ANNAL. BIOL. TIHANY | 28 | 29—47 | HUNGARIA 1961

STUDIES ON THE LIGHT- AND DARK-ADAPTATION OF THE COLOUR OF THE CRAYFISH, *ASTACUS LEPTODACTYLUS* ESCHSCHOLZ (DECAPODA) CONTROLLED BY THE SECRETORY ACTIVITY OF THE CENTRAL NERVOUS SYSTEM

ISTVÁN KONOK

Received: March 14th 1961

The colour-change of Crustaceans under the effect of light is a well-known phenomenon. However, the mechanism of the process responsible for the adaptation is still uncleared in many respects (CARLISLE and KNOWLES 1959).

KOLLER and PERKINS (KOLLER 1928, PERKINS 1928) were the first to recognize, that hormone regulation playing a part in the motion of the chromatophore pigments of Crustaceans. Chromotophorotropic hormone extracted first from the eye-stalk, was found later in the supraesophageal ganglion (BROWN 1933, KNOWLES 1939) and the postesophageal commissure (BROWN and EDERSTROM 1940, BROWN 1946). In the course of investigations it became evident that the sinus-gland lying in the eye-stalk was not the producing, but the storing and secretory organ of chromatophorotropic hormones (KLEINHOLZ 1942). The assumption that not only one kind of chromatophorotropic hormone existed but several effective substances participated in the regulation of chromatophores, has proved to be right (BROWN 1935, CARLISLE and KNOWLES 1959).

A long series of investigations into the motion of the distal retinal pigment of the compound eye, have proved, among other facts, the existence of a light- (KLEINHOLZ 1936) and of a dark-adapting factor (FINGERMAN, LOWE and SUNDARARAJ 1959), which are responsible for the light- resp. the dark-adapted state of the distal retinal pigment (KLEINHOLZ and KNOWLES 1938, KNOWLES 1950, BROWN, HINES and FINGERMAN 1952, SANDEEN and BROWN 1952, FINGERMAN and AOTO 1958, FINGERMAN, SANDEEN and MOBBERLY 1960, FINGERMAN and MOBBERLY 1960).

From the results of these tests performed mainly on different sea Crustaceans, we may conclude that the chromatophore-control system shows marked differences between the groups of Crustaceans and even within these groups.

The purpose of our experiments has been to elucidate the significance of colour-change, especially in the case of *Astacus leptodactylus*, a fresh water crayfish, and to acquire a further knowledge as to the mechanism of adaptation, resp. to collect more data concerning the role of the neurosecretory activity of the central nervous system.

Our investigations were always completed by parallel histological tests, the discussion of which, however, would go beyond the scope of the present paper.

I wish to thank Mr. KÁROLY FENYVESI Technical Assistant for his valuable help.

Material and methods

Specimens of the crayfish *Astacus leptodactylus* ESCHSCHOLZ collected from Lake Balaton were used for our investigations. This is the only species of Decapoda living in the lake and can be caught in relatively large amount by dradging and by hand from below the coastal stones.

The *Astacus* population of the lake is characterized by a much lighter colour than that of other specimens of the species living in other biotops. This phenomenon is due to the reduction of the number of chromatophores resp. of the quantity of pigments, caused by special environmental conditions. For, though the average depth of water of Lake Balaton does not exceed 3.5 m., yet owing to chemical and physical reasons, the turbidity of water is so great that the specimens not living in the coastal shallow water must be adapted to life in practically constant darkness. This adaptation explains the above-mentioned reduction phenomena, and leads to the result that the chromatophores of specimens living here are constantly in the dark-adapted physiological condition.

We tested about 340 adult specimens of 12—18 cm. average size. We tested both males and females, but paid due attention to this circumstance at the control experiments. We collected the animals in summer and autumn, using part of them immediately for testing, while the rest were kept in a through-flow type aquarium at a temperature of 10—15°C, until being used.

Since the strongly incrustated thick carapace of the animals does not allow an immediate in vivo observation of chromatophores, we elaborated an in vitro testing method which proved to be very efficient for testing different extracts on different chromatophores.

The densest pigmentation most suitable for observation can be found at the dorsal side of the carapace, on the plate above the heart and before the suture. From the opened and removed carapace, we cut out with scissors the parts shown in *Fig. 1.* We removed the ovary resp. the testes, the heart and the connective tissue located caudally of the suture of the carapace and took care not to injure the chromatophores of the cuticle. Of the parts of the carapace previously bathed in isotonic salt solution (1.2% NaCl), we cut out 2×2 mm. large disks, containing red, white and blue chromatophores resp. pigments in sufficient number. The sections proved to be good for testing for about 8 hours. Tests were carried out in embryo-dishes, in not more than 1.5 ml. of liquid, in such a way that we placed at least two sections cut out of dark- and light-adapted animals into each cup thus containing contracted and diffused chromatophores.

The elements of the nervous system which were to be tested for their chromatophore activity: the supraesophageal ganglion, the postesophageal commissure, the infraesophageal ganglion, the sinus gland, etc. were prepared under a binocular microscope. The organs were first homogenized by a POTTER-type glasshomogenizer in a 1.2% sodium chloride solution of 1.5 ml. final volume and then centrifuged. Total extracts of eye-stalks were made by the same method.

Extracts of the above-mentioned organs were made also in buthanol by the same process, and after centrifuging and warming the samples in a water-bath we distilled them in vacuo to the final volume of 0.05 ml. Then we soaked with the concentrated substance SCHLEICHER-SCHÜLL 2043/b

slips of paper, upon which we let it run for 5 hours at 300 V voltage, using for electrolyte 0.2 M sodium borate buffer (pH 9). We used for testing those parts of the electrophoretic strips which lay in a breadth of 10 cm from the point of application to the anode resp. the cathode, cutting them into small slips and eluating them in a 1.2% NaCl solution at 21°C for 2 hours.

For control we used the slips cut off from the ends of the strips. We made electrophoretic separations also on substances put directly on the paper strips.

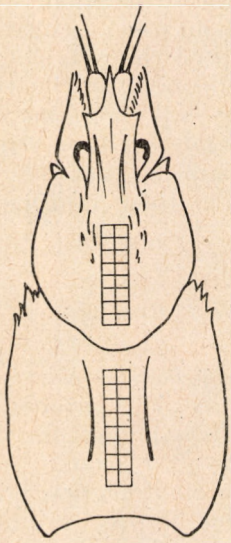

Fig. 1. Carapace of *Astacus leptodactylus*. The squares show the parts of carapace used for testing

1. ábra. Astacus leptodactylus carapaxa. A kis négyzetek a tesztelésre használt carapax részeket jelölik

When using the method of paper chromatography, we put the corresponding organs also on SCHLEICHER-SCHÜLL paper (KONOK 1960). Then we cut out of the chromatogram the spots showing UV-fluorescence, eluated and tested them by the above mentioned method.

We tested the electrophoretic strips in UV-light filtered for fluorescence and developed them with ninhydrin. Then we submitted the eluated and tested chromatophorotropic substances to pepsin resp. trypsin digestion, incubating them for an hour at 38° C. After digestion (in case of pepsin with newly stabilised pH) we tested the extracts in question a second time with the necessary control.

