A MAGYAR TUDOMÁNYOS AKADÉMIA
TIHANYI BIOLÓGIAI KUTATÓINTÉZETÉNEK ÉVKÖNYVE
(1966)
(VOL. XXXIII.)

ANNALES INSTITUTI BIOLOGICI (TIHANY) HUNGARICAE ACADEMIAE SCIENTIARUM

TIHANY, 1966

ANNAL. BIOL. TIHANY

PRELIMINARY ANNOUNCEMENT

The Biological Research Institute of the Hungarian Academy of Science at Tihany celebrates in 1967 the 40th anniversary of its foundation. In the course of the proceedings several scientific meetings will be held in the Institute:

1. From the 28th–31st of August 1967: *International symposium on paleolimnology* sponsored by the International Association of Theoretical and Applied Limnology and Hungarian Academy of Sciences.
2. From the 1rst–2nd of September, 1967: *Commemorative session* giving an account of recent research work carried on in the Institute.
3. From the 3rd–7th of September, 1967: *Symposium on invertebrate neurobiology.*

For detailed information concerning participation, please inquire of Dr. János Salánki, Direktor (Biological Research Institute of the Hungarian Academy of Science, T i h a n y, Hungary).

ELŐZETES KÖZLEMÉNY

A Magyar Tudományos Akadémia Biológiai Kutatóintézete 1967-ben ünnepli fennállásának 40. évfordulóját. Az évfordulóval egyidejűleg több tudományos összejövetel kerül megrendezésre az Intézetben:

1. 1967. aug. 28—31.: *Nemzetközi Paleolimnológiai Szimpozium* a Nemzetközi Limnológiai Társaság és a MTA védnöksége alatt.
2. 1967 szept. 1—2.: *Emlékülés*, mely az Intézetben jelenleg folyó kutatásokról ad tájékoztatást.
3. 1967 szept. 3—7.: Szimpozium *„Idegi szabályozás gerinctelen állatokon"* címmel.

Részvételre vonatkozóan bővebb felvilágosítást ad dr. Salánki János igazgató (MTA Biológiai Kutatóintézete, Tihany).

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A CYTO-TOPOGRAPHIC STUDY IN THE GANGLIA OF *ANODONTA CYGNEA L.*

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Received: March 30th 1966

In the last years the fine structure of the nervous system of molluscs was standing in the forefront of interest (SCHLOTE 1957, BULLOCK 1961, ÁBRAHÁM 1963, ROSENBLUTH 1963, ZS.-NAGY 1964, SUGAWARA 1964, etc.). This interest is due primarily to the fact that the giant nerve cells present in the nervous system of the gastropodes provide excellent possibilities for the use of electrophysiological methods. The molluscs which present an important group in the course of phylogenesis dispose also — as regards their nervous system — of several important features which little attention has been given to until now, further their specific anatomical structure provides a chance to approach problems that cannot be studied suitably in other animals.

In the mussels a neuromuscular system exists consisting of one ganglion, a nerve centre and the adductors. It is possible to examine the activity of this system also under completely physiological conditions by recording the movement of the adductors. Nevertheless, in studies of this kind it is important to learn what kind of nerve cells are localized in the different areas of the various ganglia. In earlier works a suitable impregnation technique has been developed (GUBICZA and ZS.-NAGY 1964) on *Anodonta cygnea*. In other works the chief histological features of the ganglia (GUBICZA and ZS.-NAGY 1965) and the relationship between the nerve cells and the dimension of the animal (GUBICZA 1965) is presented. The objective of the work reported here is the investigation of the cell-topography of the nerves primarily with the view to establish whether micro electrophysiological methods may be sucessfully used and in which area in the ganglia. The clearing of this problem may be of importance also in the application of micromanipulation exstirpation techniques.

Material and methods

The experiments were performed in the cerebral, visceral and pedal ganglia of 12—18 cm long specimens of *Anodonta cygnea L.* In agreement with the objective of the examinations complete serial sections were made. A modification (GUBICZA and ZS.-NAGY 1964) of the CAJAL I. block-impregnation technique which proved most suitable for this purpose was used for the demonstration of nerve cells and fibres. Embedding of ganglia was controlled

under stereoscopical microscope, whereby with regard to the in situ position of ganglia always sagittal and horizontal plain of intersection could be secured in case of cerebral ganglia and in the case of visceral and pedal ganglia respectively. Serial sections of 10 μ were prepared. To facilitate the reconstruction of serial sections a projector similar to the so-called "PALKOVITS-table" (1962) used in variation statistical studies on nuclei was utilized. On the sheet of paper placed on the screen the structural elements of several subsequent sections are outlined and the dimension of cell groups and the direction of processes and fibre fascicles of cells may be recorded in a relatively simple way.

Results

Cerebral ganglion

The two cerebral ganglia are connected with each other by the cerebral commissure, and with the other two pairs of ganglia by the cerebropedal and cerebrovisceral connectives (CVc). Besides the connectives the chief branches as nervus pallialis anterior I, II, III, and several side branches proceed from the ganglia (SPLITTSTÖSSER 1913).

The nerve cells form a 100—150 μ thick cortex on the surface of the ganglion. In the area between the site of origin of cerebropedal connective and cerebral commissure this cortex is only 50 μ thick *(Fig. 1)*. Next to the place, of origin of the commisure and the connectives, however, this cortex is thicker, about 200 μ. Groups of large cells are observable in the space between the

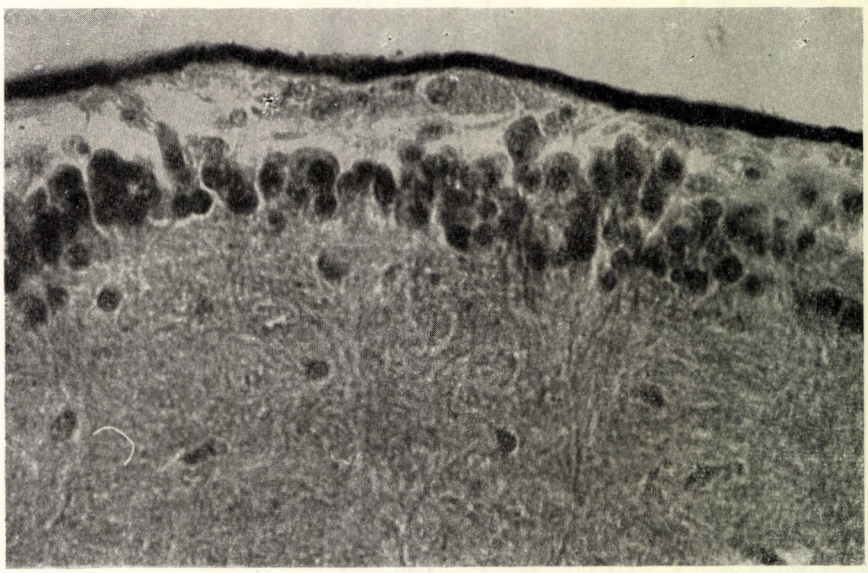

Fig. 1. Cerebral ganglion. Thin (40 μ) portion of cortex from the area between the cerebral commissure and the cerebropedal connective. × 500

1. ábra. Cerebrális ganglion. Vékony kéregrész (40 μ) a cerebrális commissura és a cerebropedalis connectivum közötti területről. Nagyítás: 500 ×.

site of origin of cerebral commissure and nervus pallialis. It has been also observed as a rule, that emerging nerves, as nervi adductoris anterioris, nervi retractoris anterioris and nervi protractoris are surrounded by the cells *(Fig. 2)*. It is seen in *Fig. 2* that there are several large cells among the cells localized at the cross-section of the emerging side branch. It is possible by serial sections to demonstrate the processes of these great cells in the initial part of emerging nerves.

Several large-sized multipolar cells independent of the cortex are also visible in the neuropile *(Fig. 3)*.

Thin and thicker (crisp) fibres are located in the neuropile. The thick fibres processing from the giant cells leave the ganglia through the nerve branches. The majority, about 60—70% of the robust fibres enter the cerebropedal connective. Only few such robust fibres entered the side branches as for instance nervus adductoris.

Large cells are very often surrounded by several layer of flat glia cells.

Visceral ganglion

The visceral ganglia which are medially completely fused are localized under the posterior adductor. The visceral ganglion is flattened in dorsoventral direction. CVc reaches it on its anterior tip and on its posterior tip originates nervus pallialis posterior major. Lobus branchialis (Zs.-Nagy 1966) originates on both sides of the anterior part of the ventrolateral surface. Besides these several thin nerve branches emerge from the ganglion (Splittstösser 1913).

The cortex is 150—200 μ thick in average, and is generally thickened close to the emerging nerves as in the case of the other two ganglia. The average dimension of cells is 15—40, μ large cells of 40—55 μ are relatively few and they are localized also here, near the emerging nerves. In lobus branchialis the dimension of nerve cells is less than 30 μ. These nerves are uni- or bipolar and are forming a cortex of varying thickness.

In the area between the spots from where the two pallial nerves originate a group of cells is observable which is wide in its middle and is narrowed laterally and separated from the cortex a by considerable neuropile area. Because of its localization it can be designated as nucleus posterior *(Fig. 4)*. This nucleus is constituted predominantly from uni-and multipolar cells, and close to the nucleus many robust fibres are observable in the neuropile *(Fig. 5)*. A cell group of similar appearance is localized in front of the area between the two CVc, this, however, is not separated from the cortex and may consequenly by regarded as its thickening.

In the neuropile of the visceral ganglion groups of thin and thick fibres passing in various directions are observable. The thick fibres may generally be traced until the initial section of emerging nerves. Groups of thin fibres passing unilaterally and also transversally between the sites of origin of nervus pallialis and the CVc are also observable. These paths are often recognizable also under stereoscopic microscope on the dorsal surface of the visceral ganglion. Minor or major groups of fibres from other nerve branches are also joining these tracks. Besides these, thin fibre groups of most varying directions are also demonstrable in the neuropile.

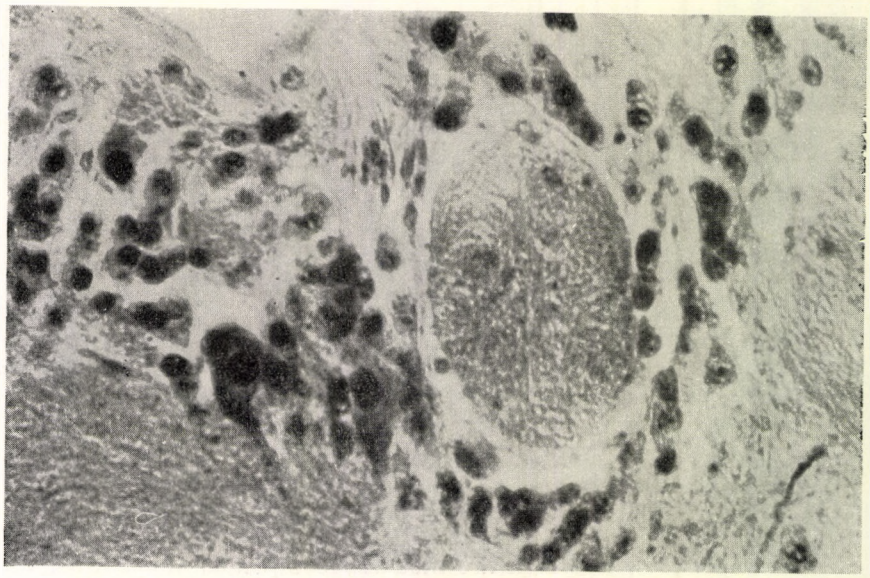

Fig. 2. Cerebral ganglion. Cross-section (N) of n. adductoris anterior surrounded by nerve cells — multipolar nerve cells. × 500

2. ábra. Cerebrális ganglion. A n. adductoris anterioris egy kilépő ágának keresztmetszete (N), amelyet idegsejtek vesznek körül — multipoláris idegsejtek. Nagyítás: 500 ×.

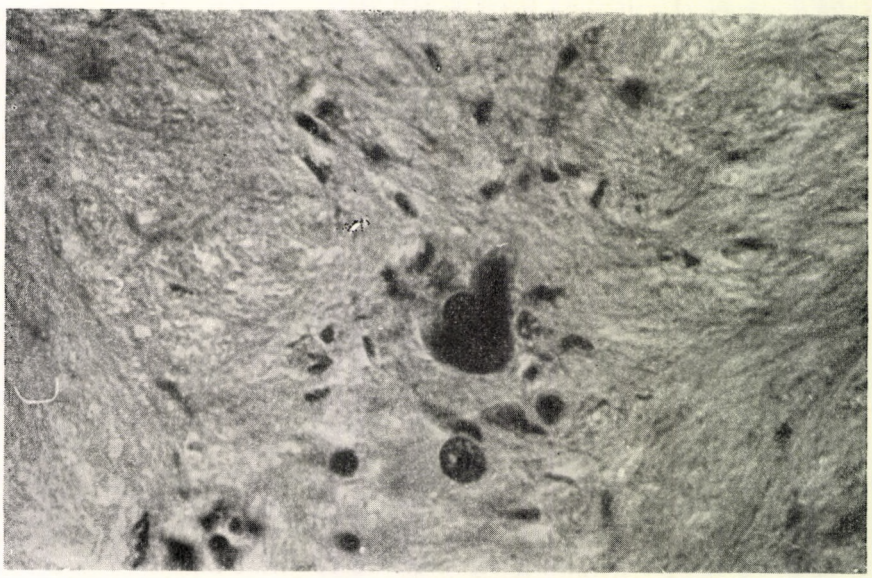

Fig. 3. Cerebral ganglion. Isolated multipolar cell in the neuropile. (The processes are readily observale in the serial sections). + 500

3. ábra. Cerebrális ganglion. Magános multipoláris sejt a neuropilben. (A nyúlványok sorozatmetszeten jól követhetők). Nagyítás: 500 ×.

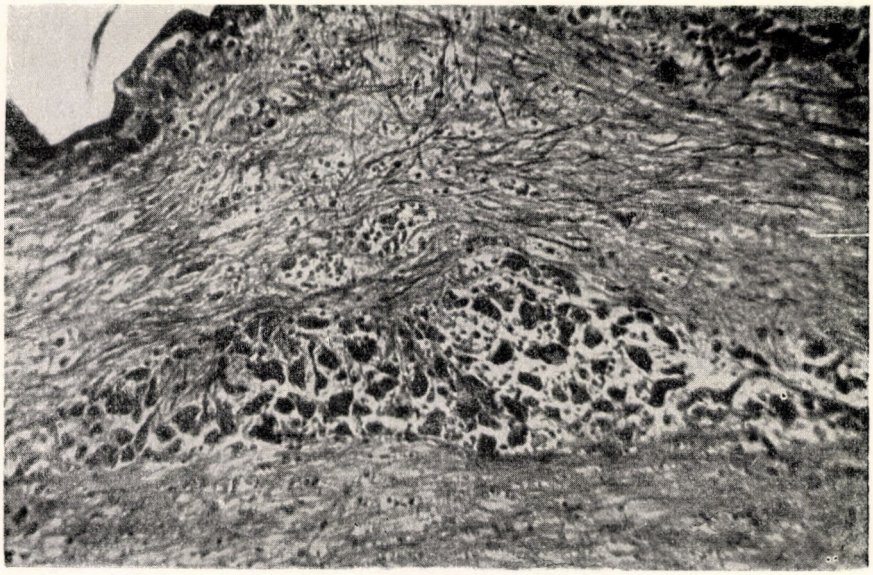

Fig. 4. General picture of the nucleus posterior of the visceral ganglion. × 160
4. ábra. A viscerális ganglion nucleus posteriorjának átnézeti képe. Nagyítás: 160 ×

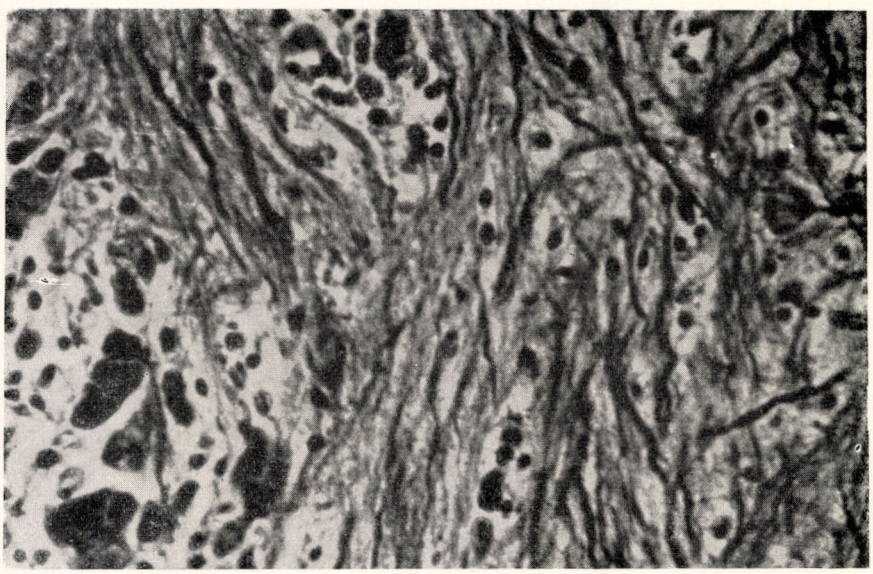

Fig. 5. Enlargement of the section of Fig. 4. The large numbered fibres are readily visible. × 500
5. ábra. A 4. ábra részlete. Jól láthatók a tömegben előforduló vastag rostok. Nagyítás: 500 ×.

Pedal ganglion

The bilateral lobes of pedal ganglion are not completely fused. Their medial surfaces are very close to each other, but organic contact exists only in the form of short commissure-kind connectives between them. On the dorsal and lateral surfaces of this pair of ganglia only very thin nerve branches originate. The cerebropedal connective arrives at the anterior tip. Nerve pedals I, II, III, and IV originate from the ventral side, moreover, from its lateral edge and from the posterior tip (SPLITTSTÖSSER 1913). It is worth mentioning that many individual variations both in number and in pattern of branching are observable in these nerves and very often differences exist also between the two sides.

On the dorsal and lateral surfaces where only thin nerves pass out from the ganglion a cortex is observable constituted chiefly of medium sized and smaller cells. A thicker cortex is found on the medial surface where large cells occur in relatively great umber. Their processes pass also to the opposite side across the connectives. The cortex is generally thicker and the emerging nerves are surrounded by coronary groups of large cells on the ventral surface, near the site of origin of the chief nerves. The thick axons of the large cells are passing towards the emerging nerves.

On this place large cells with light plasm are often observable. This area is extraordinarily rich in lamellar glia cells *(Fig. 6)*.

It should be noted that in the case of all three ganglia the thick fibres can be followed up to the initial section of the emerging nerves. Fibres of this kind are not demonstrable in sections of nerves lying at a greater distance from the ganglia. In some nerve branches, however, as for instance in the CVc *(Fig. 7)* and in nervus pallialis posterior maior these nerve cells appear solitarily or in small groups also at a greater distance from the ganglia.

Discussion

It may be established on basis of the experimental results that the cellular cortex of ganglia is far from being homogeneous everywhere. There are places where large cells are dominating, and in other parts of the cortex they are not demonstrable. A stratification, however, similar to that described by NAGY (1962) was not found in the cortex.

The structural differences in the various areas of cortex suggest that different functions are bound to its different areas. The thick axons visible around the place from where the nerves originate are observable also in the initial section of the nerves, which point to the fact, that these cells represent cetrifugal pathways i.e. they are of motoric nature. Thus, it is inferred that the areas where these giant cells and thick axons do not occur are most probably sensorial or associative areas.

No reference is made in earlier works to the nucleus posterior of visceral ganglion. This nucleus is probably of motoric character.

It would be decisive to explain the fact that in the peripherial nerves only thin fibers could be found. According to earlier electron microscopic observations of the CVc 75—80 per cent of the axons are thinner than 0.5 μ (LÁBOS et al. 1963). At the same time, it is worth mentioning that the axons of CVc are branching off in different directions (ZS.-NAGY and BENKŐ 1965).

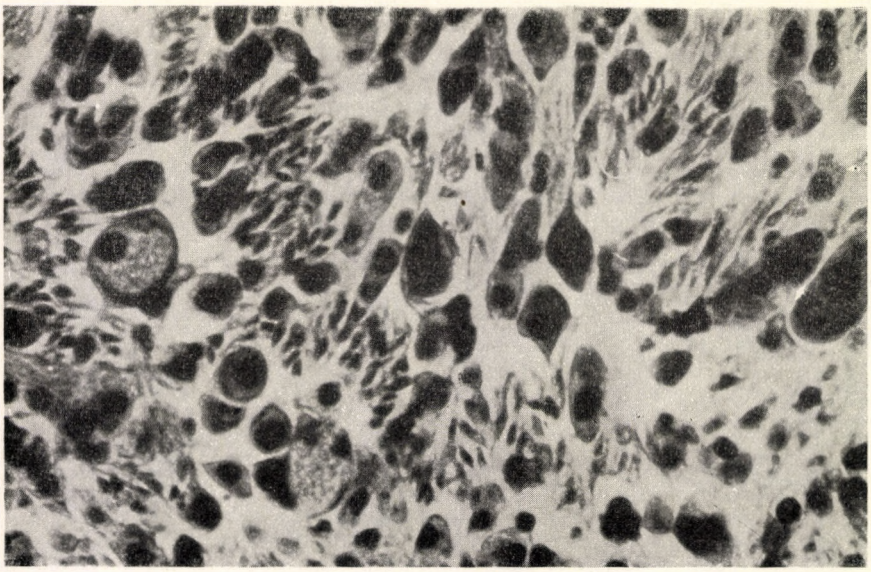

Fig. 6. Section of the ventral surface of pedal ganglion. × 500
6. ábra. A pedális ganglion ventrális felszínének részlete. Nagyítás: 500 ×.

On basis of these findings it is assumed that the thick axons are divided into thinner ones and this is why they are not demonstrable in the nerves in peripheral areas far from the ganglia.

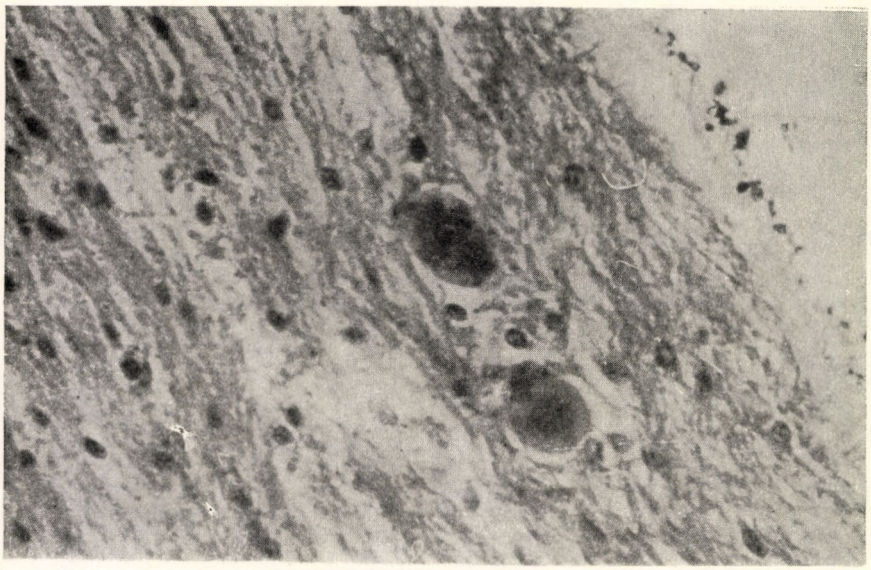

Fig. 7. Nerve cells in the CVc at 10 mm distance from the cerebral ganglion. × 500.
7. ábra. Idegsejtek a CVc állományában a cerebrális gangliontól kb. 10 mm-re. Nagyítás: 500 ×.

Summary

Authors investigated the cyto-topographic construction of ganglia of *Anodonta cygnea* by the impregnation technique. It was established that the large cells are present primarily in the immediate neighbourhood of the nerves, that originate from the ganglia and that their thick (2—3 μ) axons may be followed up until the initial section of the branches of nerves. In the cortex there are areas which contain only medium-sized or small nerve cells and are in contact only with thin axons. In the posterior part of the visceral ganglion groups of motor-like cells are observable, which are separated from the cortex and may be taken for nucleus posterior. In the visceral ganglia unilateral and transversal pathways are also observable.

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CYTO-TOPOGRÁFIAI VIZSGÁLATOK AZ *ANODONTA CYGNEA L.* GANGLIONJAIBAN

Gubicza András és Zs.-Nagy Imre

Összefoglalás

Szerzők impregnációs módszerrel vizsgálták az *Anodonta cygnea* ganglionjainak cyto-topográfiai szerkezetét. Megállapítást nyert, hogy a nagy sejtek, főleg a ganglionból kilépő idegek közvetlen környékén találhatók, vastag axonjaik (2—3μ) követhetők az degágak kezdeti szakaszáig. Vannak olyan kéregterületek, amelyek csak közepes vagy kisméretű idegsejteket tartalmaznak és csak vékony axonokkal állnak kapcsolatban. A viscerális ganglion hátsó részén motoros jellegű sejtek csoportja található, amely a kéregből lefűződött, s nucleus posteriornak nevezhető. A viscerális ganglionban azonos oldali és keresztezett átfutó pályák is találhatók.

ЦИТОТОПОГРАФИЧЕСКОЕ ИССЛЕДОВАНИЕ ГАНГЛИЕВ *ANODONTA CYGNEA L.*

Андраш Губица и Имре Ж.-Надь

Изучали цитотопографию ганглиев беззубки импрегнационным методом. Крупные нейроны обнаруживаются в непосредственной близости от места выхода нервов из ганглия, их толстые аксоны (2—3 μ) можно наблюдать до разветвления нервных стволов. В ганглиях имеются кортикальные участки, содержащие только мелкие и средние нейроны, которые связаны только тонкими аксонами. В задней части висцерального ганглия обнаруживается группа клеток моторного характера, отделяющаяся от корковых клеток; ее можно назвать nucleus posterior. В висцеральном ганглии можно видеть и прямые, и перекрещенные нервные пути.

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CONTRIBUTIONS TO THE MECHANISM OF TRYPTAMINE EFFECT ON THE ADDUCTOR ACTIVITY OF FRESH-WATER MUSSEL LARVAE

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Received: March 3rd 1966

In previous investigations it has been demonstrated that tryptamine in a 10^{-6}—10^{-3} M/lit concentration induces a rhythmic activity of considerable degree on the adductor of fresh water mussel larvae (LÁBOS et al. 1964 a). Activity is either not induced at all by serotonine (5-HT) or it leads to an initial, rapidly subsiding activity. Tryptamine effect is changing, too. It is considerably lower in the case of late glochidia.

Tryptamine effect can be inhibited by SH-inhibitors, methionine, cysteine (LÁBOS et al. 1964 b).

In view of the fact that organ preparations of adult *Anodonta* are generally 5-HT, but not tryptamine sensitive (SALÁNKI 1963; FÄNGE 1955) and according to certain assumptions the various indolealkylamines penetrate differently through the mammal blood-brain-barrier (VANE et al. 1961), it seemed necessary to further examine the existing differences and changes in the effect of 5-HT and tryptamine. Experiments were directed in the first place to the action of agonists and antagonists that proved to be effective on cholinergic, adrenergic and other mediator systems. We also examined the effect of oxidase inhibitors to elucidate the metabolic processes essential in tryptamine effect. With this purpose in view we also demonstrate a photoreactive system in the presence of tryptamine, taking into consideration that the measure of some metabolic inhibitions may be light dependent even in not photosynthesizing systems. We consider in the case of embryonal object the comparative pharmacological examination of morphogenesis and embryonal motorics as a source of useful relationships and therefore conducted the examination of tryptamine effect also in an earlier ontogenetic stage.

Method

We gained the glochidia and earlier embryos from the external gill plates of adult animals, then selected them with the aid of a fine pipette into groups of 25 and within these groups examined the activity of the various animals for at most 60' in general. With each substance — in the way described earlier (LÁBOS et al. 1964 a) — we counted the contractions per minute and the larvae in tone at least in four groups. So the activity values are to be understood

on every *Figure* and concerning each material and material mixture for at least 100 glochidia each.

The materials used in the experiments were the following: tryptamine HCl (TA; Fluka), serotonin creatinine sulhpate (5-HT; Fluka), serotonin hydrogen oxalate (Calbiochem), Indole-3-yl-propionic acid (IPA; BDH), indole-3-yl-butyric acid (IBA; Schuchardt), indole-3-yl-acetic acid (IAA; Schuchardt), melatonine, bufotenine, 5-methoxy-tryptamine, L-andrenaline, L-noradrenaline, 3,4-dihydroxy-phenylalanine (DOPA), α-methyl-DOPA, dopamine, DL-iso-propyl-noradrenaline (IPNA), dichlor-isoproterenol (DCI), dibenamine HCl, acetylcholine chloride (ACh; Sandoz), mecholyl, carbamylcholine chloride (CaCh; Fluka), acetylthiocholine iodide (Fluka), butirylthiocholine iodide (Fluka), eserine-salicylate, neostigminium bromide (Merck), Mytolon (Win-2747), picrotoxin (Fluka) γ- amino butyric acid (GABA; Reanal), histamine, cystamine HCl, tyramiten HCl (Fluka), L-glutamine (BDH) KCl, $CaCl_2$, KCN, NaN_3 (BDH), 2,4-dintrophenol (2,4-DNP), iproniazid, isonicotinic acid hydrazide (INH), ergotamine tartarate, riboflavin-5-phosphate Na (FMN; Schuchardt).

Results

1. Some characteristics of the tryptamine effect.

The group reaction of glochidia shows in the presence of effective tryptamine concentration a frequency-time diagram taking place through maximum (LÁBOS et al. 1964 a). The rhythmic contraction series produced by the individual shows the same distribution in time *(Fig. 1)*. Thus the maximum observed in the group-reaction is not only the consequence of the averaging but also of the individual reaction. Therefore the formal modeling of the rhythm taking place through the maximum may supply information on certain questions. Let us consider the adductor as a system with in- and output. In the input there is an analogous process of the binding and chemical transformation of the substance employed (tryptamine), while on the output of the system there is a rhythmic contraction series. The rhythm appears after a certain period of latency, the frequency increases, for a time the contractions follow each other in regular intervals, subsequently the frequency diminishes and the glochidia generally remain open in a relaxed condition of their adductor. If the velocity of the input process is the frequency maintaining factor then its realization in time takes place along an S-shaped diagram the median linear sector of which corresponds to the rhythm of often remarkably stable frequency. Beside the rhythm pattern indicated in *Fig. 1* we also supplied the diagram of the assumed analogous process gained with graphical demodulation.

The maximum of the tryptamine dosage-effect diagram *(Fig. 2)* is in tryptamine sensible populations around a concentration of 100 μg/ml (type A). The reduction of sensitivity in spring described previously (LÁBOS et al. 1964) may be very considerable and is presumably connected with maturity because it can be observed also on late winter glochidia and those before getting out. In the autumn the maximum frequency is generally 10/min and 100 μg/ml tryptamine elicits 2000—4000 contractions out of 100 glochidia while in the case of insensible populations 500 μg/ml tryptamine causes 400—700 contractions and the 2—3/min individual maximum frequency also appears later

(type B). The data refer to examinations conducted at room temperature with an illumination of some thousand lx.

The further experiments were conducted on populations giving tryptamine response of partly A, partly B type. The concentration employed was

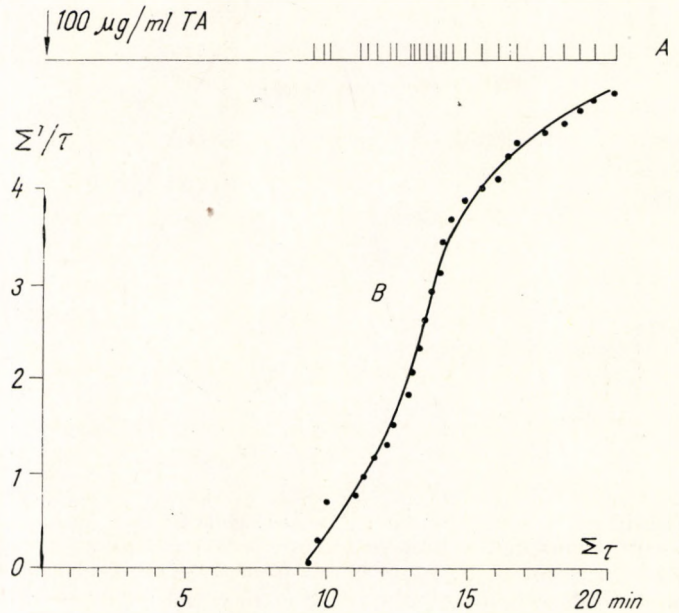

Fig. 1. Graphic demodulation of a rhythmic contraction series (upper line) induced by 100 µg/ml tryptamine on a single glochidium. *Abscissa:* time from the administration of tryptamine in minutes. *Ordinate:* sum of the $1/\tau$ values proportionate to the momentary frequency in arbitrary units, where τ_i is the time between the (i—1)th and i-eth contractions, observed after the administration. For further explanation see the text.

1. ábra Egyetlen glochidiumon 100 µg/ml triptaminnal kiváltott ritmikus kontrakciósorozat (felső vonal) grafikus demodulálása. *Abszcissza:* A triptamin adásától eltelt idő percekben. *Ordináta:* önkényes egységekben a pillanatnyi frekvenciával arányos $1/\tau$ értékek összege, ahol τ_i az adás után észlelt (i—1)-ik és i-edik kontrakció között eltelt idő. További magyarázatot lásd a szövegben.

50—100 or 500 µg/ml respectively and the value of the response pointed to the character of the population. With regard to the few months of difference between the appearance of the two populations not all examinations were carried out on both populations. Therefore in the course of the following the observation of pharmacological differences beyond the reduction of sensitivity may be expected.

2. The effect of indole derivatives

The effects of tryptamine, serotonin, melatonine, bufotenine, 5-methoxytryptamine, 3-indole acetic acid, 3-indole propionic acid, 3-indole-butyric acid were compared in 50—200 µg/ml concentrations.

Of the substances examined tryptamine and 5-methoxytryptamine are effective *(Fig. 3)*.

Serotonin and the other compounds elicit insignificant activity. Both preparations of serotonin used (creatinine sulphate and oxalate) are inefficient. In some cases and in higher concentrations serotonin induces higher initial activity which gradually subsides while in other cases this phenomenon also

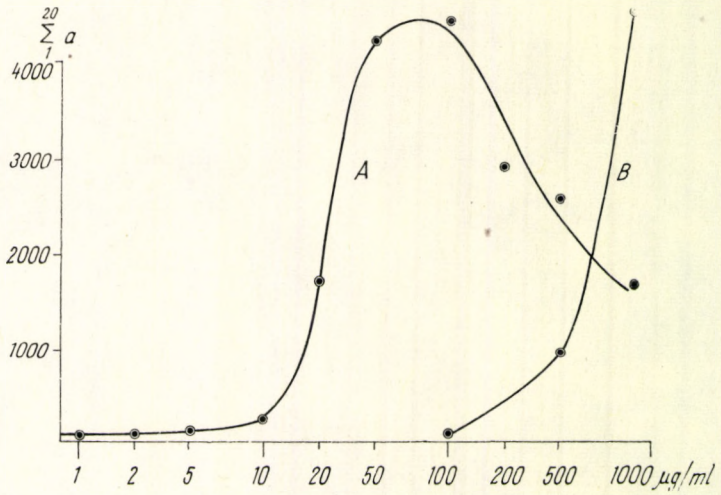

Fig. 2. Diagram of the tryptamine dosage effect in the case of populations sensitive (A) and less sensitive (B) to tryptamine. *Abscissa:* time in min., *ordinate:* the number of rhythmic contractions in the first 20 minutes following the administration. Every point signifies the number of the actions of 100 larvae each.

2. ábra A triptamin dózis-hatás görbéje triptaminra érzékeny (A) és érzéketlenebb (B) populáció esetén. *Abszcissza:* idő min-ban, *ordináta:* az adást követő első 20 perc ritmikus kontrakcióinak száma. Minden pont 100—100 lárva akcióinak számát jelenti.

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ails to come about. Even this minimum activity observed can be reduced if serotonin is administered together with flavinadenosine monophosphate (FMN) which forms a complex in the solution. The external interaction arising in the mixture diminishes the initial 5-HT rhythm which contradicts the assumption that the exchange of solution should be the cause of the activity. Tryptamine activity can be reduced too in the presence of FMN.

The effect of non-activating bufotenine and serotonin and 3-indole acetic acid was examined on 100 glochidia each upon tryptamine action. Bufotenine hardly or not at all while serotonin to a slight degree delays the tryptamine effect. The phenomenon may be based on the hardly explicit competition of the two substances — the ineffective 5-HT and the efficient tryptamine — in a penetration to the site of the action.

3-indole acetic acid given together with tryptamine potentiates its rhythm — inducing effect *(Fig. 4)*. At the same time the tone diagram observed in the presence of the mixture runs on lower values.

3. The effect of sympathicotropic pharmacons

In our previous examinations we demonstrated that adrenaline, noradrenaline, tyramine do not induce rhythmic and tonic adductor activity (LÁBOS et al. 1964 a).

In the present investigations we examined in 50—100 μg/ml concentration the action of DOPA, α-methyl-DOPA, dopamine, iso-propyl-noradrenaline (IPNA), ergotamine, dibenamine, dichloro-iso-propyl-noradrenaline (DCI), adrenaline, noradrenaline on the adductor activity of glochidia and on the rhythmic response of the adductor caused by tryptamine.

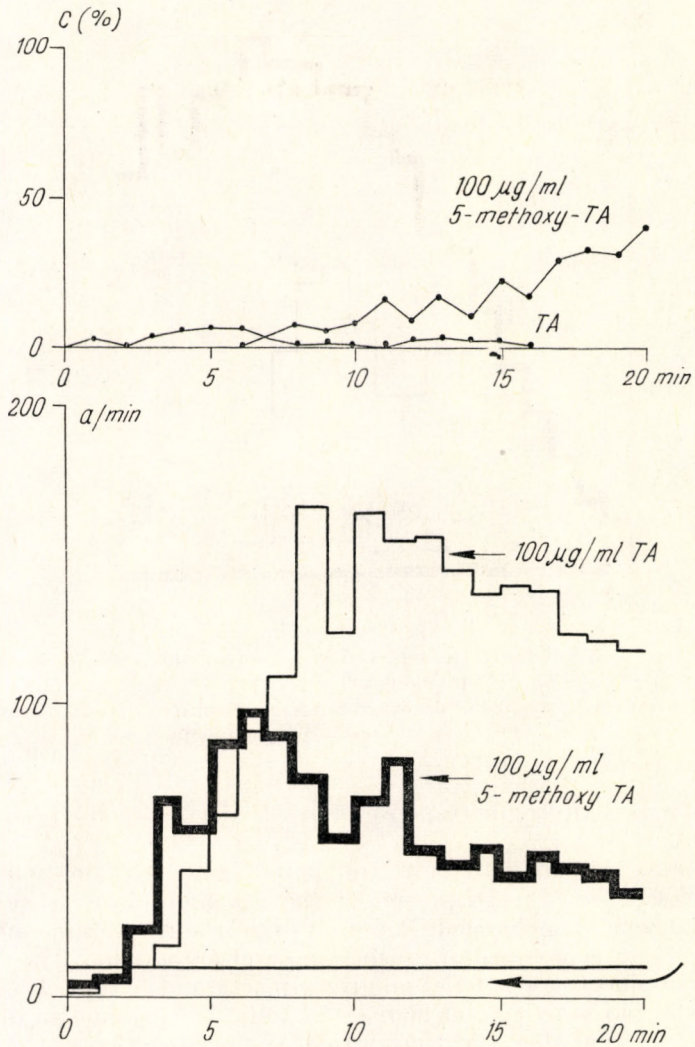


Fig. 3. Rhythmic activity and tonic closure inducing effect of 100 μg/ml tryptamine, 5-methoxy-tryptamine and 5-HT. *Abscissa:* time in min., *ordinate:* frequency of the rhythmic activity of 100 animals (a/min) and the proportion of the tonically closed larvae, c[%]. Population of intermediary sensitivity.

3. ábra 100 μg/ml triptamin, 5-methoxy-triptamin és 5-HT ritmikus aktivitást és tónusos zárást kiváltó hatása. *Abszcissza:* idő min-ban, *ordináta:* 100 állat ritmikus aktivitásának frekvenciája (a/min) illetve a tónusosan zárt lárvák aránya, c[%]. Átmeneti érzékenységű populáció.

In themselves, in concentrations of 10—100 μg/ml DOPA, α-methyl-DOPA, dopamine, IPNA on the basis of a 30 minute examination generally do not induce either rhytmic or tonic response on B-type population. Some

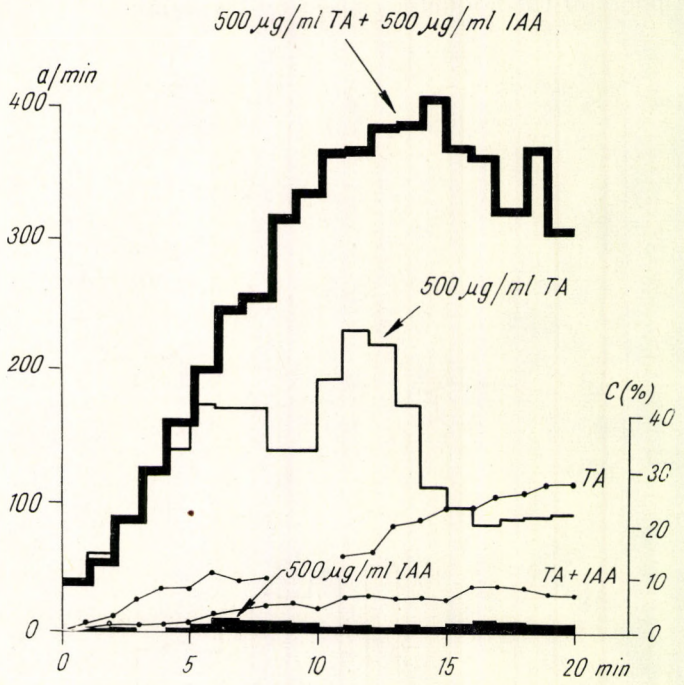


Fig. 4. Effect of 3-indole acetic acid (IAA). *Abscissa* and *ordinate* as in *Fig. 3.* Population of the B-type.

4. ábra Nagy koncentrációjú 3-indolecetsav (IAA) potencírozó hatása. *Abszcissa* és *ordináta*, mint a *3. ábránál.* B-típusú populáció.

populations gave a rhythmic response of low frequency in the presence of 100 μg/ml DOPA.

Noradrenaline, adrenaline in 100 μg/ml concentration potentiate the tryptamine response. The frequency of the rhythmic activity substantially increases, the tone is unchanged. Potentiation of the tryptamine effect can be also observed in the presence of dibenamine and ergotamine. The dibenamine effect was examined on a population giving reaction of A-type. Ergotamine in itself causes initial activity and increase of tone. Dibenamine in itself is ineffective, inducing neither rhytmic nor tonic response. Action of the α-receptor agonist and its antagonists is presented in *Figs 5* and *6.*

Examinations were conducted at room temperature with some thousand lx illumination in a neutral medium, thus, the decomposition of catecholamines accompanied by colouration took place in spite of the preparation of fresh solution.

The effect of IPNA differs from that of adrenaline and noradrenaline in that the potentiation appears later. The initial sector of tryptamine effect is

inhibited in the presence of IPNA, while the tryptamine effect extends to almost 1 hour. Potentiating dominates. The own effect of IPNA is insignificant, the tone unchanged. Results are presented in *Fig. 7.*

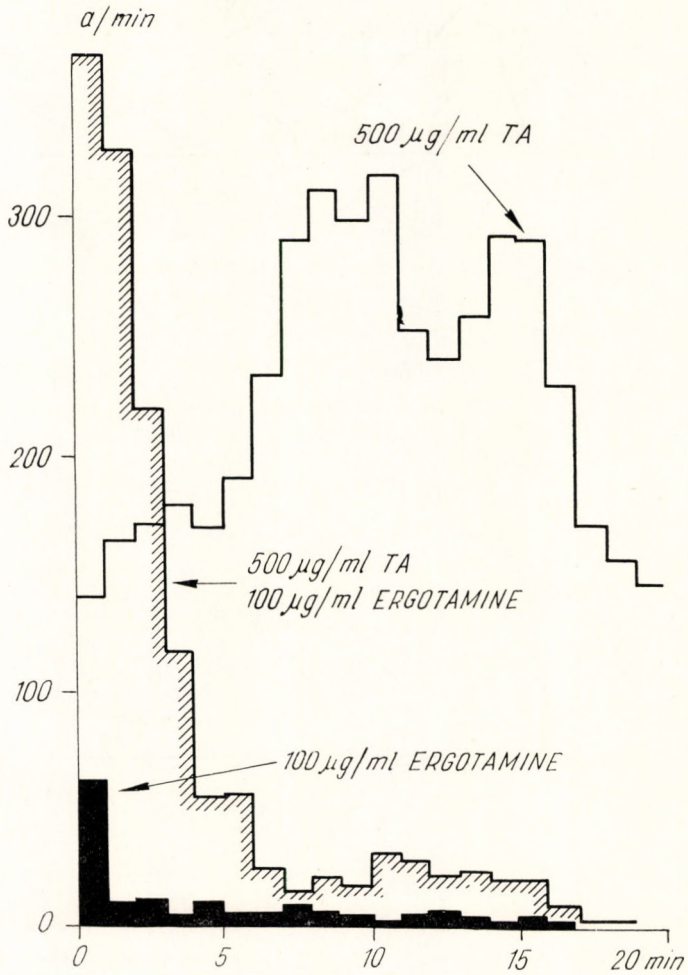


Fig. 5. Action of ergotamine on the tryptamine rhythm. *Abscissa* and *ordinate* as in *Fig. 3.*
5. ábra Ergotamin hatása a triptamin-ritmusra. *Abszcissza* és *ordináta* mint a *3. ábránál.*

DCI in itself depending on the cocentration is able to elicit a rhythmic activity of very high grade, without increasing the tone during that time. Thus 50 µg/ml DCI includes a rhythmic acitivity lasting for 10 minutes, with a peak in the 4—6th minute. This rhythmic activity almost completely subsides by the 10th minute. Until the 100—200th minute of the examination, however, almost 100 per cent of the glochidia get gradually into tonic contraction *(Fig. 8).* DCI increases the activity already in a concentration of 10 µg/ml ($\sim$ 40

μ M). This activity reaches its maximum in the 1—4th hour of the examination. No increase of tone occurs. In 100—200 μg/ml DCI the increase of activity takes place during the first 3—5 minutes of the examination. The time needed

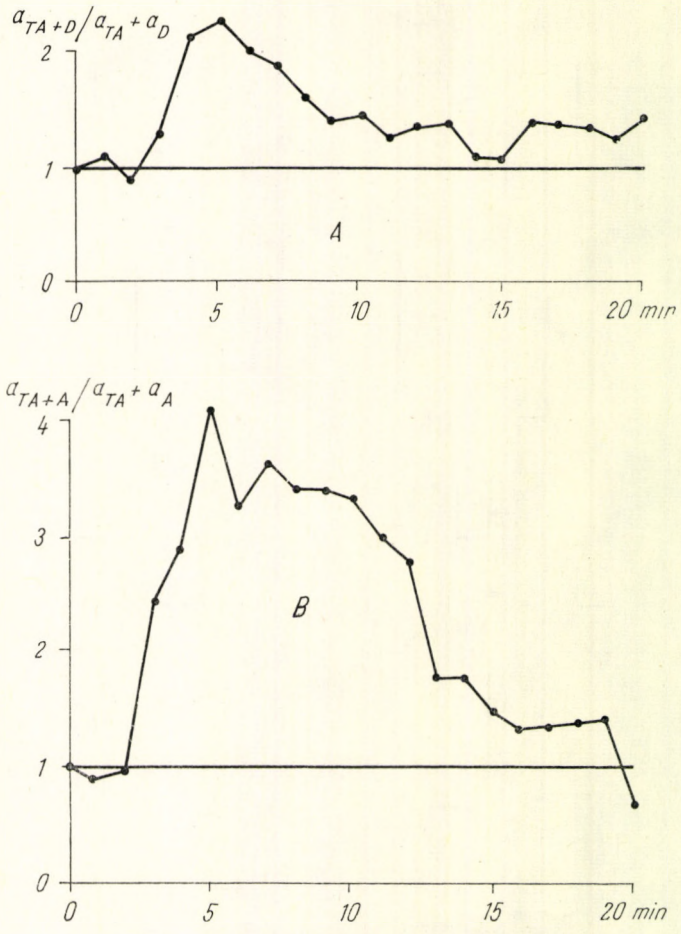


Fig. 6. A) Proportion of frequencies (a_{TA},a_D,a_{TA+D}) observed at the combined and separate administration of 50 μg/ml tryptamine and 70 μg/ml dibenamine (D)
B) Proportion of frequencies (a_{TA},a_A,a_{TA+A}) observed at the separate and combined administration of 500 μg/ml tryptamine and 100 μg/ml adrenaline (A)

6. ábra A) 50 μg/ml triptamine és 70 μg/ml dibenamine (D) együttes és külön történt adásakor észlelt frekvenciák aránya (a_{TA},a_D,a_{TA+D}).
B) 500 μg/ml triptamin és 100 μg/ml adrenaline (A) külön és együttes adásakor észlelt frekvenciák aránya (a_{TA}, a_A, a_{TA+A}).

for the closing of 50 per cent of the glochidia is 10—35 minutes. In the non-buffered system the pH was practically neutral. Thus the action of DCI may be considered as specific. When given together with tryptamine (100 μg/ml TA + 100 μg/ml DCI) a frequency exceeding the sum of the effect of the two substances measured separately can be observed.

Rhythmic activity caused by tryptamine is not substantially influenced by α-methyl-DOPA while it is somewhat inhibited by dopamine. Triptamine was employed in 500 μg/ml, α-methyl-DOPA and dopamine in 100 μg/ml concentration.

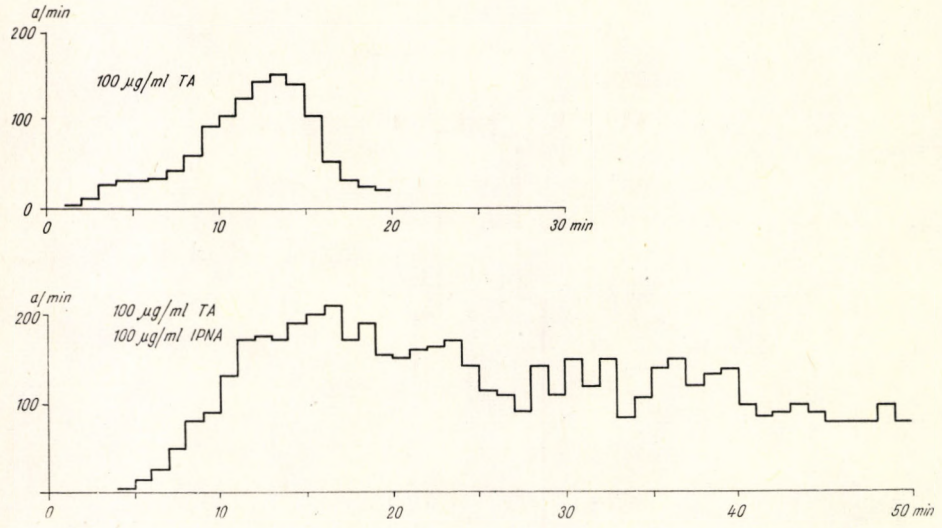


Fig. 7. Effect of isopropyl-noradrenaline (IPNA) on the tryptamine rhythm. For *abscissa* and *ordinate* see *Fig. 3.*

7. ábra Izopropil-noradrenaline (IPNA) hatása a triptaminritmusra. *Abszcisszát* és *ordinátát* lásd a *3. ábránál.*

4. The effect of cholinesters, cholinesterase-inhibitors, mytolon, picrotoxin, GABA, glutamine, histamine, cystamine, KCl, $CaCl_2$ on the rhythmic activity and the tryptamine rhythm.

Various cholinesters — acetylcholine, carbamylcholine, acetyl-β-methyl-choline, acetyl- and butyril-thiocholine, benzoylcholine in themselves induce neither lasting rhythmic activity nor tone. ACh in some cases elicits a rapidly (in 1—3 min) subsiding rhythm in high — 10^{-4} — 10^{-3} M/lit — concentrations. At room temperature and pH—7—8, with 4—5 mM substrate concentration in the complete homogenizate of 200 mg glochidium there is no measurable ACh-hydrolysis in 1—3 hours, and at 37°C only a few, which does not explain the ineffectiveness of ACh. When givin ACh in 1 m M concentration together with tryptamine, the tryptamine-rhythm arises to an unchanged degree *(Fig. 9).*

Prostigmine, mytolon by themselves are ineffective. Eserine in 1 mg/ml concentration induces considerable rhythmic activity. Given together with tryptamine, their effect generally gets summarized. The effect of UV-irradiated eserine changes depending on the dosage rate of irradiation. The growth, decrease then new increase of the effect may be in connection with the action of various photolytic and hydrolytic intermediates *(Fig. 10).* The conditions of

the irradiation are: 250 W, Hg-lamp, 50 cm, in a quartz test tube, for a period of lo" — 60'. The new increase refers only to the frequency of the rhythm.

