÉKOK KÖZPONTI IDEGRENDSZERÉBEN

Konok István

Összefoglalás

Egynapos *Astacus leptodactylus* rákivadékokkal végzett fénykísérletek makro- és mikromorfológiai értékelése alapján a szerző a következő megállapításokat tette.

Valamennyi kromatofór típus, illetve pigmentféleség — kivéve a xanthophorokat — már nagy mennyiségben megtalálható az integumentumban. Xanthophorok még — abszolút és viszonylagos értelemben egyaránt — kis számban figyelhetők meg. A kromatofórok, illetőleg pigmentek fényadaptatív átrendeződése a felnőtt állatokéhoz hasonlóan megy végbe. Az ocellusok proximális retina pigmentjeinek akkomodációs vándorlása ugyancsak megegyezik a kifejlett kecskerákok esetében megfigyeltekkel.

Kifejlett állatokkal összehasonlítva, a szemnyélben morfológiai eltérések figyelhetők meg az egyes medullák egymáshoz való orientációját illetően. A medulla terminalis X szerv még kevés szekréciós neuront, A típusú sejtet pedig egyáltalán nem tartalmaz és aktivitást nem mutat. A granulociták relatív mennyiségi változása az adaptáció egyes fázisaival összefüggésben, fiatal rákoknál is megfigyelhető.

A központi idegrendszer morfológiailag még nem alakult ki véglegesen. Részben a suboesophagealis ganglionban nem teljes még a 6 pár ganglion összeolvadása, másrészt a hasdúclánc további ganglion párjai — nagyságuk, illetve az őket összekötő konnektívumok rövidsége következtében — tömörülve, egymással közvetlen összeköttetésben helyezkednek el egymás után. Az összeolvadó, de az egymással összeköttetésben álló további ganglionokat is ventrálisan összefüggő, közös szekréciós neuron állomány borítja.

A nyelőcső két oldalán futó konnektívumokon laterálisan egy-egy paraoesophagealis ganglion található. Ezek a ganglionok más szerzők által más rákcsoportoknál leírt, hasonló elnevezésű képletektől lényegesen különböznek abban, hogy nem csupán motoros neuronokat tartalmaznak, hanem a kis méretű ganglionok nagy részét szekréciós neuronállomány alkotja.

Az agyban és a hasdúclánc ganglionjaiban nagy mennyiségben található szekréciós sejtek aktív szekréciós fázisban vannak. A hormontranszport és a szinuszmirigy morfológiai képe alapján is erős neuroendokrín aktivitás állapítható meg.

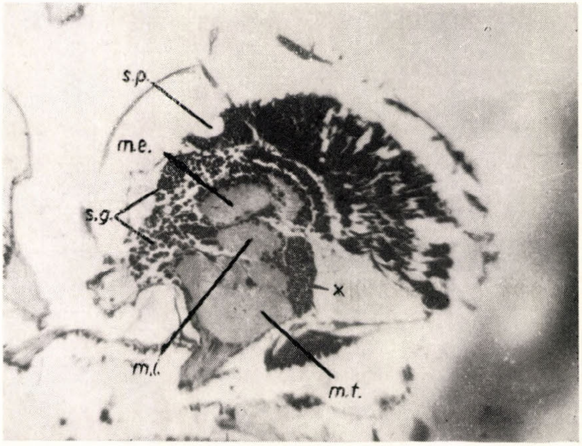

Fig. 2. Eyestalk of three days old *Astacus leptodactylus* (horizontal section). m.e. = medulla externa, m.i. = medulla interna, m.t. = medulla terminalis, s.g. = sinus gland, s.p. = sensory pore, X = medulla terminalis X organ

2. ábra. 3 napos *Astacus leptodactylus* szemnyele (horizontális metszet). m. e. = medulla externa, m. i. = medulla interna, m. t. = medulla terminalis, s. m. = szinusz mirigy, s. p. = sensory pore, X = medulla terminalis X szerv

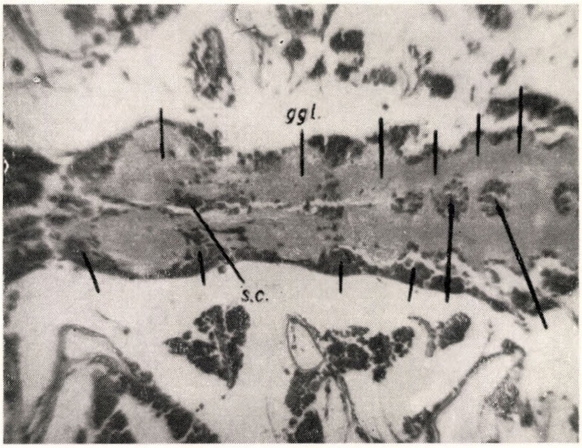

Fig. 4. Continuous secretory cell „envelope" in the ventral nerve cord (3 days old *Astacus leptodactylus*). ggl. = ganglia, s.c. = secretory cells

4. ábra. Összefüggő szekréciós sejtállomány a hasdúcláncban (3 napos *Astacus leptodactylus*). ggl. = ganglionok, s. c. = szekréciós sejtek

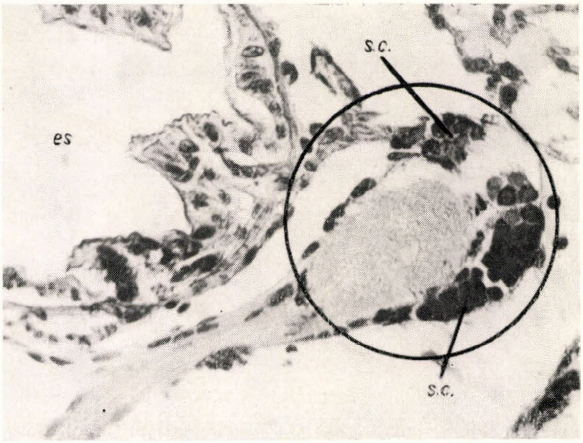

Fig. 6. Paraesophageal ganglion (3 days old *Astacus leptodactylus*). es. = esophagus, s.c. = secretory cells

6. ábra. Paraoesophagealis ganglion (3 napos *Astacus leptodactylus*). es. = nyelőcső, s. c. = szekréciós sejtek