Missing the possibility of objective means to evaluate the chromatophorotropic effect, we proceeded as follows: we observed under a binocular microscope a certain group of chromatophores resp. pigments and repeated the observations every 10 minutes during the first three hours. We divided the state of movement of the pigments in chromatophores into 5 phases, in the percentage of the maximum diffusion, marking the maximum contraction with 0%. On the ordinates we charted this approximative percentage values and of the abscissae the intervals of observation (minutes).

In light-tests we put the animals by twos into glass dishes of $14 \times 14 \times 25$ cm size. The temperature of water being 21°C, we aerated the basins strongly. Those basins into which we placed the animals to be exposed to light were placed on a white, reflecting basis and lighted by a glow-lamp. Measured under water, the animals received in the average a light quantity of 1000 lux. The other part of the basins was placed on a black basis and covered with a black-emailed metal box, which assured absolute absence of light.

We experimented also with animals, whose eye-stalks were extirpated or ligated. When carrying out the extirpation, we removed one or both eye-stalks by simple operation, without previous ligature, and then put the animals into cold water, in order to speed the coagulation of the haemolymph, and thus avoid the possibility of infection. The ligature of the eye-stalk was performed as follows: first the eye-stalks were made accessible by removing the rostrum, afterwards they were ligated with a strong thread at the segmental membrane, before the chitin plates, so that the circulation between the body and the eye-stalk was stopped, without injuring the nerves.

Results

In the case of *Astacus leptodactylus* we find two types of characteristic chromatophores, red and white ones, furthermore blue pigment granules whose motion can also be observed. These blue pigments, when diffused, are spread in the form of a fine dispersion over a large area, while they do not contract in one spot, like in the chromatophores, but concentrate in a relatively small area which is to be found mostly within a larger group of red chromatophores. In general, the movements of the red and blue pigments are antagonistic, *i. e.* when the red ones are diffused, the blue ones are contracted and the reds cover the blue ones, whereas, at the contraction of the red pigments, the animal takes a blue colour.

The first step was to determine the normal position of the pigments in the light- and dark-adapted states. We exposed twenty animals to constant darkness and as many to constant light, and tested the position of the chromatophores after 72 hours. Then we interchanged the animals in the basins and examined the motion of the chromatophores during 96 hours from the moment of the interchange. The result was as follows: Under the effect of light, the red chromatophores diffused permanently, while the white ones diffusing at the beginning, after 24 hours gradually contracted to their maximum. The blue pigments contracted permanently. Under the effect of darkness, the red and white chromatophores contracted, while the blue ones diffused. According to these observations, light-adaptation means in the case of *Astacus leptodactylus*, the diffusion of red and the contraction of blue pigments as well as the diffusion of white pigments for 24 hours followed by gradual contraction. In the dark-adapted state the contraction of red and white pigments and the diffusion of blue ones can be observed. Consequently, the basic colour of the animals is determined by the distribution of red and blue pigments. This can be observed particularly well in freshly moulted specimens, where the difference of colour is very intensive, depending on, whether they were collected from the littoral area or from the open water.

In the course of our further experiments, we worked with 40 animals per group. In one of these experimental groups we ligated the eye-stalks after having kept the animals for 24 hours under constant circumstances, and thus exposed them to light resp. to darkness. We preparated the animals after 24 hours. No significant change was observed in the distribution of chromatophores resp. pigments, as compared with the controls.

We repeated the experiments with the difference that, when the eye-stalks were ligated, we interchanged the animals in the basins. After 24 hours, in the course of preparation, we found the distribution of pigments altered; the red pigments were diffused to a maximum in the animals exposed to light, while in the animals exposed to darkness the red pigments gradually contracted, and finally the state of normal adaptation was reached. The normal adaptation could also be observed in the white pigments, which diffused under the effect of light and gradually contracted when exposed to darkness. Similarly, the distribution of the blue pigments altered according to normal adaptation.

In the next series of experiments, after having adapted the animals to light resp. to darkness, we extirpated first one of the eye-stalks. After 24 hours, the examination of the chromatophores showed the expected result, and no alteration could be observed in the situation of pigments, as compared with the controls. Similarly, after the interchange of animals adaptation went on normally.

In another group of animals we extirpated both eye-stalks after adaptation. We preparated the animals 24 hours later and could observe that the red and white chromatophores were diffused to a maximum, on the animals kept in darkness as well as on those exposed to light, while blue pigments were contracted in both groups.

In our next series of investigations, we repeated the total extirpation of eye-stalks, but immediately afterwards we interchanged the animals in the basins. No important alteration was observable in the position of the pigments.

We tested in vitro, on the preparated parts of the carapace, whether light had a direct effect on the chromatophores. Even after 12 hours of exposure to light the position of the pigments did not alter.

Next we examined in vitro the chromatophorotropic activity of the total watery extract of certain organs as eyestalk, supraesophageal ganglion, postesophageal commissure and infraesophageal ganglion on different chromatophores. The watery extract preparated from 2 eye-stalks homogenized in 1.5 ml isotonic solution of salt, when deriving from animals kept in darkness, proved to have a marked contracting effect on red and white chromatophores, and at the same time a faint diffusing effect on blue chromatophores *(Fig. 2)*. Extracts of eye-stalks from animals exposed to light showed a faintly contracting effect on red chromatophores and a very weak effect on white ones, not even a slight diffusing action was observable on blue pigments *(Fig. 3)*.

The extract made of brains (1 brain in 1.5 ml isotonic solution of salt) proved to have about the same activity as eye-stalk extracts. The brain of the animals kept in darkness had a very strong contracting effect on red and white pigments and, at the same time, a weak diffusing effect on blue pigments *(Fig. 2)*.

Brain extracts obtained from animals exposed to light have a weak activity on the red chromatophores, but a stronger one than eye-stalk extracts. They

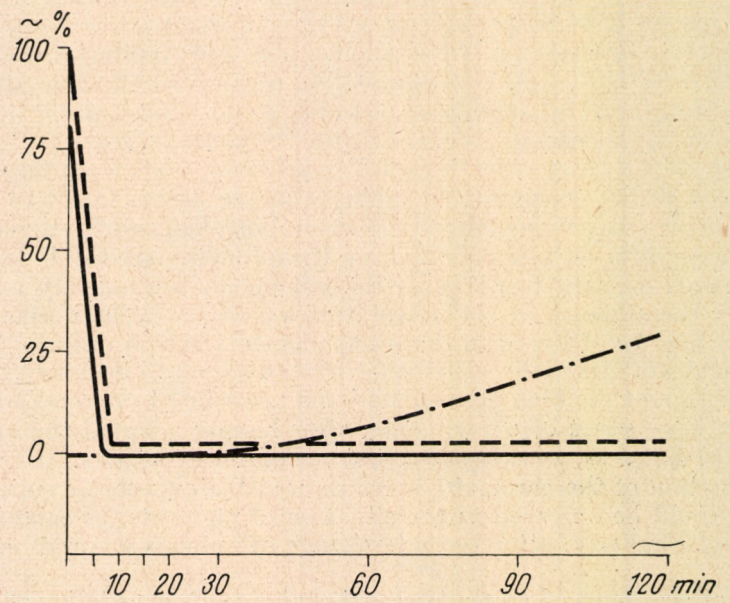

Fig. 2. Chromatophorotrop activity of eye-stalk and brain of dark-adapted animals.
——— red pigments, — — — white pigments, —.—. blue pigments
2. ábra. Sötétséghez adaptálódott állatok szemnyelének és agyának kromatoforotróp aktivitása ——— vörös pigmentek — — — fehér pigmentek —·—·— kék pigmentek