Mytolon given together with tryptamine prolongs its effect. Histamine, cystamine do not induce rhytmic activity. Cystamine potentiates the rhythm induced by tryptamine.

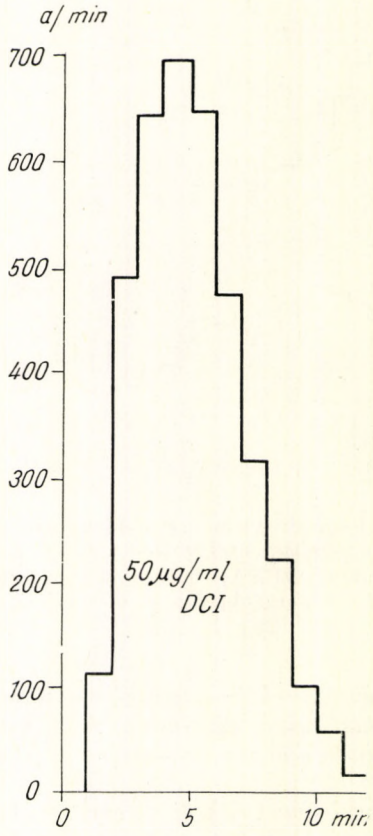


Fig. 8. Effect dichlor-iso-proterenol (DCI) For *abscissa* and *ordinate* see *Fig. 3.*
8. ábra Diklór-izo-proterenol (DCI) hatása. *Abszcissza* és *ordináta* mint a *3. ábrán.*

Picrotoxin in concentrations not higher than 100 µg/ml potentiates *(Fig. 11)* the tryptamine effect. γ-amino-butyric acid (GABA) and glutamine in 100 µg/ml concentration are ineffective by themselves and neither inhibit nor potentiate the tryptamine effect.

The KCl-tone increases with the presence of tryptamine. The rhythmic activity, when KCl and tryptamine are given togther, is indicated by the rhythm subsiding on account of the increase of tone; it lasts for a shorter time and becomes lesser as compared with the separate application of the two substances.

$CaCl_2$ in high (10—50 mM/lit) concentration blocks the rhythmic activity caused by tryptamine. The tone on account of the presence of $CaCl_2$ increases.

5. The effect on tryptamine response of agents acting on oxidative metabolic processes.

In our previous investigations we demonstrated the blocking effect of iproniazid in a short-term experiment on tryptamine-sensitive population (LÁBOS et al. 1964 a). In the course of the repetition of these experiments the

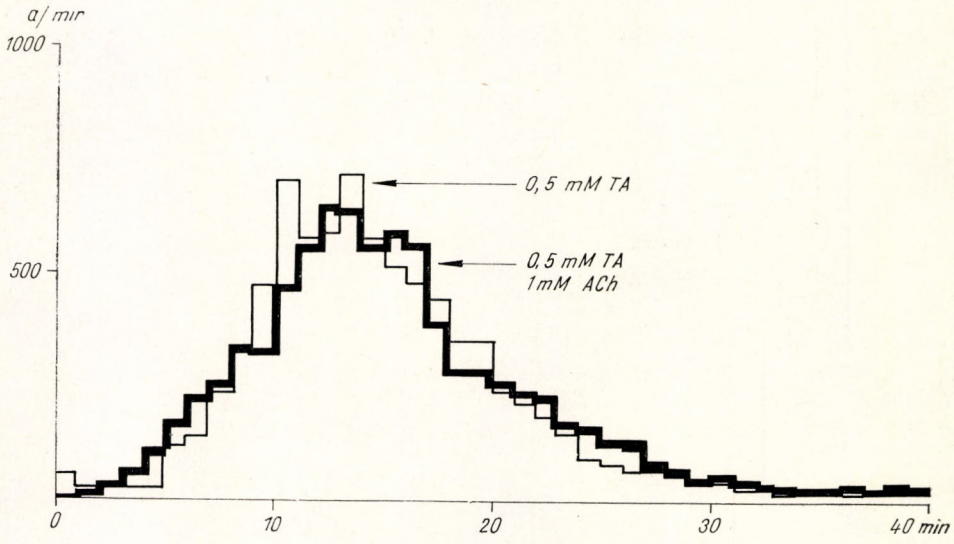


Fig. 9. Effect of acetylcholine (ACh) on the tryptamine rhythm (TA). For *abscissa* and *ordinate* see *Fig. 3.*

9. ábra Acetilkolin (ACh) hatása a triptamin-ritmusra (TA). *Abszcissza* és *ordináta* mint *a 3. ábrán.*

phenomenon proved to be essentially the delaying of the tryptamine effect. The equally monoamino-oxidase-inhibiting iso-nicotinic acid hydrazide (INH) exhibited still less blocking effect than iproniazide. In the 50—100 μg/ml concentration in a 20 minute experiment no potentiating was observed.

Conducting the examinations only on populations of the B type, 100 μg/ml $NaN_3$and 50 μg/ml KCN influence the tryptamine effect. In the case of KCN owing to its own effect the potentiating relates to the 1st and 2nd minute and manifests itself in an increase of about three times of the maximum frequency. In the case of NaN_3 potentiating of the maximum frequency is 8—10 fold*) Fig. 12)*. The own tone increasing effect of KCN dominates the tone-diagram which in the presence of tryptamine somewhat decreases. In the presence of NaN_3 + tryptamine mixture also the tone is potentiated.

From *Fig. 13* it appears that the otherwise slight effect of 5-HT is also potentiated by NaN_3. Essentially this is the appearance of a new effect, because 5-HT in itself is almost ineffective. In the presence of tyramine no potentiating comes into being.

2,4-DNP inhibits the tryptamine effect *(Fig. 14).*

6. Light sensitive system in the presence of tryptamine

The examinations outlined above were conducted in the presence of a 15 W microscopic tungsten lamp (50—70°, 120—140 mm). Measured with a mercury thermometer a heating up by 5° C/ hour was then observed. With the

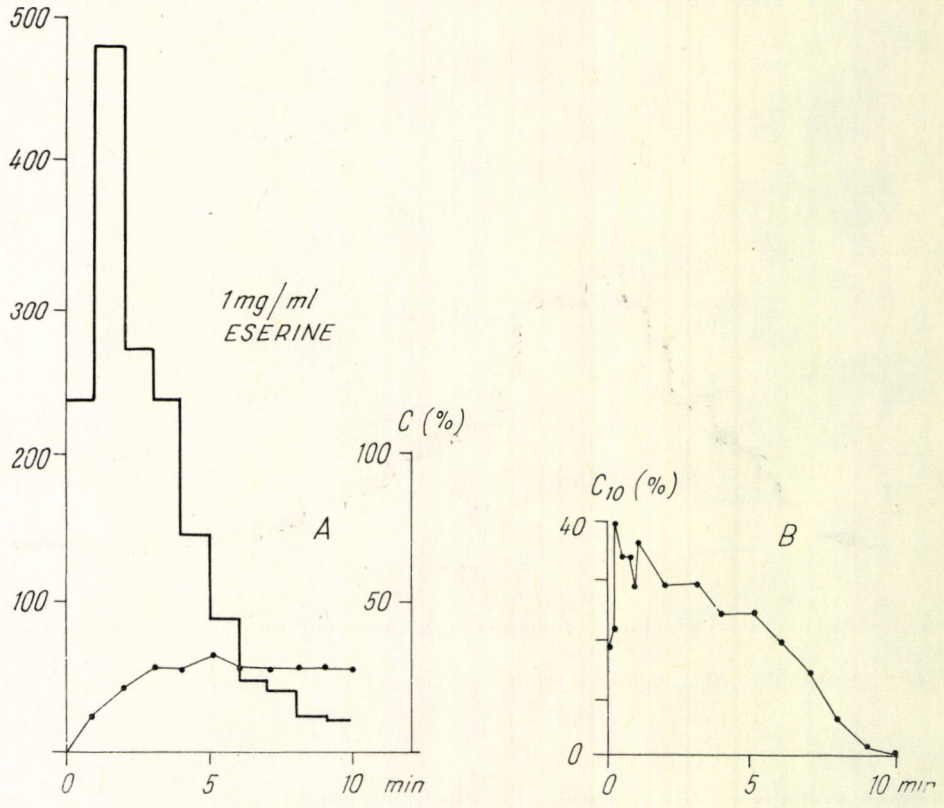


Fig. 10. A) Effect of eserine applied in high concentration. For signs see *Fig. 3.* B) Proportion of larvae found in tone in the 10th minute after the administration of irradiated 1 mg/ml eserine. On the *abscissa* the period of UV-irradiation.

10. ábra A) Nagy koncentrációban alkalmazott eserin hatása. Jelöléseket lásd a *3. ábrán.* B) 1 mg/ml eserin adása után a 10. percben tónusban talált lárvák aránya. *Abszcisszán* az UV- besugárzás ideje.

use of BG-17 infra filter this value was 3—4 C° hour. Thus the nominal temperature of the incubation mixtures rose by 3—5°C at the most.

We observed the following phenomenon: in the presence of 0.5 mM tryptamine, 1 mM monoiodide acetic acid, 10 mM methionine- after 20—50 minutes of incubation in this solution the rhythmic activity showed a very great difference depending on the illumination *(Fig. 15).* The light is of activating effect. The solution is not coloured as observed with the naked eye. The reaction takes place also in the presence of BG-17 infra filter and with ventilator cooling (evaporation). Without previous incubation the difference is less.

Of the three components only tryptamine has an activity increasing effect. Methionine and monoiodo acetic acid are, irrespective of the illumination, ineffective. The tryptamine effect on the other hand increases when the microscope lamp is lighted. This increase is lesser than in the case of the mixture, after a proper period of preliminary incubation.

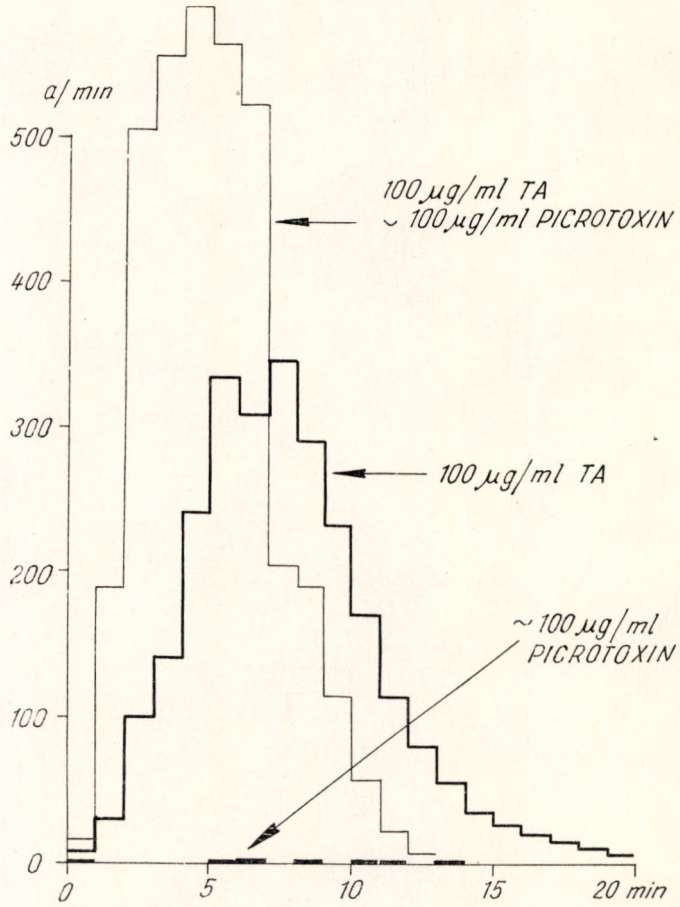


Fig. 11. Effect of 100 µg/ml picrotoxin on the rhythmic activity induced by 100 µg/ml tryptamine. Population of the A-type. For signs see *Fig. 3.*

11. ábra 100 µg/ml picrotoxin hatása a 100 µg/ml triptamin által kiváltott ritmikus aktivitásra. A-típusú populáció. Jelöléseket lásd a *3. ábrán.*

7. The effect of tryptamine on rotating forms

The glochidium stage is preceded by the so-called rotating embryonal stage when within membrane the embryo is rotating. For the rotation a ciliary zone is responsible on the surface of the embryo. The limits of the rotation are 20— 360. cph.

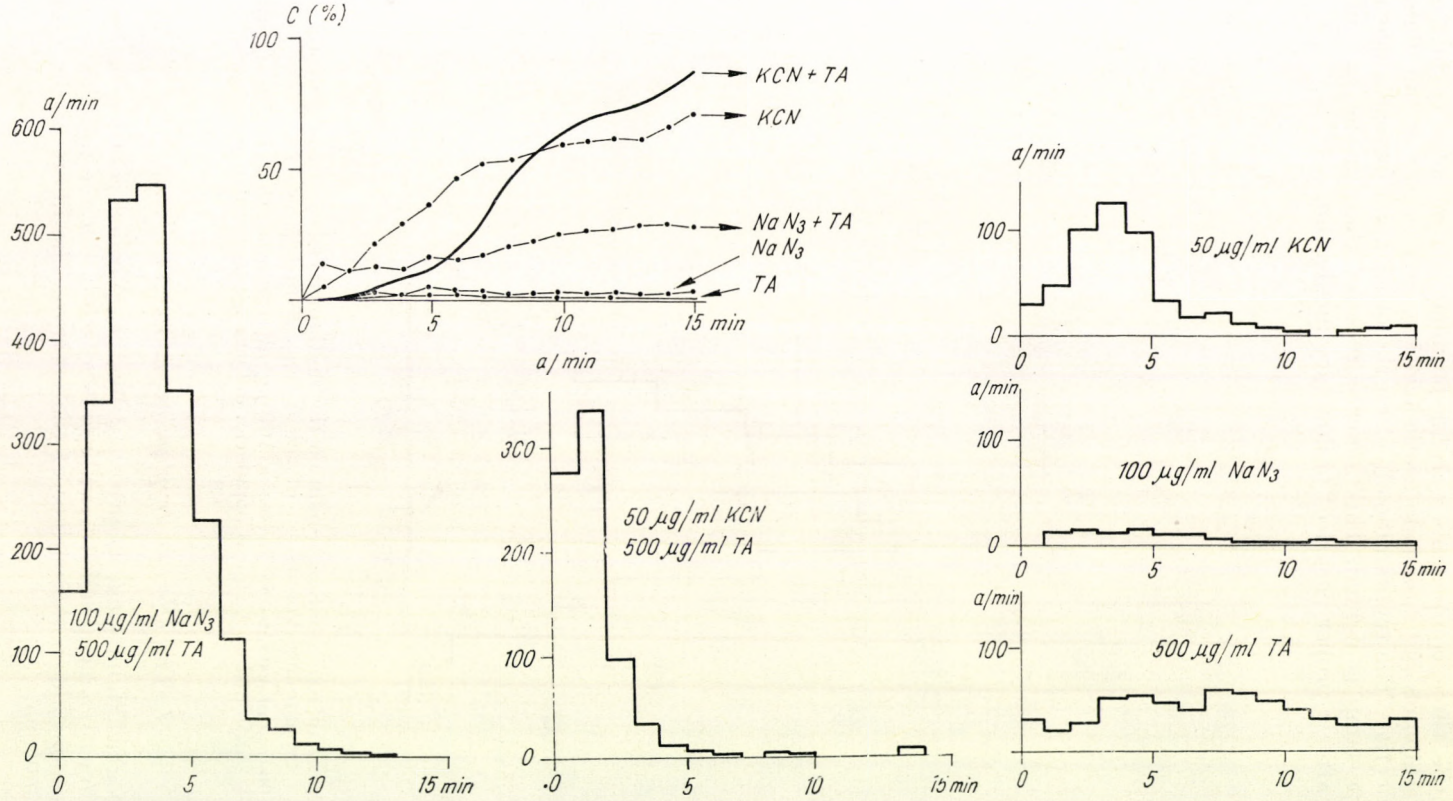


Fig. 12. Effect of KCN, NaN_3 on rhythm and tone induced by tryptamine. For sign see *Fig. 3.*

12. ábra KCN, NaN_3 hatása a triptamin által kiváltott ritmusra és tónusra. Jelöléseket lásd a *3. ábrán.*

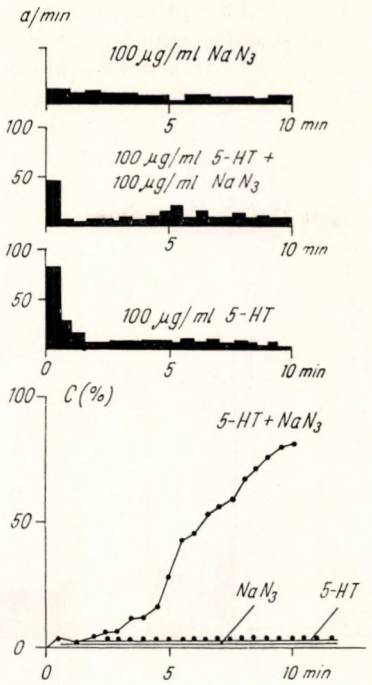


Fig. 13. Tonic closure at combined administration of 5-HT and NaN_3. For signs see *Fig. 3.*
13. ábra Tónusos zárás 5-HT és NaN_3 együttes adásakor. Jelöléseket lásd a *3. ábrán.*

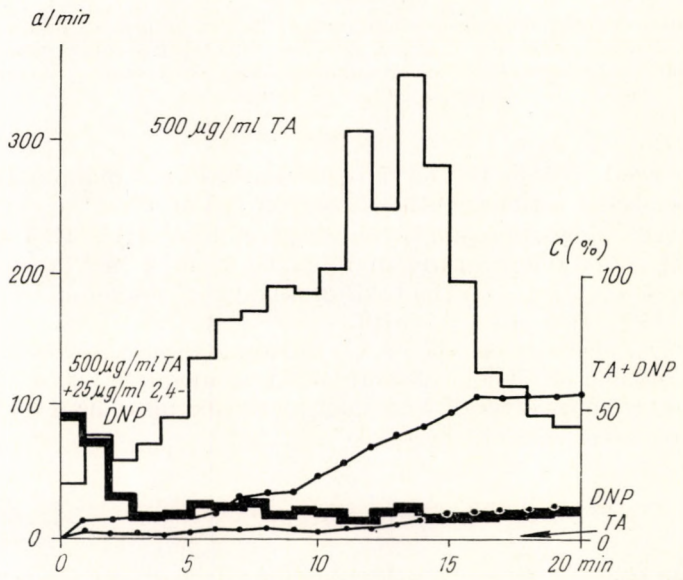


Fig. 14. The inhibition of 500 µg/ml tryptamine with 25 µg/ml 2,4DNP.
For signs see *Fig. 3.*
14. ábra 500 µg/ml triptamin hatásának gátlása 25 µg/ml 2,4-DNP-lal. Jelöléseket lásd a *3. ábrán.*

In the presence of 10 μg/ml tryptamine after a transitory acceleration (which in view of its instantaneous appearance is regarded as non specific) therotation after a few hours comes to a stillstand and the following phenom-

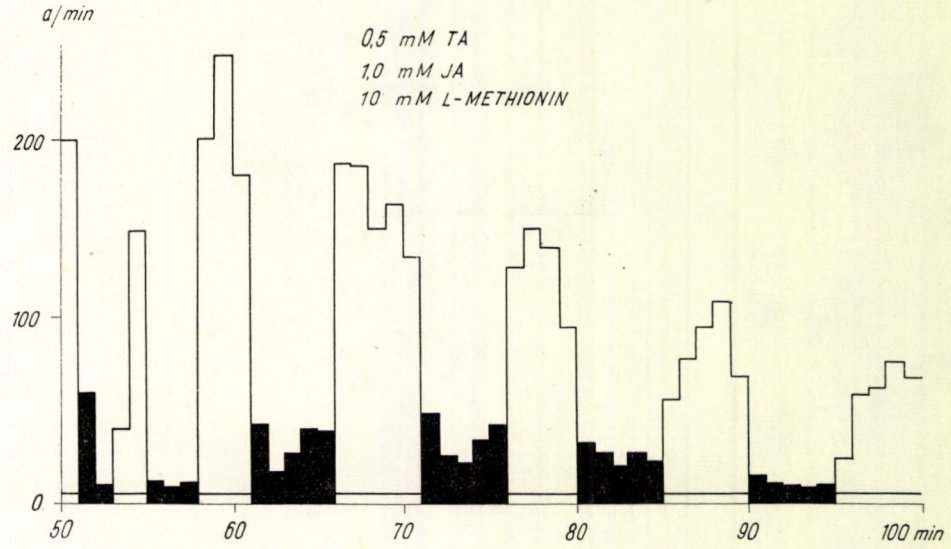


Fig. 15. Frequency of the rhythmic activity of 25 glochidia with alternating light and darkness. For signs see *Fig. 3.* Dark areas refer to dark, light areas to light time periods. Composition of incubation mixture: tryptamine, monoiodo-acetic acid JA and L-methionine

15. ábra Váltakozó megvilágítás és sötét mellett 25 glochidium ritmikus aktivitásának frekvenciája. Jelöléseket lásd a *3. ábrán.* A sötét területek a sötét, a világos területek a világos időszakaszokra vonatkoznak. Inkubációs elegy összetétele: triptamin, monojód-ecetsav (JA) és L-metionin.

enon is observed: within the larva is surrounded by a marginally branching cell mass connected with each other loosely or not at all.

Of other indole compounds the effect of IAA, IPA, IBA and 5-HT is examined at room temperature, in room light, in 2 mM/lit concentration.

The action sequence on the basis of 5 hours of mesaurement:

TA > IPA, IBA, IAA > 5-HT.

The effect also sets on when a very dense embryo suspension is standing for a few hours in a physiological solution. In an adequately diluted suspension the embryos for hours invarably conduct a rotating movement and no signs of "dysmorphogenesis" are observed.

Discussion

A main characteristic of the tryptamine effect is that the termination of the rhythm induced generally takes place in a relaxed condition of the adductor. The group reaction shows a time diagram running through maximum frequency.

Considering that in spite of this the individual reaction can be manyfold, it was desirable to examine also the individual rhythm. The individual rhythm appears after a latency period, its frequency increases and after a comparatively stable interval it decreases. We discuss this phenomenon according to a widespread digitalization principle. The development of the rhythm is conceived as a counting, similar to a frequency modulation mechanism (NEUMANN 1948). For increase and decrease of the frequency the velocity of the process is responsible. Then the modulating factor must show an S-shaped time diagram, the median, nearly linear sector of which corresponds to the stable rhythm. This mechanism does not demand exact elementary oscillators, only a nearly linear physical or chemical process for the origin of the reactions sometimes following each other with remarkably exactitude to be explained. This linear sector is the median part of the process S. This process can be manifold, e. g. permeation, oxidation or other chemical transformation.

To test whether the oxidation of tryptamine through indole acetaldehyde into indole acetic acid may explain the stop of the rhythm induced by indole acetic acid production we employed tryptamine together with indole acetic acid and found that indole acetic acid far from inhibiting even potentiates the tryptamine effect. Thus, it is not indole acetic acid production but some other chemical transformation which is responsible for the cessation of the rhythm. This is supported by the finding according to which the indole acetic acid (heteroauxin) is of lesser "morphogenetic" effect than tryptamine. Consequently some other reaction of tryptamine must be responsible also for the effect of the latter.

Similar phenomenon is the decrease of tryptamine effect with the progress of ontogeny. In this case also the development of a mediator system not based on indoles can be assumed simultaneously with the development of the innervation of the adductor. The ineffectiveness of cholinesters and that of ACh also observed when administered together with tryptamine *(Fig. 8)* argues against the cholinergic character of the inhibiting system in question. This is supported also by the slight ChE-activity. Similarly the examinations of PHILLIS (1966) on *Tapes* heart do not point, to the possibility of a cholinergic system functioning not with ACh. The activity observed in the presence of high eserine concentration is attributed to the fact that eserine is an indole derivative and on the 5th C atom of the indole nucleus, there is a methyl carbamic acid ester bond. The comparison of 5-HT, tryptamine, 5-methoxy-tryptamine pointed to the effect of the two latter compounds. According to VANE and coworkers (1961) the latter two substances penetrate easier thorugh membranes. From present observations the great effect — modifiyng role of the substitution on the C atom No 5 is clear, but we do not see the reason, steric or originating from charge, of the inhibition of permeation. The reactivity of the group on the 5th C atom seems to be more important. AXELROD (1962) pointed to the importance of the O and N-methyl transferase systems of the biogenic amines. This, beside or instead of its permeation — regulating role may supply a point of support for the effectivity of the 5-methoxy-tryptamine as against the ineffectiveness of the 5-HT. The efficiency of tryptamine is not clear. SAKHAROV and PÉCSI (1965) on *Anodonta* heart observed the protective effect of 5-HT from heat inactivation. Tryptamine and 5-methoxy-tryptamine were of considerably lesser effect in the same phenomenon. The inverse sequence of action stressed instead of permeation the importance of other factors. In the

excitatory processes of Molluscs catechol- and indole-alkylamines are supposed to be widespread mediators. Among them, concerning effect and occurrence tryptamine is less outstanding than 5-HT (CARDOT and RIPPLINGER, 1963; DAHL et al. 1962). Our present examinations on the other hand stress the specificity of the tryptaminerg activating effect against the indole and phenyl-alkylamines examined.

Among the theoretically possible causes of the decrease of sensitivity also inhibition based on gamma-amino butyric acid system could be assumed. Picrotoxin, which is of convulsive effect on Crustacean peripheric and central preparations and in the same place specific blocker of inhibitions (HICHAR 1960) increases the activity of the glochidia caused by tryptamine. The gamma-amino butyric acid, however, does not inhibit the same. FLOREY and CHAPMAN (1961) pointed out that in Crustaceans the inhibiting factor is not unconditionally GABA. Our present result also seems to point out that behind the potentiation of picrotoxin no GABA-erg mechanism should be sought. The ineffectiveness of glutamine (as a blocking agent) excludes also the presence of the glutaminergic inhibiting system.

Adrenaline and noradrenaline are of potentiating effect. DOPA α-methyl-DOPA, dopamine are ineffective, respectively the latter is slightly inhibiting. The effect of IPNA can be also regarded as potentiation.

The antagonists of the α- and β-receptors, dibenamine, ergotamine and DCI potentiate or induce tonic or rhytmic activity respectively. The effect of antagonists of this type points to the fact that the tryptamine response of the A and B type can be increased by α- and β-adrenolytic interference. Especially-explicit is the own activating effect of DCI. Since, however, no definitely inhibiting sympathic agonist was found among those examined it may be assumed that a catecholamine not applied here may be responsible for the decrease of tryptamine effect in spring.

The potentiating effect of the various catecholamines raises the possibility of the release of activating catecholamine under the influence of tryptamine. BUNAG and WALASZEK (1963) have brought the two-phase character of the vascular effects of serotonin into connection with the release of two secondary mediators of antagonistic effect. It is an open question whether in our case a so much combined system may prevail. In this case the activating effect of tryptamine could be regarded as catecholamine mobilization. In this hypothesis no inhibiting catecholamine ought to be postulated. There are simple peptides e. g. glycyl-glycine which block the tryptamine effect in 1 mM concentration.

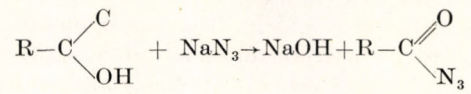
Finally it can not be left out of consideration in the evaluation of the potentianting effects of agonists that in the course of examination a number of coloured catecholamine oxidation products developed. BORG (1965) regards the development of the phenoxy- and indole derivative free radicals as an important primary step in the mode of action both of catechol and indole-alkylamines. Taking into consideration the reversibility of the one-electron oxidation, changes of action can arise in the course of oxidation as demonstrated in the photo-oxidation of phenothiazines (LÁBOS AND TURCSÁNYI 1965).

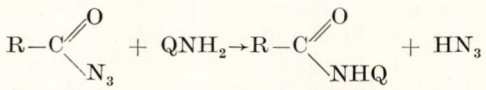
The effect of activating monoamines is potentiated by the inhibition of the oxidase ferments responsible for their decomposition. In mammal nervous system the inhibition of this widespread ferment induces excitation phenomena. Our previous finding on the inhibiting effect of the MAO-inhibiting

iproniazide (LÁBOS et al. 1964 a) and literary data (KARKI et al. 1962) on the ontogeny of the MAO-ferment (MAO level appearing and increasing with age (contradict the assumption that the energy released during tryptamine oxidation should be responsible for the increase of activity. This finding of ours on the other hand that cysteine inhibits the activity brought about by tryptamine (LÁBOS et al. 1964 b) argues for radical reactions and thus indirectly for the participation of oxidases.

The effect of KCN, NaN_3 and the activity inducing effect of KCN to the participation of an oxidase system. The effect of KCN substantially differs from that of KCl applied in the same concentration. The former leads to a tone and the rhythm simultaneously subsides (see *Fig. 12)* while the latter under a 1 mM/lit concentration does not lead to a tone. Thus the K- ion effects are not sufficient for the explanation of the phenomena accompanying the KCN action. The effect is a potentiating of a considerable degree in which the effective product argues for oxidative breakdown. More difficult to explain is the phenomenon that the NaN_3 potentiation can be observed also in the presence of the hardly effective 5-HT. The azide-potentiation can thus be interpreted also as block of relaxation. The hydrolysis of KCN also could not be neglected in the production of tonus.

The azide-potentiation is not specific for the tryptamine effect, so it can be assumed that the azide non specifically reacts with amines and the HN_3 developed in the reaction (STANNARD 1939) is responsible for the effects. The reaction is well known in organic chemistry (BRUCKNER 1965). The azide-acids in vitro react under comparatively mild conditions with amines since the N_3-group is very reactive and rich in energies.





In biochemistry for azide effects generally the development of HN_3 is made responsible for oxidase inhibitons (ref. HEWITT AND NICHOLAS 1963, STANNARD 1939). If the mechanism of the phenomenon is the outlined then binding of the amine in question must take place. The azide action led in the case of tryptamine, serotonin, to potentiating while in the case of tyramine this phenomenon failed to come about and in the case of serotonin even a tone, while in the case of tryptamine rhythm increase was observed. Therefore in our opinion the development of HN_3 in itself is no sufficient explanation and its bond with the amine — CO — NH— group might be a passible factor, too. This is of different effect in the case of the various amines.

The effect of 2,4-DNP stresses the significance of oxidative phosphorylation. The above azide-reaction also takes place more readily in the presence of activated i. e. esterified R—COOH group.

At present no other explanation is available for azido-potentiating which contains also specific for the various amines.

The photo-activation observed in the presence of methionine and monoiodo ethanoic acid can not be explained at present but it is evident that it takes

place on a coloured system. Since it is also supplied by tryptamine alone to a lesser degree, therefore the tryptamine binding can be already in connection with coloured substances. The KCN effect pointing to the participation of the cytochrome system and the coloured products which can be easily developed from tryptamine stress the possbility of the realization of a photochemical system.

BORG (1965) assumes free radicals in the first phase of the effect of biogenic amines. Our results on account of the realizability of the photoactivable system support the free radical formation. For this argues our preliminary finding according to which cysteine already in a concentration of 1—2 mM/lit readily inhibits the activating effect of tryptamine (LÁBOS et al. 1964 b). The potentiating and inhibiting effects of azide, cyanide active on oxidase systems and metalloferments and of 2,4-DNP decoupling oxidation from phosphorylation also argue for radical reactions. Consequently they support those hypotheses which assume behind the term "receptor" in the pharmacological sense a combined metabolic chain in which the electron-transfer reactions and phospholipids are deeply involved (DIKSTEIN and SULMAN 1965 a. 1965 b; WOOLEY and GOMMI 1964).

In view of the fact that in the ontogeny of glochidia the metamorphosis is preceded by a change in motorics namely lasting tone, the comparative pharmacological analysis of morphogenesis and embryo-motorics seems to be justified. BUZNYKOV (1963—64) developed incubation mixtures containing indole-compounds influencing both the motorics and morphogenesis of larvae. On the basis of his results and assumption the humoral mediators of ontogeny may include in an undifferentiated form also the specializing nervous transmitter systems. Further the fact that the oxidative desamination of tryptamine rather specifically activating the glochidium mechanics leads to the development of one of the plant growth hormones indole acetic acid (hetero-auxin) points to some common traits of morphogenesis and motorics. The development of amine incorporation and peptide bond assumed in the potentiating effect of NaN_3 motorics directs the attention also toward morhogenesis. Our unpublished data according to which chloramphenicol and aureomycin elicit motoric activity which are definitely substances influencing protein and nucleic acid synthesis, similarly require the examination of the common mechanism of embryonal motorics and morphogenesis.

According to our experiments the embryonal development preceding the glochidium stage can be disturbed with certain indole-compounds. Tryptamine also here proved to be more active than other derivatives.

Summary

The rhythmic motoric activity induced by tryptamine of the glochidia of the fresh water mussel *(Anodonta cygnea L.)* was examined alone and in the presence of various pharmacons. For the modeling of the rhythm a counting off mechanism was assumed. The cause of the subsiding of the rhythm could not be the development of indole acetic acid, because this agent potentiates the tryptamine effect.

By the examination of various indole derivatives we stress te specificity of the tryptamine effect. In concentrations of 10^{-4}—10^{-3} M/lit 5-methoxy —

tryptamine and eserine are indole derivatives of minor action but effective. Indole alkylic acids, melatonine, bufotenine are ineffective.

To explain the reduction of sensitivity observed with the progress of ontogey we assumed the development of a new mediator system. This can not be a cholinergic, GABA-ergic, glutaminergic system, since ACh,GABA, glutamine do not inhibit the activity caused by tryptamine. The application of sympathicolythica, however, argues for the development of a catecholamine inhibiting system. Dibenamine and ergotamine potentiate the tryptamine effect. Dichlor-isoproternol in itself is of activating and given together with tryptamine of potentiating effect. Of the catecholamines examined adrenaline, noradrenaline, IPNA are potentiating, while at tryptamine insensitive population dopamine, DOPA, α-methyl-DOPA are ineffective. Thus, the inhibiting catecholamine which can be made responsible for the activating effect of the sympatholytics is not among these.

It can be assumed that in the course of ontogeny also inhibiting and activating systems based on adrenergic mediation develop.

The effects of NaN_3 KCN and the inhibiting action of 2,4-DNP stress the importance of the oxidative metabolic processes. The mechanism of the azide action was discussed. The cyanide effect suggests the direct role of terminal oxidation. Photo-activation developed in a special incubation mixture (tryptamine + methionine + mono-iodo-acetic acid) also assumes the participation of a coloured (cytochromic?) system and stresses the importance of mechamisms with free radicals.

Action with indole-compounds can be demonstrated also on the morphogenesis of earlier embryonal forms. Tryptamine is also here of outstanding effect and in 100 μg/ml concentration brings about dysmorphogenesis, swel_ing and desintegration.

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ADATOK A TRIPTAMINHATÁS MECHANIZMUSÁHOZ ÉDESVÍZI KAGYLÓLÁRVÁK ZÁRÓIZOMTEVÉKENYSÉGÉN

Lábos Elemér

Összefoglalás

Édesvízi kagyló *(Anodonta cygnea L.)* glochidiumainak triptaminnal kiváltott ritmikus motoros tevékenységét vizsgáltuk egyedül és különböző farmakonok jelenlétében. A ritmus modellezésére leszámlálási mechanizmust tételeztünk fel. A ritmus csillapodásának nem lehet oka indolecetsav keletkezése, mert az a triptaminhatást potencírozza.

Különböző indol-származékok vizsgálata révén a triptaminhatás specifikus voltát hangsúlyozzuk. 10^{-4} — 10^{-3} M/lit koncentrációban kisebb hatású, de hatásos indol-származék az 5-methoxytriptamin és eserin. Indol-alkilsavak, melatonin, bufotenin hatástalanok.

Az ontogenezis előrehaladtával észlelhető érzékenységcsökkenés magyarázatára az új mediátor-rendszer kialakulását tételeztük fel. Ez nem lehet kolinerg, GABA-erg, glutaminerg rendszer. Ugyanis ACh, GABA, glutamin nem gátolják a triptamin okozta aktivitást. Azonban szimpatikolitikumok alkalmazása katecholamin-gátló rendszer kialakulása mellett szól. Dibenamin és ergotamin potencírozzák a triptaminhatást. Diklórizoproterenol önmagában aktiváló és triptaminnal együtt adva potencírozó hatású. A vizsgált catecholaminok közül adrenalin, noradrenalin, IPNA potencíroz, dopamin, DOPA, α-metil-DOPA hatástalan. Így a szimpatolitikumok aktiváló hatásáért felelőssé tehető gátló catecholamin nem ezek között van.

Feltehető, hogy az ontogenezis során adrenerg mediáción alapuló gátló és aktiváló rendszerek is kialakulnak.

A NaN_3, KCN potencírozó, 2,4-DPN gátló hatásai az oxidatív anyagcserefolyamatok fontosságát hangsúlyozzák. Az azid-hatás mechanizmusát taglaltuk. A cianid-hatás a terminalis oxidáció direkt szerepére utal. Speciális inkubációs elegyben (triptamin+methionin+monojódecetsav) létrehozott fotoaktiváció ugyancsak színes (citokrom?) rendszer részvételét tételezi fel és szabad gyökös mechanizmusok fontosságát húzza alá.

Az indol-vázas vegyületekkel történő aktiválás korábbi embrionális alakok morfogenezisén is kimutatható. A triptamin itt is emelkedő hatású és 100 μg/ml koncentrációban duzzadást, dysmorphogenesist, dezintegrációt hoz létre.

К ВОПРОСУ О МЕХАНИЗМЕ ДЕЙСТВИЯ ТРИПТАМИНА НА ЗАПИРЯТЕЛЬНУЮ МЫШЦУ ГЛОХИДИЕВ БЕЗЗУБКИ

Э. Лабош

Изучали ритмическую моторику глохидиев беззубки, вызванную триптамином. Урежение ритма не связано с образованием индолуксусной кислоты.

При сравнении действия различных производных индола выяснился специфический характер влияния триптамина. Активными, хотя и в меньшей степени, были 5-метокситриптамин и эзерин. Индол-алкильные кислоты, мелатонин и буфотенин неэффективны.

Предполагается, что понижение чувствительности на более поздних стадиях онтогенеза связано с образованием новой системы медиаторов. Она не может быть холинэргической, ГАМК-эргической либо глютаминэргической. Опыты с симпатиколитиками указывают на появление тормозной системы, связанной с катехоламинами. Изопропилнорадреналин обладает замедляющим, но потенциирующим действием, дихлоризопротеренол сам обладает активирующим действием, а совместно с триптамином дает потенциирующий эффект. Это явление связано со свойствами β-адренорецепторов. Эта рецепторная система представлена в недифференцированной форме, так как эффект триптамина потенциируется и при даче антагонистов α-адренорецепторов. Из примененных катехоламинов адреналин, норадреналин и изопропилнорадреналин потенциируют, а допамин и диоксифенилаланин неэффективны. Таким образом, в это число не входит тот катехоламин, который тормозит активирующее действие симпатиколитиков.

Можно предположить, что и активирующие и тормозящие системы развиваются в онтогенезе на основе адренэргической медиации.

Влияние NaN_3, KCN, 2,4-ДНП подчеркивает значение окислительного метаболизма. Описан механизм действия азида. Влияние цианида указывает на прямое значение терминальной оксидации. Опыты с фотоактивацией, созданной в условиях специальной инкубационной среды, указывают на участие цветной (цитохромы?) системы и важность свободно-радикальных механизмов.

Активацию индольными производными можно наблюдать и на более ранних стадиях морфогенеза. Триптамин, будучи наиболее активным и на этих стадиях, вызывает нарушения развития и дезинтеграцию.

ANNAL. BIOL. TIHANY	33	37—44	HUNGARIA 1966

HISTOLOGICAL AND CHEMICAL STUDIES ON THE YELLOW PIGMENT PRESENT IN THE NERVE- AND OTHER TISSUES OF *ANODONTA CYGNEA L.*

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It is known from literature (ref. GOODWIN 1952) that various carotenoids exist in the organs of several marine lamellibranchiates. The data refer chiefly to *Mytilus californiatus*, nevertheless, investigations were performed also on the tissues of other species as: *Pectenculus gylcimeris*, *Pecten maximus*, *Pecten Jacobaeus*, *Volsella nodiolus*, *Lima excavata* and other species belonging to the *Pleurobranchus* genus. The analyses show that beta-carotene and lutein are present in highest quantity in the tissues examined. Besides these, however, other carotenoids as glycimerin, pectenoxanthin, mytiloxanthin, hopkinsiaxanthin, zeaxanthin and astaxanthin were also demonstrable. The amount of these carotenoids in the tissues of the molluscs depend on feeding conditions and are presumably related to certain metabolic processes and even to sexual cycles (GOODWIN 1952).

It is known long ago that the central nervous system (SCHULTZE 1879) and also several other tissues of fresh-water *Anodonta* and *Unio* species contain a surprising quantity of yellow pigments. No chemical data were found in literature with regard to the analysis of this pigment. It was suggested by NAGY (1962) on basis of certain positive histochemical reactions that the yellow pigment present in the nerve cells of *Unio pictorum* is identical with lipofuscin and this author observed a parallelism between accumulation of this pigment and the age of the animal. She did not find carotenoids in the nerve cells (NAGY 1962). It was thought necessary to investigate this problem not only because of the above contradiction existing between fresh-water and marine molluscs but also because it is suggested that a relationship exists between extensive pigmentation and the function of cells. The objective of these investigations was the histological and qualitative chemical analysis of these pigments.

Material and methods

Investigations were performed on 13—20 cm long specimens of *Anodonta cygnea L.* The animals were kept in aquaria containing Balaton-water. Pigment analyses were conducted on the tissues of ganglia and feet in different seasons of the year.

The histological localization of the pigments is well observable in native cryostat-sections. For this purpose 8 μ thick sections were made. The sections were treated with concentrated acids (HCl, H_2SO_4, TCA) for the histochemical demonstration of carotenoids (PEARSE 1960). Staining with alcoholic Sudan-black was used (PEARSE 1960) for the demonstration of lipoids in general.

To avoid emulsion formation the pigments were extracted from the tissues in the presence of anhydrous Na_2SO_4 by various organic solvents. Benzene, ethanol, acetone, carbon tetrachloride, carbon disulphide, n-heptane, n-hexane, cyclo-hexane and petroleum ether were used. The absorption of UV and visible light by the extracts was measured by BECKMAN Model G-2400 spectrophotometer at 365 mμ wave-length, whereas the visible emission of the samples with the same spectrophotometer using its fluorescence device (DU/DK).

The CARR-PRICE reaction was also performed for the demonstration of the carotenoids.

A synthetic (95%) beta-carotene (Light) solution was used as standard.

Results

Differences in pigmentation were observed in the individuals examined and also in the various tissues. In some individuals the colour of the tissues were paler yellow with a shade of brown in it and the tissues of other specimens were of bright yellow colour often with a red tint. No relationship between degree of visible pigmentation and size of animals was demonstrable. Because a relationship exists between size and age of the molluscs, consequently no parallelism was observable between increased pigmentation and the age of animals. Young, small sized mussels may also be strongly and adult large ones less pigmented. Uniform pigmentation was observed in general in mussels originating from the same habitat.

In the cryostat-sections the localization of the bright yellow pigment is readily seen. The cytoplasm of nerve cells contains very much golden yellow pigment granules of 1—5 μ size. These granules occur sometimes in such a great number that they are fused along their bordering lines and are forming a mass of pigment which fills in the whole cytoplasm *(Fig. 1 A)*. Yellow granules of the same appearance are observable in the glia cells, in the epithelial cells of the foot, in the cells of connective tissue, in the epithelial cells of the duct of sexual organs and in the most varying cell formations of gametogenesis. In all these areas the yellow colour turns blue on the effect of concentrated acids and this colour is maintained for longer or shorter periods. Destruction of sections results soon after a treatment with cc. H_2SO_4, therefore cc. HCl is more suitable for examination purposes *(Fig. 1B)*. In the ganglion the bright yellow colour which is observable also by the naked eye changes blue also in Susa fixative for instance and disappears later in the dehydrating alcoholic bath series.

After 1 minute long staining with Sudan-black an intensive black colour reaction is observable in areas where the yellow pigment granules are localized *(Fig. 1/C)*.

The yellow pigment is not soluble in water, it may be extracted, however, from the tissues by any of the above mentioned solvents. The sections are also indicative of this. A yellow substance of a considerable light absorption may

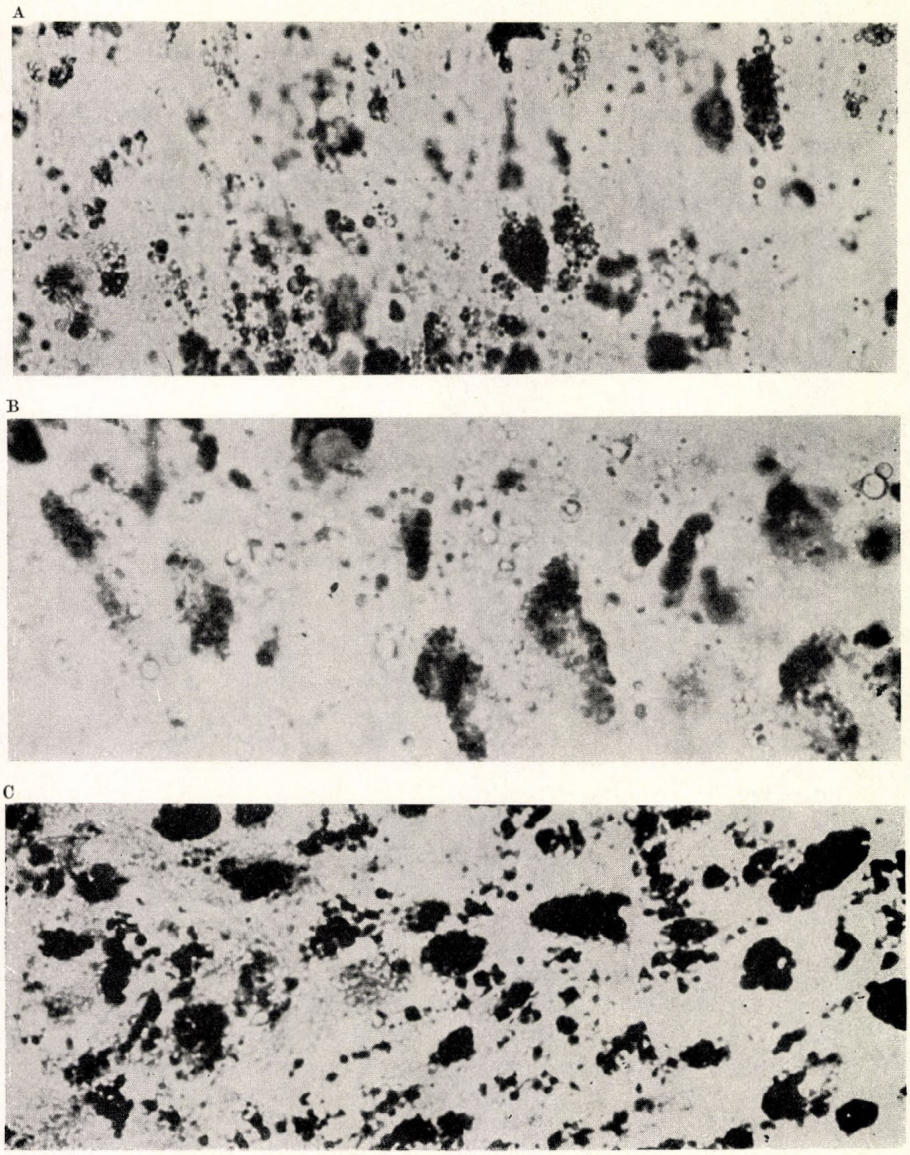

Fig. 1. A) A part of the section of visceral ganglion. Native condition. The substance observable in the cells corresponds to the yellow pigment. × 500
B) A part of the section of visceral ganglion. On the influence of cc. HCl the yellow colour of the pigment changes for blue and its granular localization becomes more distinct. × 500. C) Sudan-black staining of visceral ganglion. The localization of the intensive staining corresponds in general to the localization of the pigment. × 500

1 A. ábra Viscerális ganglion metszetének részlete. Natív állapot. A sejtekben látható anyag a sárga pigmentnek felel meg. Nagyítás: 500 ×.

B. ábra Viscerális ganglion metszetének részlete. cc. HCl hatására a sárga pigment kék színű lesz, szemcsézettsége még kifejezettebb. Nagyítás: 500 ×.

C. ábra Viscerális ganglion Sudan-fekete festődése. Az erős festődés lokalizációja nagyrészt megfelel a pigment lokalizációjának. Nagyítás: 500 ×.

be extracted by any of the above solvents. The degree of extraction by ethanol was examined also after fixation in BOUIN and in 4% formalin respectively. The time needed for complete extraction was different for the various individuals also in sections of equal thickness. In many cases, however, the amount of extracted pigments was not proportional to the time of extraction.

The absorption spectrum of the extracts has at least three maxima or inflexion points between 400—500 mμ, namely at 425—430, 450—460 and

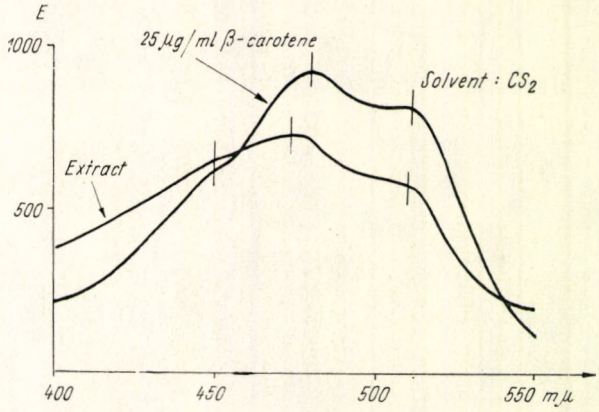

Fig. 2. The absorption of the pigment extracted from the foot by CS_2 and that of the standard beta-carotene in visible light. The amount of pigment expressed in 0.02—0.1 mg/g wet weight beta-carotene equivalents

2. ábra A talpból CS_2-vel extrahált pigment és standard beta-karotin látható fényben mért abszorpciója. A pigment-mennyiség 0,02—0,1 mg/g nedves súly beta-karotinnal ekvivalens.

470—480 mμ wavelength. The absorption measured in carbon-tetrachloride shows a considerable redward shift in comparison to other extracts *(Fig. 2)*.

Absorption maxima at 428, 448 and 476 mμ wavelengths were measured in n-hexane (Fig. 3). The synthetic beta-carotene solution used as control has absorption maxima at 428, 449 and 477 mμ. The absorption at 428 mμ of the ganglion extract seems to be an inflexion point and not a maximum, thus the absorption curve was normalized to that of the carotene solution on the basis of the most distinct maximum observed at 476 mμ and the differential curve obtained was examined. It was demonstrable by this procedure that also another chromophor exists here which is pale yellow and has an absorption maximum at about 420 mμ. This component is present also in ethanol, benzene and acetone extracts.

A light-sensitivity of similar degree was experienced after UV irradiation in the control beta carotene and in the hexane extract *(Fig. 3)*.

The activity produced by CARR—PRICE reaction is definitely characteristic of the carotenoids. Serotonine did not produce colour-changes either in the pigment extract or in the control beta-carotene solution, thus under the given conditions charge-transfer complex was not produced.

The petroleum ether extracts show considerable emission at 365 mμ excitation in the spectrum from 490 mμ to 650 mμ and at maxima of 535 and 580 mμ (corrected spectrum) *(Fig. 4)*.

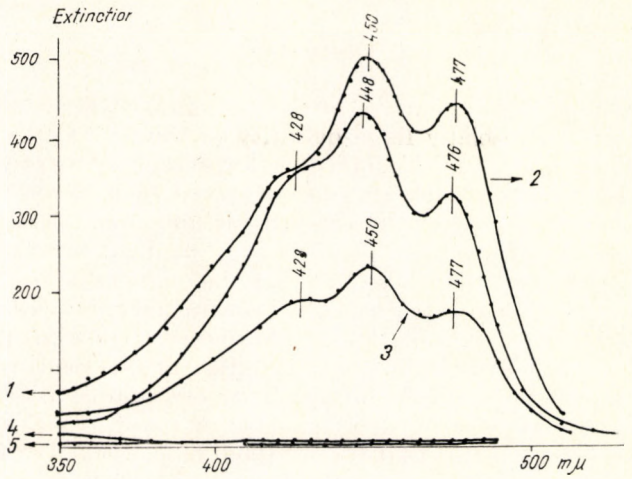

Fig. 3. The absorption spectra of the pigment extracted from the central nervous system (cerebral, visceral and pedal ganglia) (1), from the foot (3), and that of 2.5 μg/ml beta-carotene solution (2). The curves 4 and 5 were drawn after 1 hour UV-irradiation of the extract and of the standard respectively (250 watt, 100 mm, 90° incidence, mercury-arc, in quartz test tube)

3. ábra A központi idegrendszerből (1) cerebrális, viscerális és pedális ggl) és a talpból (3) extrahált pigment, valamint 2,5 μg/ml beta-karotin (2) abszorpciós spektruma.
A 4. és 5. görbe az extraktum ill. a standard 1 órás UV-besugárzás után készültek (250 watt, 100 mm, 90° beesési szög, higany-ív, kvarc-kémcsőben).