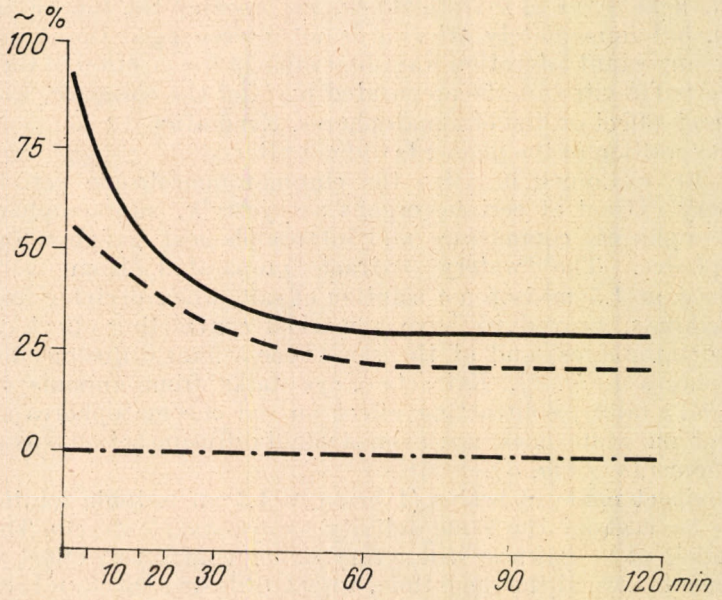

Fig. 3. Chromatophorotrop activity of the eye-stalk of light-adapted animals.
——— red pigments, — — — white pigments, —.—. blue pigments
3. ábra. Fényhez adaptálódott állatok szemnyelének kromatoforotróp aktivitása.
V. ö. *2. ábra.*

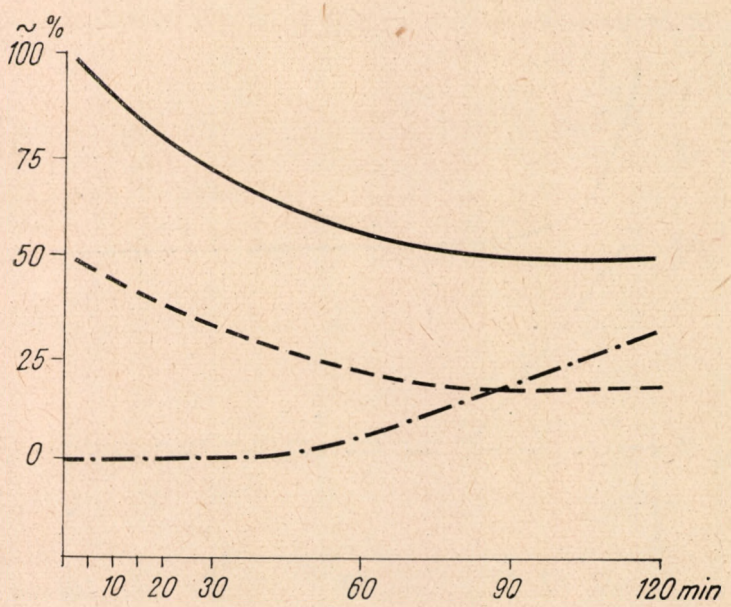

Fig. 4. Chromatophorotrop activity of the brain (supraesophageal ganglion) of light-adapted animals. ——— red pigments, — — — white pigments, —.—. blue pigments
4. ábra. Fényhez adaptálódott állatok agyának (szupraözofageális ganglionjának) kromatoforotróp aktivitása. V. ö. *2. ábra.*

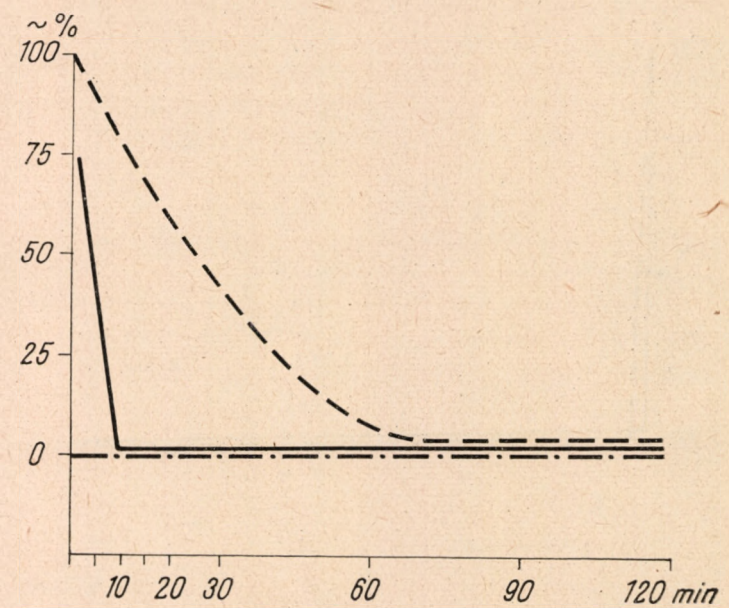

Fig. 5. Chromatophorotrop activity of the postesophageal commissure of dark-adapted animals. ——— red pigments, — — — white pigments, —.—. blue pigments
5. ábra. Sötétséghez adaptálódott állatok posztözofageális kommisszúrájának kromatoforotróp aktivitása. V. ö. *2. ábra.*

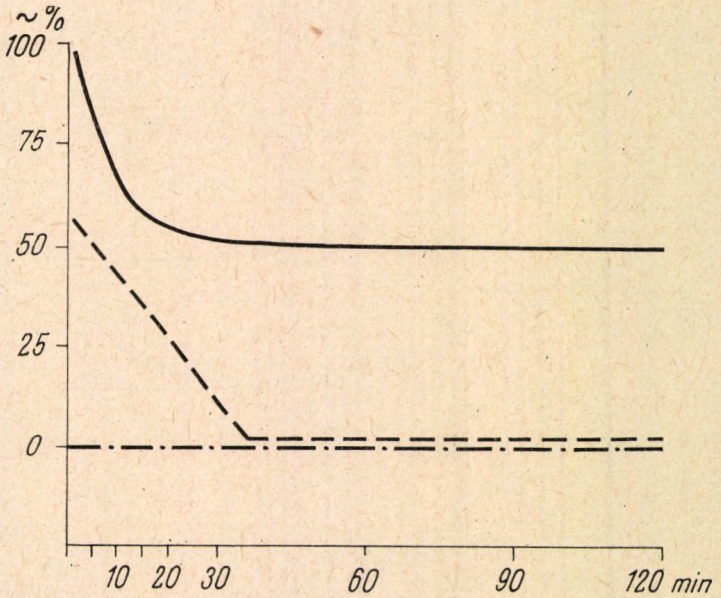

Fig. 6. Chromatophorotrop activity of the postesophageal commissure of light-adapted animals. ——— red pigments, — — — white pigments, —.—. blue pigments
6. ábra. Fényhez adaptálódott állatok posztözofageális kommisszúrájának kromatofo-rotróp aktivitása. V. ö. *2. ábra.*

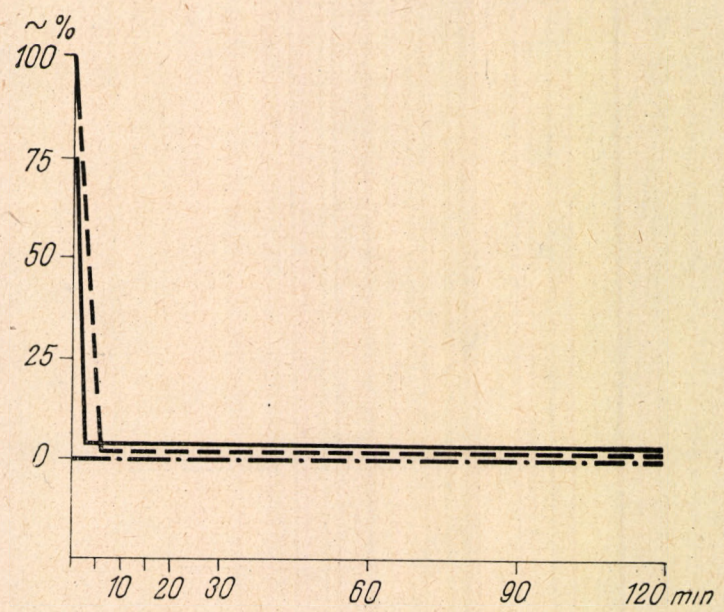

Fig. 7. Chromatophorotrop activity of the infraesophageal ganglion of dark-adapted animals. ——— red pigments, — — — white pigments, —.—. blue pigments
7. ábra. Sötétséghez adaptálódott állatok infraözofageális ganglionjának kromatofo-rotróp aktivitása. V. ö. *2. ábra.*

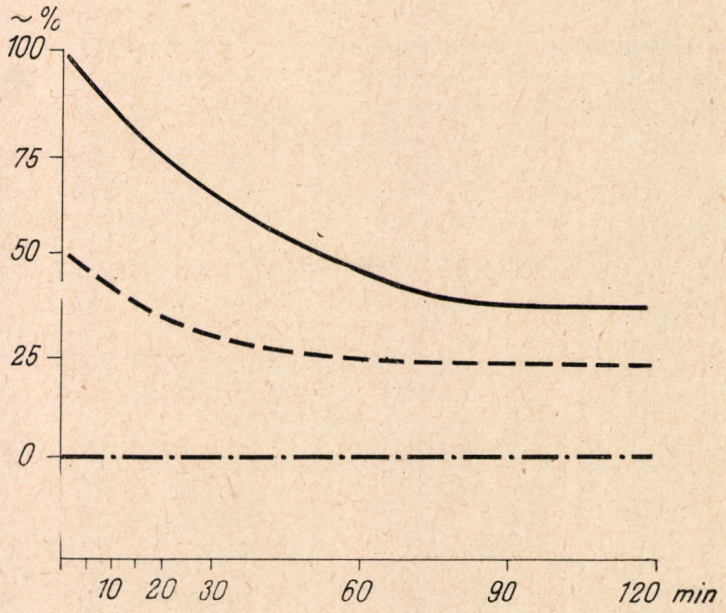

Fig. 8. Chromatophorotrop activity of the infraesophageal ganglion of light-adapted animals. ——— red pigments, — — — white pigments, —.—. blue pigments

8. ábra. Fényhez adaptálódott állatok infraözofageális ganglionjának kromatoforotróp aktivitása. V. ö. *2. ábra.*

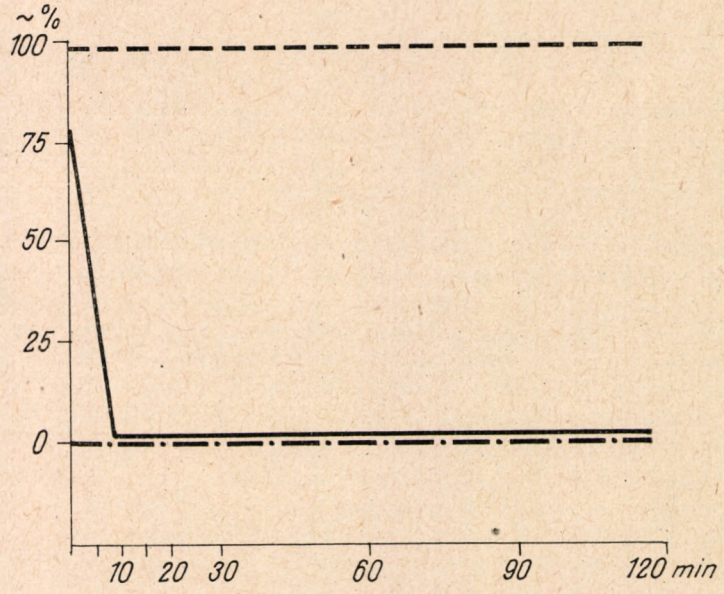

Fig. 9. Chromatophorotrop effect of isoxanthopterin isolated by means of paper-chromatography. ——— red pigments, — — — white pigments, —.—. blue pigments

9. ábra. Papírelektroforézis útján izolált izoxantopterin kromatoforotróp hatása. V. ö. *2. ábra*

act very little on white chromatophores and faintly diffuse the blue ones. Their effect upon white chromatophores is as weak as that of eye-stalk extract, while they induce a slight diffusion of the blue pigments opposite to the eye-stalks *(Fig. 4)*.

Extracts made from the postesophageal commissure (1 pc in 1.5 ml) of animals kept in darkness had the same effect as the eye-stalk extracts obtained from similar animals, except the diffusing effect on blue pigments *(Fig. 5)*.