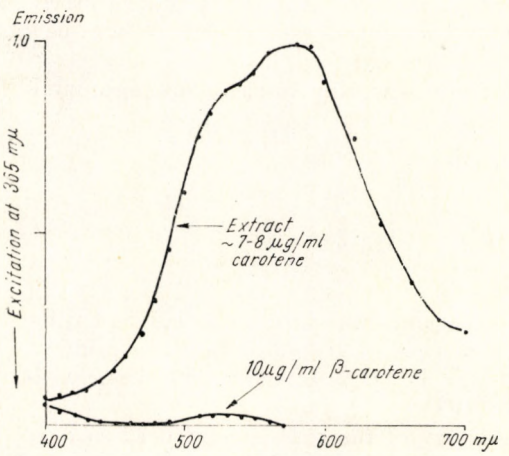

Fig. 4. The emission of petroleum ether extract and of standard between 350 and 700 mμ Corrected spectra. Excitation at 365 mμ

4. ábra Petroléteres extraktum és standard emissziója 350—700 mμ között. Korrigált spektrumok. Gerjesztés 365 mμ-nal.

Carotene content of ganglia was estimated also quantitatively on basis of hexane extracts. The average pigment-content in the total ganglia of 50 mussels computed on basis of the maximum at 449 mμ was 0,1 mg/g wet weight expressed as beta-carotene equivalent.

Discussion

The yellow pigment occurring everywhere in the tissues examined, and which is present in especially large quantity in the nerve cells changes blue with concentrated acids, and this indicates the presence of carotenoids (PEARSE 1960). The strong Sudan-black staining observed in a localization identical with the pigment proves that the carotenoids and lipoids exist together. It is inferred on basis of this observation that the pigment belongs to the group of lipochromes (PEARSE 1960). The fact that this pigment is soluble in organic solvents also supports this statement. The observation that occasionally longer time is necessary after fixation for the alcoholic extraction of the pigment from the sections may be explained by the nature of the lipochromes, for these being bound to proteins ("chromoproteid") are less soluble in organic solvents after fixation and may exist in such condition in paraffin embedded material (PEARSE 1960). It is probable that NAGY (1962) has observed in the nerve cells of *Unio pictorum* the lipochrome in this condition and took it for lipofuscin. None of the histochemical reactions performed by NAGY (1962) is specific on lipofuscin and thus the suggestion that the pigment is exclusively of lipofuscin nature is not considered correct. The negative results on the carotenoids obtained by NAGY (1965) can be explained by the fact that $SbCl_3$-reaction was performed on material embedded in paraffin.

Considering the solvent dependency of carotenoids of identified structure and that of the standard beta-carotene (KARRER and JUCKER 1949; LIAAEN, JENSEN AND JENSEN 1965) it is inferred on basis of the analytical data on the carotene-component of the pigment extracted from the tissues of *Anodonta cygnea L.* that this component stands nearest to beta-carotene. This suggestion is confirmed by the followings:

1. In hexane the absorption maxima of pigment and of standard beta-carotene was identical:

428—428 mμ
448—449 mμ
476—477 mμ

2. In carbon disulphide the kind and degree of the redward shift is similar with that observed in case of standard beta-carotene.

3. With $SbCl_3$ and H_2SO_4 a bluish-green colouring is observable in chloroform.

4. Light sensitivity of the extract and beta-carotene are similar.

In view of these considerations it seems that there is no essential difference in the chief pigments between sea-shell (GOODWIN 1952) and fresh-water mussel. In case of fresh-water mussel the pigment belongs to the group of carotenoids and its chief component is identical with beta-carotene. Its absorption

curve differs from that of the human *nerve cells lipofuscin.* HYDÉN and LINDSTRÖM 1950).

In the tissues of *Anodonta cygnea* besides beta-carotene a pale yellow chromophor is also present with an absorption maximum at 420 mμ. This substance is presumably identical with the component with an absorption at about 418—425 mμ which is present in the nerve cells of *Aplysia* species and is considered by CHALAZONITIS (1961) as belonging to the haem-protein. This may, however, as well be a carotene derivative. This phenomenon further the smaller resolving power of CS_2-extract and its smaller redward shift than that in case of beta-carotene suggest that various oxidation products may be present. This difference, however, is not sufficient for the more detailed definition of this component.

A considerable yellowish-green emission *(Fig. 4)* was also observed in the extract at an exitation of 365 mμ. This was not observed in the case of beta-carotene. The autofluorescence of nerve cells is most probably due to the presence of this component (DAHL et al. 1962; ZS.-NAGY 1967).

There are only hypotheses as regards the role of the carotenoid present in the tissues examined, but existing in greatest amount in the nerve cells. Due to its special electron configuration (PULLMAN and PULLMAN 1963) beta-carotene may be an important factor in oxidative metabolism. This is confirmed also by the observation of CHALAZONITIS and GOLA (1964) that in the nerve cells of *Helix pomatia* the respiratory ferments are concentrated exactly on areas containing carotene pigments.

Summary

Authors investigated the yellow pigment present in the nerve- and other tissues of *Anodonta cygnea L.* by histological and spectrophotometrical methods. Histochemically this pigment belongs to the group of lipochromes, where the carotene component is predominantly identical with beta-carotene.

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HISZTOLÓGIAI ÉS KÉMIAI VIZSGÁLATOK AZ *ANODONTA CYGNEA L.* IDEG- ÉS EGYÉB SZÖVETEINEK SÁRGA PIGMENTJÉN

Lábos Elemér, Zs.-Nagy Imre és *Hiripi László*

Összefoglalás

Szerzők *Anodonta cygnea L.* ideg- és egyéb szöveteiben található sárga pigmentet vizsgálták hisztológiai módszerekkel és spektrofotometriával. A pigment hisztokémiailag a lipochrom csoportba tartozik, amelyben a karotin-komponens túlnyomóan β-karotinnal azonos.

ГИСТОЛОГИЧЕСКОЕ И ХИМИЧЕСКОЕ ИССЛЕДОВАНИЕ ЖЕЛТОГО ПИГМЕНТА НЕРВНОЙ И ДРУГИХ ТКАНЕЙ БЕЗЗУБКИ

Элэмер Лабош, Имре Ж.-Надь и Ласло Хирипи

Изучали гистологически и спектрофотометрически желтый пигмент нервной и других тканей беззубки. По гистохимическим характеристикам пигмент соответствует липохромам. Его каротиновая составляющая большей частью относится к β-каротину.

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ON PRIMARY AND SECONDARY PROCESSES OF PHOTOINDUCED MUSCLE RESPONSE IN *ANODONTA*-LARVAE

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Earlier a definite correlation was pointed out between structure and effect of xanthene-type photosensitor-dyes (LÁBOS and TURCSÁNYI 1965, LÁBOS 1966a). This correlation means that dyes emitting light weakly and absorbing at longer wavelenghts are more effective in the sensitization of muscle contraction of mussel larvae. WEBER (1960) concluded similarly for photosynthesizing systems taking into account the strength in vivo quenching of fluorescent pigments. It is also known that bad luminophors are good sensitors of photopapers (ref. LJOVSIN 1951). Thus it seems obvious that energy utilization and fluorescence of sensitors are in negative correlation in different systems.

The effect of 16 dye-stuffs was observed. Our present work deals with correlations of some dyes containing at 9th and 10th positions of molecule different hetero-atoms (C, O, N, S). Results are discussed emphasizing the primary photochemical processes. Effects of some pharmacons, salts and heavy water were also investigated with basic acridine orange and with xanthenes to obtain informations on the validity of previous observations (LÁBOS 1965, 1966b) and on the role of water in the photosensitization. The role of water was interpreted as important in different electron-transfer reactions (van NIEL 1935, CALVIN 1962, KLOTZ 1962, SZENT-GYÖRGYI 1957).

Materials and method

Photosensitization was investigated on fresh-water mussel *(Anodonta cygnea L.)* larval motor system with an earlier described method (LÁBOS 1966a). The photoinduced response consists of rhythmic contractions and tonic closure of the larval adductor muscle. In general the rhythmic contractions and number of closed glochidia were counted in every minute, in 4 groups of 25 animals. Experiments were carried out at room temperature (22—25 C°), in distillated water solvent, illuminating the animals with standard tungsten light source (15W 6V, 180 mm, 75°, about 5000 lx nominal illumination). The used sensitors were the following: methylene blue (MB; Merck), toluidine

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blue O (TB; Merck), thionin (THIO; Merck), gallocyanin (GC; Reanal), nile blue sulfate (NBS; Grübler), neutral red (NR; Chroma), neutral violet (NV; Grübler), safranin G (SG; Light), pyronin (PYR; Grübler), rhodamine B (RH; Michrome 407), uranin K (U; Haen), eosin Y (EO; Michrome 533), erythrosin B (ERY; Gurr), rose bengal extra (RB; Fluka), acridine orange (AO; Reanal), acriflavine HCl (XA; EGYT). Absorption and emission spectra of the stains were controlled with BECKMAN Model G-2400 spectrophotometer and DU/DK fluorescence attachment; excitation at 365 mμ. Other substances used were: NaCl, $CaCl_2$, $MgCl_2$, chlorpromazine, (EGYT), serotonine creatinine sulfate (Fluka), L-cysteine (Fluka), $CdCl_2$, KCN, D_2O (99.8%). More than 5000 glochidia were used in the experiments. Only the compared data originate from experiments carried out under exactly identical conditions (day, population etc).

Experiments

1. Sensitor-effect of phenothiazine-, xanthene, phenoxazine-, and phenazine-type stains was investigated at 50 μM/lit concentration in groups of 100—100 larvae. Sensitor effect is characterized by t_{50} value, that is by the time passing until the closure of 50 larvae (see in *Table 1)*. The ratio of closed glochidia plotted against time is demonstrated in *Fig. 1.* At the given conditions the phenothiazines evoke the closure in 6—8 min. Rose bengal is the most effective, uranin is the least of all effective. The other stains — except the ineffective gallocyanine—are between these two sensitors. Among the acridines acridine orange is more effective than xanthacridine. In the group of phenazines the effect of safranin G and neutral red is about the same, neutral violet is less effective. The sequence of sensitor ability at the given conditions is:

RB > MB > (TB, THIO) > (NR, SG) > (NBS, AO, NV) > RH > ERY > XA > (PYR, EO) > U > GC

Rhythmic activity preceding the tonic closure is different. This phenomenon was not investigated in detail but in general the high frequency values could be observed at highly good sensitors. In case of rose bengal and thionin very high rhythmic activity could be observed. In methylene blue relaxations are relatively slow. It is probable that a sequence based on frequency of rhythmic activity would not be far from that one based on t_{50} values. To characterize rhythmic response the effect of 350 μg/ml neutral red is presented in *Fig. 2.*

2. Dose-response curve of rose bengal is demonstrated in *Fig. 3.* Abscissa shows the concentration of sensitor from 0.25 to 1000 μM/lit. Ordinate gives the effect by $1/t_{50}$ values. Both co-ordinates are logarithmic. The linear function obtained this way reminds of the adsorption isotherms.

3. Distribution in time of rhythmic contractions preceding the tonus was studied as follows. Time between the beginning of dye-administration and tonus was divided into 10 equal parts, and contractions in these intervals were counted. In *Fig. 4* data obtained from experiments carried out on 10 individual animals are demonstrated. Frequency of rhythmic activity is nearly exponentially increasing with time until closing.

4. In previous studies (LÁBOS 1965, 1966b) pharmacons and metabolic inhibitors proved to be effective inhibitors of photoresponse sensitized by

xanthene dyes. These agents were: chlorpromazine, serotonine, KCN, $CdCl_2$, L-cysteine. The effect of the mentioned inhibitors on sensitization evoked by the basic acridine orange can be seen in *Fig. 5—7*. The agents are inhibitors

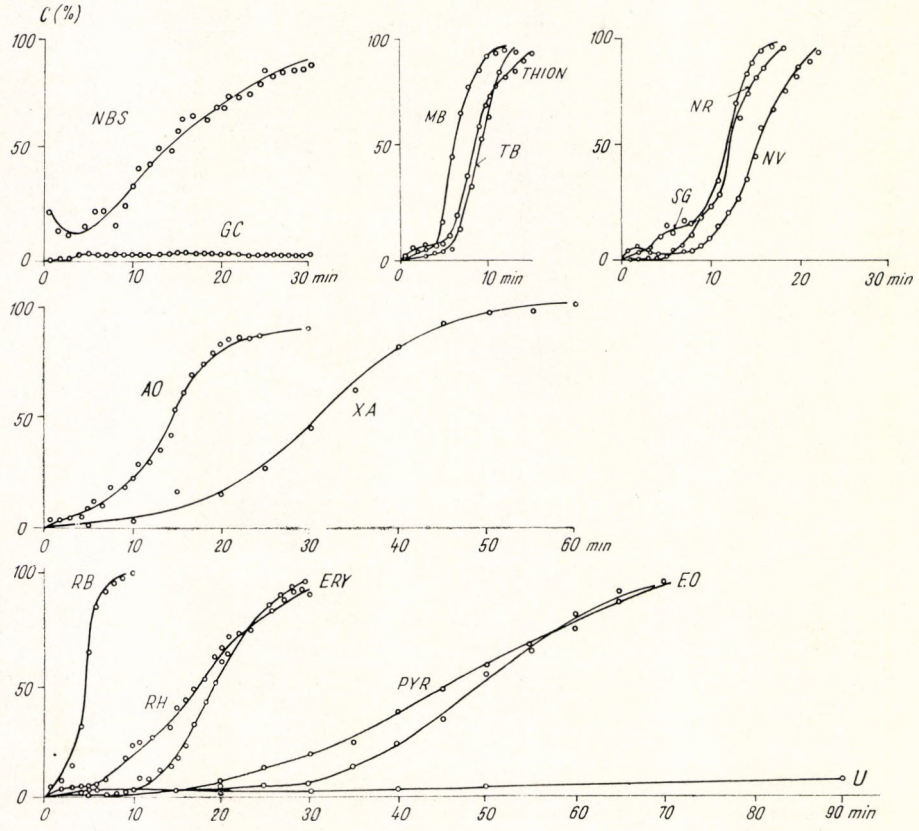


Fig. 1. Effect of 16 dyes. *Abscissa:* time in minutes; *ordinate:* ratio of glochidia in tonus, c [%]. Contractions see in Table 1 and in Method. Temperature 24° C. Illumination parameters 15 W, 6 V, 75°, 180 mm, tungsten. 100—100 animals

1. ábra 16 színezék hatása. *Abszcissza:* idő min-ban; *ordináta:* a tónusos kontrakcióban lévő lárvák aránya, c [%]. A rövidítéseket lásd az 1. táblázaton. Hőmérséklet 24° C, megvilágítás: 15 W, 6V, 75 °, 180 mm, wolfram-izzó. 100—100 állat.

of different degrees. Inhibition caused by $CdCl_2$ is rather high. Inhibitor effect of L-cysteine is not too high because of the applied high concentration. LILLIE and co-workers (1965) pointed out the potentiating effect of NaCl in frog-muscle photosensitized tonus. In the case of glochidia xanthene dyes (eosin, erythrosin, rose bengal) sensitization could be increased by NaCl, LiCl, $CaCl_2$, $MgCl_2$. The concentration of salts in 30—50. mM. In *Fig 8—9* this kind of potentiation is demonstrated. In the presence of 25 μM rose bengal t_{50} is 5,8 but in the presence of 25 μM rose bengal and 30 mM $CaCl_2$ or 40 mM $MgCl_2$ t_{50} is less than 1 min *(Fig. 9)*. In *Fig.* 8 promoting effect of 3 mg/ml NaCl is demonstrated on sensitization caused by 25 μg/ml. erythrosin.

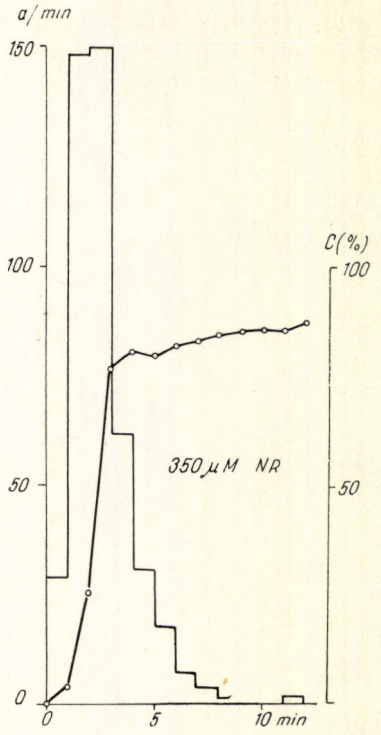


Fig. 2. Effect of neutral red. *Abscissa:* time in minutes, *ordinates:* frequency (a/min) and tonus-ratio (c[%]). 100 animals

2. ábra Neutrál vörös ritmikus aktivitást és tónust kiváltó hatása. *Abszcissza:* idő min-ban. *Ordináták:* frekvencia ill. tónusarány. 100 állat.

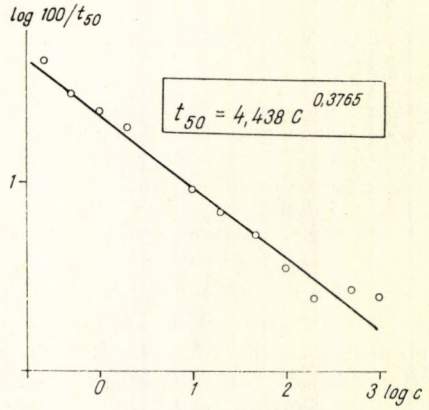


Fig. 3. Concentration-effect curve of rose bengal. *Abscissa:* concentration in µM/lit; *ordinate:* $100/t_{50}$; (explained in the text). Temperature 24° C, illumination see in *Fig. 1.* 100—100 animals

3. ábra Bengál vörös hatás-koncentráció függése. Hőmérséklet 24° C, megvilágítás mint az *1. ábránál.* 100—100 állat. *Abszcissza:* koncentráció µM/lit-ben; *ordináta:* a glochidiumok 50%-ának zárásához szükséges idő min-ban (t_{50}).

In *Fig. 10* the tonus promoting effect of NaCl is demonstrated on the sensitization evoked by acridine orange. In this case the rhythmic respons is inhibited. Note that either $CaCl_2$ or $MgCl_2$ and NaCl alone do not evoke

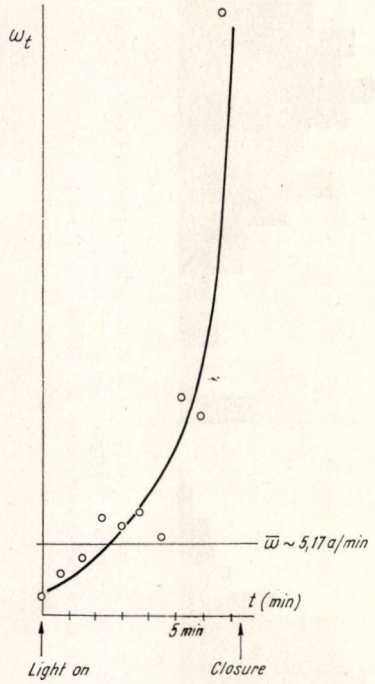


Fig. 4. Frequency of phasic contractions plotted against time. Explanation in the text

4. ábra Frekvencia-idő görbe. 10 μM/lit bengál vörös. Magyarázat a szövegben.

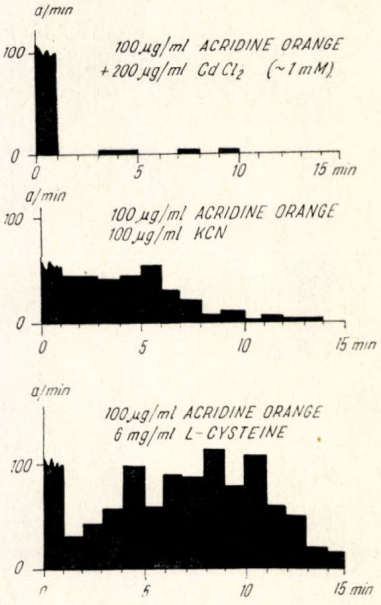


Fig. 5. $CdCl_2$,KCN, and L-cysteine effects on photoresponse in presence of 100 μg/ml acridine orange. 100—100 animals. Marking see at *Fig. 2.* Control see in *Fig. 6.*

5. ábra $CdCl_2$, KCN és L-cysteine hatása 100 μg/ml acridine orange által érzékenyített ritmikus izomválaszra. 100—100 állat. Kontrol a *6. ábrán.*

significant rhythmic or tonic response that could be responsible for the acitivity increased by them. The highly promoted rhythmic activity is decreasing with time after a maximum, due to inactivity of closed glochidia in increasing number.

6. As the photoinduced contraction is sensitive for neutral salts (Na, Li, Ca, Mg) it seemed intriguing to investigate the photoreaction in heavy water which could inform about hydratation and dehydratation taking place in the system. In literature for example quantum yield of photolysis of pyrimidines and dehydratation of photoreproducts are lower in D_2O than in H_2O. Therefore it was expected that heavy water inhibits the photinduced muscle response. In *Fig. 11* we compared curves originating from experiments carried out in H_2O (1) or in H_2O+D_2O (3). Curve marked 2 is the solvent control (50% H_2O+50% D_2O). 25 μM rose bengal in H_2O evokes rhythmic activity

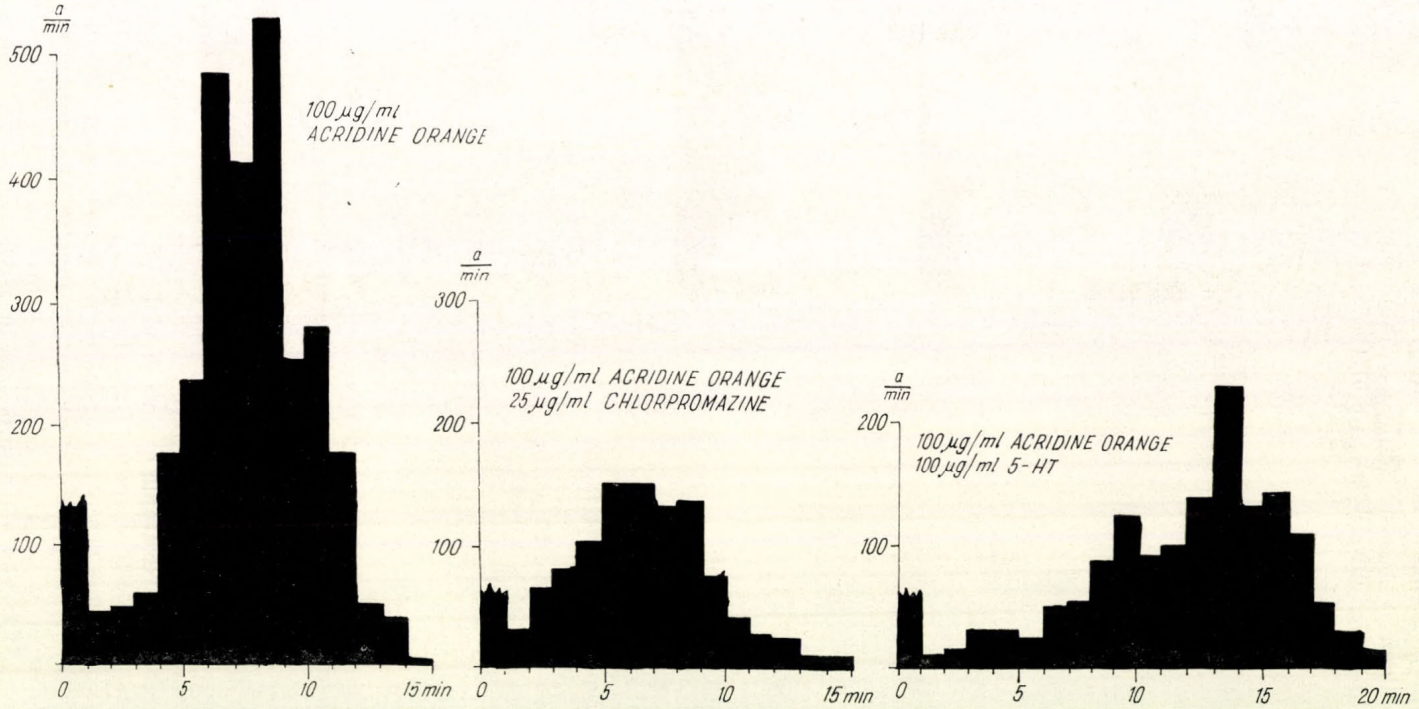


Fig. 6. Chlorpromazine and 5-HT effect on 100 µg/ml acridine orange sensitized muscle response. Control: effect of 100 acridine orange. 100—100 animals. Marking see at *Fig. 2.*

6. ábra Chlorpromazine, 5-HT hatása 100 µg/ml acridine orange által kiváltott ritmikus izomválaszra. Kontrol: 100 µg/ml acridine orange hatása. 100—100 állat. Jelöléseket lásd a *2. ábránál.*

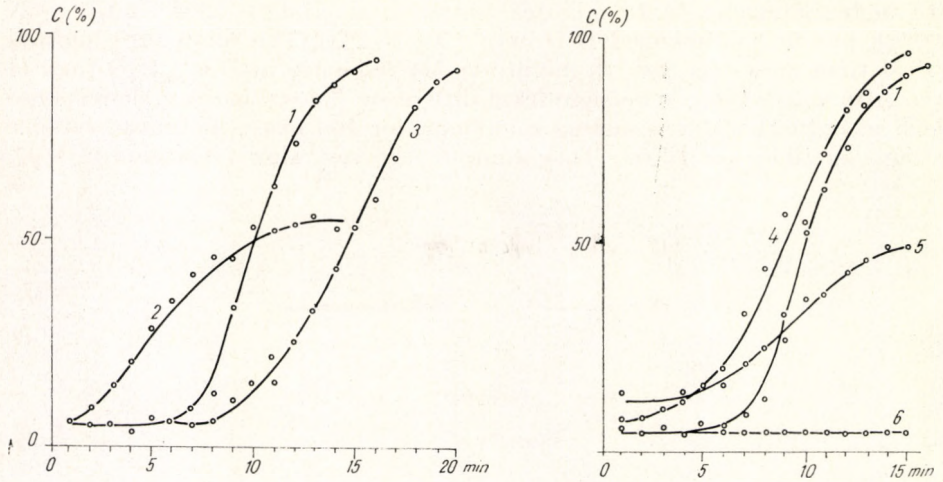


Fig. 7. Tonus-ratio (c[%]) plotted against time. 1. AO control, 2. AO + chlorpromazine, 3. AO + 5-HT, 4. AO + KCN, 6. AO + $CdCl_2$, 5. L-cysteine, Curves belong to *Fig. 5.* and *6.* Concentrations see there

7. ábra Tónusarány (c [%]) időfüggése; 1—AO kontrol, 2—AO + chlorpromazine, 3—AO + 5—HT, 4—AO + KCN ,5—L-cysteine, 6—AO + $CdCl_2$. A görbék az *5. és 6. ábrákhoz* tartoznak és a koncentrációk ezeken vannak feltüntetve.

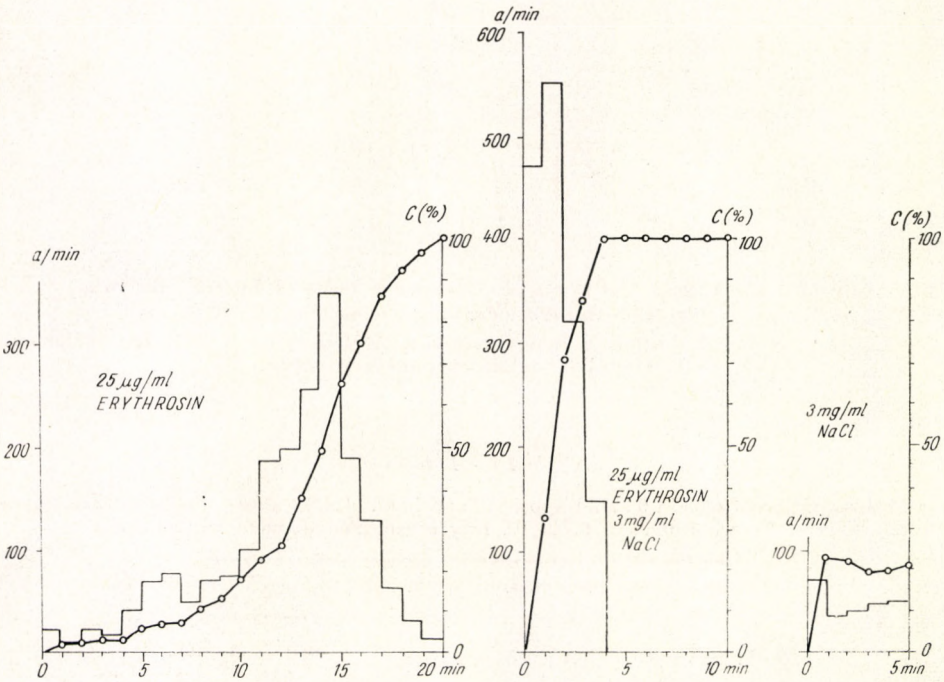


Fig. 8. Effect of NaCl on the sensitization caused by erythrosin. 100—100 animals. Marking see at in *Fig. 2.*

8. ábra NaCl hatása a erythrosin jelenlétében fotoindukált ritmikus és tónusos izomválaszra. 100—100 állat. Jelöléseket lásd a *2. ábránál.*

with high frequency. In 10 minutes 100 animals produce 3328 contractions (100%), but in half-changed D_2O only 870 (26,2%). The tonus-ratio plotted against time shows till the 7th minute higher tendency in D_2O+H_2O than in H_2O. Afterwards there is no significant difference. The phasic muscle response which in natural water is lasting sometimes for 100 msec, in heavy water is lasting sometimes for 10 sec. This is due to the very slow relaxation in D_2O.

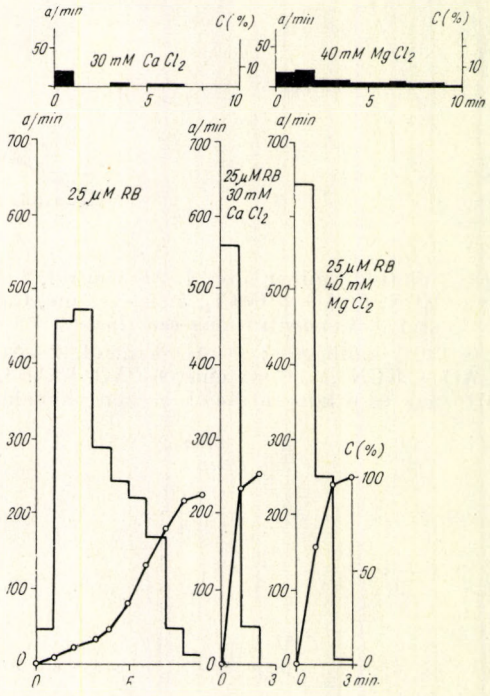


Fig. 9. Effect of $CaCl_2$ and $MgCl_2$ on photoresponse induced by rose bengal. 100—100 animals. Marking see in *Fig. 2.*

9. ábra $CaCl_2$ és $MgCl_2$ hatása a bengál vörös által fényérzékenyített izomválaszra. 100—100 állat. Jelölések mint a *2. ábrán.*

Table 1 — 1. tábla

Type of dyes. Hetero-atoms in 9 and 10 position and band of light absorption at different dyes
A festék-típusok. Hetero-atomok a 9. és 10. helyzetben. Fényelnyelés hullámhossza

Type — Alapvegyület	9.	10.	mμ
Phenothiazine	N	S	600—650
Phenoxazine	N	O	635
Phenazine	N	N	530—540
Xanthene	C	O	490—546
Acridine	C	N	440—490

Table 2

Name, marking, spectroscopic parameters and effect (t_{50}) of the sensitors

2. tábla

A szenzitorok neve, jelölése, spektroszkópiai mutatói és hatásuk (t_{50})

Dye Festék	Sign Jelölés	t_{50} [min]	λ abs. mμ	μ em. mμ corrected korrigált	Relative intensity of emission — Az emisszió relatív intenzitása
Methylene blue	MB	6.2	665		<1
Toluidine blue	TB	8.6	621		<1
Thionin	THIO	8.8	599		<1
Nile blue sulphate ...	NBS	14	640		<1
Gallocyanine	GC	∞	636		<1
Acridine orange	AO	14.8	490	565	1.5
Xanthacridine	XA	32	450	525	8.2
Rose bengal	RB	4.5	546	590	0.8
Erythrosin	ERY	18.9	525	560	0.5
Eosin	EO	48.2	515	552	19
Uranin	U	180	490	515	30
Rhodamine B	RH	16.8	555	563	100
Pyronin	PYR	46	555	580	2.3
Neutral red	NR	12.2	541	610	0.4
Safranin G	SG	12.4	530	595	1.3
Neutral violet	NV	15.4	541		<1

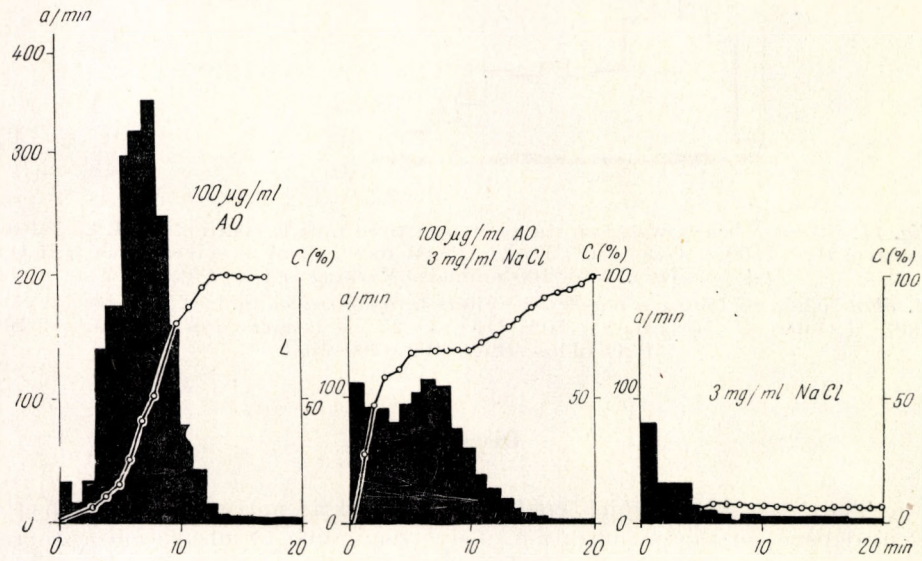


Fig. 10. Effect of NaCl on the sensitization caused by acridine orange. 100—100 animals Marking see in *Fig. 2.*

10. ábra NaCl hatása az acridine orange által fényérzékenyített izomválaszra. 100—100 állat. Jelöléseket lásd a *2. ábránál.*

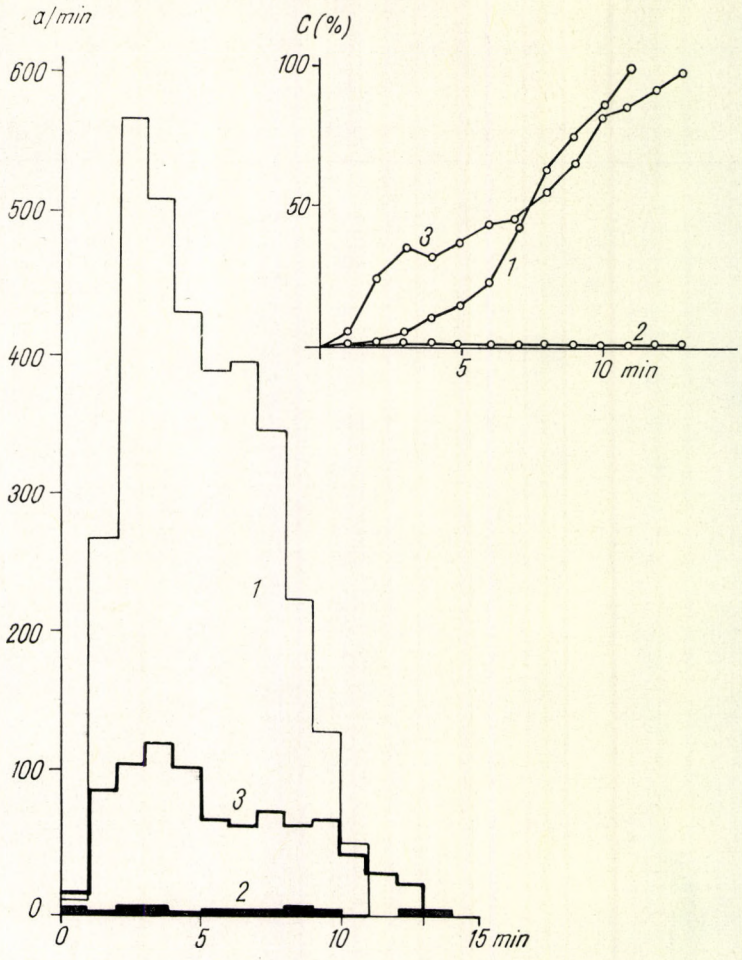


Fig. 11. Effect of heavy water on the photosensitized muscle contraction. 1. 25 μM rose bengal in H_2O, 2. 50% H_2O + 50% D_2O, 3. 25 μM rose bengal in solvent of 50% H_2O + + 50% D_2O. 100—100 animals. Marking see in *Fig. 2.*

11. ábra Nehéz-víz hatása a fényérzékenyített izomkontrakcióra. 1—25 μM bengál vörös 100% H_2O-ban, 2—50% H_2O + 50% D_2O, 3—25 μM bengál vörös 50% D_2O + 50% H_2O oldószerben. 100—100 állat.

Discussion

The $1/t_{50}$ values which could be regarded as measure of the sensitor-effect depend on the oncentration of dye reminding of adsorption isotherms (FREUNDLICH-type). It is observed that change in concentration is 4 logarithmic units and in effect only 1,7. For this reason it is improbable that differences in the effect are due to differences in the quantity of adsorbed dye. Furthermore as both basic and acidic dyes are effective, the influence of adsorption may not be significant at the given conditions. But comparing the

obtained data with those of YAMAMOTO (1958) who investigated T-phag inactivating effect of 10 dyes, — effects of neutral red and acridines deviate from our effects, clearly showing the significance of adsorption, when comparing different objects.

The energy of absorbed light-quanta is much higher than the thermal activation energy at room temperature. So the high effectivity of light is not

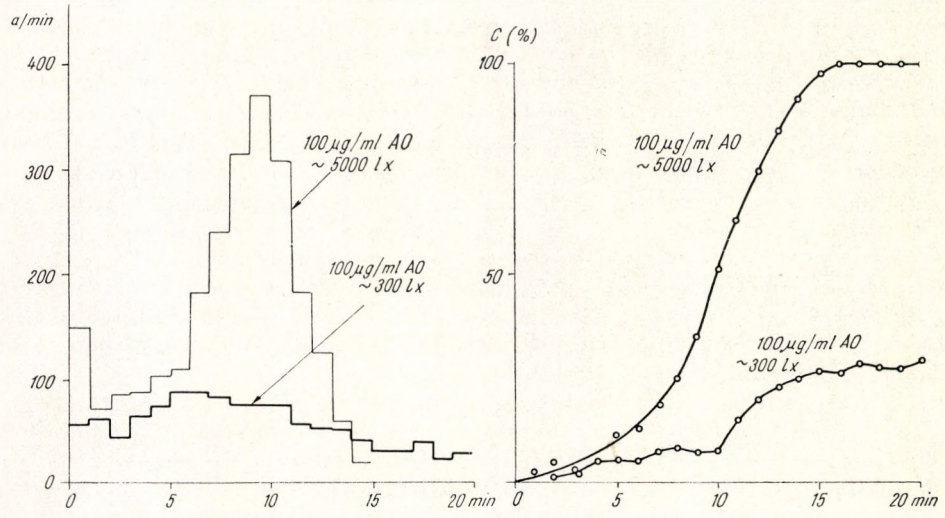
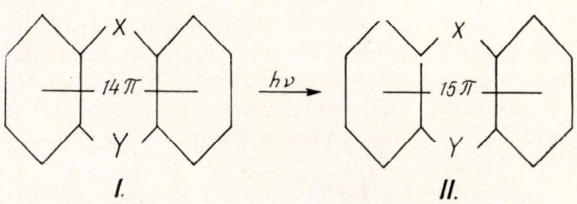


Fig. 12. Dark and photoinduced activity in 100 μg/ml acridine orange. Marking see in *Fig. 2.*

12. ábra Sötét és fotoaktivitás 100 μg/ml acridine orange jelenlétében. Jelöléseket lásd a *2. ábrán.*

surprizing. But it is questionable, how the energy can drive the biochemical system?

The used dyes have primary structures of positively charged electron acceptors containing electron-donor subtituents. The absorption in visible region is considerable as intramolecular charge-transfer corresponding to $n \longrightarrow \pi^*$ trasition



The importance of such transitions in radiobiology has been emphasized by KASHA (1960). The electron-affinity of the basic ring-compounds is the function of the electronegativity of the 9th and 10th atoms. With their increas-

ing electronegativity the wavelength of absorption band is also increasing, the energy of transition is decreasing (see in *Table 2.)*. Increase in the ionization potential of donor-groups also leads to redward shift of the band. For example among the phenothiazines the methyl-substitution of the amino-groups increases the wavelength of the absorption band. Similar changes can be observed comparing acridine orange and acriflavine.

The structure II which characterizes the excited state of molecule has two radical-centres. But the reactivity of these is highly different. Free radicals of basic ring compound are stable with 15 π electrons; it could be even crystallized (ethyl-phenazine, McIlwain 1937). On the other hand the centre at the donor-groups is very reactive and quickly captures electron, so the structure of the donor-substituent is essential in the sensitor-effect and the electron-affinity of the sensitor in excited state is much increased. This causes that stable free radicals from used dyes can not be produced (Bubnov et al. 1959). The sensitor after receiving electron transforms to "semiquinone" which can further change to reduced leukobase or reverses. The dominancy of these two processes is determined by the relative redoxi-potentials of the systems connected. From energetical points of view both processes are less significant than the excited dye semiquinone reaction. The real photoreduction can be observed by the bleaching in the dye solutions and in the area of glochidial adductor (unpublished observations).

Chemical reactions are taking place mainly in metastable excited states. Thus, the activity of a dye is determined by $S^* \rightarrow T$ transition probability. The fluorescence-yield, if the other quenching factors are neglected, is in unambiguous connection with the $S^* \rightarrow T$ transition probability. Thus, good luminophors (uranin, eosin, acriflavine, pyronine) are not good photoactivators, (see *Table 1* and *Fig 1)*, but bad luminophors are good ones (phenothiazines, eryhtrosin, rose bengal, safranin, neutral red, and neutral violet). But this correlation may be overwhelmed by the influence of direct connection of dye-substituents with donor-systems. The highly fluorescent rhodamine B may strongly sensitize for this reason.

If the biological system contains molecules with low energy triplet-levels, contact interaction is enough for energy transfer without singlet excitation of biomolecule (see the mecanism at Turro 1966).

In the groups of halogenized fluoresceine derivates parallel with the favourable change in charge-transfer, another important change takes place with the increasing halogenization. This is the increase of the spin-orbit interaction, which increases the intersystem crossing (see in Murrel 1963). The role of this mechanism is also suggested by the slight effect of magnetic fields on the photoinduced muscle response (unpublished preliminary observation). Therefore it was expected that rose bengal (containing 65% halogene) is effective even in 10^{-7} M/lit concentration. The real free radical concentration may be lower by 1—3 magnitudes. From uranin to rose bengal, t_{50} decreases from 180 to 4.5 min.

One of the possible causes responsible for the deviations from the expected negative correlation between fluorescence and effect is the activity obsrvable in dark. In case of xanthenes it is very low, but for example in case of acridine orange it is higher. From *Fig 12* one can observe that at t_{50} time the dark-effect is only about 15 per cent of the tonus observed in light. Sandow and Isaacson (1960) showed the dark-effect of acridine orange and methylene

blue on muscular contraction. The dark activity may come from impurities or vehicles. Nevertheless the dark chargetransfer can not be neglected.

After consideration of primary processes, we outline the possible biochemical mechanisms. The first emerging question is the quality of the adsorbing macromolecule. The dose-effect curve of rose bengal suggests real adsorption. The applied stains are suitable for staining diverse structures (cytoplasma, Nissl-granules, RNA, DNA, Golgi-apparatus etc; CONN 1961), and sensitize different macromolecules (ref. MACLAREN and SHUGAR 1964). For this reason the sensitization could take place on proteins and nucleic acids too. The real effect of nucleic acid decomposition could be suggested after some hours of the administration on early mussel embryos (unpublished observation). Rose bengal evokes desintegration in light but not in dark. Acridine orange does not cause similar changes. BELLIN and YANKUS (1965) report results that photodegradation of DNA is similar in case of rose bengal and methylene blue but differs with acridines.

Cytological localization is more problematic. The direct or partially indirect (through nerves) character of the sensitization is not clear. The emerging problems were discussed earlier (LÁBOS 1964, LÁBOS 1966a, 1966b). Another question is the functional localization. The inhibition observable with serotonine chlorpromazine, KCN, $CdCl_2$ argues for the role of SH-groups and for the role of terminal oxidation in the sensitor-effect. The sensitizations examined in general utilize oxygen and the dye activates it (KAUTSKY 1931). Nevertheless in a biological system it must be supposed that sensitization or relaxation involve oxygen activation partly by the cytochrome system. The increasing frequency of the activated muscle is finished by irreversible tonus. OSTER (1959) showed that reversible photoreduction of dyes could serve as explanation for the plant photoperiodical phenomenons. BUBNOV and co-workers (1959) emphasized that eosin can react with oxygen or hydrogen. Above we showed that the biradical had a dominant electron acceptor centre then its reaction with the donor would be quicker. Considering the inhibitons the proton- and electron-donor function of water and SH-groups seem to be important. In the course of reversible stage the donor-property of biradical also may stand out. The biological electron-acceptor-counting with the cyanide effect — could be the cytochromeoxidase and/or oxygene. The total photoreduction of dye and the irreversible stage may be due to asymmetric electron-mediation.

The role of water on the base of acute salts and D_2O effect seems to be distinguished. The D_2O medium leads to slower solvatation and desolvatation similarly to results of WIERZCHOWSKI and SHUGAR (1957—61) who showed even about nucleophilic addition of water. The acute salt effects can be regarded as destruction in solvate layer on photoproducts.

Summary

Authors investigated photosensitized rhythmic and tonic muscular contractions of fresh-water mussel larvae (glochidia of *Anodonta cygnea L.*). The used sensitors were different aromatic heterocyclic compounds (xanthenes, phenazines, phenoxazines, phenothiazines). The sensitor ability was characterized by the time needed to the closing of 50 per cent of the animals (t_{50}). The obtained sequence of sensitor-effectivity is

RB > MB > (TB THIO) > NR > SG > (NBS, AO, NV) > RH > ERY > XA > (PYR, EO) > U > GC

Considering the structure (effect correlations, two factors are emphasized. Firstly the intramolecular chargetransfer ($n \rightarrow \pi^*$ transition), secondly the spin-orbit coupling (in the case of xanthenes).

Dose-effect curve of rose bengal suggests adsorption of the sensitors on macromolecules. In this compact system even 10^{-7} M/lit rose bengal could well sensitize. The reaction has two stages: the reversible with increasing frequency of phasic contractions, and the irreversible-one.

In case of acridine orange inhibitor-effect of KCN, $CdCl_2$, L-cysteine, chlorpromazine, 5-HT was demonstrated. Considering these inhibitions the terminal oxidation (proton and electron mediation) seem to be important in the process. The electron transfer is supposed to take place with cytochrom-oxidase.

In about 30—50 mM concentration NaCl, $CaCl_2$, $MgCl_2$ are potentiating the effect of xanthenes. The reaction in case of acridine orange is not the same (tonus is potentiated, rhythmic response is not). Solvents containing D_2O inhibit the rhythmic activity. Tonus is not depressed by D_2O. The phenomenon is supposed to be connected to slower reversible dehydratation (hydratation of the photoproducts in D_2O.

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A FOTOINDKUKÁLT IZOMKONTRAKCIÓ ELSŐDLEGES ÉS MÁSODLAGOS FOLYAMATAIRÓL

Lábos Elemér és Turcsányi Béla

Összefoglalás

Szerzők édesvízi kagyló *(Anodonta cygnea L.)* glochidiumát különböző aromás heterociklikus festékanyagokkal érzékenyítették látható fénnyel szembe. A festékek érzékenyítő hatását a lárvák 50%-ának irreverzibilis kontraktúrájához szükséges idővel jellemezték (t_{50}). Ennek alapján a hatás-sorrend:

$$RB > MB < TB, THIO > NR, SG > NBS, AO, NV > RH > ERY > XA > PYR, EO > U > GC$$

A hatás-szerkezet korrelációk elemzése;kapcsán az intramolekuláris charge-transfer mechanizmus révén létrejövő megnövekedett elektronaffinitás és a spin-orbita kölcsönhatás szerepére mutatnak rá.

A reakció-mechanizmus elemzése azt mutatja, hogy a szenzitor — feltehetően makromolekulák felületén adszorbeálódik. Az így létrejövő kompakt rendszerben jó hatásfokú energiaátadás számára javulnak a feltételek. A reakció reverzibilis szakaszában növekvő sebességű folyamat vezet az irreverzibilis tónushoz. Acridine orange esetében demonstráltuk a KCN, $CdCl_2$ L-cisztein, chlorpromazine, 5-HT gátló hatását a fényérzékenyített izomingerületre. A gátló hatások elemzése a terminális oxidáció részvételére utal. Feltehetően a festék aktiválja a légző-fermentet és ez szükséges lépése a fényérzékenyítésnek.

10 mM/lit koncentráció-nagyságrendben NaCl, $CaCl_2$ és $MgCl_2$ potencírozza a rose bengal érzékenyítő hatását. Bizonyos koncentrációarányok mellett a NaCl gátolja az acridine orange hatását. D_2O tartalmú vizes oldatokban a relaxáció jelentősen lelassul és ennek révén a fotoindukált ritmikus izomműködés frekvenciája jelentősen esik. A jelenségeket a fotolízis-produktumok hidratációjának és dehidratációjának megváltozásával hozták összefüggésbe.

ПЕРВЫЧНЫЕ И ВТОРИЧНЫЕ ПРОЦЕССЫ ФОТОИНДУКЦИОННОЙ МЫШЕЧНОЙ КОНТРАКЦИИ

Э. Лабош и Б. Турчани

Авторы вызывали чувствительность глохидиев беззубки *(Anodonta cygnea L.)* в видимом свете с разными ароматическими гетероциклическими красками. Свойство красок вызывать чувствительность выражали временем необходимым для необротимой контракции 50% глохидиев (t_{50}). На этом основании порядок воздействия красок оказался следующим:

RB>MB>TB, THIO>NR, SG>NBS,AO, NV>RH>ERY>XA>PYR, EO>U>GC

На основе анализа взаимодействия эффекта и структуры указывают на значение (1)электронно-восприимчивости которая увеличивается вследствие внутримолекулярного ионно-транспортного механизма и (2) взаймодействие спинорбит.

Анализ механизма реакции показывал, что сензитор по всей вероятности адсорбируется на поверхности макромолекул. В системе такого ряда улучшаются условия для передачи энергии. В обратимой стадии реакции необратимый тонус наступает вследствие увеличения скорости реакции. При даче акридинораижа нашли тормозное действие KCN, $CdCl_2$, L-цистеина, аминазина и серотонина на мышечное возбуждение, чувствительность которого повышалось освещением. Анализ тормозных влияний указывает на значение терминального окисления. Предпологается, что краски вызывают активацию дыхательных ферментов и это лежит в основе процесса фоточувствительности.

NaCl, $CaCl_2$, $MgCl_2$, в концентрации 10 мМ/л увеличивают влияние бенгального красного. В определенных концентрациях NaCl тормозит эффект акридинораижа. В водных растворах, содержащих D_2O расслабление мышцы замедляется вследствие чего частата фотоиндукционной ритмической мышечной деятельности значительно снижается. Наблюдаемые явления объясняются изменениями гидратации и дегидратации промежуточных продуктов фотолиза.

ANNAL. BIOL. TIHANY	33	61—78	HUNGARIA 1966

ON THE NATURE OF THE COMPONENTS OF THE POTENTIAL COMPLEX INDUCED BY ELECTRIC STIMULATION ON *ANODONTA* NERVE

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In preliminary experiments by electric stimulation of the cerebrovisceral connective (CVc) of the fresh-water mussel *Anodonta cygnea L.* we dwelt on the stimulus-parameter-dependence of the action potential (SALÁNKI et al. 1964), on the demonstration of the morphological and electrophysiological heterogeneity of the nerve (LÁBOS et al. 1963), on the pharmacological behaviour of the nerve (LÁBOS et al. 1966) and on the analysis of the effects of some methodical conditions (LÁBOS 1965). The experiments unequivocally indicated that the evoked complex potential of the nerve consists of a part more sensitive to chemical influences and of a more resistant part. The present studies are related to the characters of the complete and resistant rest potential. The complete action potential is the action potential of the nerve activated with sufficiently great stimulation while the rest potential is the response to be led off after the block of the conduction (e. g. tetracaine or procaine treatment).

A substantial part of the previous leadings and purposefully also the present ones were conducted under conditions where one of the electrodes of the exciting and leading off circuit is common so that also the local response of the nerve was recorded (BURES et al. 1960). It is necessary to elucidate the relationship of the rest-potential and local potential to each other. In connection with the analysis of other features of these potentials we dwelt also on the issue of the decrement of the *Anodonta*-CVc described under various methodical conditions (ZHUKOV 1946; KAHN and KUSNEZOV 1938). BULLOCK (1965) believes the cause of the decremental conduction to be found in the conditions dwelt on by SCHLOTE (1955).

Method

The nerve during excitation and conduction was in paraffin oil. Excitation was carried out by DISA Multistim stimulator, recordind with the DISA 51BO1 (1MΩ, 100 pF) DC amplifier. Among the stimulating and recording electrodes one was a common and at the same time grounded electorede. The upwards directed deflection signifies an increase in positivity of the area below the active (not grounded) leading off electrode as compared with

the potential of the area below the ground electrode. Silver wire and plate electrodes were used. For reproducibility of the electrode layout the distance between the stimulating electrode and the proximal edge of the ground plate (s), the length of the ground plate (g) and the distance between the (different) recording electrode and the other marginal point of the ground plate (r) are given. Under the given conditions at distances of 1 mm minimum the potential with maximum amplitude is obtained at the sequence of values 1—10—3 mm s-g-r. We refer to ground positive stimulation when the common ground electrode at the time of the employed square-impulses is the anode, while the other stimulating electrode compared with it is the negative one (cathode). The case of the stimulation of reversed polarity is called ground-negative one.

Results

1. The difference between the response of the fresh and blocked nerve

The CVc responds after application of effectively blocking pharmacons — in the present case tetracaine — to the electric excitation with a prolonged, one-component "rest potential".

In *Fig 1.* in the cases A, C, E a ground-negative excitation, in the cases B, D, F a ground-positive excitation was employed. The non-blocked nerve retains the direction of its response with the change of the polarity of the stimulus at the given parameters of excitation but we obtain a lesser response to ground positive excitation. From *Fig. 1* it appears that after tetracaine block *(C, D, E, F)* we obtain a response turning with the polarity of excitation.

On this basis we can distinguish a resistant polarity-dependent and polarity-independent comparatively rapid wave group which is substantially more sensitive to chemical influences. The response at the fresh nerve contains both. To the turn of this polarity-dependent part is ascribed the reduction of the response of the fresh nerve with the employment of ground-positive excitation.

It should be noted that the deflection begins after the 10 ms square wave impulse employed subsequently to its ending. Thus the rest-potential does not correspond to the stimulus-artefact but is a substantially slower procedure. Using a longer stimulus *(Figs 1E* and *1F)* it becomes visible that a more inert phenomenon is involved which continues irrespective of the ending of the excitation. As pointed out previously (LÁBOS et al. 1966) similar phenomenon can be induced on dead nerve or thread wetted with electrolyte. The slow phenomenon goes also with the reaction of the living nerve and can be led off at the layout using the three electrodes. The living nerve, besides, gives a polarity-independent response, while the blocked and dead excitation exhibits only polarity-dependent phenomena. Under the given methodical conditions, besides, the stimulus-artefact appears both on living and blocked nerve.

2. The response of the nerve to ground-positive excitation.

If the nerve sector below the ground-electrode is the anode, then depending on the voltage and period of the stimulation subsequently to the ending of the latter, downwards directed signals of various size are obtained. On *Fig.*

2 we employed ground-positive excitations of short period and of various tensions. Subsequently to the wave directed downwards after various periods we observe a wave of opposite direction. The latter is the polarity-independent

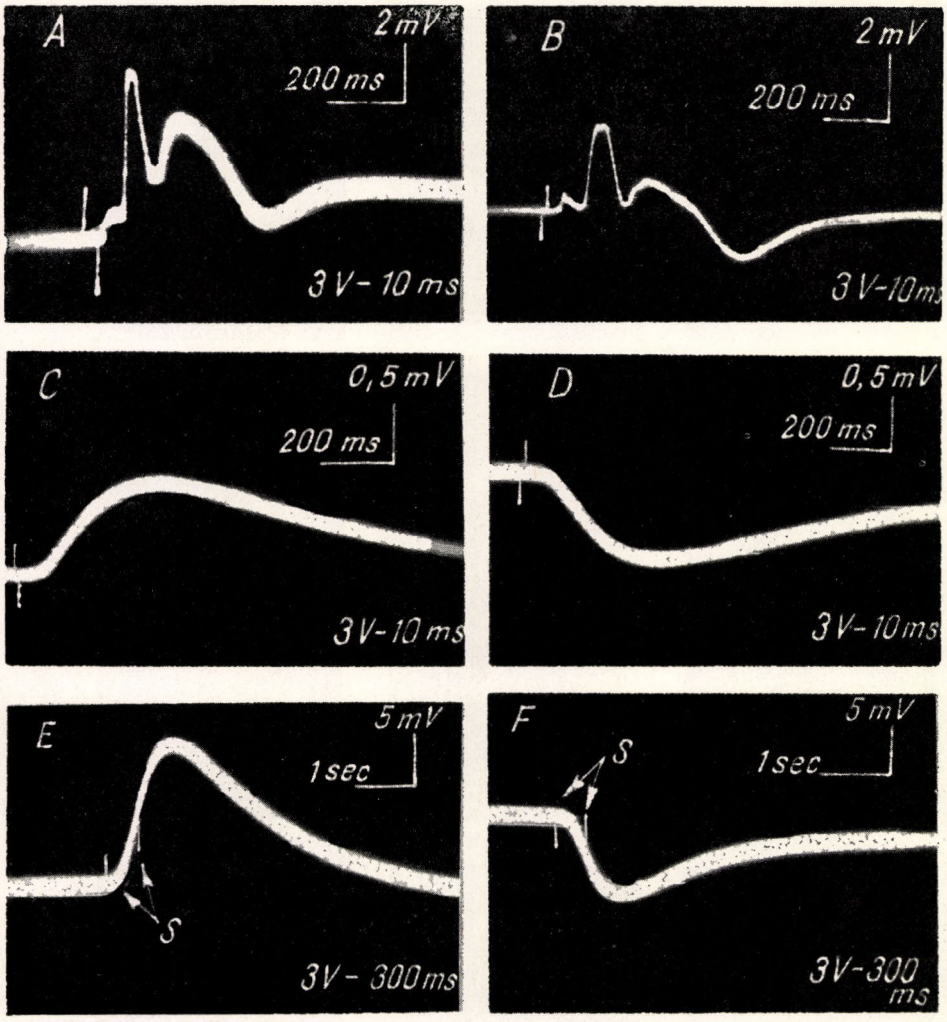

Fig. 1. Explanation in the text. (A—C—E: ground negative, B—D—F: ground positive stimulation

1. ábra Magyarázat a szövegben. (A-C-E: földnegatív, B-D-F: földpozitív ingerlés.)

response appearing during the period of the anelectrotone but after the ending of the stimulus signal. Since this is directed upwards, its appearance causes the potential to rise.

The beginning of the wave directed upwards takes place depending on the velocity of the processes of opposite trend sooner or later; therefore the real period of latency can not be exactly determined. Although the period until the

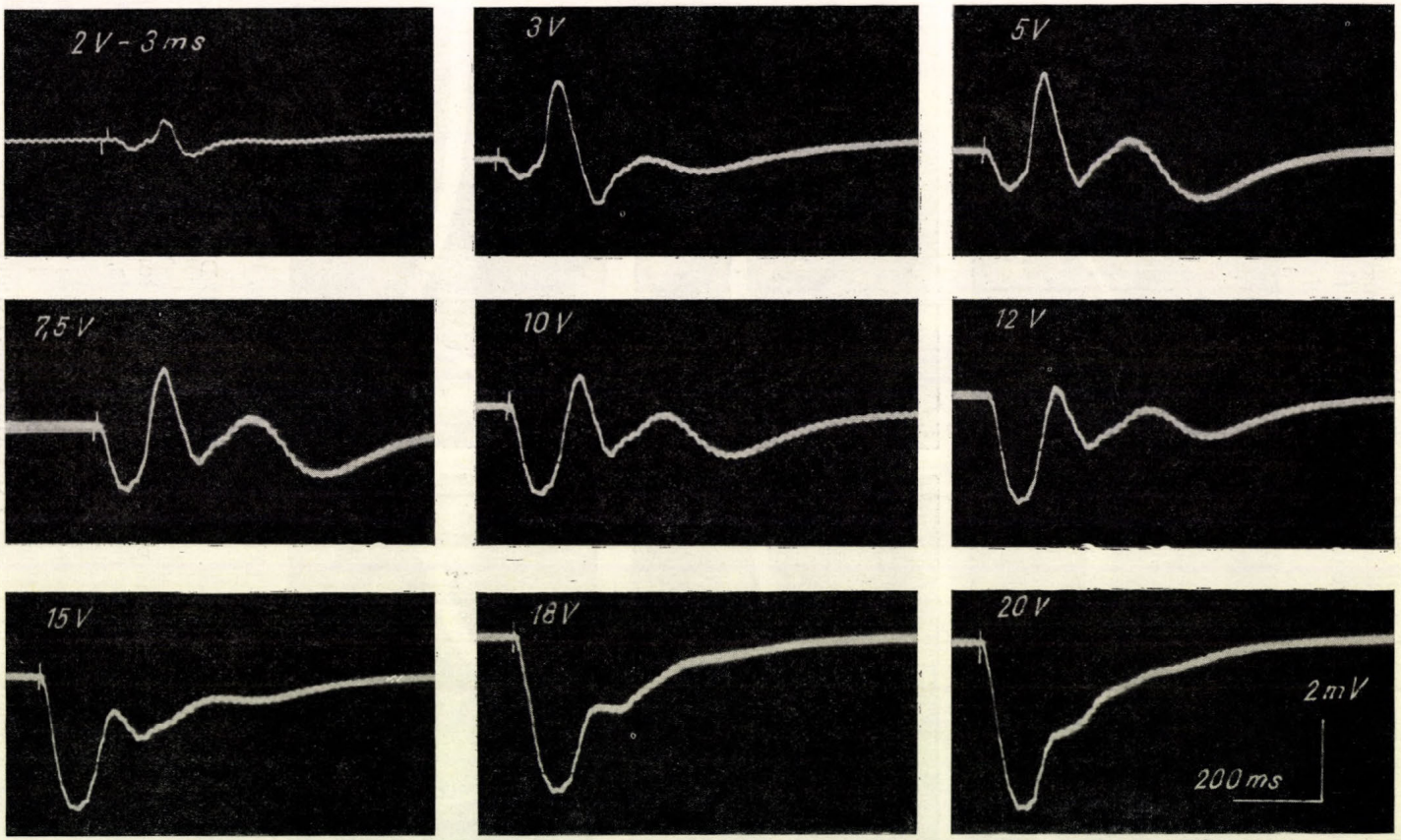

Fig. 2. Ground positive stimulation voltage is changing. Electrode layout r—g—s/3—10—1 mm; DC-recording
2. ábra Földpozitív ingerlés. Ingerfeszültség változik. Elektróda elrendezés: r—g—s/3—10-1 mm; DC-elvezetés.

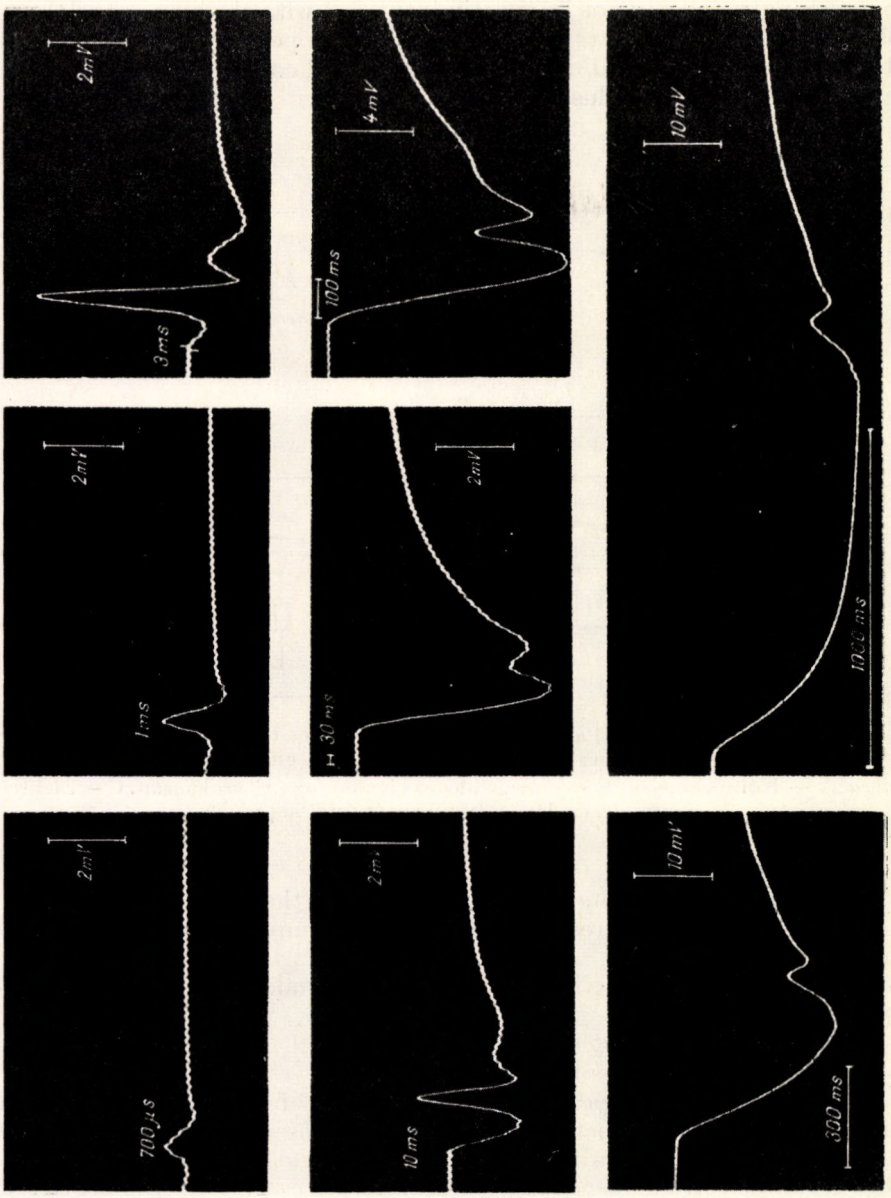

Fig. 3. Ground positive stimulation. Impulse-duration changing. Electrode layout 3—10—1 mm; DC recording; stimulus voltage 5 V

3. ábra Földpozitív ingerlés. Impulzusszélesség változik. Elektródaelrendezés 3—10—1 mm; DC elvezetés; ingerfeszültség 5 V.

turn increases both on the increase of voltage and impulse duration (see *Figs 2* and *3*), on account of the picture of composed character the beginning of the action potential can not be exactly identified with the moment of the turn. Still it undoubtedly appears from *Fig. 3* that the polarity-independent action potential begins at the ending of the stimulus and constantly lags behind with the increase of the stimulus duration.

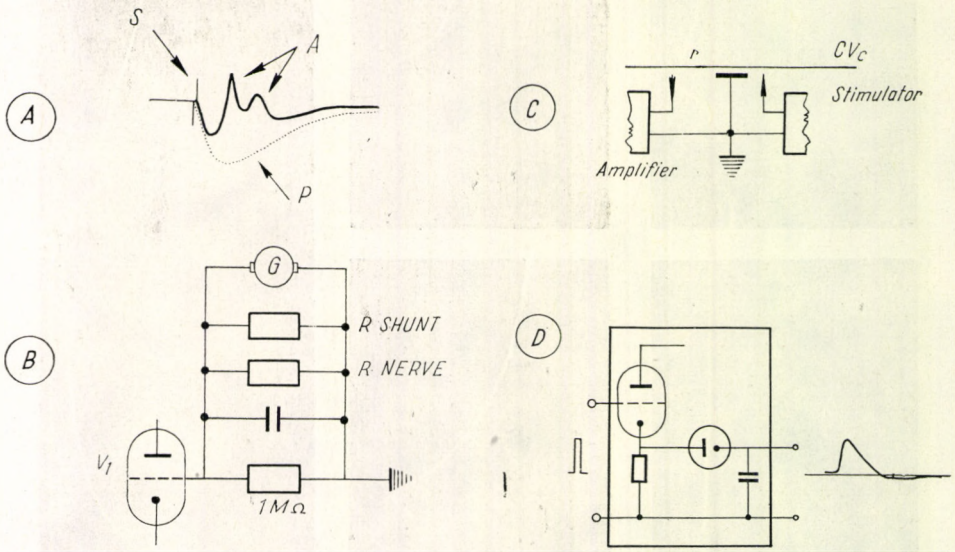

Fig. 4. A) Components; *B)* Passive nerve equivalent on the sector „r"; *C)* Electrode ayout; *D)* Impulse prolonging circuit

4. ábra A — Komponensek, B — Passziv idegekvivalens az „r" szakaszon. C — Elektróda-elrendezés D — Impulzusnyújtó kapcsolás

Summarizing the facts it appears *(Fig. 4 a)* that the potential condition can be broken down to three phenomena in the course of ground-positive and negative stimulation:

1. stimulus-artefact, which is a polarity-dependent, differentiated square impulse (S)
2. polarity-dependent slow potential (P)
3. polarity-independent action potential (A)

From all these it appears that the direction of the polarity-independent action potential is, irrespective of the trend of the excitation signal, always such that the positivity of the different recording electrode or the negativity of the ground electrode respectively is seen to increase. Since the nerve sector in excitement is negative as compared with that in rest, therefore the comparative increase of negativity of the ground electrode as compared with the different electrode can only signify the excitement of the area below the ground electrode. Thus the place of origin of the signal recorded is the ground electrode. The time of origin of the action potential falls to the period after the cessation of the anelectrotone induced in the ground-electrode.

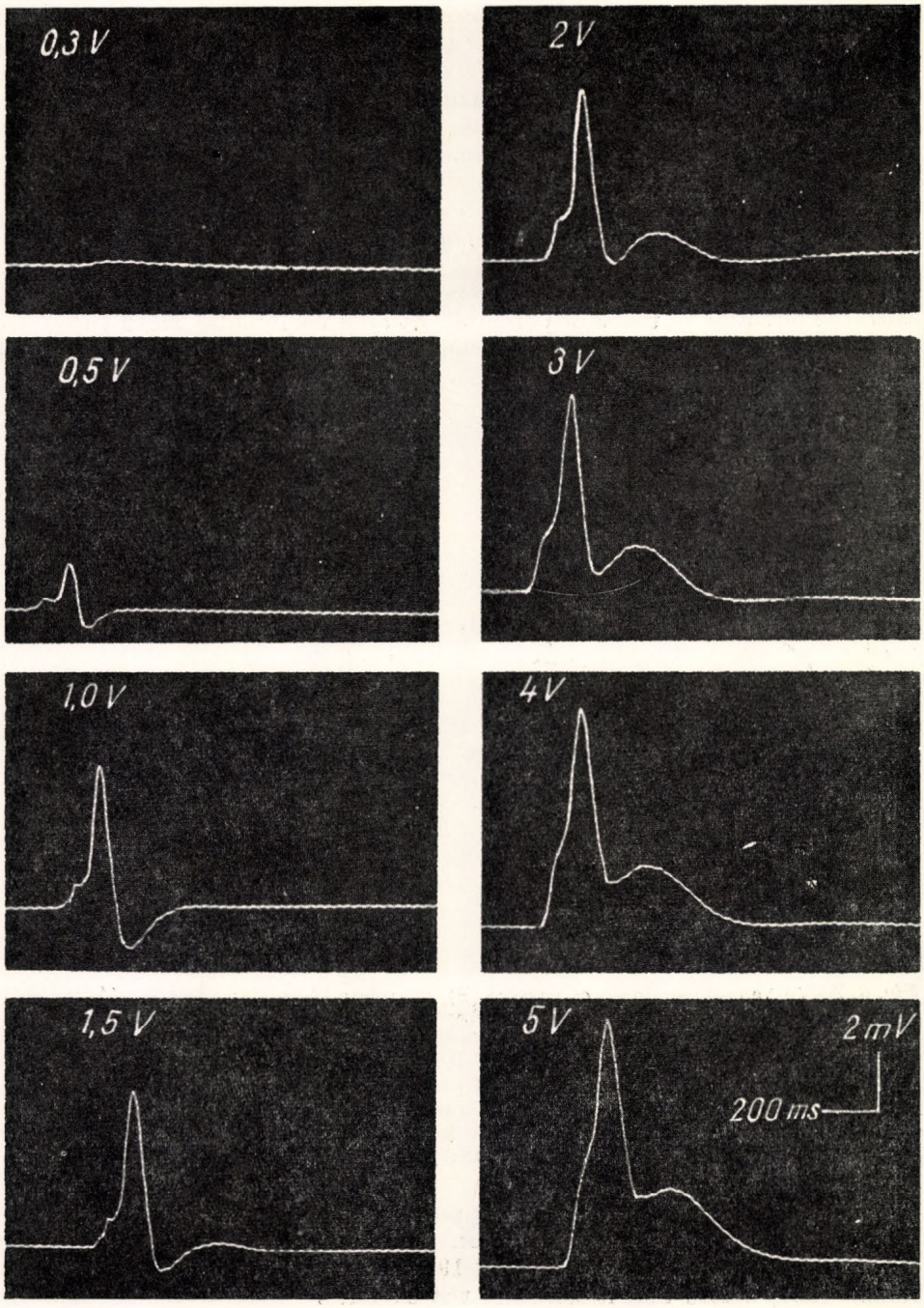

Fig. 5. The response of CVc at various voltages. Impulse duration 5 ms; r—g—s/3—10—1 mm; DC-recording; ground negative stimulation

5. ábra A CVc válasza különböző ingerfeszültségek mellett. Impulzusszélesség 5 ms; r—g—s/3—10—1 mm; DC — elvezetés; földnegatív inger.

The process is initiated by the differentiated excitation signal which according to our measurements under the given conditions (the upper limit of the amplifier is 100 kcp (a 1/100—1/1000 part of the amplitude of the excitation signal, the relation between impulse duration and time constant $d/\tau \sim 100$; e. g. upon 5V — 3 ms excitation with optimum electrode layout $\pm$ 20 mV amplitude and about 50 μ sec time constant can be observed.

3. The effect of ground-negative excitation

In this case the direction of the polarity-independent potential coincides with the direction of the slow potential. At an 8 ms impulse width with 0.1—0.4 V voltage only the slow potential can be observed *(Fig. 5)*. Further increas-

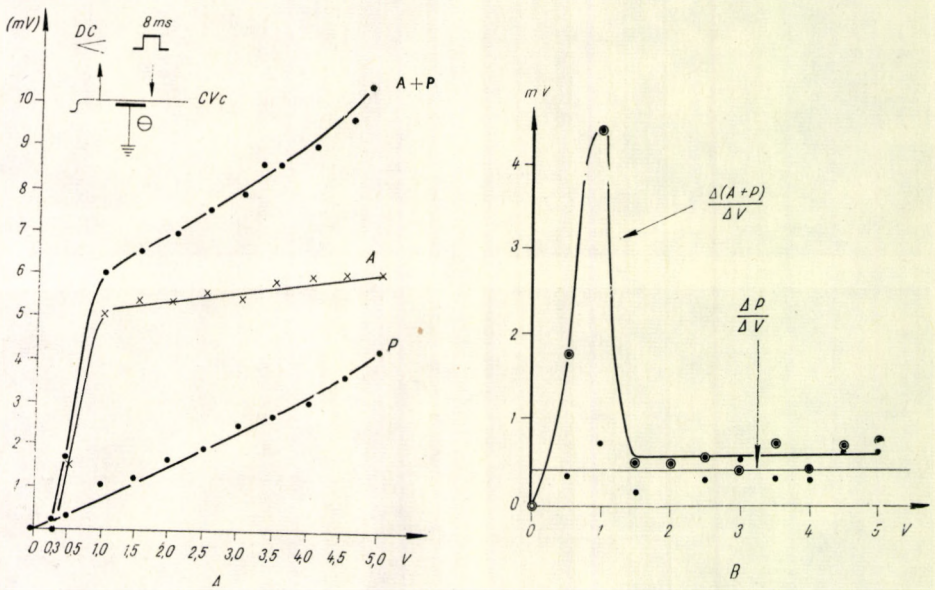

Fig. 6. A) Volt age-dependence of passive (P), complete (A + P) and active (A) potentia component. The curve P was recorded after the novocaine blocking of the nerve. B) Response amplitude increase (fibre "activation") pertaining to the unit increase in voltage. Considerable activation takes place only below 2 V.

6. ábra A. — A passzív (P), teljes (A + P) és aktív (A) potenciálkomponens feszültségfüggése. A P görbét az ideg novokain-blokkja után vettük fel. B. — Egységnyi ingerfeszültségfokozáshoz tartozó válaszamplitúdó-növekedés (rostaktiválás). Jelentős aktiválás csak 2 V ingerfeszültség alatt van.

ing the voltage there appears the first polarity-independent component practically with "all or nothing" character. Since on the CVc previous authors (Kahn and Kusnezov 1938; Zhukov 1949; Lábos et al. 1963; Salánki and Lábos 1964) stated that it conducts the excitation with decrement and the "all or nothing" character is no property of the responses of structures conducting with decrement, we will dwell on the demonstration of the appearance of response with a threshold in detail. As CVc is a complex nerve, its discrete

response appears not at a definite intensity of stimulus but in a stimulus voltage interval. It is seen *(Fig. 6 A)* that upon the increase of the voltage by 0.2 V (from 0.3 V to 0.5 V) a considerable amount of fibre is activated. The further increase of voltage is accompanied by a great increase of the amplitude Δ (A+P) Δ V high; *Fig. 6 B)*. It appears from *Fig. 6 B* illustrating the voltage dependence of fibre activation that at 1 V, 4 msec under the given conditions the fibre activation is minimal. According to measurements conducted for the first active component a stimulus of 1.5—2 V is already supermaximal.

From previous investigations (LÁBOS 1965) it is evident that the electrode removal in the stimulation circuit may increase the above value several times. Therefore, without indicating methodical conditions e. g. electrode parameters the reproduction of this data is not possible even apart from the individual dispersion.

The voltage-dependence curve of the active wave group (as measured at the first peak) *(Fig. 6 A)* is in our assumption combined out of the corresponding curve of the passive polarity — dependent potential and the first polarity-independent component. Thus the *A-curve* indicates the corrected voltage dependence.

In our previous studies owing to the AC recording it was difficult to evaluate the slow components. From *Fig. 5* it appears that the potential maximum is at the 40, 80, 220 and 720 msec moments as measured from the start of the stimulus signal. Accordingly, there is about 20-fold difference between the velocity of the most rapid and the slowest process. The after-potential indicating the positivity of the ground-electrode is present, however, also with DC-recording (see *Fig. 5*, at 1 V), but this corresponds only partly to a true after-potential and is on the other hand the consequence of a rather high degree of asymmetry in the conduction. In such sense it may be brought in connection with spreading.

4. The effect of the change in electrode distance

According to previous examinations small electrode displacements substantially influence size and shape of the recorded action potential (LÁBOS 1965).

At the empoyment of the leading off seen in *Fig. 4 C* and of ground-negative stimulation the removal of the (different) leading electrode from the ground electrode leads to the following changes:

1. with the increase of the distance r the height of the greatest component increases and then diminishes *(Figs 7* and *8)*
2. the components become indistinct
3. as measured on the basis of time at the peak of first component the velocity of this component depends on the assumed path-way of conduction; this relation subsists irrespective of whether we assume the site of origin of the excitement to be at the possible two extreme points.

When conceiving the action potential recorded as a spreading excitation, we can refer to conduction with increment and decrement. But then we must raise the question of the place of origin of the excitement to measure the distance of the way covered. On *Fig. 9.* we represented the dependence on

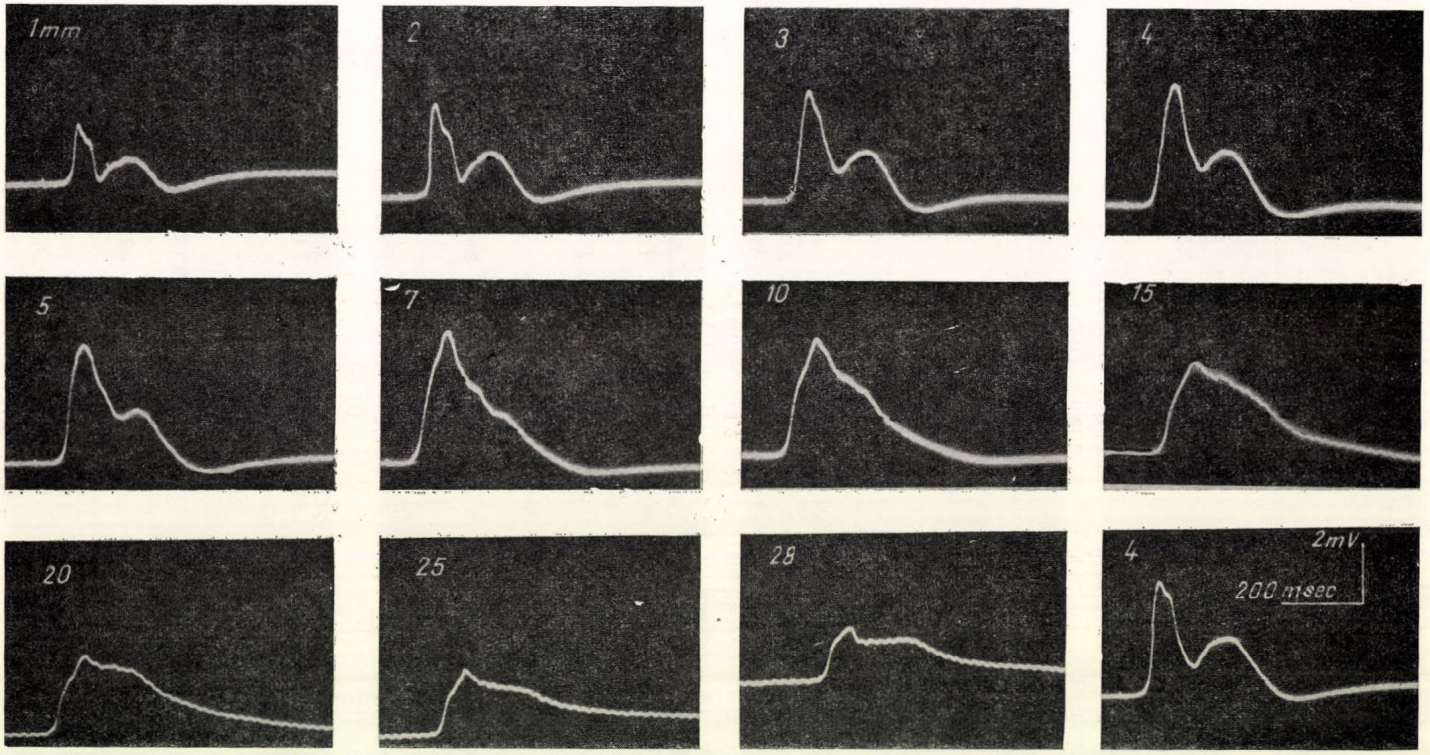

Fig. 7. Change of the action potential at the ,,r'' sector of 1—28 mm length
Stimulus parameters: 3 V, 3 msec; DC-recording; g = 10 mm, s = 1 mm

7. ábra Az akciós potenciál változása 1—28 mm hosszúságú ,,r'' szakasz mellett. Ingerparaméterek:
3 V, 3 msec; DC-elvezetés; g = 10 mm, s = 1 mm.

the way of the assumed velocity of the conduction considering two extreme hypotheses namely whether the excitement originated in points 1 or 2, respectively. From the *Figure* it appears that the hypothesis of the leading off conducted potential goes with the consequence that the velocity has an increment. This increment is of a lesser degree if the excitement would arise in the distant point 2.

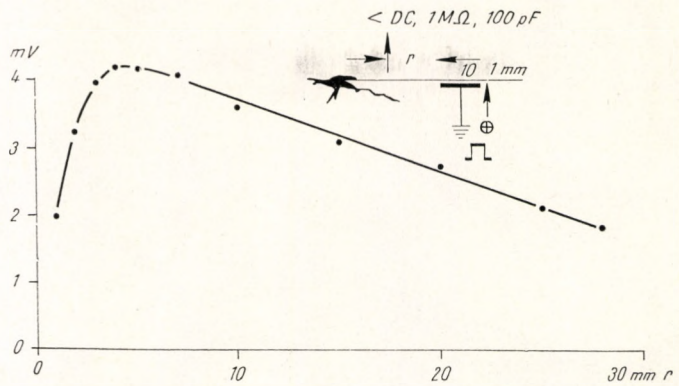

Fig. 8. The dependence of the maximum amplitude of the response seen in *Fig. 7* on the length of the sector „r"

8. ábra A *7. ábrán* látható válasz maximális amplitúdójának függése az „r" szakasz nagyságától.

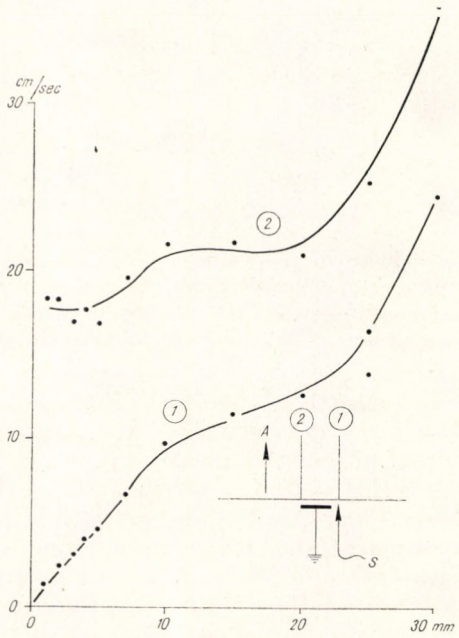

Fig. 9. Explanation in the text; S = stimulator; A = amplifier

9. ábra Magyarázat a szövegben; S = ingerlő; A = erősítő

5. *On the characters of the potential of passive origin*

The period of the passive polarity-dependent potential generally exceeds substantially the period of the excitation signal. When employing long excitations it shows a release effect, a breaking point. This phenomenon begins

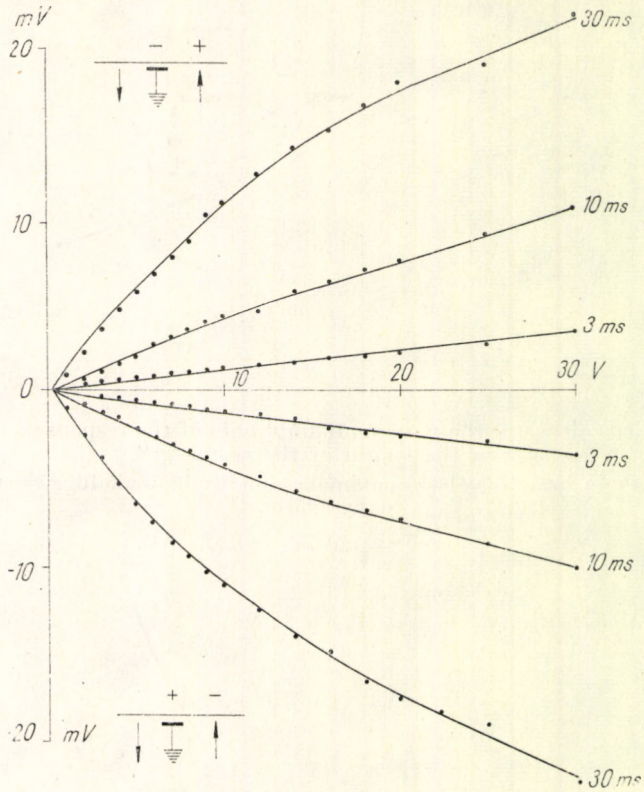

Fig. 10. The voltage dependence of the value of the passive component at various duration of impulse. Ground positive and ground negative stimulation

10. ábra A passzív komponens nagyságának feszültség függése különböző impulzusszélességek mellett. Földpozitív és földnegatív ingerlés.

to appear in excitations longer than 100 msec *(Fig. 3)*. Also with DC recording a slow overshoot can be observed which with high stimulus parameters amounts to 5—10 per cent of the main phase.

The dependence of this potential on the voltage *(Fig. 10)* at small impulse duration is linear. Consequently it differs mainly here from the similar dependence of the action potential. With increasing impulse duration the curve somewhat fattens. With optimum electrode layout (1—10—3 mm) and with impulses of 1—100 msec duration the peak value of the passive potential attains a 5.10^{-3}—10^{-4} part of the stimuli-voltage. The size of the response directed downwards and upwards on blocked nerve is uniform, but a period of several times 10 sec is needed to elicit a reaction true to form and of the original size.

The impulse duration dependence of the amplitude of the potential between 1—10 ms steeply rises to flatten subsequently.

In some cases the passive response obtained on the ground-negative and ground-positive stimulation differs to the benefit of the former. The basis of this asymmetrical behaviour can not be sought in the given electrode display, deviation of stimulating current density, conduction with decrement, inhibiting effect of anode zone, and asymmetrical reaction of amplifier. Consequently it must be ascribed either to the injurious effect of the long-term excitations or to the fact that the depolarisation of the blocked nerve takes place more readily than its hyperpolarization.

Discussion

In analysing the phenomena examined let us deal first of all with the passive polarity-dependent potential. Since thread and dead nerve also exhibit similar phenomena, the term passive response is justified. Naturally the passive reaction different from each other of thread, dead nerve and living nerve reflects also the differences of electrochemical processes taking place in these objects under the influence of electric voltage difference. With extracellular recording it is not possible to decide whether in the living nerve the local potential is only of electrolysis (SEGAL 1958) or another phenomenon. The intracellular recording clearly points to membrane potential changes (HODGKIN, and HUXLEY 1945). The analysis of this, however, exceeds our fixed purpose.

The quadripole, which most closely imitates the time course of the passive response is the impulse stretching circuit (TARNAY 1962). Besides the linear circuits some RCL circuits near limit damping also give similar response if upon their input we give a square pulse. They are not satisfactory, however, for other reasons.

The other important character of the polarity-dependent passive response is that it appears also in subthreshold excitations. As to this feature it corresponds to the subthreshold response of HODGKIN (1938). But even over the supermaximal voltage the increase of passive response could be observed.

Its relative resistance to blocking substances justifies the denomination as rest potential.

Its character of local response, local potential follows from the mode of recording (BURES 1962). It is remarkable that the synaptic potential which is formally similar to the passive potential also shows the features enumerated (TAKEUCHI and TAKEUCHI 1962, ECCLES 1957). Thus, both the natural and artificially arising excitement is introduced by a phenomenon concordant in several features. Of course beside the similarities referred to there are also substantial differences the discussion of which exceeds our purpose.

The passive potential obviously possesses such characteristics on the basis of which it may be named electrotonic potential. To this the fact is pointing that the cessation of its positivity leads to excitement.

We regard as the place of origin of the polarity-independent potential the ground electrode because its direction points to the relative negativity of the ground electrode. In this sense we recorded also the polarity-independent potential as a local phenomenon. It is evident that this does not contradict to its spreading but it can not be regarded as a signal of active change under

the different electrode, when it is directed upwards. The downwards directed and the shunt of opposite direction exceeding even the zero line *(see Fig. 5,* at 1V) may be the consequence of the action potential spread below the different electrode because it points to the increase of negativity of the area below this electrode.

Thus it can be established that we lead off the passive and active response originated under the ground electrode. The local changes taking place under the ground electrode interfere with the waves directed downwards and develop as a consequence of spreading. The excitement originates where the passive response appears as a negativity. Consequently the nerve behaves so that it always responds to a change of ascending direction of the square excitation: we have lead off cathode-on and anode-off responses. In this respect the reaction of the CVc agrees with the response of vertebrate nerves. At ground-positive stimulus the conduction of the excitement developed below the negative stimulating electrode is inhibited by the developed anodic zone. Thus the responding system behaves so as if it would react to a differentiated signal and leaves without answer the positive phase.

In judging the origin of increment and decrement observed in the course of electrode removal we must reckon with the following possibilities:

1. The measurement of the spreading velocity is not correct because the site of measurement (that is the peak of the action potential) is shifting for different reasons.

2. If we assume the fact of spreading for the explanation of the phenomenon demonstrated in *Figs 7* and *8* then the increment can be conceived as a separation phenomenon. In the course of bipolar recording the removal of the conducting electrodes may lead to increased amplitude of the originating wave if the velocity of spreading and the rising velocity of the spreading wave are in a definite relation. But then we ought to observe the increase of the amplitude of the wave spread below the different electrode which, however, is not observed.

3. Increment and decrement can be conceived as a consequence of the passive electric properties on the basis of the following simple cable model. Let us assume that the equivalent of the nervous signal source is a generator causing longitudinal polarisation between the different recording electrode conduction and the ground electrode which has an ohmic resistance and capacity depending on the length. To the reality of the assumption of capacity the differentiation of the square signal points directly. This equivalent (see*F ig. 4 B)* contains these two elements in a parallel bond.

Then, according to the 1. law of KIRCHOFF:

$$I_{\Sigma} = I_A + I_R + I_C$$

where the sum of the currents flowing through the elements R, C and the amplifier A constitute the total current. The current flowing on the amplifier on the input resistance causes the voltage corresponding to the recorded signal.

From the former equation

$$I_A = I_{\Sigma} - (I_R + I_C) = I - \left[U\frac{1}{R} + j\,\omega C\right]$$

where I_Σ is the short-circuit current which in the case of steady excitation is constant, U is the voltage drop on the parallel RC- member.

The voltage drop on the input of the amplifier is

$$E_A = R_A\left[I_\Sigma - U\left(\frac{1}{R} + j\,\omega\,C\right)\right]$$

Let the values of R and C be proportional with the inter-electrodal distance r. Then

$$E_A = K_A - UR_A\left[\frac{1}{k_R r} + j\,\omega\,k_C r\right]$$

From the model employed it follows (see *Fig. 11)*:

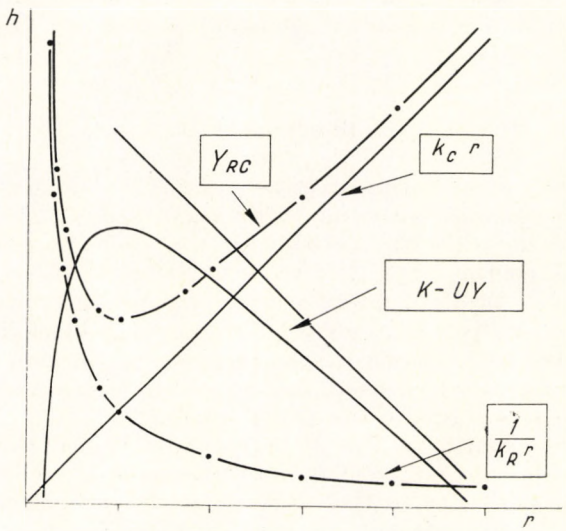

Fig. 11. Explanation in the text
11. ábra Magyarázat a szövegben.

1. On account of the growth of capacity proportional with the length a linearly increasing member ($k_C r$), on account of the growth of resistance a hyperbolically decreasing member ($1/k_R r$) is substracted from the tension dropping on the instrument. Thus from the electromotoric force constant in the case of constant stimulus on account of the inner impedance of the signal source the losses are deducted. The dependence of the value of the voltage drop on the amplifier is determined by these factors. Also the role of the shunt-resistances can not be neglected (e. g. liquid layers in the nerve; see *Fig. 4 B)*. The r-dependence assumes the shape observed in the experiment.

2. In view of the presce of the capacity its signal consuming effect will be more explicit for the rapid components and therefore the dominance of

the slow phenomena can be expected at high r values. This is what the experiments show in reality.

3. The phase of the components of different frequency must show a shift as compared to each other owing to the capacitive member.

It appears that the model used, the correctness of which must be controlled with concrete longitudinal impedance measurements, can readily be employed for the case examined of the electrode removal. Thus in the realization of the decrement observed the role of the passive electric properties can not be neglected. It is remarkable that the slow and according to literature decrementally spreading local response (see the handbook of BURES 1962, p. 243) does not exhibit an explicit decrement. This also stresses the correctness of our model exacting the decrement of lower grade and of methodical origin of the slower components.

The issue of the decrement of the response spreading forth is another problem. According to the model employed a decremental conduction is possible but it must not be assumed for the observed reduction of amplitude. Since it exhibits "all or nothing" properties, it is probable that it has no real decrement.

Summary

The response induced with electric stimulation of the cerebrovisceral connective (CVc) of the fresh-water mussel *(Anodonta cygnea L.)* was examined.

The experiments were conducted with the common grounding of the stimulating and measuring circuit. It was established that

1. The response is divided into a polarity dependent, to chemical influences less sensible passive and a polarity-independent blockable component.

2. The excitement is caused by the increased negativity of the site of its origin. The ground-positive excitation gives an off response. The site of origin of the response recorded is the ground plate.

3 During the polarity independent action potential the negativity of the ground electrode increases, that is in this sense it is accompanied by the change of relative positivity of the different leading-off electrode. Positive "after potential" can be observed also with DC-recording.

4. The increment and decrement observed in the course of the removal of the different leading-off electrode as well as filtering and phase-shift can be explained by the change of the complex admittance of nerve sector in the measuring circuit (r) without the assumption of the recording of the spreading wave.

5. One of the most simple non-linear models of the passive response is an impulse prolonging circuit.

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AZ ELEKTROMOS INGERREL KIVÁLTOTT POTENCIÁLKOMPLEXUM KOMPONENSEINEK TERMÉSZETÉRŐL, *ANODONTA* IDEGEN

Lábos Elemér és *Varanka István*

Összefoglalás

Édesvízi kagyló *(Anodonta cygnea L.)* cerebroviscerális connectivumának (CVc) elektromos ingerrel kiváltott válaszát vizsgáltuk.

A kísérletek az ingerlő és mérő-áramkör közös földelése mellett történtek. Megállapítható, hogy:

1. A válasz polaritásfüggő, kémiai behatásokra érzéketlen passzív, és polaritásfüggetlen, blokkolható komponensre oszlik.

2 Az ingerületet az ingerület keletkezési helyének negativitás-növekedése okozza. Így földpozitív inger nyitási választ ad. Az elvezetett válasz keletkezési helye a földlemez.

3. A polaritás-független akciós potenciál tartama alatt a föld-elektród negativitása nő, azaz ilyen értelemben a differens elvezető elektród pozitivitásváltozása kíséri. Pozitív „utópotenciál" DC-elvezetés mellett is észlelhető.

4. A differens elvezető elektród távolítása során észlelt inkrement és dekrement, valamint szűrés és fáziseltolódás a mérőköri idegszakasz (r) komplex admittanciájának változásával, a terjedő hullám elvezetésének feltételezése nélkül magyarázható.

5. A passzív válasz egyik legegyszerűbb modellje az impulzusnyújtókapcsolás.

О ХАРАКТЕРЕ КОМПОНЕНТОВ НЕРВНОГО ИМПУЛЬСА, ВЫЗВАННОГО ЭЛЕКТРИЧЕСКИМ РАЗДРАЖЕНИЕМ В НЕРВЕ БЕЗЗУБКИ

Элэмер Лабош и Иштван Варанка

1. Изучали реакцию цереброви сцерального коннектива беззубки на электрическое раздражение.

2. Опыты проводили в условиях общего заземления отводящего и раздражающего электродов.

3. В реакции различимы две составляющие. Одна зависит от полярности и нечувствительна к химическим воздействиям, другая не зависит от полярности и состоит из компонентов, блокируемых при химических воздействиях.

4. Возбуждение возникает при увеличении местной негативности. В этом случае при замыкании возникает импульс под заземляющей пластинкой.

5. В течение потенциала действия, независимого от полярности, увеличивается негативность под земляным электродом, иначе говоря происходит изменение позитивности под дифферентным отводящим электродом. Положительное последействие наблюдается и при использовании усилителя постоянного тока.

6. Инкремент и декремент, наблюдаемые при смещении дифферентного отводящего электрода, фильтрация и сдвиг фаз объясняются изменением полной проводимости участка нерва в измерительной цепи; нет необходимости предполагать, что при этом имеет место изменение распространяющейся волны возбуждения.

7. Самая простая модель пассивного ответа обсуждена.

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HYPOXIAL EXAMINATION OF *ANODONTA CYGNEA L.* ON THE O_2-CONSUMPTION OF GILL-TISSUES AND THE RELATION BETWEEN BODY DIMENSIONS AND THE RESPIRATION OF THE GILL-TISSUE

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The various species of the phylum Mollusca reflect an exceedingly wide toleration in time of the anaerobic or hypoxial environmental conditions. *Pisidium idahoense* e. g. survives for 90 days in water free of oxygen, on room temperature, *Anodontoides ferrusaccianus* for 14 days, *Paphia (Tapes)* for 21 days (BRAND 1946 table 12.) while *Littorina punctata* and *L. neritoides* survive for several weeks in a pure nitrogen medium (PATANÉ 1946 a, b.).

Though the tolerance values have no such great amplitude in all species, it is known from the comprehensive study of BRAND (1946) that from the viewpoint of the toleration of anaerobic conditions the order is: Lamellibranchiata > Gastropoda > Cephalopoda. This ranking represents at the same time the velocity degrees of the displacement capacity of these groups.

The higher oxygen deficiency toleration is an adaptation to the wide range of the changes of oxygen content of the natural biotope since the mussel is not able to extract itself from the oxygen deficient environment (in contrast to the organisms capable of swimming). The specific tolerance of O_2 deficiency is a genetically bound characteristic property but the properties of the biotope are of a substantial influence on the intensity of the external gas exchange. Among the shell-fish species, *Modiolus modiolus* of boreal and circumpolar area is of another intensity of metabolism than the closely related cosmopolitan *Mytilus*. Further, also within the species *Mytilus* the intensity of metabolism changes depending on topographical distribution (SCHLIEPER, KOWALSKI, and ERMAN 1958).

Of our superficial natural waters the daily or seasonal changes of oxygen content are characteristic. The values of these are determined — beside physical, chemical and other biological factors — first of all by the vital activity of the O_2-producing and O_2-consuming species of the biomass. Parallel with a substantial reduction of oxygen content, according to their specific properties — in the inferior pejus and pessimum domains — life activity and metabolic processes of the mussels are modified. The energy-requiring movements as a rule slow down or come to stillstand so e. g. the frequency of the periodic activity of *Anodonta cygnea* increases, which is accompanied by the reduction of the activity of the adductor (SALÁNKI 1965). In hypoxial environment the transport performance of the gill-cilia of *Mytilus edulis* decreases or comes to a stillstand (SCHLIEPER and KOWALSKI 1958); the filtration activity is of

course of a parallel value. In a number of other marine species there is a correlation between the bacterium filtration of the clams and the oxygen content (HARANGHY 1959).

Development in time and measure of the restitution of the oxygen debt arising under anaerobic influence raises several problems. To study these problems we conducted our following experiments namely to find out the quantitative and fine characteristics of the restitution of the mussel placed after a hypoxial effect of various duration directly and then again into aerated medium, further the threshold and maximum value of the period of hypoxial effect in the winter season. We have chosen as a measure of these effects the changes in the oxygen consumptions of excised gills which certainly characterize the oxidation conditions of the tissues of the total animal.

Beside these we examined the changes in O_2-consumption depending on body dimensions of the gill of *Anodonta cygnea* and from the average of control data measured with a difference of two months we concluded on the seasonal changes of the intensity of metabolism.

Material and method

For the experiments specimens of *Anodonta cygnea* originating from the fish ponds in Tata and stored in the basins of this Institute were used. For hypoxial examinations specimens of 14 cm, for the examination of the relation between body size and gill respiration specimens of 14, 15, 16, 18 and 20 cm shell length were employed. The water of the mussels was warmed up from the temperature of the storage (+ 4 — + 7° C) gradually (in 5—6 days) to 25°C, to the experimental temperature. During the raising of temperature and adaptation the water was abundantly aerated. The control was permanently under such conditions.

For hypoxial examinations O_2-deficient water was produced in a vacuum cabinet with evacuation and subsequently neutral paraffin oil was stratified on the surface of the water in the glass vat which hindered back diffusion of O_2. Initial oxygen content of the water was 8—8.5 mg/l which by evacuation was reduced to a value of 1—1.5 mg/l but reached shortly after placing of the mussels a value of 0.5—0.2 mg/l. Determination of the O_2-content was carried out with the semimicro method of MAUCHA (1947).

The animals were divided into three groups and these kept in evacuated water for 1, 2 and 3 days. Subsequently in each group the oxygen consumption of the gills was determined in the 1—2 hour after having been taken out of the evacuated water. The remaining members of the groups were placed in aerated water, kept in constant air bubbling and the oxygen consumption of the gills daily determined on four consecutive days. The O_2-consumption was measured with WARBURG'S method at 25° C. As regards more detailed description of the preparation of tissues and measurements we refer to an earlier paper (LUKACSOVICS and SALÁNKI 1964). Each experimental series was repeated 2—3 times, so the given average values were obtained from 12—50 individual measurements. Our results were calculated for mm^3 O_2/100 mg dry matter/hour values and controlled with statistical calculations. The amount of the evacuated water was 1 litre per mussel. Although in our experiments we concentrated our attention first of all on the effect of hypoxial conditions, we

must not leave out of consideration that in a water-space closed with paraffin oil the metabolic products discharged by the mussels influence the results. We tried to reduce this effect by exchanging 25 per cent of the water daily with freshly evacuated water containing no metabolic product.