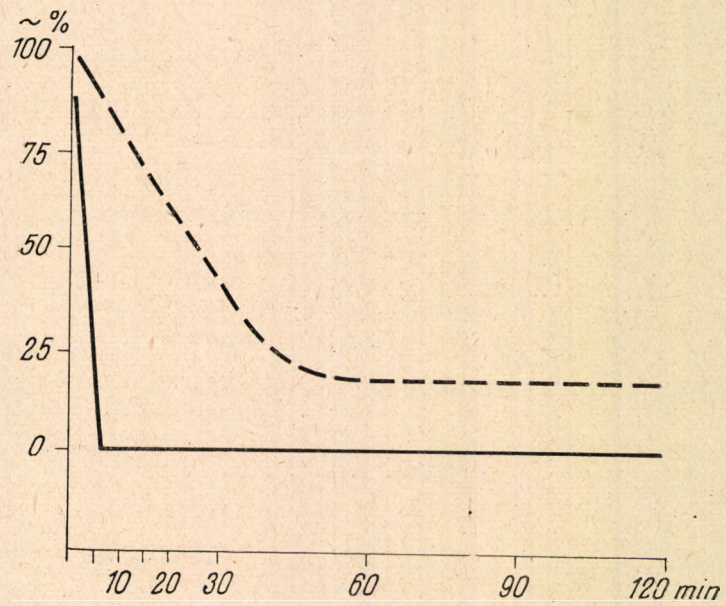

Fig. 10. Chromatophorotrop effect of RC-factor isolated from the eye-stalks of dark-adapted animals. ——— red pigments, — — — white pigments

10. ábra. Sötétséghez adaptálódott állatok szemnyeléből izolált RC-faktor kromatofo-rotróp aktivitása. ——— vörös pigmentek, — — — fehér pigmentek

The extracts obtained from animals exposed to light had a slight contracting activity on red, a strong one on white and none on blue pigments *(Fig. 6)*.

Extracts prepared from the infraesophageal ganglion (1 pc in 1.5 ml) of animals adapted to darkness, acted as those described before, with the difference that they acted more strongly on white chromatophores than brain extracts *(Fig. 7)*. The extracts obtained from animals exposed to light were faintly effective on both red and white chromatophores and ineffective on blue pigments *(Fig. 8)*.

In the corresponding control tests no change was observable.

A summing up of our observations is given in *Table 1*.

From the fluorescent substances (Konok 1960) which can be isolated by means of paper chromatography from the brain and the eye-stalks, we tested for their effect on chromatophores isoxanthopterin (Rf = 0.23) and a substance of yellow-green fluorescence (Rf = 0.30) both eluated in isotonic solution of salt. Of the two substances isoxanthopterin had a marked, strong effect on red chromatophores, but none on other pigments *(Fig. 9)*. The substance of yellow-green fluorescence was ineffective on all chromatophores.

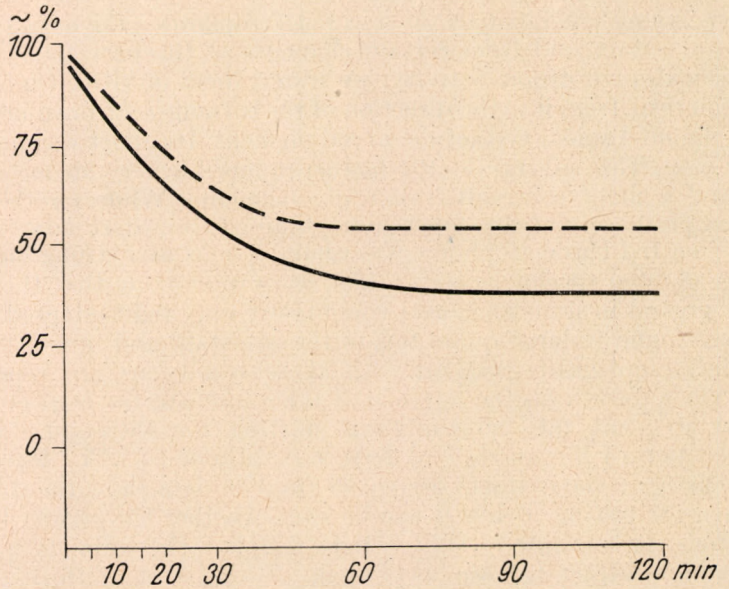

Fig. 11. Chromatophorotrop effect of RC'-factor, isolated from the supraesophageal ganglion of dark-adapted animals. ——— red pigments, — — — white pigments

11. ábra. Sötéthez adaptálódott állatok szupraözofageális ganglionjából izolált RC'-faktor kromatoforotróp hatása. ——— vörös pigmentek, — — — fehér pigmentek

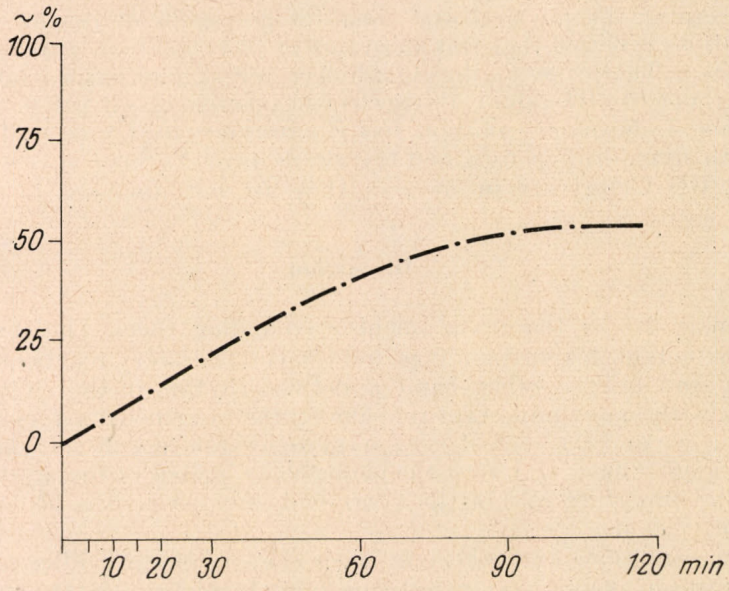

Fig. 12. Chromatophorotrop effect of BD-factor isolated from the supraesophageal ganglion of dark-adapted animals. —.—. blue pigments

12. ábra. Sötétséghez adaptálódott állatok szupraözofageális ganglionjából izolált BD-faktor kromatoforotróp hatása. —·—·— kék pigmentek

After having separated by means of electrophoresis the extracts obtained from the eye-stalk and from certain elements of the nervous system which show chromatophorotropic activity, we tested them in vitro. On the point of application resp. from it in the direction of the cathode (—), in an area of about 3 cm, we found a substance acting on red pigment. Its effect was unambiguous in every case. This substance (RC factor) induced a very strong contraction of red, and a slight contraction of white pigments. While for the latter the effect was produced only by substances obtained from dark-adapted animals, the effect on red chromatophores was unambiguous and strong on both dark- and light-adapted specimens *(Fig. 10)*. It is interesting that this RC-factor could be proved in postesophageal commissure only light-adapted animals.

It was found in large quantities in the eye-stalk and in a still larger one in the infraesophageal ganglion. All these conditions are represented in *Table 2*. The numbers on the left of the table indicate the respective parts of the paper strip cut into slips of 1 cm, starting from the point of application in the direction of the anode (+) resp. the cathode (—). The top date show, from which organ the extracts derive, the middle data show the different pigments used for testing. Below it can be seen, whether the organs in question derive from dark-adapted (D), or light-adapted (L) animals. += weak, ++=strong contracting, resp. —= weak, —— = strong diffusing effect.

As the table indicates, the weak blue diffusing substance (BD factor) observed in direct watery extracts, could also be seen from the electrophoretic strips, in the direction of the anode (+), 2—7 cm distant from the point of application *(Fig. 11)*. In the same area an electronegative substance with contracting effect on red and white pigments (RC' factor) was observable on the paper strips *(Fig. 12)*.

Concerning the physical and chemical properties of the RC factor our investigations revealed that this substance is well soluble in water and buthanol, resists to heat of 80°C during a longer period, and has a relatively high molecular weight (Rf value). Its isoelectric point is about pH 9. Neither its watery nor its buthanol solution has fluorescence in UV-light. It does not react with nynhidrin, pepsin and trypsin destroy it. Since investigations on RC' and BD factors are in progress, they are not discussed in this paper.

Discussion

Animals live in the environment surrounding them. The standard and varying actions of this surroundings induce compensatory reactions in animal organism and on the other hand a defined rhythm in the vital processes. Light plays the most important role among the exogenous factors of surroundings. The eye and the central nervous system establish contact with the light as exogenous milieu and with the endogenous milieu of the animal. In this process the important role of the neuro-endocrine system is becoming more and more evident.

Light conditions and especially their changes produce different reactions in the organism, as for instance, in many animals colour-adaptations of various degrees according to the changes in light-conditions. The importance and the mechanism of colour-adaptation in *Astacus leptodactylus* is not quite clear as yet. Most probably, chromatophores and colour-adaptation play a part during

Table 1

1	control						eye-stalk						supraesophageal ganglion						postesophageal commissure						infraesophageal ganglion					
2	red		white		blue		red		white		blue		red		white		blue		red		white		blue		red		white		blue	
3	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
4							+	+	+	+	−		+	+	+	+	−	−	+	+	+	+			+	+	+	+		
							+		+				+	+	+				+		+	+			+	+	+	+		
							+		+				+		+				+		+	+			+		+			

1. Organ of which the extract is deriving from (control: 1.2 % solution of sodium chloride)
2. Pigments
3. D = dark-adapted, L = light-adapted animals
4. + = weak contracting effect
   ++ = mean contracting effect
   +++ = strong contracting effect
   − = weak diffusing effect

Table 2

	eye-stalk						supraesophageal ganglion						postesophageal commissure						infraesophageal ganglion					
	red		white		blue		red		white		blue		red		white		blue		red		white		blue	
	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L	D	L
+8											——													— —
+7											——													— —
+6				+			+		+		——													— —
+5				+			+		+		——													— —
+4		+		++			+		++		−		+	+	+	++			++	++	+			—
+3		+		++			++		++		−		+	+	+	++			++	++	+			—
+2				+							−					+			++	++	+			—
+1																								
START	++	++					++	++						++					++	++	+			
−1	++	++	++				++	++	+					++					++	++	+			
−2	++	++	++				++	++	+					++					++	++	+			
−3	++	++	++					++											++		+			

Explanation in the text

the period of moulting, when the shell, an effective light-filter, is missing. Chromatophores resp. pigments are located in greatest quantity above the presumably most sensitive parts, like the heart, the brain or the sinus gland in the eye-stalk. These facts suggest that it is more likely that chromatophores offer protection against light than that they help the organism to adapt to the surroundings. This assumption is supported to a certain extent by the characteristic phenomenon of pigment reduction already mentioned.

Parallel experiments on *Astacus astacus* not living in Lake Balaton but only in the rivers discharging into it, raise interesting problems. The light-conditions of this biotop are quite different from those of *Astacus leptodactylus*. This is manifested also in the difference of the chromatophore-activating system so much so that in *A. astacus* the basic position and the motion of pigments is almost opposite to those in *A. leptodactylus*. This interesting divergency undoubtedly refers to the fact that, in relation to the chromatophore-control system not only the quantity but also the quality of light plays a part. This may be concluded from the fact that, in specimens of *A. leptodactylus* living at the bottom of Lake Balaton, *i. e.* in dark-adapted animals, blue colour dominates, whereas, in the shallow littoral zone, *i. e.* in case of light-adaptation, the red colour dominates. The light-conditions of the Balaton are special, at a depth of 3—3.5 m the intensity of light measured in summer is only about 350 lux (ENTZ 1961), and it is interesting that only yellow light reaches this depth in greater amount (FELFÖLDY 1958). This fact in itself may explain the blue colour.

Hence *Astacus leptodactylus* shows a definite colour-adaptation according to the variations in light-conditions. This adaptation is based on the different spatial distribution of the three types of pigments. In the dark-adapted state pigments are contracted in the red and white chromatophores, while the blue pigments are finally dispersed over a large area, thus the animal gets a blue basic colour. Upon the action of light, red and white pigments diffuse, blue ones contract; later also the white ones gradually contract, and the animals become red.

Naturally, the intensity of the basic colour may vary according to the more or less great reduction of the amount of pigments resp. of chromatophores. Specimens can be found, in which the amount of blue pigment is reduced to about 5—10% of the maximum blue pigmentation of crayfish living in Lake Balaton. The reduction of the amount of red pigments reaches 20—30% in the average, seldom 70—80%, and roughly the same holds true of white pigments. The reduction of the amount of red chromatophores may reach 50—60%, while that of the white ones reaches only 5—10%.

Our knowledge regarding the mechanism of the hormonal control system responsible for the pigment distribution of Crustaceans is rather incomplete and therefore we cannot have a clear conception of it. Most of the investigations were restricted to sea material, which offers greater variety and is more suitable for testing purposes. When considering the investigations of other authors and comparing their results with ours, a more or less marked difference can be shown in the conception of the chromatophore-activating system of different groups of Crustaceans. For, although red and white chromatophores occur in almost every group of Crustacean, they cannot be considered physiologically equivalent. The blue pigment of *Astacus* holds a special place from this point of view.

Although also in the case of *Astacus* we have to presume the presence of one or more antagonistic chromatophore-activating substances, in spite of the fact that, during our investigations we always considered the possibility of the covering over of antagonistic factors, in the central nervous system we could directly show only hormones of dark-adapting character. It is interesting to notice, that not only the direct total extracts of certain elements of the nervous system, but also the watery or alcoholic extracts derived from the same organs and separated by electrophoresis, contained such active substances, which could diffuse the pigments of the red or white chromatophores, or contract the blue pigments.

A lightly electropositive substance (RC factor) could always be found in the eye-stalk and in certain elements of the nervous system (except the postesophageal commissure of animals kept in darkness). This factor has a strongly contracting effect on the pigments of red chromatophores, and acts in the same way but less strongly on the white ones, however, only when isolated from dark-adapted animals. The hormone had no effect on blue pigments. This factor may probably be brought into connection with the A' substance (CARLISLE and KNOWLES 1959) proved in the postcommissural organ of *Squilla* and *Leander* by KNOWLES.

It has been proved that the production of the RC factor goes on continually in certain elements of the nervous system. However, the fact that the amount of these substances increases in the dark-adapted state argues against the possibility of storage, on the other hand it proves the amount of the RC factor being increased during this period. This hormone is to be found chiefly in the eye-stalk and in the infraesophageal ganglion.

On the basis of the data referring to the chemical properties of the RC factor, we may assume that this hormone is a polypeptid of a rather high molecular weight, this being in accordance with the statements of other authors (CARLISLE and KNOWLES 1959).