Results

A) *O_2-consumption of isolated gills as a function of body size.*

Before starting our experiments we deemed it necessary to measure how far our results are influenced when the gills of mussels of various size are respirated, more exactly within what dimensional limits the body size does not

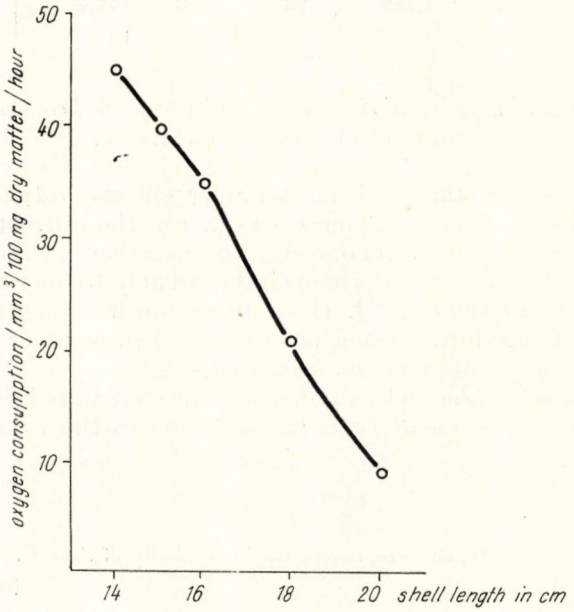

Fig. 1. Oxygen consumption of the isolated gills of *Anodonta cygnea L.* specimens of various size

1. ábra Különböző nagyságú *Anodonta cygnea L.* példányok izolált kopoltyúinak oxigénfogyasztása.

disturb our results of consumption. For this purpose in view the respiration results of specimens with 14, 15, 16, 18 and 20 cm shell length were compared *(Fig. 1)*

The mean values reveal a close connection between the mussel dimension and the O_2-consumption related to the unit weight of the gill. The relationship is inverse, because with the increase of the body dimension the consumption is reduced. The reduction of consumption per 2 cm is 26 per cent on the average.

1. táb-

Különböző ideig hypoxiásan tartott *Anodonta cygnea L.*
(mm³ O_2fogyasztás/100

Hypoxiát követő napok	Hypoxia időtartama napokban							
	1				2			
	O_2-fogyasztás átl. ($\bar{x}$)	standard deviatio (S) ±	átl. standard hibája ($S_{\bar{x}}$)	mérések száma (n)	O_2-fogyasztás átl. ($\bar{x}$)	standard deviatio (S) ±	átl. standard hibája ($S_{\bar{x}}$)	mérések száma (n)
0	48.1	5,769	1,489	15	49,1	5,187	1,132	21
1	47.4	5,677	1,467	15	67,7	8,971	2,006	20
2	50.8	13,071	3,493	14	65,6	10,709	2,395	20
3	47.7	11,079	2,477	20	70,6	8,784	2,208	19
4	50.7	7,970	1,782	20	63,2	11,492	2,786	17

B) *The effect of keeping of the mussel under hypoxial conditions on the respiration of the isolated gill tissue.*

Earlier, in connection with our summer gill respiration experiments in another direction on days following each other in the untreated groups there was between the averages from one day to the other an increase in oxygen consumption of almost 100 mm³ which on the 3rd and 4th day fell back again to the value of the first day *(Fig. 2)*. The explanation was soon found in an anaerobic effect suffered during transportation on high summer temperature the restitution of which could thus be measured.

Results of our hypoxial examinations are presented in *Table 1*, while their statistical evaluation in *Table 2*. According to the values of the Tables

Tab-

O_2-consumption of the isolated gills of *Anodonta cygnea L.* specimens
(mm³ O_2-consumption

Days following hypoxia		Period of hypoxia in days						
		1			2			
	Mean O_2-consumption ($\bar{x}$)	Standard deviation (S) ±	Standard error of mean ($S_{\bar{x}}$)	Number of measurements (n)	Mean O_2-consumption ($\bar{x}$)	Standard deviation (S) ±	Standard error of mean ($S_{\bar{x}}$)	Number of measurements (n)
0	48.1	5.769	1.489	15	49.1	5.187	1.132	21
1	47.4	5.677	1.467	15	67.7	8.971	2.006	20
2	50.8	13.071	3.493	14	65.6	10.709	2.395	20
3	47.7	11.079	2.477	20	70.6	8.784	2.208	19
4	50.7	7.970	1.782	20	63.2	11.492	2.786	17

lázat

példányok izolált kopoltyúinak O_2fogyasztása a hypoxiát követő napokon
mg száraz anyag/óra)

3				Kontrol			
O_2-fogyasztás átl. ($\bar{x}$)	standard deviatio (S) ±	átl. standard hibája ($S_{\bar{x}}$)	mérések száma (n)	O_2-fogyasztás átl. ($\bar{x}$)	standard deviatio (S) ±	átl. standard hibája ($S_{\bar{x}}$)	mérések száma (n)
58,1	9,003	2,065	19	64,8	11,124	1,966	32
51,3	8,176	1,828	20	60,2	13,957	2,737	26
60,3	9,245	2,178	18	55,8	3,826	2,115	26
66,3	8,285	1,953	18	57,1	10,094	1,784	32
62,0	6,571	1,897	12	52,0	10,684	1,806	35

the gills of the mussels kept hypoxially for 1, 2 and 3 days then they got directly in a water rich in oxygen without exeption consumed significantly less oxygen than the control. When examining the various groups separately the following statements can be made:

The specimens treated hypoxially for 1 day after 1, 2 and 3 days of aeration did not attain the level of the oxygen consumption of the control but lagged behind; only on the 4th day was the oxygen consumption almost identical.

The gills of specimens treated hypoxially for 2 days consumed already after 1 day of aeration substantially more oxygen than the control; this increased consumption could be measured also on the following days.

The specimens kept for 3 days in hypoxia in contrast to the oxygen con-

le 1

kept hypoxially for various periods, on the days following hypoxia
/100 mg dry matter/hour)

3				Control			
Mean O_2-consumption ($\bar{x}$)	Standard deviation (S) ±	Standard error of mean ($S_{\bar{x}}$)	Number of measurements (n)	Mean O_2-consumption (x)	Standard deviation (S) ±	Standard error of mean ($S_{\bar{x}}$)	Number of measurenst (n)
58.1	9.003	2.065	19	64.8	11.124	1.966	32
51.3	8.176	1.828	20	60.2	13.957	2.737	26
60.3	9.245	2.178	18	55.8	3.826	2.115	26
66.3	8.285	1.953	18	57.1	10.094	1.784	32
62.0	6.571	1.897	12	52.0	10.684	1.806	35

Tab

Statistical evaluation of O_2-consumptions of the isolated gills of *Anodonta*

Control	Period of treatment (in days))	Period of keeping in aerated water after treatment in days	t	Degree of freedom	P	Significant difference	Period of treatment (in days)	Period of keeping in aerated water after treatment in days
	1	0	5.467	45	0.01	v	1	0
0	2	0	6.034	51	0.01	v	2	0
	3	0	2.224	49	0.05	v	3	0
	1	1	3.381	39	0.01	v	1	1
1	2	1	2.091	44	0.05	v	2	1
	3	1	2.533	44	0.02	v	3	1
	1	2	1.298	38	0.10	n	1	2
2	2	2	2.124	44	0.05	v	2	2
	3	2	1.441	42	0.10	n	3	2
	1	3	3.152	50	0.01	v	1	3
3	2	3	4.691	49	0.01	v	2	3
	3	3	4.839	48	0.01	v	3	3
	1	4	0.478	53	0.50	n	1	4
4	2	4	3.459	50	0.01	v	2	4
	3	4	3.034	45	0.01	v	3	4

Sings: v = significance present n = significance absent

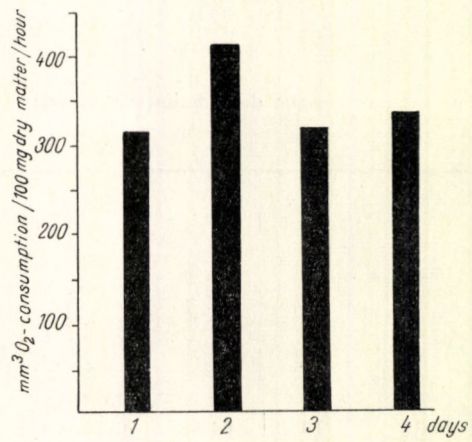

Fig. 2. Changes in oxygen consumption of the isolated gills of *Anodonta cygnea L.* specimens kept in summer for 4—6 hours on the air

2. ábra Nyáron 4—6 órát levegőn tartott *Anodanta cygnea L.* példányok izolált kopoltyúinak oxigénfogyasztás változása.

le 2

cygnea, L, measured after hypoxial treatment of various periods

t	Degree of freedom	P	Significant difference	Period of treatment (in days)	Period of keeping in aerated water after treatment in days	t	Degree of freedom	P	Significant difference
0.544	34	0.50	n	1	0	3.73	32	0.01	v
3.917	38	0.01	v	3					
7.62	33	0.01	v	1	1	1.580	33	0.10	n
6.042	38	0.01	v	3					
3.528	32	0.01	v	1	2	2.407	30	0.02	v
1.772	38	0.05	n	3					
7.127	37	0.01	v	1	3	5.803	36	0.01	v
1.528	35	0.10	n	3					
3.576	35	0.01	v	1	4	4.136	30	0,01	v
0.323	28	0.50	n	3					

sumption expected did not rise after one day of aeration above the values of the control — as in the case of the specimens kept hypoxially for 2 days — but lagged considerably behind them. After two days of aeration they surpassed though the control values but not significantly. Only after 3 and 4 days of aeration was the oxygen consumption significantly higher as compared with the control.

When comparing the oxygen consumption of the gills of the specimens treated hypoxially for 1, 2 and 3 days with each other the following results are obtained:

The consumption values of the animals treated for 1 and 2 days fail to differ from each other only on zero day while on the other aerated days they in all cases significantly differ from each other. The oxygen consumption values of the animals treated hypoxially for 1 and 3 days after 1 day of aeration do not substantially differ. The gills of the animals kept in oxygen deficient water for 2 and 3 days during aeration for 2, 3 and 4 days exhibit near oxygen cosumption values with the remarkable difference that the oxygen consumption values of the specimens kept hypoxially for 2 days are, if not significantly, invariably higher.

During the 2 months of this experimental series an almost unequivocally diminishing trend of oxygen consumption was observed in the control from 64.8 cu.mm to 48.4 cu.mm that is to 75 per cent of the starting value.

2. táb-

Anodonta cygnea L. izolált kopoltyúinak különböző időtartamú

Kontrol	Kezelés időtartama (napokban)	Kezelés után aerált vízben tartás időtart. napokban		Szabadságfok	P	Szignifikáns különbség	Kezelés időtartama (napokban)	Kezelés után aerált vízben tartás időtart. napokban
	1	0	5.467	45	0.01	v	1	0
0	2	0	6.034	51	0.01	v	2	0
	3	0	2.224	49	0.05	v	3	0
	1	1	3.381	39	0.01	v	1	1
1	2	1	2.091	44	0.05	v	2	1
	3	1	2.533	44	0.02	v	3	1
	1	2	1.298	38	0.10	n	1	2
2	2	2	2.124	44	0.05	v	2	2
	3	2	1.441	42	0.10	n	3	2
	1	3	3.152	50	0.01	v	1	3
3	2	3	4.691	49	0.01	v	2	3
	3	3	4.839	48	0.01	v	3	3
	1	4	0.478	53	0.50	n	1	4
4	2	4	3.459	50	0.01	v	2	4
	3	4	3.034	45	0.01	v	3	4

Jelzések: v = szignifikáns különbség van
n = nincs szignifikáns különbség

Comparing the mean of the controls measured between 11th and 25th October (32 measurements) with the mean of the control group from the 6—10th December measurement (38) we obtained the result that the O_2-consumption during these two months diminished by 13.3 per cent (from 57.1 cu. mm to 49.5 cu. mm.). Statistical calculation verified the significance of the differences ($P < 0.01$, $t = 3.045$, degree of freedom = 68).

Discussion

The question concerning the most proper basis of relationship for the oxygen consumption of an animal is not uniformly decided as yet. It is probable that the O_2-consumption-dependence of the different varieties can be characterized with various biometrical parameters. SCHWARTZKOPFF (1959), who carried out about 570 measurements on 17 species of the phylum Mollusca draws the conclusion that since the values of the regression constant are practically between 0.6 and 1.05 "without the recognizableness of any rational system there can be no question of surface or mass dependence of respiration. At the same time in the oxygen consumption examinations of *Mytilus edulis* an interdepence was found between surface and body weight (KRÜGER 1960).

In our measurements there was a definite connection between the body size and the oxygen consumption of the gills pertaining to them: with increasing body dimensions the amount of oxygen consumed diminished. Between 2

lázat

hypoxiás kezelése után mért O_2-fogyasztások statisztikai értékelése

t	Szabadságfok	P	Szignifikáns különbség	Kezelés időtartama (napokban)	Kezelés után aerált vízben tartás időtart. napokban	t	Szabadságfok	P	Szignifikáns különbség
0.544	34	0.50	n	1	0	3.73	32	0.01	v
3.917	38	0.01	v	3	0				
7.62	33	0.01	v	1	1	1.580	33	0.10	n
6.042	38	0.01	v	3	1				
3.628	32	0.01	v	1	2	2.407	30	0.02	v
1.772	38	0.05	n	3	2				
7.127	37	0.01	v	1	3	5.803	36	0.01	v
1.528	35	0.10	n	3	3				
3.576	35	0.01	v	1	4	4.136	30	0.01	v
0.323	28	0.50	n	3	4				

measurement groups of *Mytilus edulis* also similar body size and isolated gill O_2-consumption dependence was demonstrated (LAGERSPETZ and SIRKKA 1959). In our measurements most factors (except for body size) were constant, namely the medium (filtered water of Lake Balaton), period of heat adaptation (5 days), temperature of measurement (25° C) relating the oxygen consumption to 100 mg dry matter and finally the "nominal" surface of the gills excised and made to respire, since 30 small slices of 5 × 5 mm edge lenghth (~1500 sq. mm) were placed in each little WARBURG pot.

Of the above constant factors we must deal more in detail with the question of the actual size of the surface, since the external and internal gill plates even of the same mussel constructionally differ from each other (KILIAS 1956). The deviation manifests itself among others in that the interlamellar cavities of the external gills extend to a distance of 6—10, while those of the internal ones to a distance of 15—20 filaments and on the other hand also the number of filaments may change both concerning laterality and body size *(Table 3)*.

The density values of the filaments per unit length supply only approximately a correct picture of the actual situation because at the excision the tactic stimuli elicit muscle contraction by which the gill contracts and subsequently relaxing a few minutes after the excision its surface increases. We believe, however, that the number of filaments as related to body length readily shows the difference and at the same time represents the gill surface increase by the filament density of the smaller (younger) mussels. Therefore it was

Table 3

Number of filaments within a distance of 5 mm of the gill of *Anodonta* cygnea *L.* specimens of various size (Measurements were conducted at the edge of the gills at three sites: on the area of the broader = oral, medial and narrower = anal part)

	12.5 Length of shell cm			18		
	Number of filaments per 5 mm lenght			Number of filaments per 5 mm length		
	Site of measurement			Site of measurement		
	Oral	medial	anal	Oral	medial	anal
Exterior pair	48	55	47	35	36	34
	46	48	47	38	40	33
	46	54	48	37	44	34
	40	56	49	35	40	37
	40	54	51	38	49	31
	46	58	48	43	45	35
Interior pair	47	48	54	37	39	39
	48	51	49	37	36	43
	50	53	45	38	34	35
	45	50	49	40	43	36
	50	53	43	46	38	34
	49	54	43	46	36	34

3. táblázat

Különböző nagyságú *Anodonta cygnea L.* példányok kopoltyúinak 5 mm távolságon belüli filamentumszáma

Mérések a kopoltyúk szegélyén három helyen történtek, éspedig a szélesebb = oralis, medialis és keskenyebb = analis rész területén)

	Kagylóhéj hossza cm 12,5			18		
	5 mm hosszra eső filamentumok száma			5 mm hosszra eső filamentumok száma		
	Mérés helye			Mérés helye		
	oralis	medialis	analis	orali s	medialis	analis
Külső pár	48	55	47	35	36	34
	46	48	47	38	40	33
	46	54	48	37	44	34
	40	56	49	35	40	37
	40	54	51	38	49	31
	46	58	48	43	45	35
Belső pár	47	48	54	37	39	39
	48	51	49	37	36	43
	50	53	45	38	34	35
	45	50	49	40	43	36
	50	53	43	46	38	34
	49	54	43	46	36	34

necessary to reduce to the minimum the effects disturbing the results of the hypoxial examinations (which with 2 cm difference of length may result in a 26 per cent error on the average) by using gills of 14 cm ($\pm$5 mm) mussels.

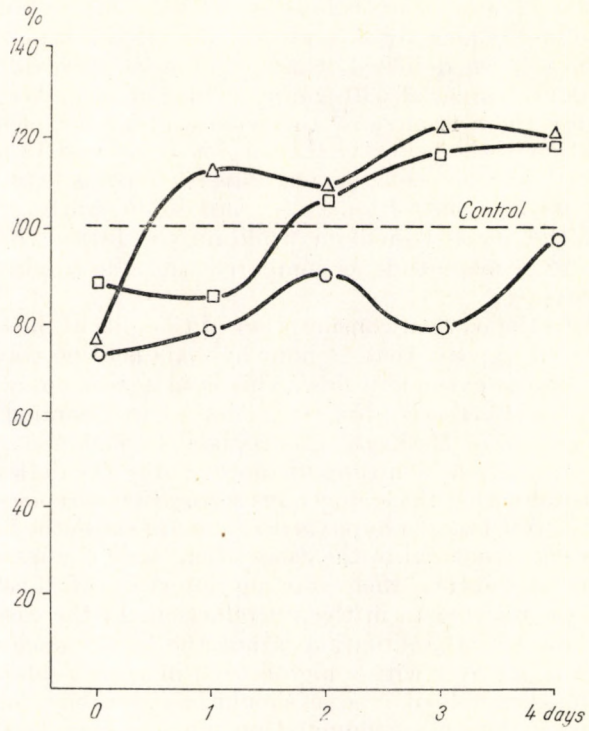

Fig. 3. Oxygen consumption values after a hypoxial effect of 1—3 days as measured in the isolated gills of *Anodonta cygnea L.* and expressed in per cent of the control
Signs: ○ = measured after 1 day of hypoxia immediately after keeping for 1—4 days in aerated water; △ = measured after 2 days of hypoxia immediately after keeping for 1—4 days in aerated water; □ = measured after 3 days hypoxia immediately after keeping for 1—4 days in aerated water

3. ábra 1—3 napig tartó hypoxiás hatás utáni oxigénfogyasztási értékek az *Anodonta cygnea L.* izolált kopoltyuin mérve és a kontrol százalékában kifejezve. ○ = 1 napig tartó hypoxia után azonnal, illetőleg 1—4 napos aerált vízben tartás után mérve. △ = 2 napig tartó hypoxia után azonnal, illetőleg 1—4 napos aerált vízben tartás után mérve. □ = 3 napig tartó hypoxia után azonnal, illetőleg 1—4 napos aerált vízben tartás után mérve.

O_2-consumption values developed upon hypoxial action are most readily illustrated by the representation expressed in per cent of the control *(Fig. 3)*. Directly after hypoxia — independently of its 1—3 day period — in contrast to expectations the oxygen consumption values do not increase but diminish as compared with the control. Thus the amortization of the O_2-debt does not start immediately, inspite of the fact that in the WARBURG pots the gill slices get into water rich in oxygen. This means that the anaerobic oxydation enzyme system of the gills is replaced with a certain delay by

an aerobic enzyme system. While the gills of the mussels which spent 1 day in hypoxia consume about 26 per cent below the control, after placing them in water abundant in O_2 on the first day the O_2-consumption is 22 per cent, on the second day 10 per cent, on the third day again 20 per cent and after the fourth day still a few per cents below the control value, but not significantly. The gills of the mussels kept for 2 days in hypoxial conditions directly after taken out of the oxygen deficient water (that is on zero day) are indebted with 10 per cent as compared with the control, but after keeping in aerated water for one day there is already an increase of 12 per cent and after the 8 per cent reduction of the next day the surplus is 22 and 18 per cent respectively as compared with the control. The gills of mussels kept hypoxially for three days surpass the control values — not significantly — only after 48 hours action of the aerated medium while on the further days there is no difference in order of magnitude as compared with the respiration of mussels after 2 days of hypoxy.

Reverting to the oxygen consumption of the gills of mussels kept hypoxially for one day it appears that 24 hour hypoxia is of no considerable effect since the restitution is extremely slow. This is in agreement with the general statement of BRAND (1964) according to which the Lamellibranchiata as compared with other groups of Mollusca (Gastropoda, Cephalopoda) on account of their ecological adaptations can support more readily the deficiency of oxygen and in this hypoxial period the endogenous respiration is replaced by an energy production which after 1 day of hypoxia does not appear in the form of intensive O_2-hunger. This seems natural in the cases when there is a question of Lamellibranchiata species showing such starting intervals of tolerance limits as *Pisidium idahoense* referred to in the introduction. In the case of the species chosen by us, however, the situation is not the same, since already the 72 hours hypoxia was got over with a high rate of mortality (about 95 per cent). That the stimulus threshold of hypoxia should be more than one day is contradicted by our measurements conducted on mussels kept in the summer for a few hours on the air when they readily responded to anaerobic action lasting for 4—6 hours. The values of the anaerobic toleration of *Anodonta cygnea* given by BRAND (1946. *Table 12)* are in reality no demonstrably anaerobic data, so the exceedingly different intervals (1, 7, 8, 30 days) are easy to understand. At the measurement of the hypoxial effect examination of the control values reveals two reductions in time of its O_2-consumption values:

1. On the days after raising the temperature of the environment (+ 4, + 7° C) to the experimental temperature (25° C) the oxygen consumption per 100 mg dry matter gradually diminishes. The reduction is almost even *(Table 1)* and its value in 9—10 days attains 25 per cent. It is well known that also. *A. cygnea* as a poikilothermic organism has in the winter a period of rest during which dug in into silt it makes little movement and its metabolism in accordance with the temperature is reduced to a low value. Heating up to 25° C is an "abiological" heat effect manifesting itself first in an increased and subsequently in a diminishing oxygen consumption.

2. The seasonal changes of the oxygen consumption of *A. cygnea* were measured by the comparison of the October and December means of the control groups. That the reduction of oxygen consumption is actually a seasonal influence is made probable by the results of several chemical analyses. Glycogen content of *Ostrea circumpicta* and *O. edulis* gradually increases from a minimum

in September or June—July respectively until March (OKAZAKI and KOBAYASHI 1929, MITCHELL 1915, RUSSEL 1923) and the protein content of *Mytilus edulis* also undervent a seasonal change (HENSCHEL 1952). Still nearer to our theme stand the data pointing — although they are not results obtained from fresh water organisms — to the alterations of the external gas exchange as the oxygen consumption of the Long Island oysters and the seasonal changes of their RQ-values (GALTSOFF 1964) and of the oxygen consumption of *Mytilus edulis* (KRÜGER 1960). In the latter animal the relative minimum appears in the months of October—November.

The influence of the anaerobic or hypoxial environment manifests itself also in other respect than in oxygen hunger but the effect is not always unequivocal. Under anaerobic conditions a reduction of the glycogen content in the tissues of *Mya arenaria* (RICKETTS and CALVIN 1948) and of *Crassostrea gigas* (USUKI 1962) could be demonstrated; at the same time in the tissues of the snail *Busycon* (Neogastropoda) the amount of oxygen was unchanged (SCHEER 1948). In the clams *Ostrea* and *Venus* after anoxybiosis lactic acid accumulation took place, but in the gills and tissues of *Anodonta cygnea* the lactic acid concentration did not change (SALÁNKI 1964). Thus the biochemical and physiological disclosure of the mechanism still belongs to the tasks of the future.

Summary

Fresh-water mussel *(Anodonta cygnea L.)* specimens were kept under hypoxial conditions and the effect of hypoxia was measured on the respiration of gill tissues in the winter months (October, November, December). The measurements were conducted on the gills after different periods of hypoxia immediately or after 1—4 days of aeration respectively.

It was further examined how far the oxygen consumption per 100 mg dry matter of the gill slices of a total of about 1500 sq.mm changes with the increase of the body dimensions (shell length) of the mussel.

Results obtained were as follows:

1. In the months of October-December the mussels, with a high (about 95 per cent) mortality tolerated the hypoxial medium for no more than 3 days.

2. Irrespective of the 1—3 day period of hypoxia in the first hours the respiration values still lagged below the control level.

3. The isolated gills of the mussels which were only for 1 day in hypoxial environment reached the oxygen consumption values of the control only after 4 days of aeration; i. e. one day of hypoxia did not result in a measurable oxygen deficit.

4. When the mussels were kept hypoxially for 2 days and then placed into aerated water for 1 day, the oxygen consumption of their isolated gills was more intensive than that of the control, i. e. signs of replacement of the oxygen deficiency suffered appeared, which phenomenon was measurable also on the 2nd 3rd and 4th day.

5. The gills of the mussels kept hypoxially for 3 days exhibited a restitution only after 2 days of aeration and also on the further days remained above the control values.

6. The oxygen consumption value of the controls during the two months of the measurement decreased by about 13 per cent which is presumably a seasonal effect.

7. Determination of the oxygen consumption values of *Anodonta cygnea* specimens of 14, 15, 18 and 20 cm shell length revealed that with increasing body dimensions the intensity of respiration related to the surface and weight unit diminished by 26 per cent per 2 cm.

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ANODONTA CYGNEA L. HYPOXIÁS VIZSGÁLATA A KOPOLTYÚSZÖVETEK O_2-FOGYASZTÁSÁN, VALAMINT TESTMÉRET ÉS A KOPOLTYÚSZÖVET LÉGZÉSÉNEK RELÁCIÓJA

Lukacsovics Ferenc

Összefoglalás

Szerző hypoxiás körülmények között tartott tavi kagyló *(Anodonta cygnea L.)* példányokat és a hypoxia hatását a kopoltyúszövetek légzésén mérte, a téli hónapokban (október, november, december). A méréseket a kopoltyúkon a különböző ideig tartó hypoxia után közvetlen, illetőleg 1—4 napos aeráció után végezte.

Vizsgálta továbbá, hogy a kagyló testméretének (héjhosszúságának) növekedésével hogyan változik az összesen kb 1500 mm² felületű kopoltyúszeletkék 100 mg szárazanyagra vonatkoztatott oxigénfogyasztása.

A következő eredményeket kapta:

1. Október—december hónapokban nagy mortalitási % mellett (kb 95%) mindössze 3 napig bírták a kagylók a hypoxiás miliőt.

2. A hypoxia 1—3 napos időtartamától függetlenül az első órákban a légzési értékek még a kontrol szintje alatt maradtak.

3. Azon kagylók izolált kopoltyúi, amelyek 1 nap időtartamig voltak hypoxiás környezetben csak 4 napos aeráció után érték el a kontrol oxigénfogyasztási értékeit, tehát egy nap hypoxia nem eredményezett mérhető oxigén deficitet.

4. Ha a kagylókat 2 napig hypoxiásan tartották, majd 1 napra aerált vízbe helyezték, akkor izolált kopoltyúik oxigénfogyasztása a kontrolnál élénkebb volt, azaz az elszenvedett oxigénhiány pótlási jelei mutatkoztak, amely jelenség a 2. 3. és 4. napon is mérhető volt.

5. A 3 napig hypoxiásan tartott kagylók kopoltyúi csak 2 nap aeráció után mutattak restitutiót és a további napokon is a kontrol értékei felett maradtak.

6. A kontrolok oxigénfogyasztási értéke a mérés két hónapja alatt mintegy 13%-kal csökkent, mely feltehetően szezonális hatás.

7. 14, 15, 16, 18 és 20 cm héjhosszúságú Anodonta cygnea példányok izolált kopoltyúinak oxigénfogyasztásait meghatározva kitűnt, hogy növekvő testmérettel csökkent a felület és súlyegységre eső légzés-intenzitás. Ennek mértéke 2 cm-enként 26% volt.

ИССЛЕДОВАНИЕ ПОТРЕБЛЕНИЯ КИСЛОРОДА ЖАБЕРНЫМИ ЛЕПЕСТКАМИ *ANODONTA CYGNEA L.* В ГИПОКСИЧЕСКИХ УСЛОВИЯХ И НАБЛЮДЕНИЯ НАД ВЗАИМООТНОШЕНИЕМ МЕЖДУ РАЗМЕРОМ ТЕЛА И ЖАБЕРНЫМ ДЫХАНИЕМ

Ференц Лукачович

Измеряли дыхание жабер беззубки в зимние месяцы (октябрь — декабрь).Измерения проводили сразу после гипоксических условий или после выдерживания беззубок, вслед за гипоксией, в воде, насыщенной кислородом, в течение 1—4 дней. Также изучали, как меняется потребление кислорода в расчете на 100 мг сухого веса, в зависимости от размеров беззубки (длина створок).

Получены следующие результаты:

1. Беззубки выдерживают в октябре — декабре гипоксические условия не более 3 дней, при относительно высокой смертности (95%).

2. В первые часы после гипоксии, независимо от ее продолжительности (1—3 дня), потребление кислорода ниже контроля.

3. Изолированные жабры беззубок, выдержанных в гипоксических условиях в течение суток, достигали контрольного уровня дыхания только после 4-дневного содержания в оксигенированной среде. Значит, одни сутки гипоксии не приводят к измеримому недостатку кислорода.

4. Если после двух суток пребывания в гипоксических условиях беззубок на сутки помещали в оксигенированную воду, то потребление кислорода изолированными жаберными лепестками было выше контроля, то есть произошло восполнение кислородных потерь. Сходное явление наблюдалось и на 2, 3, 4 день после аноксибиоза.

5. Жаберные лепестки беззубок, перенесших 3-дневную гипоксию, восстанавливали дыхания только после двух дней аэрации и в последующие дни оставались выше контроля.

6. В течение сезона измерения произошло понижение дыхания в контроле на 13%, что по-видимому связано с сезонными изменениями.

7. При сравнении дыхания жаберных лепестков у беззубок длиной 14, 15, 16, 18 и 20 см. обнаружено, что при увеличении размеров тела снижается интенсивность дыхания в расчете на единицу веса и поверхности. При изменении длины на 2 см дыхание меняется на 26%.

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CILIARY ACTIVITY EXAMINATIONS AFTER ANOXYBIOSIS ON THE ISOLATED GILLS OF *ANODONTA CYGNEA L.*

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Beside the recent comprehensive studies which appeared after the monograph of GRAY "Ciliary movement" (1928) (RIVERA 1962, SLEIGH 1962) a number of papers dealt with the ciliary activity of Molluscs. Of these the investigation of the gill cilia of Lamellibranchiata emerges quantitatively. The epithelium of comparatively large surface and intensive ciliary activity of the clams which can be conceived also as bioindicator marks with the change of velocity of movement the changes of physiological and biochemical processes taking place in tissue and cells spontaneously or induced by agents. Thus e. g. the effect of the agents influencing the intermediary metabolism (USUKI 1956 a, b, c, d) and the heat adaptation conditions (VERNBERG, SCHLIEPER and SCHNEIDER 1963, PRECHT and CHRISTOPHERSEN 1965, LAGERSPETZ and DUBITSCHER 1966) were recorded with the motion activity of gill cilia. Valuable knowledge was published also on the mode of action of osmoregulation, anoxybiosis and other environmental factors (GRAY 1928, SCHLIEPER, KOWALSKI and ERMAN 1958, SCHLIEPER and KOWALSKI 1958).

It is a well known fact that after anoxybiotic effects (=AOB SCHLIEPER et al. 1958) in the tissues an O_2 debt of such dimensions arises which under oxybiotic conditions is compensated by the organism with an oxygen consumption surpassing the normal level. In the course of this process also the activity of the gill cilia is intensified (SCHLIEPER and KOWALSKI 1958). Since in our AOB examinations conducted during the winter semester, in the cold water period (LUKACSOVICS 1966), the onset of the O_2 restitution of isolated gills exhibited a delay, and started 6—24 hours after the AOB, we examined still in this season the behaviour of the ciliary movement of gills to establish whether they also show a similar phenomenon in their intensity.

Material and method

For these experiments specimens of 14 cm shell length of *Anodonta cygnea* were used. During the winter the animals were stored in Lake Balaton, under natural conditions. The temperature of the lake in the experimental period (8th February — 21st March 1966) was +4—+7° C. The temperature of the water of the animals brought into the aquarium was gradually raised —

— in 5 days — to 25° C and at the same time ciliary activity was measured per 5° C. At the time of heat adaptation the water of the mussels was abundantly aerated.

Oxygen-free water was produced in a vacuum cabinet when the initial 8—8.5 mg/l oxygen content was reduced to a value of 1—1.5 mg/l and then shortly after placing in the mussels to an amount which could not be demonstrated. The oxygen content determinations were conducted with the semimicro method of MAUCHA (1947). The evacuated water was closed up with neutral paraffin oil. Accumulation of metabolic products was diminished with the daily exchange of freshly evacuated water. 1 liter water was calculated for 1 mussel.

The mussels were kept for 1—3 days under AOB conditions, then taken out and on the 0., 1st and 2nd day the velocity of the gill-cilia was measured, but on the 1st and 2nd day after AOB the animals were kept under OB conditions, at 25° C temperature.

Ciliary activity was measured according to SCHLIEPER and KOWALSKI (1958) on the transport performance of the gill-cilia. The excised gills were laid in PETRI dishes cast with paraffin, fixed with pins, then Balaton water rich in O_2 was stratified over them and a celluloid ruler standing on 40 mm long legs laid across so that it did not touch the gill. Subsequently small 1 sq.mm aluminium foil pieces were dropped on the lateral surface of the gill near the line of intersection and the time that passed during the 15 mm distance was covered has been measured with a stopper. The transport performance was calculated over into min/mm value. Each average result was obtained from 12—30 individual measurements.

Since in the experimental period the external gill of several mussels may be full of developing glochidium, the measurements were always carried out on the interior side of the interior gills.

Results

The experimental temperature of 25° C was obtained from the temperature of storage (+4 — +7° C) with a daily rise of 5° C. During heat adaptation ciliary activity of the isolated gills was measured at the intermediary temperatures *(Fig. 1)*. At 10, 15 and 20° C with rising temperature the transport performance of the cilia increased. When taking the value performed at 10° C as a basis, then at 15° C the measured activity was 21 per cent and at 20° C 74 per cent higher. At 25° C we found a 35 per cent inhibition as compared with the previous temperature grade that is the value of heat stimulation was no more than 39 per cent. Upon AOB effects lasting for various periods (2—3 days) on the gill of the fresh water mussel after isolation the following transport performances were measured *(Fig. 2)*. As compared with the control values after one day of AOB the intensity of the movement of the gill cilia increased by 25 per cent, after 2 days by 39 and after 3 days of AOB by 88 per cent. When after AOB the animals were placed for one day in aerated water, the measure of hyperventilation did not change in the case of animals kept in AOB for one or two days, that is they showed the motion velocity that could be measured immediately after AOB. Only the transport performance of the gill-cilia of animals that passed 3 days in AOB decreased after 24 hours aeration

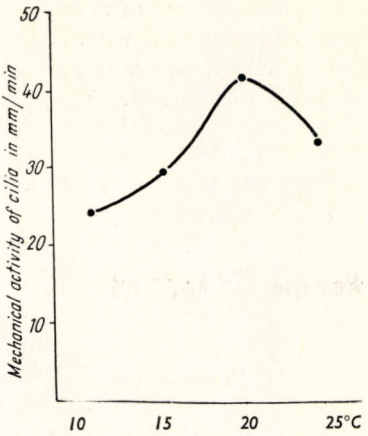

Fig. 1. Ciliary activity of the isolated gills of *Anodonta cygnea L.* specimens kept at different temperatures

1. ábra Különböző hőmérsékleten tartott *Anodonta cygnea L.* példányok izolált kopoltyúinak a csillóaktivitása.

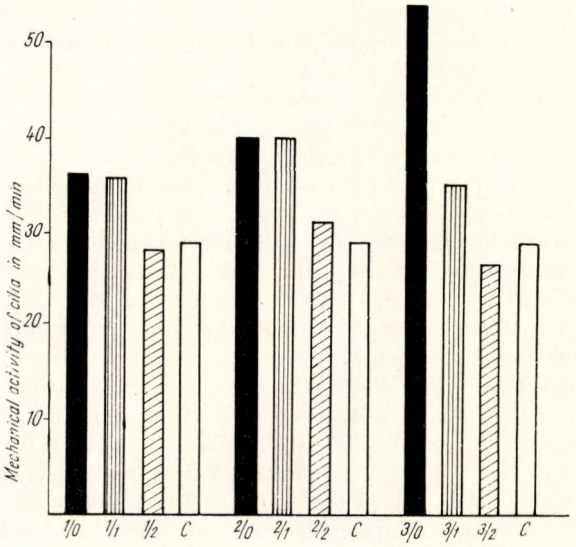

Fig. 2. Mechanical activity of the gill cilia of *Anodonta cygnea L.* specimens kept for various periods in oxygen deficient and subsequently in water with high oxygen content
Signs: Figure above the fraction-line: time spent in an oxygen-deficient medium, in days
Figure below the fraction-line: time spent in water abundant in oxygen, in days.
C = control

2. ábra Különböző ideig oxigén-szegény, majd oxigén-dús vízben tartott *Anodonta cygnea L.* példányok kopoltyúcsillóinak a mechanikus aktivitása.
Jelzések: Törtjel fölötti szám: oxigén-szegény közegben eltöltött idő napokban.
Törtjel alatti szám: oxigén-dús vízben eltöltött idő napokban.
C = kontrol

was substantially below the performance of zero day, but it still exhibited a surplus value of 22 per cent as compared with the control. After 48 hours of OB conditions the transport performance was already practically in all three groups equal with the value of the control.

When the transport velocity values measured immediately after AOB were compared not with their control but with each other, the statistical calculations showed the differences to be real *(Fig. 2)*:

1/0—2/0	$P < 0.01$	D. F. = 35	t = 4.53
2/0—3/0	$P < 0.01$	D. F. = 35	t = 8.94

Discussion

The motion activity of the gill-cilia of Lamellibranchiata that is the flow of the water necessary for respiration and with it the filtration activity is influenced by many factors. In a natural environment the most general active agent according to GRAY (1928, cit. ap. BUDDENBROCK 1939) is temperature, but they are also effectively influenced by the pH conditions and by the amount of dissolved gases, cations and anions.

With the rise of temperature according to van't HOFF's law the O_2 consumption of the isolated gills of *Unio tumidus* ZELEBOR increased (LUKACSOVICS and SALÁNKI 1964) and as measured on the gill of *Mytilus edulis* upon heat effect parallel with the increasing O_2 consumption curve rose also the ciliary acivity (GRAY 1928 pp 88, *Fig 68)*.

From the viewpoint of ecological physiology the fact is not contradictory that on account of its adaptation to the environment the cilial motorics of the gills of the fresh water mussel of higher temperature tolerance rose exponentially only until 20° C *(Fig. 1)* and at 25° C already showed decreasing activity. At the same time on the gills of *Mytilus edulis* living, as it is well known, in brack and sea water with lower annual and daily temperature-fluctuation, exponentially rising cilial activity can be measured up to 35° C. These can be explained by two factors:

1. The temperature effect above the optimum has an inverse time dependence that is during the short action of pessimum temperature (10 minutes) the ciliary movement is stimulated, but during a longer high temperature (e. g. 1 hour) "heat shock" that is blocking effect arises. These results agree with those of SCHLIEPER et. al. (1958) obtained on *Modiolus*.

2. The value of temperature resistance, optimum and tolerance changes seasonally as changes the vital activity, chemical composition etc. (GALTSOFF 1964, KRÜGER 1960, OKAZAKI and KOBAYASHI 1929, MITCHELL 1915, RUSSEL 1923 and HENSCHEL 1952). We conducted the experiments in the winter, so-called "Cold water" period when the Lamellibranchs, as the poikilothermic organisms generally, exercise a vital activity of reduced metabolic level.

Although we carried out heat adaptation before the experiments, the "plafond value" of the heat resistance of *Anodonta* is lower in the winter season, that is its temperature tolerance is lesser. Under the conditions of our experiments the "plafond" referred to evaluated on ciliary activity may be at a temperature of about 20° C.

As mentioned in the Introduction, the examination of ciliary activity after AOB was induced by the experimental result gained earlier on *Anodonta* gills that on the isolated gill of mussels kept under AOB conditions there was no restitution demonstrable within a few hours. The consumption of the gill of mussels kept hypoxially for one day surpassing the oxygen consumption value of the control could not be measured even after 4 days of aeration. In the case of 2 days of hypoxia restitution manifested itself only after 24 hours of aeration and after 3 days of hypoxia the restitution could be measured only in 48 hours (LUKACSOVICS 1966). In contrast to the above "time shift" on AOB effect the gill cilia immediately responded to the cessation of AOB with intensive mechanical activity. On the single days of 3 consecutive hypoxial days the increase of the ciliary movement was 25, 39 and 88 per cent. The order of increased activity agrees with that published by SCHLIEPER and KOWALSKI (1958) on *Mytilus edulis* of the North Sea, but the percentual growth values are much higher in *Anodonta*. Hyperventilation subsisted for about 24 hours on specimens which suffered AOB for 1—2 days. In the specimens kept under AOB conditions for 3 days, after 24 hours the transport performance diminished as compared with the 0 hour performance, but even so a substantial hyperventilation (22 per cent) could be measured as compared with the control.

The contradiction of this kind between respiration and ciliary activity which arose upon AOB effect allows several assumptions of which the following seems to be most obvious:

The members of the class Lamellibranchiata, as it is well known, have a higher capacity of adaptation as compared with several classes of the phylum Mollusca (e. g. Gastropoda, Cephalopoda) since in their natural biotope the change of the oxygen content can be substantial even daily, either as regards the conditions of ebb-tide + temperature (sea water) or of the dissimilating or O_2-consuming organisms + temperature (fresh-water). In the course of the oxygen impoverishment of the natural water the mussel as a consequence of the given conditions of its movement is unable to escape and therefore closing its shells it carries out only movements requiring less energy (BRAND 1946, SALÁNKI 1964) shifting to anaerobic energy production (anaerobic glycolysis). The water enriching itself with oxygen by assimilative activity of the phytoplankton or by the phenomenon of high tide exciting the receptors of siphonic or pallial edge (HERBERS 1914, LUCAS 1931, ORLOV 1930) opens the carapaces of the animal and the epithelial cilia of the gill begin to ventilate. It is possible that the energy necessary for ciliary activity originates still from the energy of the earlier anaerobic glycolysis. The oxydative enzyme activity of the tissues of the gill and the tissues of the body start only in the second stage. This process is ensured by the ciliary activity set on when the O_2 diffusion of the flowing water rich in oxygen reaches a considerable value. This may be the explanation of the "time contrast" between the respiratory and ciliary activity after AOB. The above considerations should be of course verified by further ecological-physiological and biochemical analyses.

Summary

Anodonta cygnea specimens were kept for 1—3 days under anoxybiotic (AOB) conditions and then after AOB immediately or in 1—2 days, during which the mussels were kept in water rich in oxygen, the mechanical activity

(transport performance) of the lateral cilia on the isolated gills was measured, in February-March 1966 with the metal plate method of SCHLIEPER et al. (1958). While raising the temperature from that of the natural environment (+4 — +7° C) gradually to 25° C the activity of the gill cilia was measured also at every 5° C. The following results were obtained:

1. At temperatures of 10, 15 and 20° C the ciliary activity increased together with the temperature, but at 25° C already a decreasing activity could be measured *(Fig. 1)* the cause of which may be "heat shock" which occurs also in other, marine Lamellibranchiata species.

2. On the zero day after each 1—3 day of AOB effect a substantial increase of transport velocity appeared as compared with the control, in direct proportion with the period spent in AOB.

3. Upon a one day aeration effect after AOB the mechanical activity of the cilia generally did not decrease, only in the mussels kept for 3 days under AOB conditions, but also here the performance was still significantly higher than in the control *(Fig. 2)*.

4. Upon the effect of 2 days aeration after AOB the transport performance agreed with the value of the control.

5. It is assumed that the contradiction between the O_2 hunger of the gill tissue appearing with a time shift after AOB and the immediately appearing ciliary transport arises from the more rapid response disposition of the cilia in contrast to the slower start of the mechanism of the oxidatory enzyme activity.

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CSILLÓAKTIVITÁS VIZSGÁLATOK ANOXYBIOZIS UTÁN AZ *ANODONTA CYGNEA L.* IZOLÁLT KOPOLTYÚJÁN

Összefoglalás

Lukacsovics Ferenc

Szerző *Anodonta cygnea* példányokat tartott 1—3 napon keresztül anoxybiotikus (AOB) körülmények között, majd AOB után közvetlen, vagy 1—2 napon keresztül oxigéndús vízben tartott kagylók izolált kopoltyúin mérte a lateralis csillók mechanikus aktivitását (transzportteljesítményét), 1966. febr.—márc. hónapokban, SCHLIEPER et. al. (1948) fémlapos módszerével. A természetes környezet hőmérsékletéről (+4— + 7° C) fokozatosan 25 °C-ra való hőmérséklet emelés közben 5 °C-onkint is megmérte a kopoltyúcsillók aktivitását. A következő eredményeket nyerte:

1. 10, 15 és 20 °C-hőmérsékletnél a csillóaktivitás a hőmérséklettel együtt nőtt, 25 °C-nál azonban már csökkenő aktivitás volt mérhető *(1. ábra),* amelynek oka — más, tengeri Lamellibranchiata fajoknál is fellelhető — „hősokk" lehet.

2. Minden 1—3 nap AOB hatást követő nulladik napon a kontrollal szembeni jelentős transzportsebesség növekedés jelentkezett éspedig az AOB-ban eltöltött idő tartamával egyenes arányban.

3. AOB utáni egynapos aerációs hatásra általában nem csökkent a csillók mechanikus aktivitása, csupán a 3 napot AOB viszonyok között tartott kagylóknál, azonban itt is még szignifikánsan magasabb volt a teljesítmény mint a kontrolnál. *(2. ábra)*

4. AOB utáni 2 napos aeráció hatására a transzportteljesítmény a kontrol értékével egyezett.

5. Szerző feltételezi, hogy a kopoltyúszövet AOB utáni időeltolódással jelentkező O_2-éhsége és az azonnal jelentkező csillótranszport közötti ellentét oka a csillók gyorsabb reakciókészségéből adódik, szemben az oxidációs enzimtevékenység lassabban beinduló mechanizmusával.

ИЗУЧЕНИЕ АКТИВНОСТИ РЕСНИЧЕК ИЗОЛИРОВАННЫХ ЖАБЕР *ANODONTA CYGNEA L.* ПОСЛЕ АНОКСИБИОЗА

Ференц Лукачович

Взрослых беззубок выдерживали 1—3 дня в условиях отсутствия кислорода. Вслед за этим либо сразу после аноксибиоза, либо после выдерживания беззубок в течении 1—2 дней в воде, насыщенной кислородом, определяли механическую активность латеральных реничек изолированных жабер. Опыты проводились по методу *Шлипера* и др. (1958) в феврале — марте 1966. Температуру естественной среды (+4—+7° С) постепенно повышали до 25°С, активность ресничек измеряли через каждые 5°С. Получены следующие результаты.

1. При повышении температуры до 15 и 20°С активность ресничек усиливалась, а при 25°С снижалась. Это, возможно, объясняется тепловым шоком, как это известно для морских двустворчатых.

2. Активность ресничек у беззубок, выдержанных 1—3 дня в условиях отсутствия кислорода, повышена, причем повышение пропорционально времени пребывания в аноксибиотических условиях.

3. Реснички беззубок, выдержанных в течение суток после аноксибиоза в воде, насыщенной кислородом, также проявляли повышенную активность в сравнении с контролем.

4. Реснички беззубок, выдержанных в течение двух суток после аноксибиоза в воде, насыщенной кислородом, совпадает с контрольной.

5. Высказывается предположение, что повышение активности ресничек после кислородного голодания, наступающего вследствие аноксибиоза, объясняется их более высокой реактивностью в сравнении с системой окислительных ферментов.

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NEW DATA ON THE ANATOMY OF THE VISCERAL GANGLION OF FRESH-WATER MUSSEL

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The detection of the nervous system of fresh-water mussel is associated with the names of CUVIER, RATHKE and MANGILI. Of these authors CUVIER discovered the cerebral and visceral ganglia, and the others the pair of pedal ganglia (cit.: SPLITTSTÖSSER 1913). Later the works of KEBER (1851) and DUVERNOY (1853) denoted a progress in the neuroanatomy of mussels, and SPENGEL (1891) described the sense organs of molluscs. The works of THIELE (1889), FREIDENFELD (1897), STEMPELL (1912) and HERBERS (1914) also contributed essentially to our knowledge on the anatomy of the central nervous system of fresh-water mussel. SPLITTSTÖSSER (1913) presented a comprehensive picture on the anatomy of central and peripheral nervous system of *Anodonta cellensis*, and with this the period of macroscopic examinations is actually ended. Later works (HANSTRÖM 1928, BULLOCK and HORRIDGE 1965) refer in general to the works of the above authors on the anatomy of the nervous system of mussels.

It was observed in the course of histological studies on the central nervous system of *Anodonta cygnea* that besides those parts as described by the above authors in the visceral ganglion of this animal, other constituents exist, namely on both sides of the ganglion a lobe of considerable dimension is found which does not differ in its histological properties from the structure of the other parts of the ganglion, but is of considerably larger size than the osphradial ganglion described by SPENGEL (1991) and a direct contact exists between it and the visceral ganglion.

Material and method

The experimental animals used were *Anodonta cygnea, A. anatina* and *Unio pictorum*. Preparation of the animals was carried out under a stereoscopic microscope. For histological examinations the samples were embedded in paraffine after SUSA-fixation. The serial sections were stained with haemalaun-eosine and kresylviolet. For the demonstration of nerve cells a modified version (GUBICZA and ZS.-NAGY 1964) of the CAJAL I. impregnation technique was used.

Results

In each species investigated a pair of visceral ganglia is situated beneath the posterior adductor. These are completely fused in their middle part and resemble one single ganglion. The anatomical conditions of the visceral ganglion are presented in *Figure 1* according to SPLITTSTÖSSER (1913). This ganglion is easily disclosed by removing the valves from the direction of the cloacal sac and by intersecting the gills. This disclosed picture is presented in *Fig. 2*. Between the two osphradia lies the visceral ganglion covered by a thin connective tissue and by the epidermis. A livid yellow fascicle is visible through the oral margin of the osphradium. By intersecting carefully the covering layer in mediansagittal direction the bright yellow ganglion becomes disclosed *(fig. 3)*. Several nerves are originating from this ganglion. The most important of them are rendered visible by pulling aside the interstitial and epidermal lobe. Forward the two cerebrovisceral connectives (CVc), backward the two nervus pallialis constitute the most robust branches. The detailed description of the nerve branches is not the objective of this study. This yellow ganglion tissue is demonstrable also in the area where according to our present knowledge the nerve branchiale leaves the ganglion namely in the anterior third section of the ventrolateral area, whereas it ceases at the site of origin of other nerve branches. This yellow tissue proceeds in lateral direction and forward in the form of thick fascicles *(fig. 4)*. Towards to front this fascicle forms a convex arch then turns backwards and stops at the posterior margin of the internal lamellae of the gill. Its consistence and colour remain all along the same as observed in the ganglia and do not resemble at all other nerves which are white, mechanically more resistant than the ganglion tissue. From this lobe several nerve branches pass forward towards the gill. This lobe is tightly attached along a considerable section of its course to the osphradial epithelium, and its preparation without injury in this place is difficult. The epithelium of the osphradium is also yellow and it occupies backwards from the lobe of the ganglion a considerably larger space than the lobe itself. As regards its localization and topography this lobe of the ganglion corresponds in general to the nerve branch described as nervus branchialis and will be named accordingly in the followings lobus branchialis.

From the histological point of view the structure of the lobus branchialis is completely identical with the structure of other areas of the visceral ganglion. The cellular cortex of the visceral ganglion continues without interruption on the surface of this lobe and forms a cortex of varying thickness, and the center is occupied by the neuropile. In the neuropile occasionally nerve cells localized in groups are visible. Here too the majority of cells is unipolar. The lobe has a definite connective sheath everywhere with the exception of the area where the lobe is closely connected on its ventral part to the epithelium of the osphradium. Here the sheath breaks up, becomes indistinct and it is not possible to draw a sharp line of distinction between epithelium and nerve tissue. Several lymphatic spaces are observable in this area. The epithelium of osphradium is not ciliated, whereas other areas in the neighbourhood are covered by ciliated cylindrical epithelium *(Fig. 5* and *6)*.

The dimension of the lobe suggests that the lobe may contain large numbers of nerve cells constituting the central nervous system of the mussels.

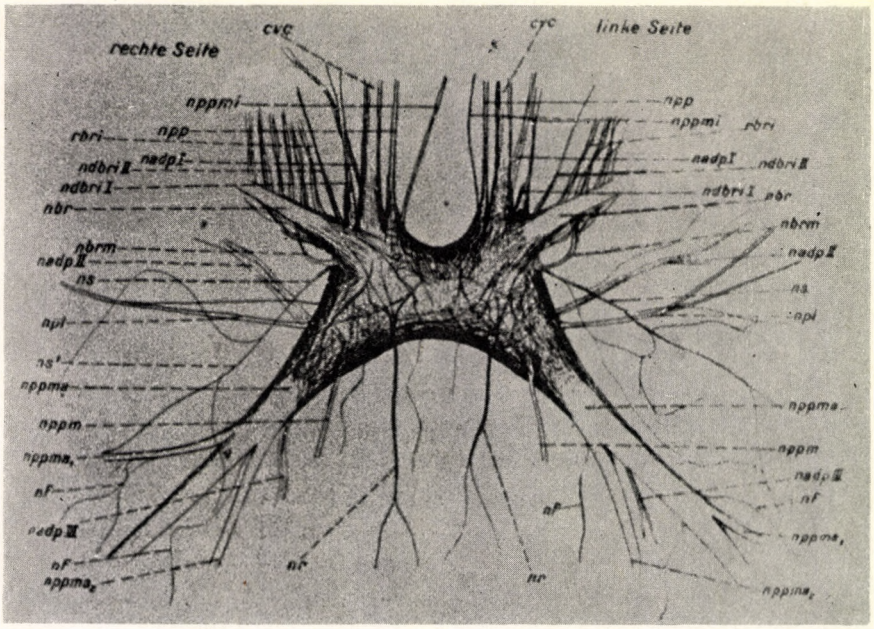

Fig. 1. The anatomy of the visceral ganglion according to SPLITTSTÖSSER (1913) (photocopy). The most important abbreviations are: CVc — cerebrovisceral connective; nbr — nervus branchialis; nppma — nervus pallialis posterior maior

1. ábra A viscerális ggl. anatómiája SPLITTSTÖSSER (1913) szerint. (Fotomásolat) Fontosabb rövidítések CVc — cerebroviscerális connectivum nbr— nervus branchiális, nppma — nervus palliális posterior maior

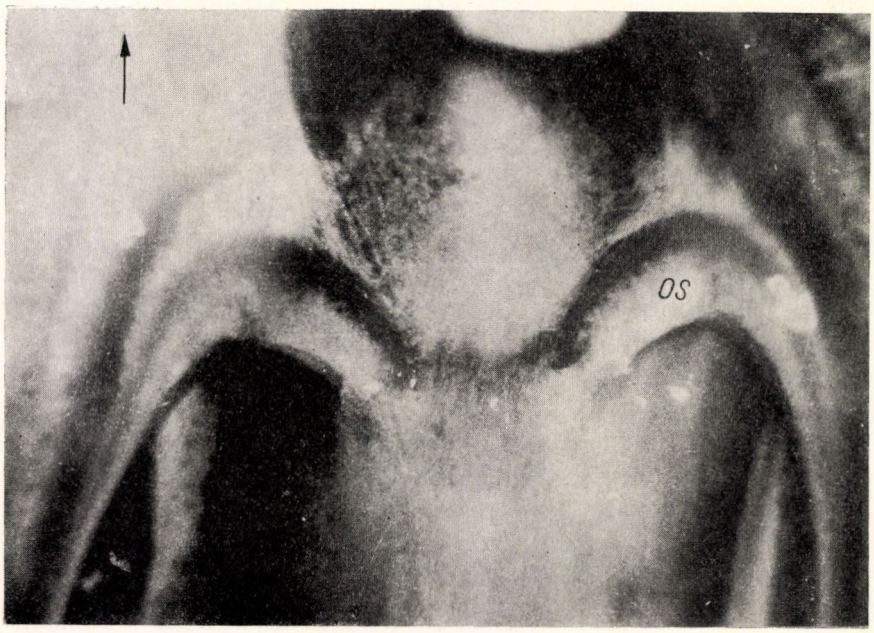

Fig. 2. The approach of the visceral ganglion from the cloacal sac. The gills were intersected mediansagittally and were turned aside. OS — osphradium, along its border a dark fascicle is situated. The arrow denotes the oral direction

2. ábra A vicerális, ggl. megközelítése a kloakális kamra felől. A kopoltyukat mediansagittálisan szétvágtuk és oldalra hajtottuk. OS — osphradium, amelynek szélén sötét köteg húzódik. A nyíl az orális irányt mutatja.

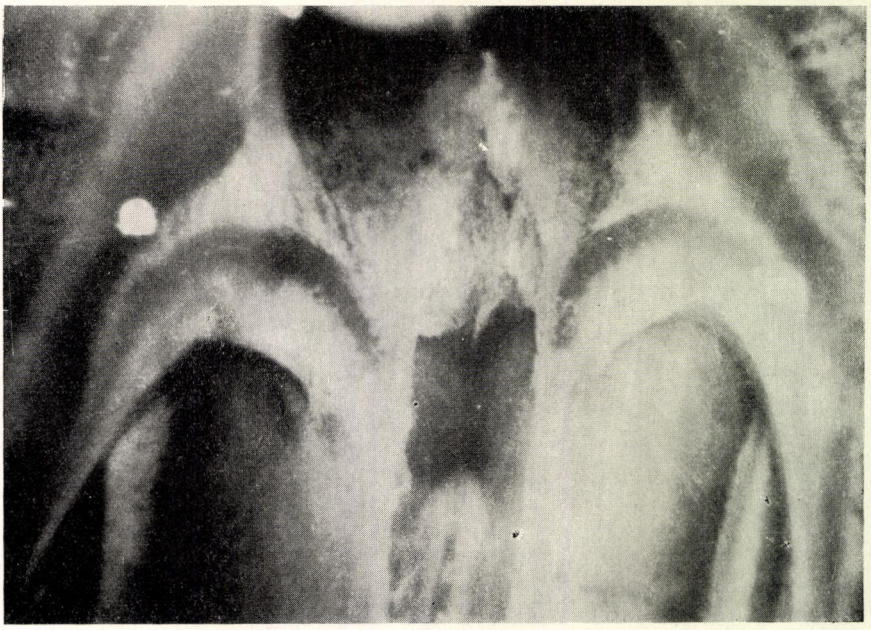

Fig. 3. The same as in *Fig. 2* after the dissection of the epithelial and connective tissue of the visceral ganglion

3. ábra Ugyanaz, mint a *2. ábra*, a viscerális ggl-t fedő hám- és kötőszövet felhasítása után.

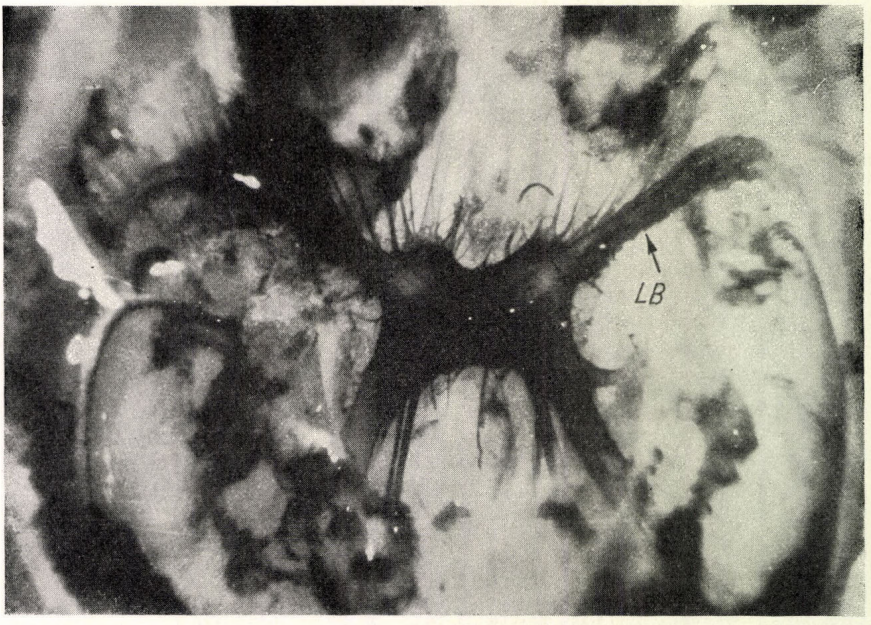

Fig. 4. Picture of the excised visceral ganglion. For obtaining a more distinct picture the ganglion was stained by 1% methylene blue. LB = lobus branchialis

4. ábra A kiboncolt viscerális ggl. képe. A jobb kontraszt elérése céljából néhány percig 1%-os methylenkékkel megfestve. LB — lobus branchiális.

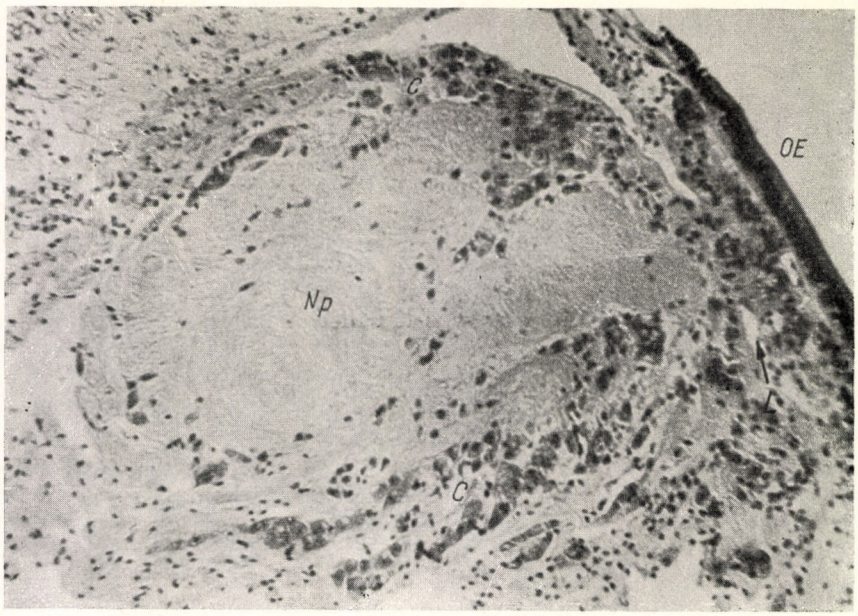

Fig. 5. Histological picture of lobus branchialis near the inflection. Np — neuoropile, C — cellular cortical substance. OE — osphradial epithelium, L — lymphatic interstices. × 500

5. ábra A lobus branchiális szövettani képe, a görbület tájékán. Np. — neuropil, C — sejtes kéregállomány OE — osphradiális hám, L — lymphatikus rések. Nagyítás 500 ×.

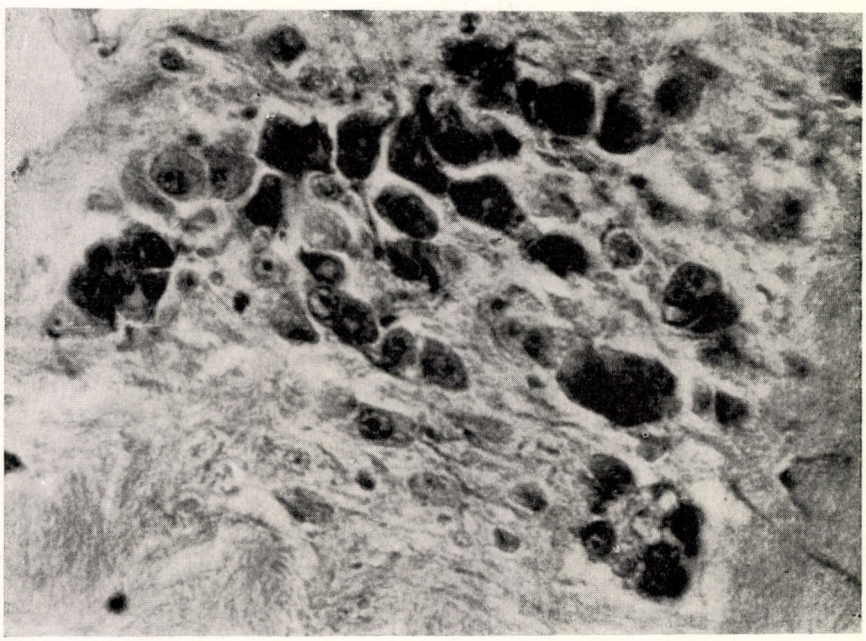

Fig. 6. Nerve cells in lobus branchialis. silver impregnation. × 750

6. ábra Idegsejtek a lobus branchiálisból. Ezüstimpregnáció. Nagyítás 750 ×.

Discussion

The findings obtained suggest that the section of the visceral ganglion designated in earlier works as branchial nerve is similar both in its macroscopic anatomical features and in its histological construction to other parts of the visceral ganglion indicating that it should be taken for the lobe of the visceral ganglion. The name branchial lobe is suggested for its designation.

It is a question to be answered why was this lobe classified as the part of the peripheral nerve, and why was it called a nerve when SPLITTSTÖSSER (1913) himself also observed that unlike other nerves it is very flimsy and is throughout pigmented. At that time it was not known that this bright yellow colour is due to the carotene of the lipochrom pigment of the nerve cells (GOODWIN 1952, LÁBOS et al. 1966). This misstatement can be explained by the fact that in the place where the lobe is contiguous with the osphradia epithelium the layer of nerve cells is most possibly removed also when removing the epithelium and only the white mass of fibres constituting the neuropile remains.

SPENGEL (1881) the first to describe osphradium mentions that an osphradial ganglion exists beneath the osphradium in the lamellibranchiates, but he ignores the possibility that this might be an organic part of the visceral ganglion. On the analogy of snails he suggests that osphradium is most possibly localized near the visceral ganglion and gives, therefore, the name visceral ganglion to the ganglion designated before him pleural ganglion. Subsequent literature uses the osphradial ganglion designation but only in the aspect that it is connected with the branchial nerve (STORK 1935). Other are of the opinion that several nerve cells are present in the branchial nerve which itself or the branches of it embody the osphradial ganglion (BULLOCK and HORRIDGE 1965). FÖRSTER described in *Pholas dactylus* a previsceral ganglion, which is most probably analogous to osphradial ganglion (cit.: HANSTRÖM 1928). It is undoubtful that one part of the branchial lobe is identical with the formation designated as osphradial ganglion. Nevertheless, this designation suggests an independent ganglion which is connected only by the branchial nerve to the visceral ganglion. In reality the opposite situation prevails, namely the osphradial ganglion constitutes an organic part of the visceral ganglion and this may also justify the designation as lobus branchialis.

In the sense of these considerations it seemes that occasionally a critical examination of old data is necessary also in the field of macroscopic structure. Lobus branchialis and other parts of the central nervous system should be subjected to detailed examination in order to learn the function of the nerve cells in these organs. The topographic vicinity and contact of lobus branchialis with the organ designated as osphradium suggests that if this organ is functioning indeed as a receptor then lobus branchialis most possibly contains analyser centres. All these, however, do not change the simple anatomical fact, that this lobe constitutes an organic part of the central nervous system and cannot be regarded as a part of the peripheral nervous system. The situtation is different from that of other nerves as e. g. nervus pallialis, namely that some nerve cells are demonstrable far from the ganglion in the periphery of the nerves. The number of these cells is few, and they are very seldom detectable. Lobus branchialis forms an organic part of the visceral ganglion and has a characteristic ganglial structure.

Summary

It was established in case of *Anodonta cygnea, A. anatina* and *Unio pictorum* that the nerve known as nerve branchiale disposes of the properties of the central nervous system as regards its macroscopic anatomical and histological construction. It is considered incorrect to regard it as an organic part of the peripheral nervous system and must be taken for a lobe of the visceral ganglion. For its designation the name lobe branchiale is suggested. This lobe contains those formulae which were designated previously as osphradial ganglion, but its dimension is greater and it contains a considerable part of the nerve cells of the central nervous system.

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ÚJABB ADATOK AZ ÉDESVÍZI KAGYLÓK VISCERÁLIS GANGLIONJÁNAK ANATÓMIÁJÁHOZ

Zs.-Nagy Imre

Összefoglalás

Anodonta cygnea, Anodonta anatina és *Unio pictorum* esetében megállapítást nyert, hogy a nervus branchiálisként ismert ideg mind makroszkópos anatómiai, mind szövettani szerkezete tekintetében a központi idegrendszer jellegzetességeit mutatja. Ezért

indokolatlan a perifériás idegrendszerhez való sorolása, s mint a viscerális ganglion egy lebenyét kell felfognunk. Elnevezésére a lobus branchiális nevet javasoljuk. E lebeny magába foglalja a korábban osphradiális ganglion néven ismert képletet, de annál nagyobb kiterjedésű, és a központi idegrendszer idegsejtjeinek jelentős hányadát tartalmazza.

НОВЫЕ ДАННЫЕ ОБ АНАТОМИИ ВИСЦЕРАЛЬНОГО ГАНГЛИЯ БЕЗЗУБКИ

Имре Ж.-Надь

На *Anodonta cygnea*, *Anodonta anatina* и *Unio pictorum* показано что жаберный нерв по своей макроскопической анатомии и гистологической структуре соответствует харектеристикам центральной нервной системы. Тем самым не оправдано отнесение его к периферической нервной системе, следует считать этот нерв долей висцерального ганглиа. Предлагается называть это образование lobus branchialis. Эта доля включает в себя осфрадильный ганглий, но по размерам она больше последнего и содержит значительную уасть нейронов центральной нервной системы.

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ANALYSIS OF THE ACTION OF SOME CHOLINERGIC COMPOUNDS ON THE HEART OF FRESH-WATER MUSSEL (*ANODONTA* sp.)

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In the heart of fresh-water mussel, similarly to several other molluscs (PROSSER 1940, WELSH and TAUB 1948, TEN CATE 1955, NISTRATOVA and YUZHANSKAIA 1966) acetylcholine is a mediator of inhibitory nervous impulses. In this respect the heart of molluscs does in no way differ from those of vertebrate animals. The investigation of pharmacological reactions has, however, shown that the muscles of molluscs, including those of the heart, exhibit specific peculiarities (see KRIJGSMAN and DIVARIS 1955, CRESCITELLI and GEISSMAN 1963). So eserine and other cholinesterase inhibitors in many cases do not change the reaction of the heart to ACh (PROSSER 1940, GHIRETTI 1948, GADDUM and PAASONEN 1955, SAKHAROV and NISTRATOVA 1963). Nicotine has an excitatory effect on heart activity to which the heart responds with increased amplitude (YUNG 1881, CHONG and PHILLIS 1965). Atropine, which is an ACh antagonist in the heart of vertebrates does not influence the effect of ACh in the case of molluscan heart (BACQ 1934, PROSSER 1940, GADDUM and PAASONEN 1955, FREDERICQ 1947, WELSH and TAUB 1953, SAKHAROV and NISTRATOVA 1963, CHONG and PHILLIS 1965).

On the other hand, in contrast to vertebrates, in the heart of molluscs a curare-like compound, mytolon, is known to be the most potent ACh antagonist (LUDUENA and BROWN 1952, WELSH and TAUB 1953, CHONG and PHILLIS 1965).

These data point to the fact that ACh receptors in the heart of molluscs differ from those in vertebrates. The investigation of some pecularities of these receptors greatly helps us to understand the mechanism of the action of mediators.

For this purpose in this paper we attempted to study the effect of some cholinergic compounds on the heart of a bivalve mollusc *(Anodonta cygnea)* in normal state and also when ACh was applied or when the visceral ganglion was being stimulated.

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Methods

Our experiments were conducted on a fresh-water mussel *(Anodonta cygnea L.)* in the Biological Research Institute of the Hungarian Academy of Sciences in Tihany and in the Institute of Animal Morphology, Academy of Sciences USSR, Moscow. Before the experiments the mussels were kept in aerated aquariums at temperatures of 7—10° C. The experiments were conducted at room temperature both in the case of isolated and in situ ventricles.

Visceral ganglion was stimulated by a series of square wave impulses (1—3 V, 10 Hz, 30 sec) using an Alvar stimulator. All compounds were injected into the interior of the ventricle by means of a cannula. For this reason the in situ preparation was made as follows: the dorsal part of the shell was removed, the pericardium cut up, the anterior and posterior aortas and also one of the auricles at the atrioventricular junction were loosely tied down without damaging the nerves. The cannula was inserted into the ventricle through the auricle and special care was taken to keep its fluid-level constant.

The isolated ventricle was prepared according to the method described earlier (SAKHAROV and NISTRATOVA 1963). The perfusion fluid was taken out of the ventricle immediately or one minute after the end of the stimulation of visceral ganglion, and was tested on an isolated mussel heart and on an isolated frog heart prepared according to the Straub method. In the latter case the obtained perfusion fluid was adjusted to the ionic concentration of frog RINGER solution. To 3 volumes of perfusion fluid one volume of solution was added with the following ionic content: NaCl 21.5 g, KCl 0.44 g, $CaCl_2$ 0.8 g, $NaHCO_3$ 0.28 g to 1 litre distilled water (SAKHAROV and NISTRATOVA 1963). The mussel-physiological solution suggested by MARCZYNSKI (1959) was used.

In the course of experiments the following agents were used: acetylcholine chloride, arecoline iodmethylate, nicotine, trimethylammonium chloride, trimethylammonium iodide (TMA), tetraethylammonium iodide (TEA), mixed mononitrogenic cholinolytics — mesphenal and arpenal — and mytolon (2,5-bis—(3-diethylaminopropylamino)-benzoquinon-bis-benzylchloride) (WIN 2747) (HOPPE 1950).

In addition to these the effect of 5-hydroxytryptamine (serotonin) and that of its antagonist 2-bromo-d-lysergic acid diethylamide or BOL-148 was examined.

Results

The isolated ventricle of *Anodonta* responds to the applied ACh with an increase in tone and a decrease in amplitude. With the increase of ACh concentration the above mentioned effects become more and more explicit. Still in the case of very high ACh concentrations causing a complete arrest the heart gets over this stoppage and overcomes the inhibition. In the case of the stimulation of visceral ganglion similar results have been obtained.

It is interesting to note that ACh of about threshold concentration or a weak electric stimulation of short duration of the visceral ganglion may cause an excitatory effect on the heart.

Arecoline at $1 \cdot 10^{-6}$—$1 \cdot 10^{-3}$ g/ml concentrations stimulates heart activity whereas at $1 \cdot 10^{-3}$ g/ml a small tonic effect is also observable. But after an arecoline treatment of the heart ACh produces its usual effect.

Nicotine about in the same concentrations as ACh almost completely repeats the effect of the latter. Thus low nicotine concentrations ($1 \cdot 10^{-8}$—$5 \cdot 10^{-9}$ g/ml) result in an increase of amplitude and the activity of heart muscles improves *(Fig. 1a)*. At high concentrations (10^{-6}—10^{-5} g/ml) a sharp increase in tone and the fall of amplitude to zero can be observed and consequently

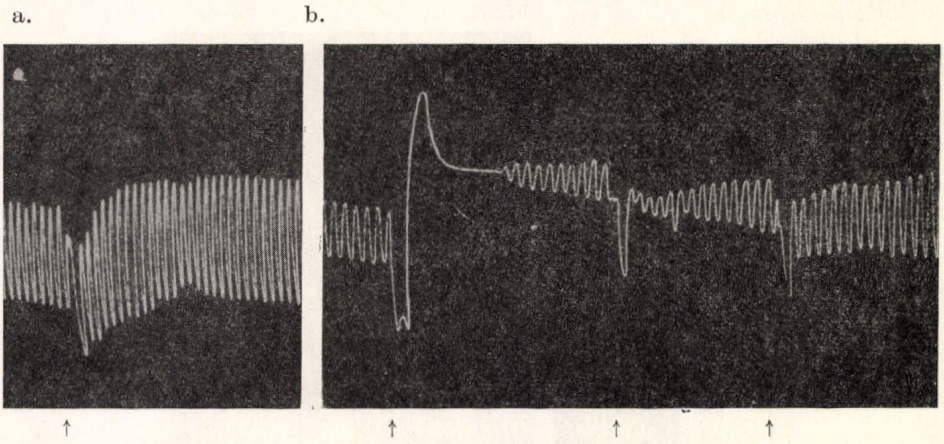

Fig. 1. The effect of nicotine at $1 \cdot 10^{-8}$ g/ml (a) and at $1 \cdot 10^{-5}$ g/ml (b) on the isolated ventricle of the mussel. Arrows indicate the moment of nicotine applying. In the case of repeated treatment with nicotine the "desensitization" is clearly observable.

1. ábra $1 \cdot 10^{-8}$ g/ml (a) és $1 \cdot 10^{-5}$ g/ml (b) koncentrációjú nicotin hatása a kagyló izolált szívén. A nyílak a nicotin applikálás pillanatát mutatják. Ismételt nicotin adásakor jól megfigyelhető a "desensitisatio".

the heart overcomes the inhibition. A repeated application of nicotine of the same concentration leads to „desensitization" or even to a slight increase of the amplitude *(Fig. 1b)*. At this stage ACh has no inhibitory effect.

Trimethylammonium chloride and tetramethylammonium iodide at $1 \cdot 10^{-5}$ — $1 \cdot 10^{-4}$ g/ml have an effect similar to ACh, although they are much weaker cholinomimetics. As a contrast, tetraethylammonium iodide (TEA) at $1 \cdot 10^{-4}$ — $1 \cdot 10^{-3}$ g/ml produces a slight decrease in tone and a considerable increase in amplitude. If the concentration of ACh is lower than that of TEA — the difference being of one order of magnitude — ACh produces only an increase in amplitude, i. e. no inhibition is observable. In the case of equal concentrations of TEA and ACh the effect of the latter becomes weaker but does not disappear completely.

Mesphenal does not influence the heart activity. At $1 \cdot 10^{-5}$ g/ml it diminishes the effect of ACh at a concentration of $1 \cdot 10^{-6}$ g/ml. A great difficulty is produced by the fact that mesphenal does not easily dissolve in water. Arpenal at $1 \cdot 10^{-5}$ g/ml stimulates the work of heart and reduces the inhibitory effect of ACh at a concentration of $1 \cdot 10^{-6}$ g/ml easily and for a long time. After arpenal treatment ACh produces a considerable stimulation of heart activity *(Fig. 2)*. In equimolar concentrations arpenal reduces the effect of ACh almost completely.

Mytolon at $1 \cdot 10^{-5}$ — $1 \cdot 10^{-4}$ g/ml brings about a considerable increase in amplitude without any observable change in tone during the first 10—20 minutes. On the other hand, after repeated mytolon treatments of 1.5—2

hours, the strength of contractions diminishes slightly but the tone of the heart rises.

After a treatment of isolated mussel heart with mytolon at $1 \cdot 10^{-5}$ g/ml for a period of 20—30 minutes, the heart does not show any symptoms of being inhibited by ACh of the same concentration, but rather exhibits an

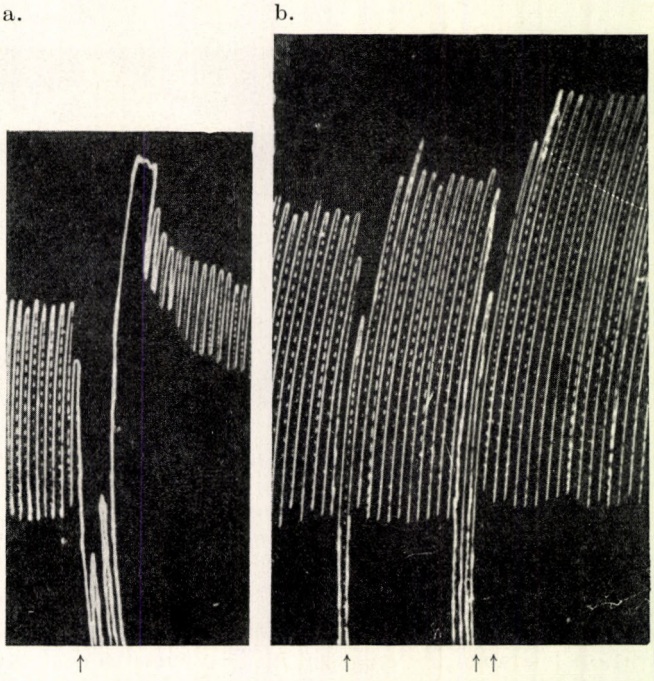

Fig. 2. Reaction of the isolated mussel heart to ACh at $1 \cdot 10^{-6}$ g/ml before (a) and after (b) arpenal treatment of the heart at the concentration of $1 \cdot 10^{-5}$ g/ml ↑ ↑ = acetylcholine; ↑ = arpenal. ACh was given after 15 min. subsequent to the treatment of the heart with arpenal (b)

2. ábra Az izolált kagylószív reakciója az $1 \cdot 10^{-6}$ g/ml koncentrációjú ACh-ra a szív arpenallal ($1 \cdot 10^{-5}$ g/ml) való kezelése előtt (a) és után (b).
↑ ↑ = acetylcholin ↑ = arpenal Az ACh 15 perccel az arpenallal való kezelés (b) után adva.

increase in amplitude *(Fig. 3b)* If, however, the concentration of ACh was 10 times as high as that of mytolon, the normal reaction of the heart to ACh returned *(Fig. 3c)*. But when the concentration of mytolon is also raised to $1 \cdot 10^{-4}$ g/ml, the sensitivity of the heart to ACh of the same concentration diminishes again.

It seems probable that there is a competition between mytolon and ACh for receptors as the affinity of mytolon to the receptor is about 10 times greater than that of ACh. An analogous phenomenon has been observed by several authors between ACh and atropine in the case of vertebrate hearts (Clark 1926, Cullis and Lucas 1936, Jacob 1956, Hall 1959, Takenaka 1959, Nistratova 1961).

In contrast to the heart of *Venus mercenaria* and *Tapes watlingi* mytolon can be washed out of the heart of *Anodonta cygnea* fairly easily with the help

of mussel-physiological solution, after which the heart becomes sensitive to ACh again.

Similar results were obtained with electric stimulation of visceral ganglion. Indeed, injecting mytolon of a concentration of $1 \cdot 10^{-5}$ g/ml into the in situ mussel's ventricle for 20 minutes created a considerable positive inotropic

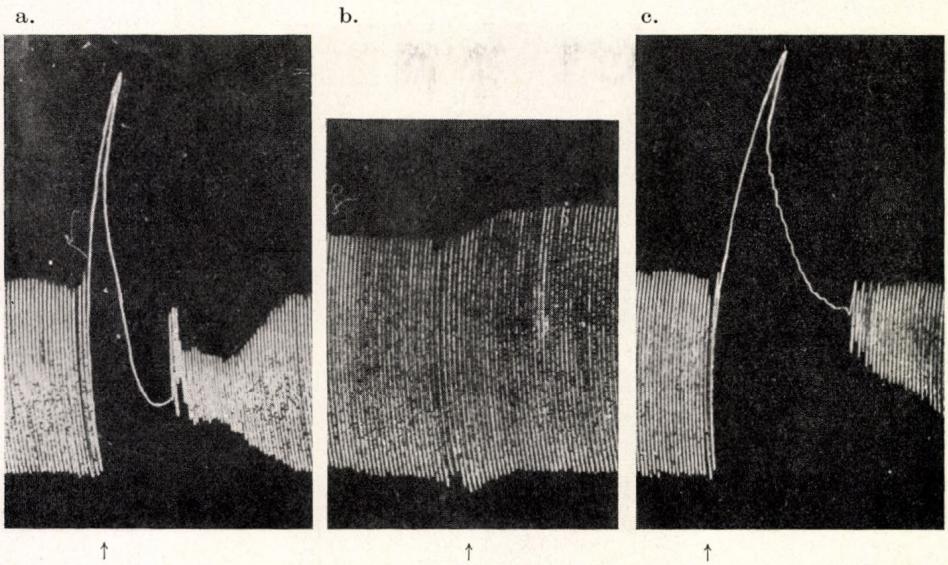

Fig. 3. The influence of mytolon to ACh effect on the isolated ventricle of mussel. a = Reaction of the heart to ACh at $1 \cdot 10^{-5}$ g/ml; b = Reaction of the heart after its treatment with mytolon at $1 \cdot 10^{-5}$ g/ml for 20 min. c = The effect of ACh at $1 \cdot 10^{-4}$ g/ml after the treatment of the heart with mytolon at $1 \cdot 10^{-5}$ g/ml

3. ábra A mytolon hatása az acetylcholin hatásra az izolált kagyló szíven. a = A szív válasza az $1 \cdot 10^{-5}$ g/ml ACh-ra. b = A szív válasza az $1 \cdot 10^{-5}$ g/ml ACh-ra 20 perces mytolon ($1 \cdot 10^{-5}$ g/ml) előkezelés után. c = Az $1 \cdot 10^{-4}$ g/ml ACh hatása a szív mytolonnal ($1 \cdot 10^{-5}$ g/ml) való előkezelése után.

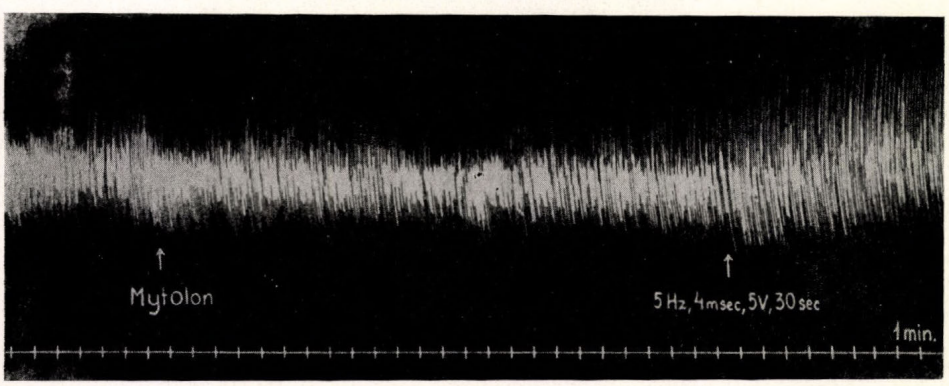

Fig. 4. Response of the in situ mussel heart to the electrical stimulation of the visceral ganglion after the treatment of the heart with mytolon at $1 \cdot 10^{-5}$ g/ml. Mytolon was left to act in the ventricle for 20 min.

4. ábra Az in situ kagylószív válasza a visceralis ganglion elektromos ingerlésére mytolonnal ($1 \cdot 10^{-5}$ g/ml) való előkezelés után. A mytolont 20 percig hagytuk hatni a szíven.

effect and a subsequent stimulation of visceral ganglion brought a further increase of the strength of contraction without any change in tone *(Fig. 4)*. What can be the cause of this increased amplitude if the treatment of the heart with mytolon is followed by another treatment with ACh? In order to solve this problem the perfusion fluid obtained from a donor mussel heart

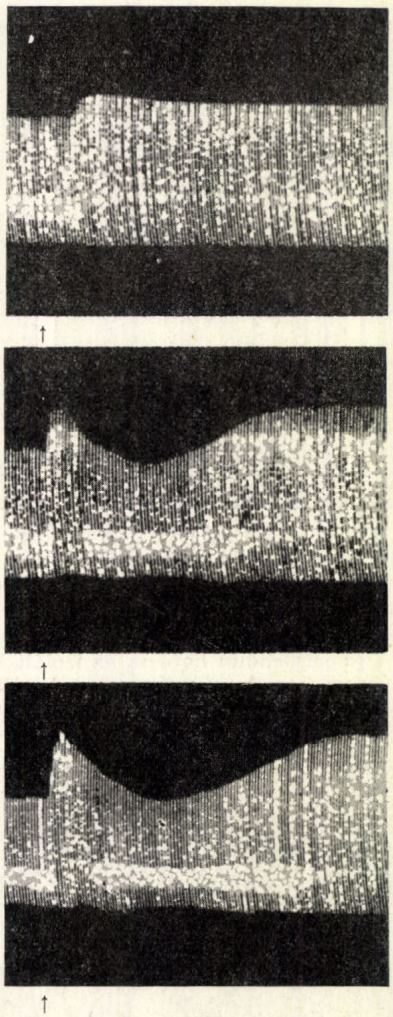

Fig. 5. Effect of perfusates obtained from the isolated mussel heart on the atropine-treated isolated frog heart. a = control, b = after the treatment of the heart with mytolon at $1 \cdot 10^{-5}$ g/ml, c = after ACh applied to the mytolon-treated ($1 \cdot 10^{-5}$ g/ml) heart
All perfusates were gained after 5 min. following the treatment. Arrow indicates the moment of fluid exchange

5. ábra Az izolált kagylószívből nyert perfuzátumok hatása az atropinnal előkezelt izolált békaszíven. a = kontroll, b = a szív mytolonnal ($1 \cdot 10^{-5}$ g/ml) való kezelése után, c = a mytolonnal előkezelt szíven való ACh adás után. Mindegyik perfuzátumot 5 perccel a kezelés után vettük le. A nyíl mutatja a folyadékcsere pillanatát.

was tested on an atropine-treated recipient frog heart isolated by STRAUB's method. Both the control and experimental perfusates were collected after equal periods of time. It appeared that the control perfusates exercised a slight excitatory effect on the frog heart *(Fig. 5a, 6a)*.

The perfusion fluid originating from the mussel heart treated with mytolon, produced a greater excitatory effect on the frog heart which response,

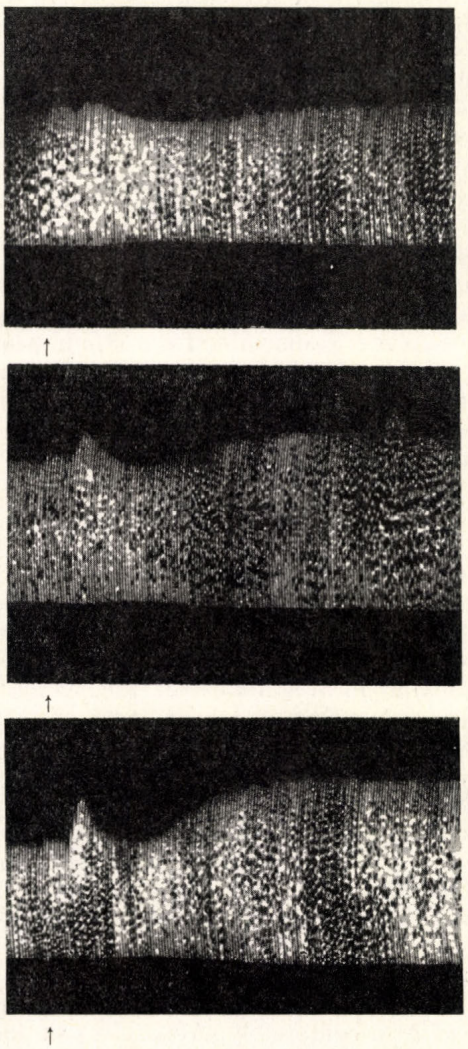

Fig. 6. Effect of perfusates obtained from in situ mussel heart on the atropine-treated isolated frog heart. a = control, b = after the treatment of the heart with mytolon at $1 \cdot 10^{-5}$ g/ml, c = after the electrical stimulation of visceral ganglion. Perfusates were collected after 90 sec. subsequent to the treatment of the heart with mytolon.

6. ábra Az in situ kagylószívből nyert perfuzátumok hatása az atropinnal előkezelt béka szíven. a = kontroll, b = a szív mytolonnal. ($1 \cdot 10^{-5}$ g/ml) való kezelése után, c = a visceralis ganglion elektromos ingerlése után. A perfuzátumokat 90 másodperccel a szív mytolonnal való kezelése után vettük le.

by its character, resembles the effect of macroergs of the adenilic or uridinic type *(Fig. 5b, 6b)*. But the greatest increase in amplitude was observed in the case of perfusion fluid gained after the stimulation of visceral ganglion *(Fig. 6c)* or after the injection of ACh into the mytolon-treated heart *(Fig. 5c)*. This effect on frog heart, in its form, is similar to that of ATP in a considerable degree. In every case the excitatory effect caused by ACh cannot be attributed to serotonin because the heart of frog is not sensitive to this compound. In addition to this the simultaneous treatment of mussel heart with BOL-148 of the concentration of $1 \cdot 10^{-5}$ g/ml and with mytolon of the same concentration did not diminish the excitatory effect on mussel heart subsequent to the stimulation of visceral ganglion either. In our control experiments, however, BOL-148 blocked completely the action of serotonin of a concentration of $1 \cdot 10^{-5}$ g/ml.

In the course of experiments the investigation of the effect of various compounds revealed that nicotine, trimethylammonium chloride and tetramethylammonium iodide act on the heart of *Anodonta* in a similar way as ACh. Mytolon, tetramethylammonium iodide and arpenal increase the amplitude of heart beat and are ACh antagonists. After the treatment of the heart with these compounds ACh is responsible for a stimulating effect which arises from the release of a macroerg compound (or compounds). Mesphenal also diminishes the effect of ACh without changing the amplitude.

And lastly arecoline, which is known as a M-cholinomimetic compound in vertebrates, does not influence the effect of ACh even in high concentrations.

Discussion

The effect of cholinergic compounds on *Anodonta* heart has not been thoroughly investigated so far. But recently there has been a gradual growth in experimental material and on the basis of these findings the following classification of investigated compounds seems to be possible:

1. Compounds similar in action to ACh, i. e. which increase the tone of heart muscles and diminish the amplitude of contractions. In the case of their repeated application "desensitization" sets in with a simultaneous increase of the strength of contractions. The following compounds are included in this group: choline, carbocholine, nicotine, trimethylammonium chloride, tetramethylammonium iodide. With the exception of nicotine these compounds, however, are much weaker cholinomimetics than ACh itself.

2. Compounds having no or very little effect on the activity of heart muscles which do not influence the effect of ACh. The following compounds belong to this group: atropine, arecoline, hexamethonium and d-tubocurarine.

3. And at last a fairly large group of pharmacological agents exists which are able to increase the amplitude on their own and in addition to this block the inhibitory effect of ACh completely. After the treatment of heart muscles with these compounds ACh displays a strong excitatory effect. The following compounds may be mentioned here: TEA, mytolon, arpenal and nicotine. It is interesting to note that either ACh itself of about threshold concentration or the slight stimulation of the visceral ganglion may produce a positive inotropic effect, but it does not come about so often as the inhibitory one. The excitatory effect of ACh and some other cholinergic compounds has been observed both in normal condition and after a treatment of the heart with mytolon

and has been reported also for other species of molluscs (WELSH and TAUB 1948, CORDA 1955, LOVELAND 1963, DITADI 1964, CHONG and PHILLIS 1965, GREENBERG 1965, TURPAEV, NISTRATOVA and PUTINTSEVA 1966).

What is the explanation of this phenomenon? It has been demonstrated in earlier investigations that under the influence of ACh (SAKHAROV and NISTRATOVA 1963) or following the stimulation of visceral ganglia (NISTRATOVA and YUZHANSKAIA 1966) a macroerg compound is released into the perfusion fluid, which was identified later as ATP (NISTRATOVA and MALINOVSKAIA, in preparation). In the ventricle of the mussel heart where according to some authors there is no cholinesterase (TURPAEV, NISTRATOVA and PUTINTSEVA 1966; NISTRATOVA and YUZHANSKAIA 1966) — but recently with Hestrin's method was revealed (HIRIPI, VARANKA and SALÁNKI 1966) — this stimulating compound is able to cease the inhibition of the heart since it is a competetive antagonist of ACh (NISTRATOVA 1965, NISTRATOVA and TURPAEV 1965).

It has been demonstrated in this paper that the stimulatory effect of mytolon is also connected with the release of the above mentioned compound. After a treatment with mytolon, ACh has an additional increasing effect on the amplitude of heart beat because of the great amount of this released macroerg compound. Similar results were obtained after a treatment of the heart with mytolon if the visceral ganglion was stimulated. Only the inhibitory phase of the ACh effect was lost, whereas its excitatory effect was accompanied by the accumulation of a large amount of macroerg compounds in the perfusion fluid.

On the basis of these data it may be concluded that the inhibitory and excitatory phases involved in the ACh effect are connected with not only one but with at least two different systems and that the release of the excitatory compound is not caused by the interaction of ACh with "inhibitory" cholinoreceptors of the mussel heart. It seems possible that both in the case of injecting sufficently high ACh concentrations into the heart and also when the visceral ganglion was stimulated the mediator reacts with the cholinoreceptor on the one hand producing a rise in tone and an inhibition of contractions, or influences some sort of an enzymatic system which leads to the accumulation of excitatory compounds in the perfusion fluid on the other. If the accumulation of this compound is of a sufficiently high concentration, it can be considered as a competetive ACh antagonist which is able to cease the inhibition of the heart.

The possibility of finding two types of ACh receptors in the heart of molluscs is not totally excluded, since the interrelation of one of them with ACh leads to inhibition and the reaction of ACh with the other one creates a positive inotropic effect. This possibility was pointed out by FRAY (cit. KRUTA 1936), WELSH (1948), DITADI (1964), CHONG and PHILLIS (1965), etc.

Several authors also observed the excitatory effect of ACh and of the stimulation of visceral ganglion after a treatment of the heart with mytolon, and explained this effect with the release of serotonin (WELSH 1953, LOVELAND 1963, PHILLIS 1966). This suggestion, however, does not seem to be the only possible explanation in our case, since the simultaneous treatment of the in situ ventricle with mytolon and BOL-148 did not eliminate the excitatory effect, they did not even change its magnitude. In the control experiments at the same time BOL-148 completely blocked the effect of serotonin (PÉCSI 1966).

An analysis of published data and of our results prove the specific peculiarities of ACh receptors both in the heart of *Anodonta* and in other molluscs On the basis of their nature they seem to be very near to "nicotinic" receptors and also to those of autonomic ganglia of vertebrates (WELSH and TAUB 1953). The analogy, however, is not complete, since mytolon, for example, influences, the synapses of autonomic ganglia only to a small degree. But, on the other hand, it is one of the most active ACh antagonist in the case of molluscan heart. Considering some characteristics of cholinoreceptors of the heart, they appear to be different from those of other molluscan muscles, including the most investigated ABRM of *Mytilus* (CAMBRIDGE, HOLGATE and SHARP 1959, TWAROG 1960, MAGAZHANIK and MIKHELSON 1963, MIKHELSON and KHROMOV–BORISOV 1964). It seems also probable that connections between receptor and ACh or its antagonists can be of other types as well since the latter can be washed out fairly easily (CAMBRIDGE, HOLGATE and SHARP 1959, MAGAZHANIK and MIKHELSON 1963).

Although similar features can be observed in the structure and peculiarities of ACh receptors of both vertebrates and invertebrates, still there are also great differences between them the investigation of which will enable us to understand the mechanism of ACh effect better.

Summary

The effect of various cholinergic compounds on the in situ heart of *Anodonta cygnea* and also on the isolated heart of this mollusc was studied.

It was found that nicotine, trimethylammonium chloride and tetramethylammonium iodide have an effect similar to ACh: in low concentrations they increase the amplitude of contractions, in high concentrations they increase the tone and diminish the amplitude completely. In the case of repeated application of these compounds "desensitization" sets in both to themselves and also to ACh injected from outside.

Mytolon, tetraethylammonium iodide and arpenal increase the amplitude of contractions of the heart and work as ACh antagonists. After a treatment of the heart with these compounds, ACh or the stimulation of the visceral ganglion produce a stimulatory effect in consequence of released macroerg compound (or compounds).

Mesphenal also diminished the effect of ACh but did not change the strength of contractions of the ventricle.

M-cholinomimetic arecoline produced a slight increase in amplitude but did no influence the effect of ACh.

The pecularities of ACh receptors in the heart of molluscs are discussed in comparison with cholinoreceptors of vertebrates.

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BIZONYOS CHOLINERG TÍPUSÚ VEGYÜLETEK HATÁSÁNAK ANALÍZISE A TAVIKAGYLÓ *(ANODONTA* sp.*)* SZÍVÉN

Szerafima N. Nyisztratova és Pécsi Tibor

Összefoglalás

Az *Anodonta sp.* in situ és izolált szívén különböző cholinerg típusú anyagok hatását vizsgáltuk.

Megállapítást nyert, hogy a nicotin, trimethylammoniumklorid és a tetramethylammoniumjodid az acetylcholinhoz (ACh) hasonlóan hatnak: kis koncentrációkban növelik az amplitudót, míg nagy koncentrációkban a tónust növelik, viszont az amplitudót egészen a leállásig csökkentik. Ezen anyagok ismételt bevitelére „desensitisatio" lép fel mind a saját, mind a kívülről bevitt ACh-ra.

A mytolon, tetraethylammoniumjodid és az arpenal növelik az amplitudót és mint ACh antagonisták szerepelnek. A szív ezen anyagokkal való kezelése után az ACh, illetve a visceralis ganglion elektromos ingerlése stimuláló hatást eredményez a szíven makroerg természetű anyag (vagy anyagok) kiszabadulása következtében.

A mesphenal szintén blokkolja az ACh hatást, de az amplitudót nem változtatja meg.

Az M-cholinomimetikus arecolin kis amplitudónövekedést eredményez, de az ACh hatására nem hat.

Megbeszéltük a molluszkasziv ACh receptorának sajátosságait, összevetve a gerincesek cholinoreceptorával.

АНАЛИЗ ДЕЙСТВИЯ НЕКОТОРЫХ ВЕЩЕСТВ ХОЛИНЕРГИЧЕСКОГО РЯДА НА СЕРДЦЕ БЕЗЗУБКИ *(ANODONTA* sp.*)*

С. Н. Нистратова и Т. Печи

На сердце пластинчатожаберного моллюска *Anodonta* in situ, а также на изолированном сердце этого моллюска было испытано действие различных веществ холинергического ряда.

Никотин, триметиламмоний хлорид и тетраметиламмоний иодид действуют подобно ацетилхолину: в малых концентрациях увеличивают амплитуду сокрашений, а в больших — повышают тонус и снижают амплитуду вплоть до полной остановки. При повторном введении этих веществ наступает «десенситизация» как ним самим, так и к внесенному извне ацетилхолину.

Митолон, тетраэтиламмоний иодид и арпенал увеличивают амплитуду сокращений сердца и являются антагонистами ацетилхолина. После обработки сердца этими веществами ацетилхолин или раздражение висцерального ганглия начинают оказывать стимулирующее действие за счет высвобождения вещества (или веществ) макроэргической природы.

Месфенал также снимает ацетилхолиновый эффект, но не изменяет силы сокращений желудочка.

М-холиномиметик ареколин вызывает небольшее увеличение амплитуды, но не влияет на ацетилхолиновый эффект.

В статье обсуждается вопрос об особенностях рецептора ацетилхолина в сердце моллюсков по сравнению с холинорецепторами позвоночных.

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ADAPTATION OF THE HEART OF *HELIX POMATIA L.* TO THE INHIBITORY EFFECT PRODUCED BY THE EXTRACARDIAC NERVE

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As earlier investigations show (S.—RÓZSA and GRAUL 1964) it is possible to produce by the stimulation of the extracardiac nerve of *Helix pomatia L.* both inhibitory and stimulatory effects in the heart. The effects depend on the stimulation parameters applied. It has been also established that serotonine and a ninhydrine-positive second factor standing close to arginine chromatographically (S.—RÓZSA and PERÉNYI 1966) is demonstrable by chemical methods in the perfusate collected from the heart stimulated.

The objective of the study reported here was to establish the conditions of the realization of these nervous effects, with special regard to the earlier observation that beside stimulation parameters seasonal changes may also be involved in these inhibitory and stimulatory effects. The observations obtained may be explained either by the alterations occurring in the synthesis of transmitter substances or by sensitivity changes of excitable structures through which the effect of chemical agents released during stimulation of the nerve is realized.

Method

The experiments were performed on *Helix pomatia L.* Activation of the inactive animals before the experiments was conducted by providing the animals with water and food. The calcareous shell of the animals was removed together with the foot and the central nervous system. Thereafter the visceral bag was opened and a thin canule was inserted into the aorta through which MENG'S solution and the agents investigated were perfused through the heart in a way described previously (S.-RÓZSA and GRAUL 1964). In every case the direction of perfusion was identical with the natural direction of the hemolymph i. e. the physiological solution was being circulated from the pulmonal vena towards the aorta.

Special care was taken that the hydrostatic pressure weighing on the heart should not change during the experiment. The heart-innervating intestinal nerve was exposed and placed on stimulating electrodes and stimulated with a square-wave stimulator. At the beginning of the experiment the parameters necessary for producing stabile inhibition and stimulation were determined for every preparation and they were used during the experiment in

question. Heart activity was registered by a kymograph. Under the given experimental conditions the perfused hearts were capable of functioning for 1—2 days.

As a physiological solution MENG'S solution (1960) was used and the agents examined were similarly diluted in it. In these experiments the following substances were used: acridine orange, neutral red, adenosine monophosphate (AMP), adenosine diphosphate (ADP) and adenosine triphosphate (ATP). The adenosine preparates were REANAL products.