Isoxanthopterin, a substance fluorescent in violet, isolated from the eye-stalks by means of paper chromatography (KONOK 1960), was acting somewhat similarly to the RC factor, having had a strongly contracting effect on pigments, but only on red ones. The RC factor did not show any UV-fluorescence on the electrophoretic strips, and so the problem remains unsolved, whether or not there is any relation between this hormone and isoxanthopterin.

We detected a less active substance, resembling the RC factor, moving towards the anode. This RC' factor of medium electronegativity was just as effective in the case of red as in that of white chromatophores. This substance was found in the postesophageal commissure of dark- and light-adapted specimens, further in the eye-stalk of light-adapted and in the supraesophageal ganglion of dark-adapted animals. In the infraesophageal ganglion of both groups it was found in a large amount.

We detected further a third chromatophore-active substance (BD factor) in the supra- and infraesophageal ganglion. It is of a medium electronegative character (pH 9). It was found in large amounts in the infraesophageal ganglion of light-adapted and in the brain of dark-adapted animals and it diffused strongly blue pigments.

It is apparent that all these substances induce pigment movements of dark-adapting character. There is no direct proof regarding a factor of light-adapting character which could be made responsible for the diffusion of red

and white pigments resp. for the contraction of blue pigments. Indirectly the presence of these hormones is proved partly by the fact that, light has no direct influence on chromatophores. A further proof is furnished by the result of those experiments which showed that, after the extirpation of both eye-stalks red and white pigments diffused permanently while the blue ones contracted. This fact can be explained most obviously by the deficiency of the dark-adapting factors, when the action of the antagonistic light-adapting factor or factors prevail.

The experiments of the extirpation and ligating of the eye-stalk and their variation prove naturally the well known neurohormonal storing role of the sinus-gland, but seem to prove at the same time that the secretion of effective substances into the haemolymphe does not proceed through the sinus-gland only. This conclusions are supported by our histological investigations (KONOK 1961), and might prove the similar hypothesis of KNOWLES (CARLISLE and KNOWLES 1959).

Summary

1. According to the variations of light-conditions, definite colour-adaptation can be observed in the crayfish *Astacus leptodactylus*.

2. The role of pigments, the importance of light-adaptation probably consists in constituting a suitable light filter layer, especially in relation to moulting.

3. It can be proved that not only the quantity but also the quality of light plays a role in colour-adaptation.

4. In connection with the quantitative and qualitative conditions of light, phenomena of pigment- and chromatophore reduction can be observed in various degrees on the population of *Astacus leptodactylus* living in Lake Balaton.

5. The distribution of the three types of pigments is such as to give to *Astacus leptodactylus* a blue basic colour in the case of dark-adaptation. The red and white pigments contract, the blue ones diffuse.

6. Due to the action of light, red and white pigments diffuse, blue ones contract. After 24 hours of constant exposure to light, white pigments gradually contract again. The basic colour of the light-adapted animal will be red.

7. In the case of *Astacus astacus*, the function of the chromatophores is approximately antagonistic, red chromatophores diffusing in the dark-adapted state and contracting on the action of light. The same holds true of blue pigments.

8. Light is not acting directly on the chromatophores resp. on the movement of pigments.

9. In the case of *Astacus leptodactylus* certain elements of the eye-stalk and of the nervous system show only an activity of dark-adapting character.

10. No antagonistic chromatophorotrop effect could be demonstrated in the postesophageal ganglion, the factor of dark-adapting character was to be found here too.

11. The sources of the dark-adapting factors are eye-stalks and the ganglia of the central nervous system, where they are produced continually.

12. The chromatophorotrop activity of certain organs is growing in dark-adapted state. Most of the hormones can be found in the eye-stalk and in the infraesopageal ganglion.

13. The dark-adapting chromatophorotrop hormone is not only excreted through the sinus gland, but also directly from every ganglion into the haemolymph.

14. By the means of electrophoretic separation, an electro-positive (pH 9) RC factor could be isolated from certain elements of nerves and from the eye-stalk, effecting strong contraction of the red, and a weaker one of the white pigments. This factor is probably identical with the A' substance shown by KNOWLES in the post-commissure organ of *Squilla* and *Leander*.

15. The RC factor is a heat-resisting substance well soluble in water and buthanol, presumably a polypeptid of a rather high molecular weight.

16. By electrophoresis another electro-negative (pH 9) RC' factor could be isolated from the eye-stalk and from elements of the nervous system, having a weakly contracting effect on both red and white pigments.

17. An electronegative BD factor (pH 9) could be isolated from the supra- and infraesophageal ganglia, diffusing strongly the blue pigments.

18. In connection with the investigations, a useful process for in vitro chromatophore-testing has been elaborated.

LITERATURE

BROWN, F. A. JR. (1933): The controlling mechanism of chromatophores in Palaemonetes. — *Proc. nat. Acad. Sci., Wash.*, **19**, 327—329.

BROWN, F. A. JR. (1935): Control of pigment migration within the chromatophores of Palaemonetes vulgaris. — *Jour. Exper. Zoöl.*, **71**, 1—14.

BROWN, F. A. JR. (1946): Endocrine activity of the tritocerebral commissure of Crago (Crustacea). — *Anat. Rec.*, **94**, 405.

BROWN, F. A. JR. and H. E. EDERSTROM (1940): Dual control of certain black chromatophores of Crago. — *Jour. Exper. Zoöl.*, **85**, 53—69.

BROWN, F. A. JR., M. N. HINES and M. FINGERMAN (1952): Hormonal regulation of the distal retinal pigment of Palaemonetes. — *Biol. Bull.*, **102**, 212—225.

CARLISLE, D. B. and F. G. W. KNOWLES (1959): Endocrine control in Crustaceans. — Cambridge University Press. — *Cambridge*, VII + 120.

ENTZ, B. (1961): Personal communication.

FELFÖLDY, L. and ZS. F. KALKÓ (1958): The rate of photosynthesis and underwater radiation in lake Balaton. Observations of summer 1957. — *Annal. Biol. Tihany*, **25**, 303—329. (In Hungarian with English summary)

FINGERMAN, M. and T. AOTO (1958): Electrophoretic analysis of chromatophorotropins in the dwarf crayfish, Cambarellus shufeldti. — *Jour. Exper. Zoöl.*, **138**, 25—50.

FINGERMAN, M., M. E. LOWE and B. I. SUNDARARAJ (1959): Dark-adapting and light-adapting hormones controlling the distal retinal pigment of the prawn Palaemonetes vulgaris. — *Biol. Bull.*, **116**, 30—36.

FINGERMAN, M. and W. C. MOBBERLY, JR. (1960): Investigation of the hormones controlling the distal retinal pigment of the prawn Palaemonetes. — *Biol. Bull.*, **118**, 393—406.

FINGERMAN, M., M. I. SANDEEN and W. C. MOBBERLY, JR. (1960): Hormonal regulation of the distal retinal pigment of Custaceans with special reference to the shrimp Crangon septemspinosus. — *Physiol. Zoöl.*, **33**, 260—270.

KLEINHOLZ, L. H. (1936): Crustacean eye-stalk hormone and retinal pigment migration. — *Biol. Bull.*, **70**, 159—184.

KLEINHOLZ, L. H. (1942): Hormones in Crustacea. — *Biol. Rev.*, **17**, 91—119.

KLEINHOLZ, L. H. and F. G. W. KNOWLES (1938): Studies in the pigmentary system of Crustacea. III. Light intensity and the position of the distal retinal pigment in Leander adspersus. — *Biol. Bull.*, **75**, 266—273.

KNOWLES, F. G. W. (1939): The control of the white reflecting chromatophores in Crustacea. — *Publ. Staz. zool. Napoli*, **42**, 174—182.
KNOWLES, F. G. W. (1950): The control of retinal pigment migration in Leander serratus. — *Biol. Bull.* **98**, 66—80.
KOLLER, G. (1928): Versuche über die inkretorischen Vorgänge beim Garneelenfarbwechsel. — *Zeitschr. vergl. Physiol.*, **8**, 601.
KONOK, I. (1960): Studies on the neurosecretory activity of the brain in the fresh water Crustacean, Astacus leptodactylus Eschscholz (Decapoda). — *Annal. Biol. Tihany*, **27**, 15—28.
KONOK, I. (1961): In perparation.
PERKINS, E. B. (1928): Color changes in Crustaceans, especially in Palaemonetes. — *Jour. Exper. Zoöl.*, **50**, 71—195.
SANDEEN, M. I. and F. A. BROWN, JR. (1952): Responses of the distal retinal pigment of Palaemonetes to illumination. — *Physiol. Zoöl.*, **25**, 223—230.

VIZSGÁLATOK A KECSKERÁKON, *ASTACUS LEPTODACTYLUS* ESCHSCHOLZ (DECAPODA), A KÖZPONTI IDEGRENDSZER SZEKRÉCIÓS TEVÉKENYSÉGE ÁLTAL SZABÁLYOZOTT FÉNY- ÉS SÖTÉTSÉG-ADAPTÁCIÓVAL KAPCSOLATBAN

Konok István

Összefoglalás

A Balatonból fogott, mintegy 340 darab ivarérett kecskerákon végzett vizsgálatok és kísérletek eredményeképpen a következő megállapításokat tettük.

Az *Astacus leptodactylus* esetében három különböző színű pigment található, melyek közül a piros és a fehér típusos kromatofórokban helyezkedik el. A kromatofórok, illetve a pigmentek túlnyomó többségben a szív, az agy, a szemnyélben a szinuszmirigy stb. felett helyezkednek el. Az ezek felett az érzékeny szervek felett való koncentrálódás fényszűrő szerepükre utal, ami különösen a vedlések idején nyer jelentőséget, amikor az egyébként hatásos fényvédelmet biztosító vastag páncél hiányzik.

A változó fényviszonyoknak megfelelően a pigmentek mozgásán alapuló meghatározott színadaptáció figyelhető meg, mely az *Astacus leptodactylus* esetében olyan értelmű, hogy megvilágítás hatására a piros és fehér pigmentek szétterülnek (tartós megvilágítás alatt 24 óra múlva a fehérek fokozatosan ismét összehúzódnak), a kékek ellenben összehúzódnak. A sötétséghez való alkalmazkodásnál viszont éppen a fordított folyamat játszódik le, amikoris a piros és fehér pigmentek koncentrálódnak a kromatofórokban, a kékek pedig szétterülnek, miáltal az állat alapszíne kék lesz. Különösen jól látszik mindez a frissen vedlett állatoknál, amikor az állatok intenzív vörös vagy kék színűek.

A Balatonba ömlő folyóvizekben is élő *Astacus astacus*on végzett összehasonlító vizsgálataink azt mutatták, hogy a fényviszonyokkal összefüggő színadaptáció, illetve a pigmentek mozgása és elrendeződése ennek a fajnak az esetében éppen a fordítottja annak, amit az *A. leptodactylus* esetében megfigyeltünk. Ennek a ténynek, valamint a Balaton vizének zavarossága következtében, a tó fenekén 3—3,5 méter mélységben uralkodó speciális fényviszonyoknak ismeretében megállapítható, hogy nemcsak a fény mennyiségi, de minőségi viszonyai is lényeges szerepet játszanak a fényadaptációt illetően. Az elmondottakkal hozható szoros kapcsolatba az *Astacus leptodactylus* balatoni populációjánál tapasztalható különböző mértékű pigment- és kromatofór-szám redukció jelensége, mely az állatok szokásostól eltérő halvány színezettségében jut kifejezésre.

A fénynek nincsen közvetlen hatása a kromatofórokra, illetőleg a pigmentek mozgására. A színadaptációs folyamatokat a központi idegrendszerben termelődő neurohormonok szabályozzák.

A szerző által kidolgozott in vitro kromatofór-teszt segítségével a szemnyélből, továbbá különböző idegrendszeri elemekből (supraoesophagealis- és infraoesophagealis ganglion, postoesophagealis commissura) nyert extraktumokban kromatoforotróp hatású hormonok voltak kimutathatók. Ezekből az extraktumokból papírkromatográfiás és elektroforézises módszerekkel háromféle hatóanyagot sikerült izolálni. A gyengén elektropozitív (pH 9) RC-faktor a piros pigmentekre erős, a fehérekre gyengébb összehúzó

hatást fejt ki. Ez a hormon egyébként valószínűleg azonosítható a KNOWLES által a *Squilla* és *Leander* postcommissuralis szervéből kimutatott A'-substance-szal. Az RC-faktor vízben, butanolban jól oldódik és termostabil vegyület. Feltehetően magasabb molekulasúlyú polypeptid.

Elektroforézis útján a szemnyélből és az említett idegrendszeri elemekből izolálható volt még egy hasonló hatású, elektronegatív (pH 9) RC'-faktor, mely gyenge összehúzó hatást fejt ki a piros és fehér pigmentekre egyaránt. A supra- és infraoesophagealis ganglionból végül izolálható volt még egy harmadik hatóanyag is, egy elektronegatív (pH 9) BD-faktor, mely erős szétterítő hatást fejt ki a kék pigmentekre. A papírkromatográfiás úton izolált isoxanthopterin erős összehúzó hatást fejtett ki a piros kromatofórokra.

Jóllehet a fent említett, kimutatott hatóanyagok kivétel nélkül mind a sötétséghez való alkalmazkodás létrejöttében játszanak szerepet, szemnyél lekötéses és extirpációs kísérletekkel, indirekt úton igazolva látszik az antagonista hatású hormonok megléte is. Az *Astacus leptodactylus* esetében azonban ezekre a hatóanyagokra vonatkozólag, melyek a fényhez való adaptáció létrejöttéért felelősek, közelebbi ismereteink még nincsenek.

A kimutatott kromatoforotróp hatóanyagok termelése a központi idegrendszerben folyamatosan történik, de szükség esetén a termelés fokozódik. Ezek a hormonok, az eddigi ismeretekkel szemben, úgy látszik, nemcsak a szinusz-mirigyen keresztül, de valamennyi ganglionból közvetlenül választódnak el a hemolimfába. A legnagyobb kromatoforotróp aktivitás a szemnyélben és az infraoesophagealis ganglionban volt kimutatható.