Experimental results

1. Inhibiting and stimulating effects produced by lasting and repeated stimulation of the intestinal nerve

a) *Inhibitory effect.* — A gradual cessation of the inhibitory effect produced by lasting excitation of the intestinal nerve was observable during the experiments. Despite continued stimulation the heart thus liberated from the inhibitory effect continues to operate rhythmically for longer or shorter periods, during which a rhythmic alternation of periodic activity and rest periods is observable. In case of repeated and lasting stimulation the duration of inhibition periods gradually decreases and the restored activity of the heart dominates. This is well demonstrated in *Fig. 1* which illustrates also the typical responses of the heart in case of stimulation at parameters producing inhibition. In this case the stimulation of the intestinal nerve was performed at the following parameters: frequency: 5/sec; duration of the impulses 1 msec; amplitude: 10 V. The duration of stimulation was 2—3 minutes. The liberation of the heart from the inhibitory effect of the nerve at the 20th—25th minute after the onset of stimulation is well visible *(A* in *Fig. 1)*. At that time, namely, the heart begins to move rhythmically with an increasing amplitude, which is still followed here by a new inhibition. Further on a nearly regular alternation of active and rest periods is observable during the stimulation of the nerve. At unchanged parameters the duration of inhibition decreases to its half after repeated stimulation and thereafter the heart is more infrequently inhibited than in the case of the first stimulation *(B* in *Fig. 1)*. — After the fourth- fifth stimulation *(C* in *Fig. 1)* the duration of inhibition is even shorter and does not practically return again despite repeated stimulation. Moreover, about the first minute after the onset of stimulation the frequency of cardiac action does not differ either from that observed before stimulation. If the parameters were left unchanged it was achieved by further repeated stimulation that the heart ceased to respond. The longer the duration of the single stimulations the sooner the inhibitory effect disappears.

b) Stimulatory effect. — Fundamental difference exists between inhibitory and stimulatory effects induced by lasting and repeated stimulation of the heart of the snail. In the case of parameters producing stimulatory effect adaptation never occurs after lasting or repeated stimulation, and the heart is not liberated from the stimulating effect during the time of excitation. The stimulatory responses to repeated excitation remain practically unchanged for 4—5 hours at unchanged parameters. Of course intervening rest periods between the single stimulations are important for the preparations.

In that period of the year when the experients were performed it was found in accordance with previous experiments (S.-Rózsa and Graul 1964) that increased activity stopped immediately after the cessation of stimulation.

Figure 2 illustrates the above finding obtained at 8/sec frequency, 1 msec duration of impulse and 15 V. This figure presents the lst, 5th and 10th

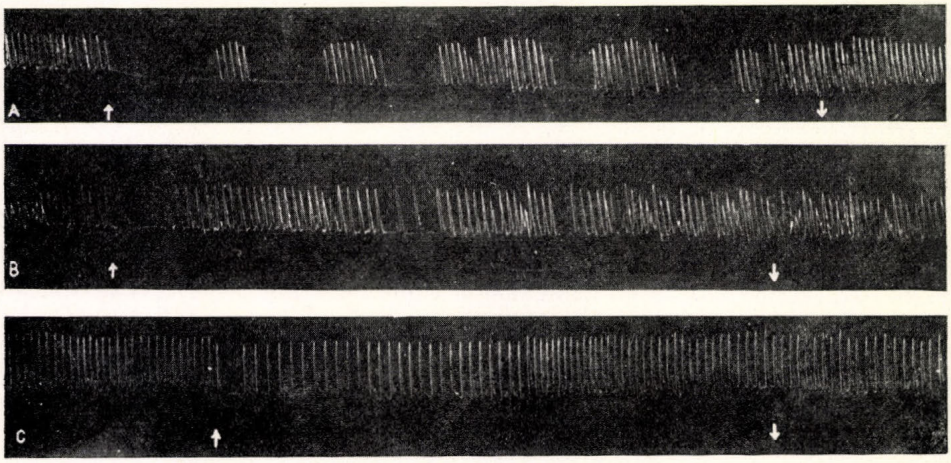

Fig. 1 Inhibitory effects produced on the heart of *Helix pomatia Z.* by long lasting and repeated stimulation of the intestinal nerve. Parameters of stimulus: frequency: 5/sec; duration of the impulses: 1 msec; amplitude: 10 volt. A — stimulation of intestinal nerve on the first occasion, B — second stimulation, C — fifth stimulation

1. ábra Gátló hatások *Helix pomatia L.* szívén az intestinális idegének hosszan tartó és ismételt ingerlése esetén. Ingerparaméterek: frekvencia 5/sec, impulzusszélesség 1 msec, feszültség 10 V. A — az intestinális ideg első alkalommal történő ingerlése — B — másodszori ingerlés — C — ötödik ingerlés

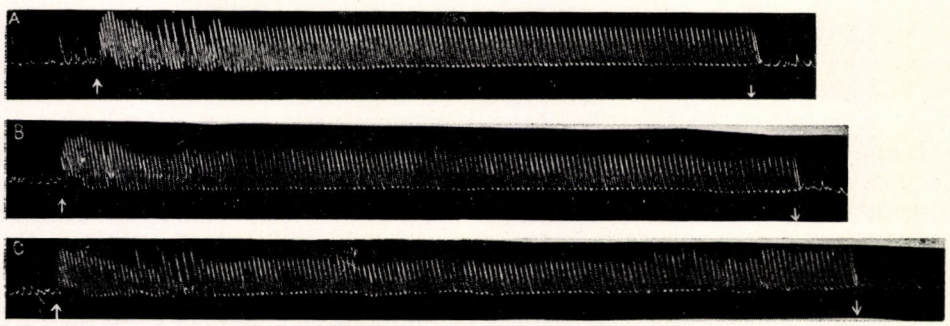

Fig. 2. Stimulatory effects produced by long lasting and repeated stimulation of the intestinal nerve in the heart of *Helix pomatia L.* Parameters of stimulus: 8/sec, 1 msec, 15 V. A — first stimulation of the intestinal nerve, B — fifth stimulation, C — tenth stimulation

2. ábra Stimuláló hatások *Helix pomatia L.* szívén az intestinális ideg hosszantartó és ismételt ingerlése esetén. — Ingerparaméterek: frekvencia 8/sec, impulzusszélesség 1 msec, feszültség 15 V. — A — az intestinális ideg első alkalommal történő ingerlése — B — ötödik ingerlés — C — tizedik ingerlés.

stimulations performed at unchanged parametrs. It is visible that after an initial, somewhat greater increase in amplitude the heart is stabilized to an activity of higher amplitude which remains practically unchanged till the end of the stimulation *(A, B, C* in *Fig. 2)*. When stimulation stops the amplitude rapidly decreases to the original level. In case of repeated stimulation the effect produced remains in its entire course the same as at the first stimulation. During the experiment MENG'S solution was perfused through the heart and thus the released mediators could not accumulate in the heart.

The course of inhibitory and stimulatory effects produced by lasting and repeated excitation suggests that a basic difference may exist in the sensitivity of structures through which these effects are realized. The heart becomes easily adapted to the kind of stimulation which produces inhibition and consequently stimulation becomes ineffectual hereafter. In case of stimulatory effect this kind of adaptation was not observed.

The studies on the different behaviour of inhibitory and stimulatory influences experienced in case of lasting and repeated stimulation are based on the suggestion that this phenomenon cannot be due to the exhaustion of inhibitory mediator reserves, for there is no reason to assume that they exist in a lesser amount than the stimulating transmitters. It was assumed, therefore, that the adaptation to inhibitory stimulation is due to the fact that the heart becomes insusceptible to the inhibitory substances released during stimulation because the released chemical substances change those surface structures through which the effects of the inhibiting nerve are realized. Accordingly, studies were performed to investigate the course and realization of inhibitory influence by agents that produce alterations in surface structures by changing the proportion between free and bound nucleotides and nucleic acids.

2. Effect of agents influencing the structure of nucleotides and nucleic acids on the course of the inhibitory effect.

Dyes were used (acridine orange and neutral red) as agents influencing nucleotides and nucleic acids. It is known that free nucleic acids and nucleotides present on the surface of the cell are adsorbed by acridine orange and that in the resulting chemical bonds adenyl groups are involved (SZENT—GYÖRGYI 1957). Neutral red, on the other hand, enters into interaction with RNS and influences extracellular nucleic acid content (KOSHTOYANTS 1963).

In the presence of acridine orange the inhibition of the heart ceases and at constant parameters the effect produced was only stimulatory. These observations are demonstrated in *Fig. 3*. The inhibitory effect stops when $1 \cdot 10^{-4}$ g/ml acridine orange is perfused and from the fifth minute of perfusion stimulating influence was observed at the parameters which produced inhibition previously *(C* in *Fig. 3)*. The original inhibitory effect was not restituted by washing with physiological solution. Neither could AMP or ADP restitute the inhibition eliminated by acridine orange. Perfusion of the heart with $1 \cdot 10^{-4}$ g/ml ATP, however, effectuates again complete inhibition within 10 minutes during nerve stimulation *(E* in *Fig. 3)*.

Neutral red did not influence essentially the course of the inhibitory effect. Inhibition is not realized, nor was the transition from inhibitory to stimulatory effect observable at the parameters left unchanged.

If snail heart was perfused with $1 \cdot 10^{-4}$ g/ml acridine orange before stimulation for 20—30 minutes inhibition never took place, only stimulating effect was observed. Similar phenomena due to seasonal changes were reported earlier (S.-Rózsa and Graul 1964). It was observed namely that in certain periods of the year only stimulatory effects could be produced. It is inferred on basis of the present data that alterations in structure of nucleotides and nucleic

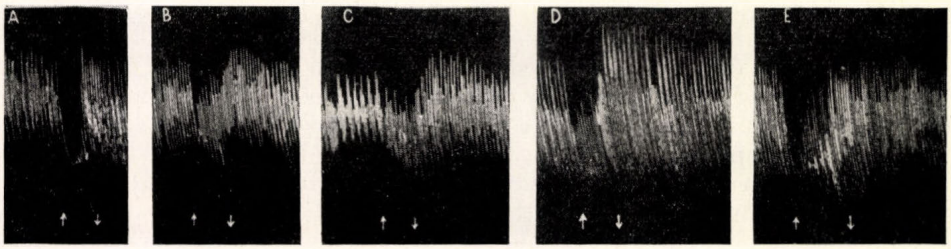

Fig. 3. Effect of acridine orange and ATP on the response reaction produced by the stimulation of the intestinal nerve. Parameters of stimulus: 5/sec, 1 msec, 10 V. A — initial inhibitory effect produced on the heart by stimulation; B — reaction of the heart on stimulation at the same parameters 3 minutes after the perfusion of $1 \cdot 10^{-4}$ g/ml acridine orange; C — the same after 5 minutes; D — reaction of the heart after 5 minutes long perfusion of $1 \cdot 10^{-4}$ g/ml ATP; E — the same after 10 minutes

3. ábra Acridine orange és ATP hatása a szív intestinális ideg ingerlésére fellépő válaszreakcióra. — Ingerparamétek: frekvencia 5/sec, impulzusszélesség 1 msec, feszültség 10 V. — A — kezdetben a sziven ingerléssel kiváltott gátló effektus — B — a szív reakciója ugyanazon ingerparaméterekkel történő ingerlés esetén 3 perccel $1 \cdot 10^{-4}$ g/ml acridine orange perfuzálása után — C — ugyanaz 5 perc mulva — D — a szív reakciója $1 \cdot 10^{-4}$ g/ml ATP 5 percig tartó perfuzálása után — E — ugyanaz 10 percig tartó perfuzió után.

acids are influencing the receptors of the inhibitory transmitter, and by means of this influence the heart becomes insensitive towards inhibitory influences.

Isolated snail heart does not respond practically to AMP and ADP introduced from outside. Its response to ATP cannot be taken for physiological either, for this agent produces only small increase in amplitude and only at a high concentration ($1 \cdot 10^{-3} - 1 \cdot 10^{-2}$ g/ml). Nevertheless, this kind of increase in amplitude is similar to that induced by most amines and amino acids at similar concentrations.

Discussion

The liberation from the inhibiting influence produced by stimulating the extracardiac nerve is a phenomenon known long ago in the heart of vertebrates. The inhibition produced by lasting stimulation of n. vagus in the heart of the frog is not continuous and active periods are observable periodically (Poskonova 1961, Beregszászi et al 1957). It has been demonstrated by Poskonova (1961) also that uridine and uracyl change the inhibitory effect on frog heart to stimulatory effect. Putintseva and Turpaev (1959), Putintseva (1961) suggest that a chemical agent producing stimulatory effect is released during long lasting stimulation in frog heart on the influence of acetylcholine. It is belived by these authors that the chemical agent released

is identical with uridinediphosphate in opposition to the suggestion of BEREGSZÁSZI et al (1957) that this stimulatory effect is attributable to the presence of a contraregulatory mechanism and to the liberation of an adrenaline-like substance.

Of Molluscs *Venus mercenaria* was studied by PROSSER (1940) from this point of view, who observed the liberation of the heart of this animal from the effect of inhibitory nerve and attributed it to the exhaustion of transmitter reserves. SAKHAROV and NISTRATOVA (1963) observed an adaptation to acetylcholine in the heart of *Anodonta*, and explained this by the presence of ATP. The ATP which is released by acetylcholin in the heart of *Anodonta* becomes involved, as suggested by these authors, in the elimination of inhibitory effect because they act as competing antagonists on the acetylcholine receptors and displace acetylcholine from them. They believe that cholinesterase is not present in the heart of *Anodonta*, and the heart is liberated from the influence of the inhibitory transmitter in the above discussed manner. In special studies considerable amount of cholinesterase was demonstrated in the heart of both *Helix* and *Anodonta* especially in the area of the auricle (unpublished data) and thus, it is considered more probable that released ATP might much rather be involved in the energy turnover and in the regulation of ion migration (CALDWELL et al 1964). It seems improbable that the cessation of inhibitory influence on the heart of *Helix* by continuous stimulation is due to the complete consumption of the inhibitoray transmitter (PROSSER 1940), because a stimulating mediator is practically inexhaustible in preparations of normally operating heart nerve.

It is suggested that the above discussed effects are due to structural alterations occurring on the receptors. This explains the different behaviour of both inhibitory and stimulatory effects in case of lasting stimulation, because the acetylcholine receptor is a proteinaceous substance (TURPAEV 1962), whereas the serotonine receptors are considered lipid-like substances (VANE et al, 1961, WOOLLEY 1966). A smaller change in the protein receptors may take place more easily also on the influence of the transmitter substance released under natural conditions. If, however, simultaneously with the transmitter another substance is also liberated, then this may have a role in the alteration of the sensible structure which turns it insusceptible towards the liberated transmitter. This might explain why snail heart is set free despite continuous stimulation from the inhibitory effect of intestinal nerve.

The observation that nervous stimulation becomes of opposite sign on the influence of dye-stuffs forming complexes with nucleic acids and nucleotides, further that this effect is restorable by the administration of ATP indicate that perhaps adenyl groups are being inactivated on the influence of acridine orange. Under this condition inhibitory effect cannot be realized either because of the fixation of adenyl groups or because of their structural alterations. The observation that instead of inhibitione a stimulating effect is produced makes two suppositions probable:

a) The effect produced by acetylcholin released by means of nerve stimulation does not take place in the usual manner, and not inhibition but stimulation is produced by the excitation of the altered structure.

b) It is possible that the release of stimulatory mediator takes place also under inhibitory effect, if, however, the inhibitory effect predominates this mediator cannot manifest itself. It is prevailing only if inhibition is eliminated.

The fact that ATP restores the inhibitory effect produced by nerve stimulation after the addition of dye stuffs suggests that structural alterations of certain proteins are responsible for this transition from inhibition to stimulation. Thus it is inferred that ATP might promote the restitution of structures that are involved in stimulation transfer between nerve-terminations of the intestinal nerve and cardiac muscle.

On basis of these considerations it may be concluded that the effect of transmitter agents depends on the condition of the surface structures of cells. In case of snail heart this is closely related to the localization of nucleotides and of nucleic acids and in case of inhibitory effects primarily to the adenyl groups of these substances.

Conclusions

1. The long lasting stimulation of the intestinal nerve of *Helix pomatia L.* liberates the heart from inhibitory influences. By repeated stimulation the time of inhibition periods is shortened and finally ceases completely.

2. In case of lasting and repeated excitation stimulating effects occur with unchanged intensity.

3. It is assumed that the release from the inhibitory effect is due to the adaptation of acetylcholine receptor protein. A similar adaptation was not observable in case of serotonine receptors involved in stimulatory effects of lipid nature.

4. Acridine orange which influences surface structures stops the inhibitory effect produced by the intestinal nerve and produces an effect of opposite sign (stimulating).

5. In the heart treated with acridine orange ATP ($1 \cdot 10^{-4}$ g/ml) restores the eliminated inhibitory effect of the intestinal nerve. This fact emphasizes the importance of adenyl groups in the realization of inhibitory effects.

6. In the realization of the inhibitory effect of intestinal nerve on snail heart an important role is ascribed to nucleotides and nucleic acids.

Summary

Author investigated the responses of the heart of *Helix pomatia L.* to long lasting and repeated stimulation of intestinal nerve. It was established that in case of long lasting and repeated nerve stimulation with inhibitory parameters the heart becomes liberated from the inhibiting effect, whereas at other parameters stimulating effect is demonstrable which is of unchanged intensity even in case of long lasting and repeated stimulation. The liberation of the heart from the inhibitory effects might be explained by the adaptation of acetylcholine receptors of protein nature. In case of 5-HT receptor of lipid nature an adaptation of this kind was not observed. Acridine orange which forms complexes with nucleotides and nucleic acids stops the inhibition produced by the intestinal nerve and makes it stimulatory even if the parameters are left unchanged. ATP restores the inhibitory effect. It is suggested that in the realization of inhibition produced by the intestinal nerve surface structures and primarily structural changes in the adenyl groups might be of importance.

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EXTRAKARDIÁLIS IDEG GÁTLÓ HATÁSÁHOZ VALÓ ALKALMAZKODÁS VIZSGÁLATA *HELIX POMATIA L.* SZÍVEN

S.-Rózsa Katalin

Összefoglalás

Szerző vizsgálta *Helix pomatia L.* szívének viselkedését az intestinális ideg hosszantartó és ismételt ingerlése esetén. Megállapítást nyert, hogy gátlást kiváltó paraméterekkel történő hosszantartó és ismételt idegingerlés esetén a szív kiszabadul a gátlás alól, míg más paraméterekkel kiváltott stimuláló hatások változatlan intenzitással jelentkeznek ugyancsak hosszantartó és ismételt ingerlés mellett is. A szív gátló hatások alól való kiszabadulása a fehérjetermészetű acetylcholin-receptor adaptációval magyarázható. A lipid-természetű 5-HT-receptor hasonló jellegű adaptációja nem figyelhető meg. A nukleotidokkal és nukleinsavakkal komplexet képező acridine orange megszünteti az intestinális ideg gátló hatását és változatlan ingerparaméterek mellett stimulálóvá alakítja azt. A gátló hatást az ATP helyreállítja. Az intestinális ideg gátló hatásának realizálódásában a felületi struktúráknak, elsősorban az adenyl-csoportok struktúraváltozásainak van jelentőségük.

ИССЛЕДОВАНИЕ АДАПТАЦИИ СЕРДЦА ВИНОГРАДНОЙ УЛИТКИ К ТОРМОЗНЫМ ВЛИЯНИЯМ ЭКСТРАКАРДИАЛЬНОГО НЕРВА

Каталин Ш.-Рожа

Изучено поведение сердца при длительном и повторяющемся раздражении интестинального нерва виноградной улитки. При применении параметров раздражения, вызывающих тормозной эффект, происходит выход сердца из торможения в условиях продолжающегося раздражения. При применении параметров раздражения, вызывающих стимуляцию, уменьшения реакции сердца не наблюдается. Выход сердца из-под тормозящих воздействий объясняется адаптацией холинорецепторов, имеющих белковую природу. Сходной адаптации не удается наблюдать на 5-ОТ-рецепторах, имеющих липидный характер. Акридинораж, образующий комплекс с нуклеотидами и нуклеиновыми кислотами, снимает тормозное влияние интестинального нерва и при неизменных параметрах раздражения превращает тормозной эффект в стимулирующий. Предполагается, что в реализации тормозного эффекта интестинального нерва имеют значение поверхностные структуры, прежде всего изменения адениловых групп, так как добавление АТФ восстанавливает тормозной эффект, подавленный акридинораже.

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DAILY ACTIVITY RHYTHM OF TWO MEDITERRANEAN LAMELLIBRANCHIA *(PECTEN JACOBAEUS* AND *LITHOPHAGA LITHOPHAGA)* REGULATED BY LIGHT-DARK PERIOD

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In the course of research on biological rhythms the greatest attention has been devoted to investigations and hypotheses concerning the regulatory mechanisms of endogenous *circadian* rhythms both in plants and animals (BROWN 1959, BÜNNING 1963, HARKER 1964, SOLLBERGER 1965). Most of the work performed on invertebrates tried to prove that rhythms are less dependent on environmental factors and are regulated by a so-called "clock" mechanism. With the animals placed under "constant conditions", observations have been made on the length of time, that previously observed rhythms have continued, without normal environmental stimulation. In spite of numerous investigations the essence and functioning of the "clock" mechanism is not known in detail, therefore the question, how does the "clock" regulate the rhythm of the living processes hardly arises.

Investigations of the basic mechanisms of the nervous system in animals — on the other hand — have given more and more informations on the problem of its integrative and correlative functions. Correlation takes place, without doubt, first of all between environmental factors and the organism. In most of cases it is obvious, that the daily rhythm helps the organism to adapt to major environmental factors and that this is one of the modes of regulating equilibrium with the environment. Therefore, in spite of the fact that most of the "circadian rhythms" continue under constant conditions, thus suggesting the presence of an "internal clock", in normal life these rhythms run parallel with changes of very effective stimuli from the environment, such as light, t°, humidity and others.

For this very reason, these factors affecting the sense organs must play a continuously important role. This is proven by the fact, that in many cases the rhythms which do not change under constant conditions, will change by altering the rhythm of the controlling factor (light-dark conditions, etc.).

To understand the central regulatory mechanisms responsible for animal behaviour, including rhythmic behaviour, it seems to be more convenient to investigate the mode of action of factors maintaining the rhythm and at the

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same time directly affecting animal behaviour. In other words, it seems very important to investigate entirely and in detail the mechanisms affecting rhythm including long term environmental factors. Such an investigation could produce data and conclusions which on the one hand lead to an understanding of the nervous control of behaviour, on the other hand to the discovering of the basic mechanism of the "internal clock".

Concerning the papers dealing with the Lamellibranchia, some of them insist on the independence of their rhythms from usual environmental factors (BARNES 1955, RAO 1959) others have proved that they are in close correlation with the changes of the outside world (LOOSANOFF 1958, BROWN 1956, KOSHTOYANTS and SALÁNKI 1958, SALÁNKI 1965). The effects investigated in these experiments were the tide, the pressure, the t°, the O_2-level and the influence of some chemicals. There are a few allusions that illumination has also some effect on the behaviour of mussels (DODGSON 1928, SALÁNKI 1964). Nevertheless, owing to the fact that these animals usually have no eyes, this problem was not investigated in detail, although photosensitive structures are known in some Lamellibranchia species (HECHT 1919, 1920, KENNEDY 1960, 1964).

The aim of the present work was to obtain data on whether illumination and its changes play a role in the regulation of the rhythmic activity of Lamellibranchia, and if they do, is there any daily periodicity in the activity, corresponding to diurnal changes. It was also investigated, whether these factors have a direct role, or are coincident with an independent, persistent, internal daily rhythm.

Investigations were carried out on two different species. The one has well developed mantle eyes *(Pecten Jacobaeus)*, the other has no eyes *(Lithophaga lithophaga)*.

Material and methods

Marine Lamellibranchia, *Pecten Jacobaeus* and *Lithophaga lithophaga* were used. The animals were collected in the Gulf of Naples and kept for several days in tanks supplied with running, aerated water.

During the experiments the rhythmic movements of the shells were recorded. These are corresponding to the rhythmic contractions and relaxations of the closer muscles. For recording the activity, one of the shells was fixed to the bottom while the other was connected by a thread to a lever. The records were made on a kymograph which could run at very slow speeds, thus assuring continuous recording for six days, but at the same time making it possibile to distinguish between separate contractions. During this period the animals were placed in separate vessels with 5—6 litres of see water, without additional aeration. The room temperature was kept constant (22 ± 0.5°C).

The experiments were performed in ordinary daylight. When required, the room was darkened by covering the window with a black blind (which yet did not result in total darkness in the room); at night, light periods were obtined by using a 60 Watt bulb situated at one meter from the animal. The experiments were carried out in May and June 1965.

Results

During the investigations both *Pecten* and *Lithophaga* showed quite regular rhytmic activity. There was a rhythmicity with a frequency 1—5/hour, which continued for several days without any interruption by long adductions of the valves as noted elsewhere (SALÁNKI 1966).

Two types of daily fluctuations occurred in the rhythm:

a) the frequency of the fast contractions and relaxations were different during the night and the day, the difference being about 100%. The higher frequency occurred with *Pecten* in the night while in *Lithophaga* this peak was observed during daytime.

b) The amplitude of the shell movements also showed marked differences, which were manifest for both species in about 30—50% greater amplitude at night compared with day-time records.

This augmentation of the amplitude was caused by the increased opening of the valves, which was about twice as much at night as in the day. At night the valves usually failed to close completely, in contrast to daytime, when maximum adduction could be observed. With *Lithophaga*, at night, regular and more powerful contractions were also observable *(Fig. 1)*.

It must be noted, that the changes in the amplitude of the rhythmic movements strictly correspond with the changes of room-illumination, getting light between 6—7 o' clock in the morning and beginning the nightfall at about 5—6 p. m. (This was a shorter period of daylight, than normal, and was caused by trees overshadowing the window of the laboratory).

Since illumination was the most noticeable diurnal factor to change, this was investigated further. It was planned to examine whether changes in the light-dark period produce a shift or complete change in the rhythm of activity or not. For this purpose, in one case, the daylight period was lengthened by illumination of the animal during the night, in another experiment the dark period was extended to the next day by darkening the room.

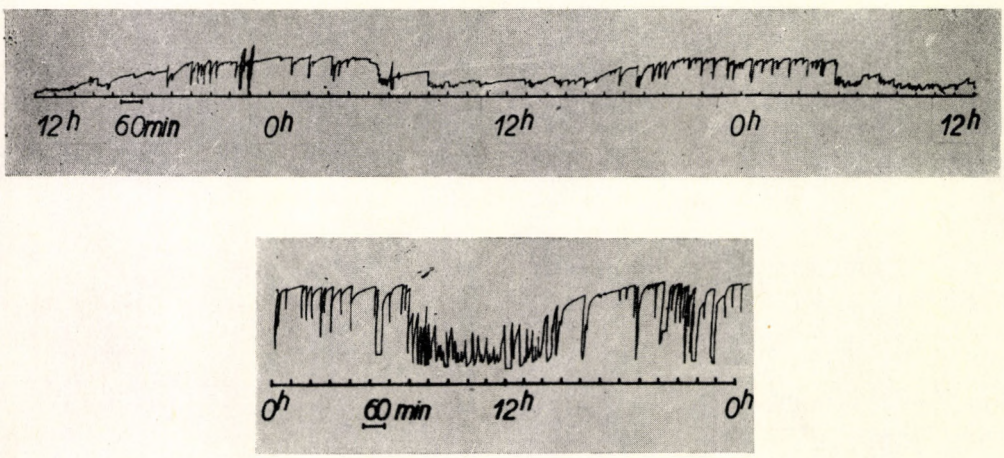

Fig. 1. Diurnal rhythm of activity: *a) Pecten Jacobaeus*; b) *Lithophaga lithophaga*
1. ábra Az aktivitás napi ritmusa

If the dark period continued for 36 hours, in both *Pecten* and *Lithophaga*, the frequency and amplitude of the rhythmic valve-movements assumed nearly completely the night time characteristics *(Fig. 2)*.

T is means that the change to the typical daytime pattern of activity was prevented merely by the lengthening of the dark period.

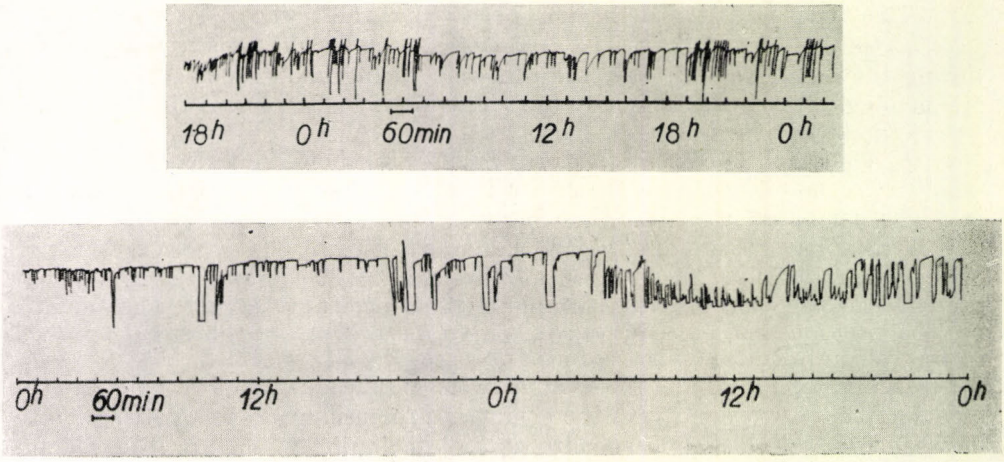

Fig. 2. Effect of 24 hours darkness on the activity of a) *Pecten Jacobaeus*; b) *Lithophaga lithophaga* (on the second day returning to the normal light)
2. ábra 24 órás sötét hatása a napi ritmusra A — *Pecten Jacobaeus* B — *Lithophaga lithophaga* (második nap visszatérés a normál megvilágítási ritmusra)

On the following days, when natural light-dark conditions were allowed to operate, the daily periodicity usually returned. However, in some cases *Pecten* did not return exactly to the pattern shown before the long dark period, but as can be seen in *Fig. 3*, during the subsequent light interval there was only a slight decrease in amplitude, and about a 50% decrease in frequency of the rhythmic contractions.

Fig. 4. shows the effect of a long light period. Continuous light caused in general the lengthening in the period of the partially open position of the valves. A lengthening of the "daily" frequency of the rhythmic contractions was also observed. In the case of *Lithophaga* some decrease in the frequency

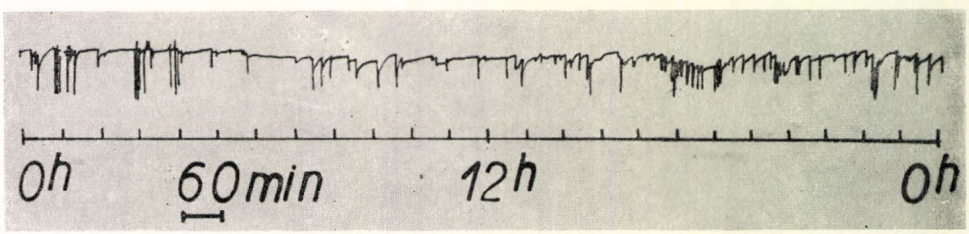

Fig. 3. Rhythmic activity of *Pecten* in normal light after 24 hours continuous dark period
3. ábra Pecten ritmikus aktivitása 24 órás sötét periódus után normál megvilágításban

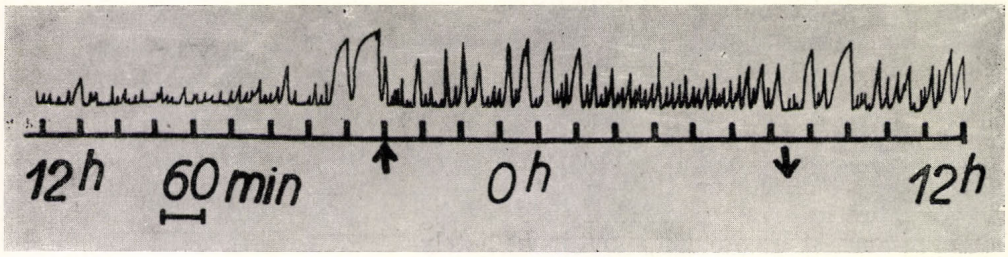

Fig. 4. Effect of light (↑ — ↓) on the activity of *Lithophaga lithophaga*
4. ábra Fény hatása (↑ — ↓) *Lithophaga* aktivitására

of the rhythmicity occurred but it is very different from that which could be seen in darkness. This was probably caused by the inadequate illumination, using bulb-light instead of natural light, being more diffuse and of a broader spectrum.

Discussion

The photosensitivity of Lamellibranchia is a well-known phenomenon (MILNE 1959), though except in the case of *Pecten* it has not been investigated in detail. HARTLINE'S classical work (1938) on the visual reception of *Pecten* gave evidence that two photosensitive layers exist in the eyes, one giving discharges in darkness and the other stimulated by light. There is also good evidence that in *Mya* the syphons respond by retracting when stimulated by light (HECHT 1919, 1920). In the pallial nerve of *Spisula solidissima* also photosensitive nerve elements were found (KENNEDY 1960). It seems probable that the non specialized "general" or "dermal" photosensitivity is widespread among Lamellibranchiates and that photoreception is located in specially pigmented areas of the mantle (KENNEDY 1964).

The general significance of the light-dark periodicity as the signalizing system of the daily rhythm is well established, but there are only a few references to this problem in the case of Lamellibranchia (DODGSON 1928, SALÁNKI 1964).

The present investigation shows that on *Pecten* and on *Lithophaga* the illumination level really play a significant role in the regulation of the rhythmic activity. This rhythm depends directly on the effect of illumination. Both in the case of *Pecten* which has well developed photoreceptors — the eyes — and *Lithophaga*, having no eyes at all, it is probable that long-term photoreception takes place in the receptor structures which, by way of nervous impulses, influence the centres controlling the rhythm. It is noteworthy, that the effect of illumination is the same in both species, pointing to the similar function of the primary photoreceptors in this respect. It may be suggested therefore that the well-developed eyes of *Pecten* might be capable not only of perception of light differences, but also of more complex functions.

The question arises whether the activity of the rhythmic centres is affected by light or by darkness. Maybe both light and dark have their own regula-

tory influence. The results of HARTLINE (1938) and KENNEDY (1960) showing that both light and dark cause changes in the activity of the photoreceptor elements support the latter possibility.

The changes in the daily rhythm of activity caused by lengthening the light or dark periods show the close relationship between illumination and activity, and prove that the activity rhythm depends to a large extent upon daily changes of light and darkness. In the case of the two Lamellibranchia investigated it can be concluded that the regulation of rhythm is external, and the changes are brought about immediately after changing the light conditions. The fact, that in some cases after long dark periods the former periodicity did not return in an analogous form shows that disturbances in the external rhythm can produce a prolonged effect on the regulatory mechanism. It is difficult to decide, whether this effect is due to some alterations of the primary photoreceptor or to that of the nervous control. However, starting from the fact that under normal conditions changes in illumination cause changes both in frequency and in amplitude of the rhythmic contractions, it may be suggested that if only one of these is disturbed, there must be probably a central mechanism involved.

Two questions remain to be answered:

(1) what is the mechanism for the regulation of the rhythm and how can it be altered by changes in illumination;

(2) what is the ecological significance of this reaction? Neither of these questions can be answered at the moment. By investigating the effect of continous light and darkness on the photoreceptive elements and especially on the integrative and coordinative centre of the rhythmic activity i.e. the central nervous system it should be possible to get data concerning the mechanism of this regulation not only in Lamellibranchia but in other light sensitive rhythm too.

Summary

The rhythmic activity of the closer muscles of *Pecten* and *Lithophaga* was recorded and a daily rhythm in the amplitude and frequency of the contractions as well as a difference in the degree of opening of the valves was established. The daily rhythm depends on the illumination changes and can be shifted by lengthening the light or dark periods.

It is concluded that basic functions of the well developed eyes of *Pecten* and the primitive dermal photoreceptors of *Lithophaga* do not differ from each other from the viewpoint of regulation by light-dark periodicity. This means that primitive photoreceptors are also capable of perception of long-term light and dark changes.

It seems probable that the effect of illumination realizes, through the receptors, on the tonus- and rhythm-regulating centre of the nervous system, which latter in the absence of external periodicity do not have a diurnal "clock" mechanism.

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KÉT MEDITERRÁN KAGYLÓ *(PECTEN JACOBAEUS* ÉS *LITHOPHAGA LITHOPHAGA)* MEGVILÁGÍTÁSFÜGGŐ NAPI AKTIVITÁSI RITMUSA

Salánki János

Összefoglalás

Szerző vizsgálta két tengeri kagylófaj *(Pecten Jacobaeus* és *Lithophaga lithophaga)* ritimikus aktivitását. A kísérletek során a héjmozgásokat (záróizomműködést) napokon át folyamatosan regisztrálta. Napi ritmust állapított meg a ritmikus aktivitás frekvenciájának és az egyes kontrakciók amplitúdójának változásában. Ugyancsak napi ritmust észlelt a maximális héjnyílás (izomrelaxáció) mértékében is. A ritmus megvilágításfüggő, minthogy a világos vagy sötét periódus megnyújtásával befolyásolható.

A vizsgálatok azt mutatják, hogy a *Pecten* kifejlett szeme és a Lithophaga fényérzékeny köpenyterülete a megvilágításfüggő napi aktivitás szabályozása szempontjából nem különbözik egymástól. Ez azt is jelenti, hogy a primitív fotoreceptor képes tartós megvilágítás ill. sötét érzékelésére.

Valószínű, hogy a megvilágítás a receptorokon keresztül az idegrendszer ritmus- és tónusszabályozó központját befolyásolja, mely utóbbi környezeti periodicitás hiányában nem rendelkezik napi ritmust fenntartó független belső mechanizmussal.

РИТМ СУТОЧНОЙ АКТИВНОСТИ У ДВУХ СРЕДИЗЕМНОМОРСКИХ ДВУСТВОРЧАТЫХ МОЛЛЮСКОВ *(PECTEN JACOBAEUS* И *LITHOPHAGA LITHOPHAGA)* РЕГУЛИРУЕМЫЙ ПЕРИОДИЧНОСТЬЮ СВЕТА И ТЕМНОТЫ

Янош Шаланки

При регистрации ритмической активности запирательных мышц у *Pecten Jacobaeus* и *Lithophaga* была установлена суточная периодичность в амплитуде и частоте сокращений, а также различия в степени раскрывания створок. Суточная периодичность зависит от смены света и темноты и может быть сдвинута продлением периодов света или темноты.

Делается вывод, что основная функция хорошо развитых глаз *Pecten* и примитивных дермальных фоторецепторов *Lithophaga* не различается в отношении регуляции этой периодичности. Это значит, что примитивные фоторецепторы также способны к восприятию длительных изменений света и темноты.

Возможно, что освещение действует, через посредство рецепторов, на центры нервной системы, регулирующие тонус и ритм, которые в отсутствие периодичности во внешней среде не обладают внутренним «часовым» механизмом.

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CHOLINESTERASE ACTIVITY IN THE CENTRAL NERVOUS SYSTEM OF *ANODONTA CYGNEA L.*

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The studies on the central and peripheral nervous system in molluscs refer primarily to the gastropods and cephalopods. Due to experimental and methodical difficulties much less is known of the construction and acitivity of the nervous system of Pelecypods (Lamellibranchiates). Thus, we have no satisfactory knowledge and exact data on the chemical specificities of active substances involved in the activity of cells and synapses of the central nervous system, though the presence of 5-HT and of catecholamines in the ganglia and the activating effect of these agents when administered from outside has been demonstrated by several authors (DAHL et al. 1962, SALÁNKI 1963, SWEENEY 1963, PUPPI 1964, ZS.-NAGY et al. 1965).

Studies performed by HORRIDGE (1961) on *Mya*, and PUPPI (1963) on *Anodonta* with the objective of establishing the possibility of the presence of a cholinergic mechanism in the central nervous system ended with positive results. The same indications were obtained by the electron microscopic studies of ZS.-NAGY (1964) demonstrating in the synapses of central nervous system the presence of structures corresponding to the ACh vesicles of vertebrates.

Nevertheless, it was not possible to histochemically demonstrate the presence of cholinesterase in the ganglia of *Anodonta* either by the thiocholine or by the indoxyle acetate methods (ZS.-NAGY and SALÁNKI 1965).

It is generally accepted that the presence of ACh-decomposing enzyme in the postsynaptic field is incident to the cholinergic mechanism, thus, in order to obtain more knowledge on the central neuronal regulation it is essential to decide whether acetylcholinesterase is present in the ganglia (which represents the central nervous system in *Anodonta)* or not. In certain cases this might offer some basis in the indetification of the above mentioned structures which are of 200—300 Å diameter and ultrastructurally resemble ACh-vesicles.

Material and methods

The whole central nervous system (cerebral, visceral, and pedal ganglia) of *Anodonta cygnea L.* was examined. The samples were homogenized for 10—15 minutes with distilled water in glass-potter placed into icy water. Incubation conditions: 1 ml homogenized sample consisting of the whole central

nervous system of two animals (about 40 mg) and the required quantity of substrate was added into 5 ml incubation solution, and the mixture obtained was adjusted to the wanted pH (5.0—8.5) value by the addition of trismaleate buffer. Incubation temperature was 37 $\pm$ 0.1 °C and the duration of incubation 3 hours. The self-hydrolysis of enzyme-free samples was determined. In case inhibitors were used they were added both to the incubation solution and the control tube. This was necessitated by the colour change of eserine during its decomposition.

Measurement: The decomposition of substrate was determined by the method of HESTRIN (1949). Calibration curve was taken with $AChClO_4$. The coloured product was measured photometrically with BECKMAN G 2400 spectrophotometer at 530 mμ wavelength.

In the experiments the following substrates and inhibitors were used:

acetylcholine Cl (ACh; Merck)
acetylthiocholine J (AThCh; Fluka)
butyrylthiocholine J (BuThCh; Fluka)
acetyl- β-methylcholine (MeCh; Light)
benzoylcholine (BeCh; Light)
eserine salicylate (BDH)
diizopropyl-fluoro-phosphate (DFP)

To avoid the differences between individuals a homogenizate was prepared of the central nervous system of 50—100 animals and this was used in the measurements. Nitrogen determinations were performed according to KJELDAHL'S method. NH_3 was distilled into saturated boric acid solution and was titrated with 0.01 n HCl solution.

Results

1. Rate of the enzyme activity with various substrates and the effect of inhibitors.

In every case incubation was performed at pH 8 and lasted for 3 hours. The concentration of substrate was 4 mM. Considering the relatively long time needed for the excision and collection of ganglia (2—3 hour's) from the animals and becasue of changes in wet-weight content occurring also in iced physiological solution, the decomposition rates obtained are expressed in terms of N content. From this value the rate of ChE-activity might be expressed also approximatively on wet-weight basis by computation (N content of ganglia = 8—10 mg N/g wet-weight).

Rate of hydrolysis

Substrate	μg substrate/mg N/hour	mg substrate/g wet-weight/hour
ACh	226	1.8—2.3
MeCh	184	1.5—1.8
AThCh	214	1.7—2.1
BuThCh	217	1.7—2.2
BeCh	no hydrolysis	—

The effect of inhibitors was measured in the presence of 4 mM ACh at 37°C and at pH 8.

Cholinesterase activity was completely inhibited by 10^{-5} M eserine and 10^{-6} M DFP. An about 40—60% inhibition was produced by 10^{-6} M eserine and 10^{-7} M DFP and no inhibition was observed in case of 10^{-7} M eserine and 10^{-8} M DFP.

2. Substrate-inhibition

Because the activity of acetyl cholinesterase is specifically inhibited by high substrate concentrations, which phenomenon is not characteristic of non-specific ChE (AUGUSTINSSON 1949) the activity of ChE was measured at different ACh concentrations. Incubation series was made at 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 mM AChCl concentrations. The results were related to the rate of decomposition at 4 mM concentration. *Fig. 1* illustrates decomposition rates

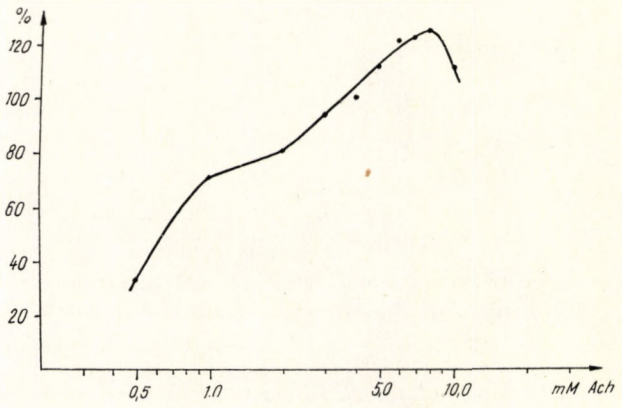

Fig. 1. The ACh splitting of homogenized ganglion depending on the substrate concentration (pH 8, 37° C)

1. ábra Ganglionhomogenizátum ACh bontása a szubsztrátkoncentráció függvényében (pH 8, 37° C)

in the percentage of values obtained at 4 mM. It appears that decomposition has a maximum which is followed by a decrease. Maximum was, however, obtained at about 8 mM substrate concentration. The shape of the curve is not identical with the bell-shaped curve generally accepted for specific ChE in literature (AUGUSTINSSON 1963), moreover, by increasing concentration further the descending slope of the curve becomes undefinite because of great deviation of data. For that very reason we shall abstain here from presenting these data. Besides the maximum at about 8 mM concentration, an inflexion point is observable on the ascending slope near to 1 mM.

3. pH dependency of ChE activity

The experiments were performed at different pH-values (5—8.5 pH), at 4 mM AChCl concentration and at 37°C, and decompostion rates were related to values obtained at pH 8. The results are presented in *Fig. 2.* A maximum activity is observable at pH 7 and only a decrease of pH below 5.5 or a rise above 8.5 produces considerable loss of activity.

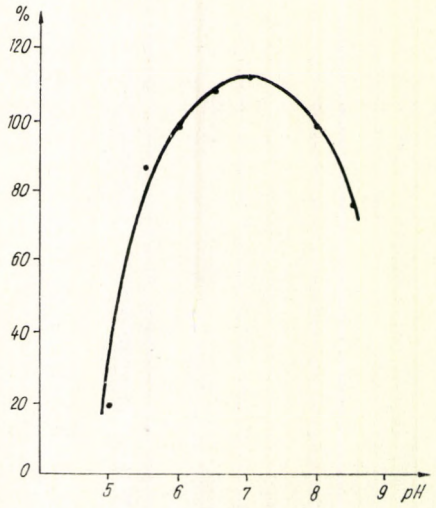

Fig. 2. pH dependency of ACh decomposition (4 mM substrate concentration, 37° C)
2. ábra ACh bontás pH függése (szubsztrátkoncentráció 4 mM, 37 °C)

4. The effect of formalin fixation on ChE activity

Previous histochemical studies on ChE in the ganglia of *Anodonta cygnea* (Zs.-Nagy and Salánki 1965) resulted in negative reactions. In these methods formalin fixation and also acide pH range were used. To elucidate the source of these negative results experiments were performed at different pH values also on homogenizates of ganglia pretreated by formalin.

Excised ganglia were treated with 4% neutral formalin solution for 1 hour. They were washed subsequently in running tap-water for 2 hours and were rinsed with distilled water. After homogenization the samples were incubated in the presence of 4 mM AChCl at 8.7 and 6 pH values. It was found that incubation at pH 6—8 after formalin fixation showed decreased activity with about 50%.

Discussion

As it is evidenced by the experiments cholinesterase exists in the central nervous system of *Anodonta cygnea L.* On basis of data obtained this is most probably a specific cholinesterase, though the properties of the demonstrated

enzyme do not comply with every criteria of it. The following phenomena are indicative of the presence of ChE: a considerable acetyl-β-methylcholine (MeCh) decomposition, the absence of benzoylcholine decomposition and the fact that highest values were observed in case of acetylcholine decomposition. Examining the rate of decomposition in the function of the substrate a maximum was observable at 8 mM and not at 4 mM regarded characteristic of AChE. Definite inhibition was not observed above 10 mM concentration either. Here decomposition rate was equal to that observed at 5 mM concentration. Occasionally a 70—100 per cent inhibition or no inhibition at all was observed at concentrations higher than 10 mM. The strong deviation of data obtained at high substrate concentration is obviously of methodical origin.

The dependency of decomposition on substrate concentration is not linear even at low concentrations and an inflexion is observable at 1 mM. It is suggested that perhaps two or more ChE-es of different nature exist in the homogenizate and they may be responsible for the deviation of the substrate curve from the conventional one. It was evidenced by previous studies (Zs.-Nagy and Salánki 1965) that non-specific esterase content in gastropods fluctuates considerably according to the functional condition of the animal. A similar phenomenon exists presumably in case of *Anodonta* and the alterations in the amount of this enzyme, induced by changes in the functional condition of the animal, may explain the undefinite descending slope of the substrate curve at concentrations above 10 mM.

Rate of enzyme activity was relatively low, 226 μg ACh/mgN/hour at pH 8 and at 4 mM substrate which expressed on wet-weight basis corresponds to about 2 mg ACh /g/hour. Considering that substrate concentration has an optimum at 8 mM where the observed activity is about 25% higher as compared to values obtained at 4 mM, further that an about 10% activity increase was obtained by incubation at pH optimum (pH 7) in comparison to pH 8, it is inferred that under optimum conditions we might reckon with an about 30% higher enzyme activity (i. e. about 2.6 mg ACh/g wet-weight/hour).

It might be of interest to compare the ACh activities of different invertebrates. The rate of the actvity of ChE expressed in mg ACh/g wet-weight/hour unit was in case of *Loligo* brain 2—4 mg, in the ganglion of *Limulus* heart 0.14 mg and in case of the mantle nerve of *Loligo* 0.006 mg (Prosser and Brown 1961).

In accordance with literary data (Taxi 1952) a decrease of activity by 50% was observed after formalin treatment and a considerable decrease of similar dimension was demonstrable below pH 6 and above pH 8.

The fact that ChE was not demonstrable histochemically in the ganglia of *Anodonta cygnea* can be attributed probably to the relatively low rate of activity, to the inhibitory effect of formaldehyde treatment and to the influence of acidic pH. It is assumable, however, that with a more suitable technique (without formaldehyde treatment or electron microscopic examination respectively) the chemically demonstrable amount would suffice for the demonstration of the localization of this enzyme.

We have no knowledge of the localization of ChE-activity demonstrated in these experiments, nonetheless the results obtained and the literary data (Horridge 1961, Puppi 1963, Zs.-Nagy 1964) suggest that it has a synaptic localization. The actual activity values suggest, however, that in the neural processes of the ganglia of *Anodonta cygnea* primarily not a cholinergic mecha-

nism, but chemical compounds different from ACh are involved. This assumption is supported by investigations demonstrating considerable amounts of dopamine (DAHL et al. 1962), catecholamine (PUPPI 1964) and 5-HT (DAHL et al. 1962; ZS.-NAGY et al. 1965) further the effect of 5-HT and of catecholamines on the central nervous system of *Anodonta* (SALÁNKI 1963). It is not to be ignored, however, that ACh may possibly have a more important role. Its decomposition may take place not in loco, but outside of the nervous system in the lymph, and the cessation of its effect in the synapses is due possibly to the adaptation of the postsynaptic structure and not to the decomposition by ChE (SAKHAROV and NISTRATOVA 1963).

The possibility of the presence of cholinerg mechanism in the central nervous system of Pelecypods is still an unsolved problem and further extensive studies are needed for the satisfactory solution of this problem.

Summary

The cholinesterase activity of the central nervous system of *Anodonta cygnea* (Pelecypoda) was investigated by biochemical methods. The cholinesterase activity observed is characterized by the followings:

1. ACh decomposition does not show linear increase parallel with the rise of substrate-concentration.
2. ACh-decomposition is most definite.
3. Acetyl-β-methylcholine (MeCh) decomposition is considerable.
4. There is no benzoylcholine decomposition.
5. 50% inhibition by 10^{-6} M eserine and by 10^{-7}M DFP.
6. There is an optimum at pH 7, and a considerable decrease in activity below pH 6 and above pH 8.
7. An about 50% decrease in activity after one hour treatment in 4% formaldehyde.

The above results indicate that a specific cholinesterase exists in the homogenized samples of the cerebral, visceral and pedal ganglia. At pH 8, at 37°C temperature and in case of 4 mM substrate concentration the rate of activity is 226 μg ACh /mg N/ 1 hour, which is equivalent to about 1.8—2.2 mg ACh/1 hour/1 g wet-weight.

The results suggest that cholinergic synapses may also be present in the ganglia of *Anodonta*. The low rate of decomposition and other factors indicate that they constitute only the smaller part of synapses in this animal and probably the central mechanisms are realized by chemical transmitters different from ACh.

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CHOLINESZTERÁZE-AKTIVITÁS *ANODONTA CYGNEA L.* KÖZPONTI IDEG RENDSZERÉBEN

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Összefoglalás

Anodonta cygnea (Pelecypoda) központi idegrendszerének cholineszteráze aktivitását vizsgáltuk biokémiai módszerrel.

A talált cholineszteráze aktivitás jellemzői:

1. A szubsztrát-koncentráció emelésével az ACh bontás nem mutat lineráis emelkedést
2. Legkifejezettebben az ACh-t bontja
3. Acetyl-β-methilcholint (MeCh) jól bontja
4. Benzoilcholint nem bontja
5. Eserin 10^{-6}, DFP 10^{-7} M koncentrációban 50%-os gátlást okoz
6. Optimuma pH 7-nél van, s az aktivitás pH 6 alatt, valamint 8 felett jelentősen csökken
7. 4%-os formaldehidben történt egy órás kezelés az aktivitást kb. 50%-kal csökkenti.

Fentiek alapján valószínű, hogy a cerebrális, viscerális és a pedális ganglionok homogenizátuma specifikus cholineszterázét tartalmaz. Az aktivitás mértéke pH 8-nál, 37° C-on, 4mM szubsztrát-koncentráció esetén 226 μg ACh/mg N/óra, ami 1 g nedves súlyra számítva kb. 1,8—2,2 mg ACh/órának felel meg.

Fentiek alapján feltehető, hogy *Anodonta* ganglionjaiban cholinerg szinapszisok is vannak. A bontás alacsony foka — más tényezőkkel együtt — arra utal, hogy ezek a szinapszisoknak csak kisebb részét képezik, s a központi ingerületáttevődés zömében az ACh-tól eltérő kémiai transzmitter-anyag részvételével valósul meg.

ХОЛИНЭСТЕРАЗНАЯ АКТИВНОСТЬ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ *ANODONTA CYGNEA L.*

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Исследовали холинэстеразную активность центральной нервной системы *Anodonta cygnea L. (Pelecypoda)* биохимическим методом. Характеристики обнаруженной холинэстеразной активности:

1. Отсутствует линейная зависимость между концентрацией субстрата и расщеплением ацетилхолина.
2. Из всех использованных субстратов наиболее выраженное расщепление проявлял ацетилхолин.
3. Ацетил-β-метилхолин расщепляется хорошо.
4. Бензоилхолин не расщепляется.
5. Эзерин 10^{-6} М и ДФП 10^{-7} М вызывают 50%-ное торможение активности фермента.
6. Оптимум активности при pH 7; ниже pH 6 и выше pH 8 активность значительно снижается.
7. Обработка 4% формальдегидом в течение часа снижает активность примерно наполовину.

На основе вышеизложенного представляется вероятным, что гомогенаты педального, висцерального и церебрального ганглиев содержат специфическую холинэстеразу. При pH 8, 37° С и концентрации субстрата 4 мМ активность соответствует 226 μg АХ /mg N/ час, что в пересчете на 1 г сырого веса равно примерно 1,8—2,2 мг АХ/час.

Полученные данные позволяют предполагать, что в ганглиях беззубки имеются и холинэргические синапсы. Низкий уровень расщепления ацетилхолина и другие факты указывают на то, что холинэргические синапсы составляют лишь небольшую часть синапсов и что в других случаях центральная передача возбуждения осуществляется каким-то другим медиатором, отличающимся от ацетилхолина.

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COMPARATIVE STUDY ON THE FATTY ACID COMPOSITION OF THE TISSUE LIPIDS IN THE FISH *CYPRINUS CARPIO L.*

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Numerous papers have been published on the fatty acid composition of fishes. Earlier reports were reviewed by HILDITCH (1956). More recently the exact chemical characterization of the fatty acids in fish (STOFFEL and AHRENS 1960), the metabolic pathways of their synthesis (MEAD et al. 1960) and the dietary factors influencing the fatty acid composition (KELLY et al. 1958) have been reported.

Many of these works were restricted to the study of the total lipids of a single organ or of the whole animal and did no throw light on the role of the different organs in the lipid metabolism of fish. In this communication we aimed at studying the fatty acid composition of both the total and different fractions of lipids from various organs hoping that our findings will form a step in the proper metabolic studies of lipids in fishes.

Material and methods

The fish studied was a two year old carp *(Cyprinus carpio L.)* of half kilogram weight, caught from the fish pond of Soponya (Hungary). The blood was collected in a heparinized tube by cutting the caudal vein. The plasma was separated. Plasma lipids were extracted with BLOOR'S mixture (ethanol-ether 3 : 1). The fish was then dissected and 1 gr liver, muscle and intestinal adipose tissue were excized, homogenized and extracted immediately with chloroform-methanol 2 : 1 after FOLCH. The extracts were divided into two portions. One portion was evaporated representing the total lipids. The other portion was fractionated by thin layer chromatography on silica gel. The developing mixture consisted of heptane — diethylether-acetic acid (40 : 10 : 0.5). The total lipids and the different lipid fractions without being eluted from the silica gel were interesterified by a rapid method as described by SZŐKE et al. (1965).

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Table 1.

The fatty acid composition of total lipids of the various tissues of the fish

	14 : 0	14 : 1	16 : 0	16 : 1	17 : 0	18 : 0
Plasma — Plazma	2.2	0.6	26.4	12.5	0.5	4.1
Adipose tissue — Zsírszövet	2.8	tr	19.0	11.5	tr	5.3
Liver — Máj	1.1	0.2	22.0	14.0	tr	4.6
Muscle — Izom	0.7	0.5	14.6	9.4	tr	4.8

Table 2.

The fatty acid composition of the cholesterol ester fraction of the various tissues of the fish

	10 : 0	12 : 0	14 : 0	14 : 1	16 : 0	16 : 1	17 : 0
Plasma — Plazma	7.0	tr	7.6	0.5	14.2	9.5	tr
Adipose tissue — Zsírszövet	tr	tr	0.5	0.3	17.6	8.7	1.6
Liver — Máj	tr	tr	0.4	0.5	15.0	6.4	0.9
Muscle — Izom	0.3	0.6	0.3	tr	13.3	10.7	1.3

Table 3.

The fatty acid composition of the triglyceride fractions of the various tissues of the fish

	14 : 0	14 : 1	16 : 0	16 : 1	17 : 0	18 : 0
Plasma — Plazma	2.2	0.7	21.9	14.7	1.4	3.2
Adipose tissue — Zsírszövet	1.9	0.3	19.9	10.4	tr	4.2
Liver — Máj	0.6	0.2	17.4	12.7	tr	2.2
Muscle — Izom	0.7	0.2	14.1	13.0	tr	3.9

The methyl esters were then analysed by an Aerograph GLC apparatus Type 90, using hydrogen as a carrier gas on a column packed with diethylene glycol-succinate coated on Chromosorb W, 30—60 mesh. The column temperature was 180° C. The flow rate was 60 ml/min. The fatty acid distribution was determined by measuring the relative areas under the curves. For the identification of the peaks the National Heart Institute fatty acid standards were used. The data in the Tables represent the mean values of three analyses.

1. táblázat

Az összzsiradék zsírsav összetétele a hal különböző szöveteiben

18 : 1	18 : 2	18 : 3	20 : 0	20 : 2	20 : 3	20 : 4	20 : 5	22 : 6
33.6	5.6	1.4	2.6	0.8	1.1	2.1	4.7	1.8
47.1	11.5	tr	tr	tr	2.8	tr	tr	tr
42.0	7.1	0.3	4.1	0.5	tr	1.8	1.4	0.9
45.5	10.8	0.5	1.9	tr	0.7	8.7		1.9

2. táblázat

A koleszterinészter frakció zsírsav összetétele a hal különböző szöveteiben

18 : 0	18 : 1	18 : 2	18 : 3	20 : 0	20 : 2	20 : 3	20 : 4	20 : 5	22 : 6
3.5	36.4	6.7	3.1	6.2	tr	tr	3.1	2.2	tr
6.5	54.4	6.9	1.6	1.1	tr	tr	0.8	tr	tr
3.2	54.5	9.1	2.3	6.8	0.9	tr	tr	tr	tr
6.3	50.5	9.5	1.6	4.4	tr	1.2	tr	tr	tr

3. táblázat

A triglicerid frakció zsírsav összetétele a hal különböző szöveteiben

18 : 1	18 : 2	18 : 3	20 : 0	20 : 2	20 : 3	20 : 4	20 : 5	22 : 6
38.5	7.1	2.7	3.6	tr	tr	2.2	1.7	0.3
50.7	8.1	0.6	2.2	tr	1.7	tr	tr	tr
50.5	12.0	tr	4.4	tr	tr	tr	tr	tr
52.0	12.1	1.2	1.2	tr	0.7	0.9	tr	tr

Results and discussion

Fishes, as compared to land animals, are characterized by high percentages of palmitoleic and C_{20}, C_{22} fatty acids. From *Table 1* it is evident that the total lipids of all of the tissues examined show these characteristics.

However the C_{20}, C_{22} polyenoic acid levels were lower in this carp than in fishes in general. This can be explained by the fact, that in the pond where

Table 4.

The fatty acid composition of the diglyceride fraction of the various tissues of the fish

	14 : 0	14 : 1	16 : 0	16 : 1	17 : 0	18 : 0
Plasma — Plazma	tr	tr	26.0	10.4	tr	23.5
Adipose tissue — Zsírszövet	1.2	0.2	17.5	10.3	tr	6.1
Liver — Máj	0.9	0.2	30.1	8.5	0.7	5.0
Muscle — Izom	0.5	tr	18.7	7.0	3.1	12.4

Table 5.

The fatty acid composition of the phospholipid fraction of the various tissues of the fish

	14 : 0	14 : 1	16 : 0	16 : 1	17 : 0	18 : 0
Plasma — Plazma	0.8	0.4	38.5	10.3	0.4	5.4
Adipose tissue — Zsírszövet	0.9	0.2	18.0	12.8	0.4	7.8
Liver — Máj	0.5	0.3	28.3	9.3	tr	11.7
Muscle — Izom	0.5	0.5	25.2	6.7	0.2	6.0

our fish was caught the crustacean plankton does not contribute much to the diet of fishes. Such plankton has been claimed to be the main source for the C_{20}, C_{22} polyenoic acids in fish (FARKAS and HERODEK 1964.)

On the other hand, we observed a high level of palmitoleic acid in all tissues examined, indicating that this fatty acid in fish lipid is mainly of endogenous origin, i. e. the conversion of palmitic to palmitoleic acid is very intensive in the fish.

In the muscle lipids there were less palmitic somewhat less palmitoleic and stearic but more linoleic, arachidonic and docosahexaenoic acids. Perhaps this drop in palmitic and stearic acids may be explained as a preferential utilisation of these acids by the muscle.

The plasma lipids had a low content of oleic acid and were rich in all polyenoic acids.

The differences existing between the different tissues are due in part to the disproportionality of the different lipid-franctions in the tissues examined and in part due to differences within the individual fractions. *Tables 2—5* compare the fatty acid composition of the different fractions from various organs.

In human (LINDGREN et al. 1961), and rat (GÖRANSON and OLIVECRONA 1964) sera the linoleic and arachidonic acids respectively constitute about half of the fatty acid components of cholesterol esters. This finding could lead

4. táblázat

A diglicerid frakció zsírsav összetétele a hal különböző szöveteiben

18 : 1	18 : 2	18 : 3	20 : 0	20 : 2	20 : 3	20 : 4	20 : 5	22 : 6
32.3	5.2	tr	2.6	tr	tr	tr	tr	tr
53.1	7.8	tr	3.4	tr	0.4	tr	tr	tr
41.2	4.6	0.9	3.9	1.1	tr	2.5	0.4	tr
42.0	6.2	2.3	2.3	tr	1.6	3.9	tr	tr

5. táblázat

A foszfolipid frakció zsírsav összetétele a hal különböző szöveteiben

18 : 1	18 : 2	18 : 3	20 : 0	20 : 2	20 : 3	20 : 4	20 : 5	22 : 6
25.0	4.7	1.1	1.8	tr	2.3	1.5	5.1	2.7
48.0	6.3	tr	1.5	tr	2 1	2.0	tr	tr
28.5	4.7	0.7	4.7	1.9	tr	9.4	tr	tr
31.8	8.9	0.2	3.7	tr	4.2	8.9	3.2	tr

to some hypothesis on the role of these acids in cholesterol metabolism. According to *Table 2* neither of these fatty acids are present in significant amounts in the cholesterol esters of our fish. The fatty acid pattern of the cholesterol ester franction of liver, muscle and adipose tissue is almost the same, and differs considerably from that of the plasma. The latter is characterized by a high percentage of $C_{10:0}$, $C_{14:0}$ and the polyenoic acids, while the oleic acid content is less than that in other tissues.

Concerning the triglyceride fraction *(Table 3)* we found no important differences between the various tissues. The only one to be mentioned is the lower oleic and higher polyenoic acid content of the plasma.

The fatty acids of diglyceride fractions *(Table 4)* showed great similarity in all tissues with the exception of the unexpected high level of stearic acid in both plasma and muscle diglycerides.

The fatty acid composition of the phospholipids are shown in *Table 5*. They differ significantly from those of the neutral lipids in that they contain higher saturated and polyenoic acid levels, and a lower monoenoic acid level. This can be markedly seen in *Fig. 1.*

The fatty acid composition of the liver and plasma phospholipids are similar. This points to the liver as the origin of plasma phospholipids. On the other hand the adipose tissue phospholipids show a picture which is very similar to that of neutral lipids, indicating that the phospholipids of the adipose

tissue are synthesized in situ. The pattern of muscle phospholipid franction lies in between that of liver and plasma and adipose tissue. From *Fig. 2* it is evident that the neutral fraction contained much less stearic and palmitic

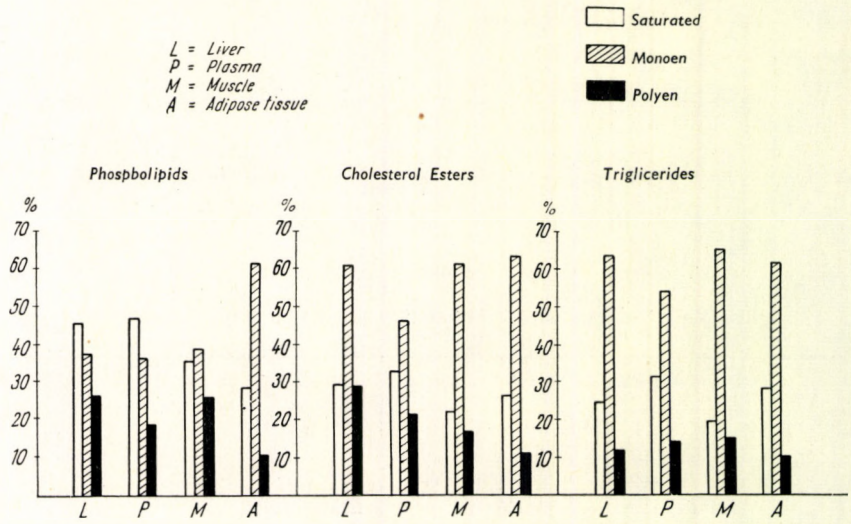

Fig. 1. The percentage of saturated, monoenoic and polyenoic fatty acids in the different tissues and fractions

1. ábra A telített, egyszer és többszörösen telítetlen zsírsavak százaléka a különböző szövetekben és frakciókban.

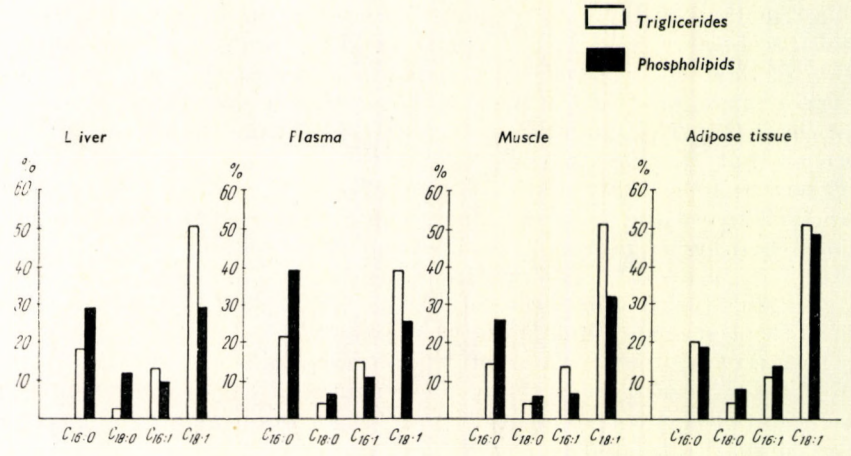

Fig. 2. The percentage of palmitic, stearic and the corresponding monoenoic acids in the triglyceride and phospholipid fractions of the different tissues

2. ábra A palmitin, sztearin és a megfelelő egyszer telítetlen zsírsavak százaléka a különböző szövetek trigliceridjeiben és foszfolipidjeiben.

acids than did the phospholipids. On the other hand the palmitoleic and oleic acid contents are higher in the neutral fractions than in the phospholipids. This picture clearly demonstrates a preferential incorporation of saturated fatty acids — palmitic, stearic — into the phospholipids and incorporation of the corresponding monoenoic acids — palmitoleic, oleic — into the neutral lipids. This relation is manifested in all tissues investigated except the adipose tissue.

Similar observations were reported recently for the stearic- oleic acid distribution in rats (GÖRANSSON and OLIVECRONA 1964).

Acknowledgement:

We wish to express thanks to Prof. R. TARJÁN, Dr. K. LINDNER, Dr. M. KRÁMER and Dr. K. SZŐKE for their interest in this work and for their kind advice.

Summary

The lipids of liver, plasma, muscle and adipose tissue were separated by thin layer chromatography. The fatty acid compositions of the cholesterol esters, triglyceride, diglyceride and phospholipid fractions were determined by gasliquid chromatography.

In our fish the percentage of C_{20},C_{22} polyenoic acids was lower than in fishes in general due to the lack of planktonic crustaceans as food constituents. The concentration of palmitoleic acid was high in the lipid franctions of all tissues.

The plasma cholesterol esters in the carp, in contrast to mammals, contain no significant amounts of linoleic or arachidonic acids. The concentrations of palmitic and stearic acid were higher in the phospholipids than in the triglicerides while with the corresponding monoenoic acids there was an inverse relationship.

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ÖSSZEHASONLÍTÓ VIZSGÁLATOK A PONTY *(CYPRINUS CARPIO L.)* KÜLÖNBÖZŐ SZÖVETEIBEN TALÁLHATÓ LIPIDFRAKCIÓK ZSÍRSAV-ÖSSZETÉTELÉN

A. Abdel Hay és Herodek Sándor

Összefoglalás

Egy ponty májának, vérplazmájának, izmának és zsírszövetének zsiradékait rétegkromatográfia segítségével szétválasztottuk. A koleszterin észter, triglicerid, diglicerid és foszfolipid frakciók zsírsavösszetételét gázkromatográfia segítségével meghatároztuk. A vizsgált halban a C_{20}, C_{22} többszörösen telítetlen zsírsavak százalékos mennyisége alacsonyabb volt, mint a halakban általában, amit az okoz, hogy jelen esetben a Crustacea plankton nem szerepel jelentős mértékben a táplálékban. A halakra jellemző palmitoleinsav viszont nagy koncentrációban volt jelen minden vizsgált szövet összes lipid frakciójában. Az emlősökkel szemben ebben a pontyban a plazma koleszterinészterek nem tartalmaztak jelentősebb mennyiségű linol vagy arachidonsavat. A palmitin és sztearinsav koncetnrációja magasabb volt a foszfolipidekben, mint a trigliceridekben, míg a megfelelő egyszer telítetlen zsírsavak fordított viszonyt mutattak.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ СОСТАВА ЖИРНЫХ КИСЛОТ ЛИПИДНЫХ ФРАКЦИЙ РАЗЛИЧНЫХ ТКАНЕЙ КАРПА

А. Абдель-Хай и Шандор Херодек

С помощью тонкослойной хроматографии фракционировали липиды жировой ткани, мышцы, сыворотки крови и печени карпа. Определяли посредством газовой хроматографии состав жирных кислот следующих фракций: эфиры холестерина, триглицериды, диглицериды и фосфолициды. У изученного вида процентное содержание насыщенных жирных кислот (C_{20} и C_{22}) ниже в сравнении с другими рыбами, это объясняется отсутствием планктонных ракообразных в пище карпа. Пальмитиновая кислота, характерная для рыб, в большом количестве присутствует во всех липидных фракциях всех исследованных тканей. В отличие от млекопитающих, эфиры холестерина плазмы карпа не содержат значительных количеств линолевой и арахидоновой кислот. Концентрация пальмитиновой и стеариновой кислот была выше в фосфолипидах, чем в триглицеридах, а насыщенные жирные кислоты проявляли обратную зависимость.

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EFFECT OF NOREPINEPHRINE ON ADIPOSE TISSUE LIPSAE AND PHOSPHORYLASE ACTIVITY OF THE FROG *RANA ESCULENTA L.*

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Catecholamines have different effects on the adipose tissue of mammals. Beside their effect on the uptake of glucose in the adipose tissue (CAHILL et al. 1960) they stimulate the phosphorylase enzyme and through this the decomposition of the glycogen stored in the adipose tissue (VAUGHAN 1960, FRERICHS et al. 1962). Their other important effect is to stimulate the hydrolysis of the neutral triglycerides into fatty acids and glycerine (WHITE et al. 1958). The catecholamines exercise this effect through the "hormon sesitive" lipase (RIZACK, 1960), which in several respects differs from the triglyceride lipase present in the adipose tissue (RUBINSTEIN et al. 1964).

In examinations conducted on a fresh water fish, *Cyprinus carpio L.* it was demonstrated that in the adipose tissue of fish — similary to that of mammals — intesive lipolysis takes place. Several lipolytic hormones very effective in mammals (catecholamines and adreno-corticotroph hormone) have, however, no effect on the extent of the hydrolysis of glycerides under in vivo or in vitro conditions. (FARKAS, in press). It was concluded from these results that the hormone sensitive lipase is absent in the adipose tissue of fish and it appears only later in the phylogeny.

For further elucidation of the problem investigations were started on other lower vertebrate organisms. The present paper describes the effect of norepinephrine on the lipase and phosphorylase activity of the adipose tissue of the frog, *Rana esculenta L.*

Material and method

Adult male frogs *(Rana esculenta L.)* weighing 50—80 g were used for the experiments. The frogs were purchased from the official firm MAVAD and kept in aquarium containing a few cm of water. The animals were used up within a week from the purchase. Food was denied from the animals during that time.

The frogs were brought into the laboratory the evening before the experiment, to adapt them to the experimental temperature. Norepinephrine diluted in physiological saline (0.48 per cent NaCl) was injected into the ventral

lymph sac. The animals were killed by decapitation two hours after the injection and the blood was collected in prechilled heparinized tubes. The adipose tissues were rapidly removed and placed into physiological saline.

In vitro experiments.

The finger-like branching parts of the adipose tissues were cut into 100—150 mg portions. From one piece the free fatty acid content of the adipose tissue was determined while the other parts were incubated in 3 ml physiological solution in the presence or absence of norepinephrine (2 μg/ml) for 60 minutes at room temperature. The composition of the physiological solution was the following: NaCl:0.083 M, $NaHCo_3$:0.025 M, KCl:0.002 M, KH_2PO_4:0.012 M, CaCl:0.025 M, $MgCl_2$:0.003 M. (FENN, 1936). The pH of the solution was adjusted to 7.4. After the incubation period the tissues were removed from the medium, blotted gently and the free fatty acid content or the phosphorylase activity were determined.

Analytical procedures

The free fatty acid level of the blood plasma was determined according to DOLE (DOLE, 1956) from 0.5 ml blood plasma. The blood sugar was measured colorimetrically according to HYVÄRINEN (HYVÄRINEN et al. 1962). For determination of free fatty acid content of the adipose tissue the tissues were homogenized in POTTER homogenizator in the presence of n-heptane. The homogenizate was filled up to 5 ml. 1 ml of aliquots were titrated with 0.01 N NaOH in the presence of bromthymol blue indicator. The results are given in μM free fatty acid/g adipose tissue.

For the assaying the phosphorylase activity the SUTHERLAND'S method was chosen (SUTHERLAND 1955). The adipose tissues were homogenized in the presence of 0.1 M NaF. The end volume of the homogenizate was 2.5 ml. Then the homogenizate was centrifuged at 4°C for 15 minutes at 1500 r. p. m. and the separated lipoid layer removed. 0.5 ml of the supernatant was incubated in the presence of 4.0 mg glycogen, 31.5 μmoles glucose — 1 — phosphate, 1.4 μmoles 5 — adenosine monophosphate and 85 μmoles NaF for 60 minutes at pH 6.3 and room temperature. End volume of the reaction mixture was 1.3 ml. The reaction was stopped in the 0 and 60 minute by the addition of 0.25 ml 0.3 M $HClO_4$. The precipitated proteins were removed with centrifuging for 15 minutes at 6000 r. p. m. Phosphorylase was measured into the direction of glycogen synthesis. The glycogen was determined according to VAN der VIES (1954). Incubations and glycogen measurements were conducted in 3 parallels.

Results

The adipose tissue of the frog contains a substantial amount of free fatty acid. The amount of the free fatty acid in frog adipose tissue seems to be higher than in mammals. Free fatty acid contents of the right and left side adipose tissue are different. From determinations made on 5 animals the following mean values were obtained: right side adipose tissue: 3.969 ± 0.52 μM/g, left side adipose tissue: 2.648 ± 0.26 μM/g. The difference is significant ($p < 0.05$).

For the experiments the left side adipose tissues were used which were larger than the pair on the opposite side.

Fig. 1. shows that in the adipose tissue an intensive lipolysis takes place under in vitro conditions. The fatty acids produced accumulate in the adipose tissues. The accumulation is linear with time in the first 90—120 minutes.

Effect of norepinephrine on the free fatty acid production of the adipose tissue.

Norepinephrine significantly increases the intensity of the lipolytic processes in the adipose tissue of mammals under in vivo or in vitro conditions. To study its effect on the fatty acid production of the adipose tissue of the frog 2 series of experiments were conducted. In the first series of experiments the hormone was administered in vivo (500 μg/animal) and both the plasma and adipose tissue free fatty acid levels were determined. The adipose tissues of the animals treated with norepinephrine in vivo were incubated in vitro to establish whether the adipose tissue of the treated animals produces more free fatty acid than that of the control animals. In the other esxperimental series the adipose tissue of untreated animals were incubated in vitro in the presence or absence of norepinephrine (2 μg/ml incubation medium) and the free fatty acid production of the adipose tissues was determined.

Fig. 2. shows that the in vivo administration of norepinephrine did not lead to the increase of the free fatty acid level of the blood plasma. In fish, after the administration of the hormone both the plasma free fatty acid level and the adipose tissue free fatty acid content were significantly decreased. These effects of the hormone could not be observed in frogs. It is evident from both the increased blood glucose levels and from its effect on the chromatophores that the hormone entered the blood circulatory system. 20—25 minutes after the injection of norepinephrine the animals have lost their brownish green colour and became pale green. This effect of the hormone subsisted for hours.

From *fig. 2.* it appears also that norepinephrine under in vitro conditions did not stimulate the production of the free fatty acids in the frog adipose tissue. The presence of the hormone leads to a decreased free fatty acid production, the difference is however not significant as compared with the control. The adipose tissue of the animals treated with norepinephrine in vivo does not produce significantly more free fatty acid than that of the untreated animals. These experiments suggest that norepinephrine one of the most effective lipid mobilizing hormone in the mammals is inactiv in stimulating the breakdown of triglycerides in the adipose tissue in frogs.

The effect of norepinephrine on phosphorylase acivity.

Fig. 3. demonstrates the presence of phosphorylase acivity in the adipose tissue of frog. The hormones stimulating the breakdown of triglycerides in mammalian adipose tissue increase also the activity of the adipose tissue phosphorylase. Since norepinephrine has no effect on the lipolytic activity of the adipose tissue of frog, it seemed interesting to examine whether the phosphorylase of the adipose tissue is sensitive to the hormone.

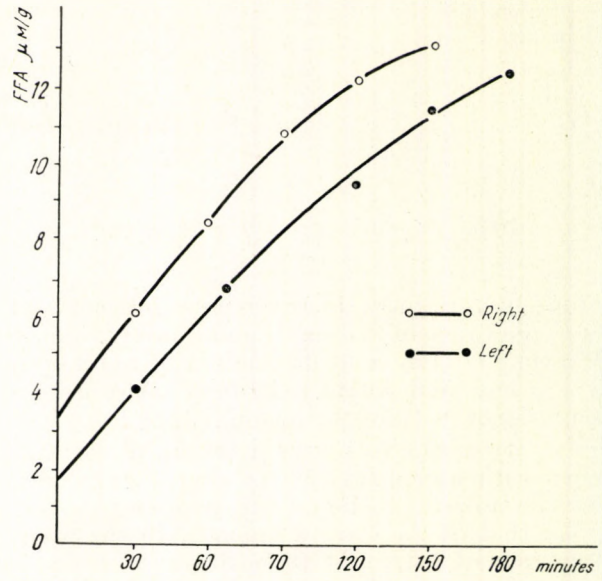

Fig. 1. Free fatty acid production in the adipose tissue of frog. The points are the mean values of measurement obtained from 3 animals

1. ábra Szabadzsírsav termelés kecskebéka zsírszövetében. A pontok 3 állatból kapott mérési eredmények átlagértékei.

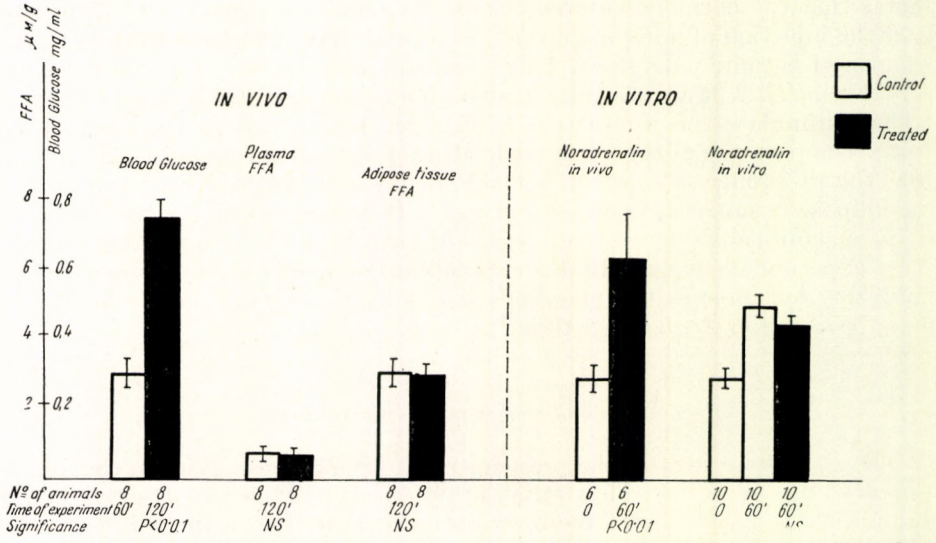

Fig. 2. The effect of noradrenaline on plasma free fatty acid level and on the free fatty acid production of the adipose tissue

2. ábra Noradrenalin hatása vérplazma szabad zsírsav szintjére és a zsírszövet szabad zsírsav termelésére.

To decide this question two experiments were conducted. In the first one norepinephrine (500 μg/animal) was administered in vivo 2 hours before the death of the animals. The adipose tissues were rapidly removed and their phosphorylase activity was compared with that of the adipose tissue of the

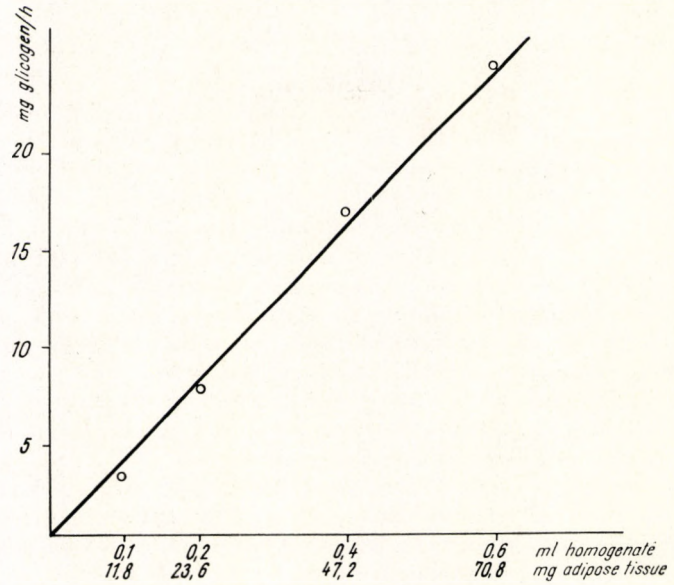

Fig. 3. Phosphorylase activity of the frog adipose tissue. The homogenizate was prepared from the right side adipose tissue of 3 frogs. Every point is the mean result of 6 measurements

3. ábra Béka zsírszövet foszforilase aktivitása. A homogenizátumot 3 béka jobb oldali zsírszövetéből készítettük. Minden pont 6 mérés átlageredménye.

untreated animals. In the second experiment the adipose tissue of untreated animals was incubated both in the presence or absence of norepinephrine (2 μg/ml incubation medium) and their phosphorylase activity has been determined.

Table 1.

The effect of noradrenaline on the phosphorylase activity of frog adipose tissue

1. táblázat

Noradrenalin hatása béka-zsírszövet foszforilase aktivitására

In vivo			In vitro		
Control	Noradrenalin	Note	Control	Noradrenalin	Note
glycogen mg/g/h	glycogen mg/g/h	Megjegyzés	glycogen/mg/g/h	glycogen mg/g/h	Megjegyzés
21.10	117.5		14.62 ± 0.25	26.24 ± 0.15	$P < 0.01$
16.15	79.0		51.94 ± 0.24	73.90 ± 0.40	$P < 0.01$
48.25	143.0		1.98 ± 0.35	8.11 ± 0.41	$P < 0.01$
18.15	92.5		4.41 ± 0.26	8.20 ± 0.14	$P < 0.01$
22.50	83.5		42.81 ± 0.30	64.59 ± 0.25	$P < 0.01$
Átlag 26.3	103.1	$P < 0.01$			

Table 1. demonstrates that after in vivo or in vitro administration of norepinephrine the phosphorylase activity is increased. This observation indicates that the hormone under the experimental conditions employed reached the adipose cells and proves that the lipolytic system of the adipose tissue of frogs is insensible to norepinephrine.

Discussion

The mammals mobilize the triglycerides stored in their fat depot in the form of free fatty acids which entering the blood circulatory system bound to protein are transported to the site of their utilization. The mobilization of fats is under a complicated metabolic, endocrine and nervous control. The facts that in both fish and amphibians an intensive lipolysis takes place in the adipose tissue and their blood contains also a considerable amount of free fatty acids suggest that the mobilization of triglycerides in the form of free fatty acids is general in vertebrates. It is also common in all groups of vertebrates that the plasma free fatty acid level is controlled by the carbohydrate metabolism of the adipose cells. Peroral administration of glucose to carp (FARKAS, in press) and to frog (study in progress) has the same effect on the plasma free fatty acid level as in mammals.

Catecholamines and probably all other adipokinetic hormones exercise their effect on the free fatty acid production of the mammalian adipose tissue through the stimulation of the "hormone-sensitive" lipase described by RIZACK (RIZACK 1964). It seems very probable that the hormones regulate the breakdown both of glycogen and triglycerides in the adipose tissue through the same mediator substance. KLAINER (1962) demonstrated that epinephrine enhances the tissue level of cyclic 3',5'-adenosine monophosphate which through a mechanism already known increases the phosphorylase activity of various tissues including the adipose tissue. It has been also demonstrated that cyclic 3',5'- adenosine monophosphate increases the free fatty acid production of the rat adipose tissue homogenizates (RIZACK 1964). BUTCHER (1965) found that the amount of this nucleotide in the adipose cells between certain limits is proportional to the lipolytic activity of the adipose tissue and that the inhibition of its formation influences the free fatty acid production.

The formation of the cyclic 3', 5'-adenosine monophosphate under the influence of various hormones was described in several animal species (SUTHERLAND 1962) and the presence of this nucleotide has been demonstrated in many animals. There is no doubt that its norepinephrine stimulated formation was involved also in the increase of the phosphorylase activity of the frog adipose tissue. On the other hand the fact that norepinephrine in frogs, norepinephrine and other catecholamines in fish were ineffective on the lipolytic activity of the adipose tissue, supports our earlier assumption i. e. the "hormone sensitive" lipase is absent from the adipose tissue of lower vertebrates.

The hormone-sensitive lipotlytic system has an importance in mammals in "emergency conditions" as with its aid it is possible to mobilize a great amount of energy within a short time. The absence of the "hormone-sensitive" lipase from the adipose tissue of lower vertebrates points to the fact that this form of gaining energy appears only later in the vertebrate phylogeny. Our present investigations do not exclude the possibility that in emergency condi-

tions the energy stored in the adipose tissue of lower vertebrates could be mobilized in some other forms than free fatty acids but they also raise the possibility that in such circumstances these organisms may mobilize other compounds (carbohydrates). The latter assumption may be supported by the observation that — as far as it can be concluded from the hyperglycemic effect of norepinephrine — the hepatic phosphorylase of fish and amphibans seems to be more sensitive to norepinephrine than that of mammals.

Summary

Norepinephrine administered in vivo (500 μg/animal) increased the blood sugar level in adult male frogs, but it had no demonstrable effect on the free fatty acid level of the plasma. The adipose tissue of frogs treated with norepinephrine in vivo did not contain more free fatty acid than that of the untreated animals and such adipose tissues did not produce more free fatty acid in vitro. Norepinephrine in vitro (2μg/ml incubation medium) did not increase the lipolytic activity of the adipose tissue of the frog. The in vivo or in vitro administration of the hormone increased significantly the phosphorylase activity of the adipose tissue. The results seem to indicate that the "hormone-sensitive" lipase is absent in the frog adipose tissue.

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NORADRENALIN HATÁSA KECSKEBÉKA *(RANA ESCULENTA L.)* ZSÍRSZÖVETÉNEK LIPASE ÉS FOSZFORILASE AKTIVITÁSÁRA

Farkas Tibor

Összefoglalás

In vivo adagolt noradrenalin erősen megemelte a kecskebéka *(Rana esuclenta L.)* vércukorszintjét, de nem volt kimutatható hatással a plasma szabad zsírsav szintjére. Noradrenalinnal in vivo kezelt békák zsírszövete nem tartalmazott több szabad zsírsavat, mint a kezeletlen állatoké, és az ilyen zsírszövetek in vitro sem termeltek több szabad zsírsavat. Noradrenalin in vitro sem növelte kecskebéka zsírszövetének lipolytikus aktivitását. In vivo, vagy in vitro adagolása után lényegesen megnövekedett a zsírszövet foszforilase aktivitása. Az eredmények arra utalnak, hogy a béka zsírszövetéből hiányzik a ,,hormon- érzékeny'' lipase.

ВЛИЯНИЕ НОРАДРЕНАЛИНА НА АКТИВНОСТЬ ФОСФОРИЛАЗЫ И ЛИПАЗЫ ЖИРОВОЙ ТКАНИ *RANA ESCULANTA L.*

Тибор Фаркаш

Норадреналин *in vivo* значительно повышает уровень сахара в крови лягущки, но не влияет на уровень свободных жирных кислот в сыворотке. Жировая ткань лягушки, получившей *in vitro* норадреналин, не содержит больше свободных жирных кислот, чем в контроле. Нет повышения уровня жирных кислот и при обработке такой ткани *in vitro*. Норадреналин *in vitro* не увеличивает липолитической активности жировой ткани. После дачи норадреналина *in vivo* или *in vitro* значительно увеличивается фосфорилазная активность жировой ткани. Полученные данные указывают на отсутствие гормончувствительной липазы в жировой ткани лягушки.

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COMPARISON OF THE TRIGLYCERIDE SYNTHESIS OF CARP AND RAT IN ADIPOSE TISSUE AND LIVER SLICES INCUBATED WITH 1—^{14}C PALMITIC ACID

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A great progress can be stated in the studies on the metabolism of adipose tissue in the last few years. A detailed review on the subject was supplied by VAUGHAN (1961). The same problem was discussed by 2 Conferences, the matter of both being published under the titles: "Adipose tissue as an organ" (KINSELL ed. 1962) and "Fat as a tissue" (RODAHL ed., 1964) resp. In contrast to such extensive studies on the metabolism of the adipose tissue of homoiotherm animals we are in the knowledge only of a single paper dealing with the metabolism of the adipose tissue of fishes. This work was carried out by T. FARKAS (1966) in the same laboratory where the present investigations took place. FARKAS investigated the release of free fatty acids from the adipose tissue into the medium. The subject of our study is the opposite process — the incorporation of the fatty acids into the adipose tissue and liver of the fish. For the sake of comparison the same investigations were carried out also with rats.

Material and methods

The carps were males of 0.5 kg weight, originating from the fish ponds of Balatonlelle. For 1 day before the experiments they were kept in aquarium. The rats were Wistar males weighing 150—200 gr and were fed ad libitium on their standard diet.

The animals were killed by a blow on their head. Their liver and a piece of adipose tissue were separated. The adipose tissue was represented in fish by the intestinal adipose tissue, in the rat by the epididymal fat pad. The liver of both animals was cut into cca 1 mm thick slices and 1 g of them was incubated in 10 ml medium. 150 mg of adipose tissue was incubated in 5 ml medium.

Each incubation lasted for 10 min by gentle shaking. The tissues of fish and rat were incubated at 20° C and 37° C resp. The incubation medium for the rat tissues consisted of a KREBS-RINGER phosphate buffer (pH 7. 4, Ca^{2-} omitted) which was 5 mM for glucose and contained 5% human serum albumin. The medium for fish differed only so far as instead of 0.9% it contained only 0.48% NaCl.

The specific activity of the 1—^{14}C palmitic acid (Reanal, Hungary) was 1 mC/mmol. Its concentration in the medium was 2 μC/ml. The labeled palmitic

acid was complexed to albumin. For this purpose the fatty acid was neutralized with 0.1 N NaOH and warmed up and mixed thoroughly with the preheated albumin solution.

After incubation the tissue slices were rinsed quickly with 0.48% NaCl and 0.9% NaCl solution for carp and rat resp., in order to remove the adherent fatty acid activity. The tissues were homogenized in a glass POTTER with chloroform-methanol 2 : 1, and extracted with 25 ml of the same mixture. An aliquot of the filtrate was evaporated under CO_2 and dissolved in chloroform. This solution then was applied on thin layer plates. 1 mg/ml diglyceride and 1 mg/ml inactive palmitic acid were also added to the chloroform to enable on the chromatograms the detection of these compounds present in the tissues only in very small quantities.

The thin-layer chromatography was carried out on 0.3 mm thick silica gel plates with the developing solvent mixture of petroleum ether (b. r. 40—70°-C)-diethyl ether — acetic acid 70 : 30 : 01. Detection by spraying with 0.2% Rhodamin B in ethanol. The location of the bonds was marked under UV light, and they were separately scraped off. This way the lipid extract was separated into the following fractions: cholesterol esters, triglycerides, free fatty acids, diglycerides, monoglycerides and phospholipids.

The lipids were eluted from the silica gel by 2 hour's extraction with diethylether in a microsoxhlet apparatus. The radioactivity measurements were carried out by a Tri-Carb liquid scintillation spectrometer. The scintillation liquid consisted of 0.3% PPO and 0.04% POPOP in toluene.

Results and discussion

The amounts of fatty acids incorporated from the medium into the three investigated lipid classes in the two different tissues of the two species are given in *Table 1.*

Of course the in vivo fatty acid uptake of these tissues can not be calculated from the data. The absolute intensity of the triglyceride synthesis is neither calculable because there are different fatty acid pools within the tissue and the specific activity of the triglyceride synthesis pool is unknown. There is no basis to compare the data of the adipose tissue and those of the liver.

Table 1

The incorporation of 1 —^{14} C palmitic acid into the various lipid fractions of the adipose tissue and liver of rat and carp

	Adipose tissue		Liver	
	rat	carp	rat	carp
Triglycerides	5317 ± 319	93 ± 7	522 ± 63	151 ± 47
Diglycerides	1527 ± 135	25 ± 2	429 ± 53	163 ± 6
Free fatty acids	728 ± 42	2147 ± 623	1065 ± 287	1145 ± 87

The values are given in 10^{-10} mol fatty acid/g wet tissue. Each value is the average of 3 animals ± standard error of the mean.

1. Táblázat

Az 1—^{14}C palmitinsav beépülése a patkány és a ponty zsírszövetének és májának zsiradékaiba

	Zsírszövet		Máj	
	patkány	ponty	patkány	ponty
Triglicerid	5317 ± 319	93 ± 7	522 ± 63	151 ± 47
Diglicerid	1527 ± 135	25 ± 2	429 ± 53	163 ± 6
Szabad zsírsav	728 ± 42	2147 ± 623	1065 ± 287	1145 ± 87

A táblázat adatai 10^{-10} mol zsírsav/g élő szövetet jelentenek. Minden érték három állat átlaga, ± az átlag középhibája.

A semiquantitative comaprison of the 2 identical tissues of the two species seemed reasonable.

The triglycerides show a measurable radioactivity in all the four tissues, there is a triglyceride synthesis in all of them.

The diglycerides show also a radioactivity in all the four tissues, and in the same order of magnitude as that of the triglycerides. More detailed the two adipose tissues are similar, the diglycerides showing one fourth of the activity of triglycerides, whereas in the livers of both species the activity of diglycerides equals that of triglycerides. Regarding the distribution of the labeled fatty acids between the diglyceride and triglyceride fraction, the carp and rat showed no differences. We studied the behaviour of the radioactive diglycerides in the adipose tissue of rat in another work (HERODEK 1966) in details, where the adipose tissue first incubated with labeled palmitic acid as above was transferred in an inactive medium for a second 10 min incubation. During this incubation in inactive medium no significant decrease of the radioactivity of the diglycerides took place, indicating, that there must be at least two different triglyceride pools within the fat cells, — in one the diglycerides are rapidly transformed into triglycerides, in the other the diglycerides have a much lower turnover rate. Our present results, showing a high diglyceride/triglyceride activity ratio in both tissues of both animals, suggest the existence of diglycerides with a lower turnover rate to be a general phenomenon of the triglyceride synthesis. In this respect the time curve of the radioactive diglycerides in more tissues of more species seems to be worthy of study.

For free fatty acids it seems to be a regularity, that the more the labeled fatty acid is incorporated by the tissue, the less is the radioactivity of the free fatty acids of the tissue. The entrance of the fatty acids into the tissue is a physico-chemical process, its intensity is regulated by the concentration gradient.

Owing to the general lack of knowledge on the lipid metabolism of fishes, we are restricted in the interpretation of the intesity of the incorporation of the fatty acids into the triglycerides only to different hypothesis.

The triglycerides of the fish liver contained only one third of the radioactivity of the rat liver triglycerides. Here not so much this difference but the identical order of magnitude is perhaps to be emphasized. On the other hand in the radioactivity of the adipose tissue the carp is surpassed about fifty times by the rat, indicating some kind of basic difference between the adipose tissues of the two species. As mentioned above the mammalian tissues

were at 37° C, those of the fish at 20° C incubated as 37° C could scarcely represent a physiological temperature for a carp. The experiments of FARKAS (1966) were carried out also at 20° C, and under this condition the adipose tissue of the carp released even more free fatty acids than the rat adipose tissue at 37° C. And just as seen above also the two livers took up the fatty acid — inspite of the temperature difference — in the same order of magnitude. There is no reason to put the medium under suspect, as FARKAS (1966) applied the same in his experiments. Still to test the most physiological medium the adipose tissues of three carps were incubated in their own plasma with the addition similar to the synthetic medium of glucose and labeled fatty acid. Also this incubation did not result in more significant incorporation into the triglycerides. 92% of the activity uptake remained in the form of free fatty acids.

It is not probable that the slow incorporation is caused by absence of glucose because glucose was added to the medium in a quantity sufficient to restore completely the triglyceride synthesis in the adipose tissue obtained from starving rats. Moreover FARKAS (1966) found the adipose tissues of carps from the same catch to be rich in glycogen. The entrance of the fatty acids into the tissue is not involved in this problem as the radioactivity of the free fatty acids is higher in the adipose tissue of the carp than in that of the rat. The possibility might be brought up that the adipose tissue of the carp incorporates into triglycerides mainly fatty acids of endogenic origin, while the exogenic ones play only an unimportant role. The gas chromatographic demonstration of a high amount of linoleic acid in this tissue (ABDEL-HAY and HERODEK 1966) excludes this possibility as this fatty acid, similarly to the mammals, can not be synthetized by fish.

It might be supposed that the triglycerides get without previous hydrolysis into the adipose tissue of fishes from the circulation. Such mechanism was suggested earlier for mammals, however, according to the more recent experiments (RODAHL 1964) both the uptake and release of triglycerides are bound to hydrolysis. Yet it is not sure that the same is true for fishes. The uptake of intact triglycerides by the cell would render unnecessary an extensive triglyceride synthesis within the cell. The incubation of the tissue with triglycerides labeled by ^{14}C in glycerol and ^{3}H in fatty acid followed by the determination of the two isotopes in the tissue could give decisive answer to this question. Such experiment is in the present out of scope of our technical possibilities. The demonstration of the presence or absence of lipoprotein lipase could provide also important informations in this problem.

A permanent exchange between the fatty acids of triglycerides and free fatty acids in the adipose tissue of rat was demonstrated (VAUGHAN and STEINBERG 1963) by comparing the release of glycerol and free fatty acid. This means that part of the lipolyzed fatty acids instead of release into the medium is re-esterificated. Therefore the total triglyceride synthesis is higher than the net increase of the amount of triglycerides, going on also under conditions where the amount of triglycerides is unchanged or even decreasing. The lack of such a dynamic relation in fish could explain the difference observed in the incorporation of labeled fatty acids even if the net change of the amount of triglycerides is similar in the two animals. According to CAHILL's suggestion (KINSELL 1962) the energy requiring re-esterification of the fatty acids can play an important role in the heat economy of the homoiothermic animals,

The relatively low oxygen consumption of the adipose tissue of homoiotherms, at least under in vitro conditions, speaks against this theory. But if CAHILL is right it could be supposed that in a poikilothermic animal, where the heat production has no meaning, we do not find an extensive re-esterification.

Summary

Adipose tissue and liver slices of the carp and rat were in vitro incubated in media containing labeled plamitic acid. The lipid fractions were separated by thin layer chromatography, and their radioactivity was determined.

Triglyceride synthesis took place in each tissue. Its intensity had similar order of magnitude in the liver slices of the two species. On the other hand the synthesis was fifty times lower in the adipose tissue of the carp than in that of the rat under the experimental conditions. For interpretation we are restricted to hypothesis.

The diglycerides showed in each tissue a radioactivity of the same order of magnitude to that of the triglycerides. Probably besides the diglycerides, rapidly transformed to triglycerides, there exists a diglyceride pool of much slower turn over.

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A PONTY *(CYPRINUS CARPIO L.)* ÉS A PATKÁNY TRIGLICERID SZINTÉZISÉNEK ÖSSZEHASONLITÁSA 1—^{14}C PALMITINSAVVAL INKULBÁLT ZSÍRSZÖVET ÉS MÁJ METSZETEKBEN

Herodek Sándor

Összefoglalás

Patkány és ponty zsírszövetéből és májából készített metszeteket jelzett palmitinsavat tartalmazó közegben inkubáltuk. A lipid-frakciókat rétegkromatográfia segítségével szétválasztottuk, és mértük radioaktivitásukat.

Mindegyik szövetben volt triglicerid szintézis. Ennek erőssége a májszövetekben azonos nagyságrendű, a ponty zsírszövetében viszont az adott kísérleti feltételek mellett ötvenszer alacsonyabb, mint a patkányéban. Ennek magyarázatára csak feltevésekre szorítkozhatunk.

A digliceridek az összes szövetben a trigliceridekéhez hasonló mértékű rádioaktivitást mutattak. Feltehető, hogy a gyorsan triglicerriddé alakuló digliceridek mellett, egy hosszabb életű diglicerid raktár is van.

A szabad zsírsavak annál alacsonyabb százalékát tartalmazták az összes aktivitásnak, mennél magasabb volt ez az összes aktivitás az egyes szövetekben.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ СИНТЕЗА ТРИГЛИЦЕРИДОВ В ЖИРОВОЙ И ПЕЧЕНОЧНОЙ ТКАНЯХ, ИНКУБИРОВАННЫХ С 1—С^{14}-ПАЛЬМИТИНОВОЙ КИСЛОТОЙ, У КАРПА И КРЫСЫ

Шандор Херодек

Срезы из жировой и печеночной тканей карпа и крысы инкубировали в среде, содержащей 1—С^{14}-пальмитиновую кислоту. Измеряли радиоактивность липидных фракций, полученных с помощью тонкослойной хроматографии.

В каждой из тканей обнаружен синтез триглицеридов. Интенсивность синтеза в тканях печени одинакова, а в жировых тканях у карпа, при данных экспериментальных условиях, в 50 раз ниже, чем у крысы. Этому явлению пока не нашли объяснения.

Во всех тканях диглицериды проявляли такую же активность, как триглицериды. Предполагается, что помимо диглицеридов, быстро превращающихся в триглицериды, имеется более стабильное депо диглицеридов.

Активность свободных жирных кислот тем неже, чем выше в данной ткзни тотальная активность.

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ADATOK A BALATONI DÉVÉRKESZEG (*ABRAMIS BRAMA L.*) NÖVEKEDÉSÉHEZ

PÉNZES BETHEN

Fővárosi Állat- és Növénykert, Budapest

Érkezett: 1966 március 15-én

A Balatoni Halászati Vállalat statisztikája szerint az elmúlt tíz évben évi 8—14.000 q dévérkeszeget fogtak a halászok. Ez a mennyiség 70—80%-át teszi ki a BHV összeredményének, így méltán nevezik a dévérkeszeget a Balaton „kenyérhalának". Ennek ellenére a felszabadulásig annyira lenézett, silány halnak minősítették, hogy 1925-ben — amikor nagyarányú pontyosítást terveztek — teljesen ki akarták irtani a Balatonból. Napjainkban megbecsülik, mert pl. a konzervipar kitűnő minőségű olajoshalat készít belőle (PÉNZES 1964, SEBESTYÉN 1943).

A szakirodalomban SEBESTYÉN, WOYNAROVICH és WUNDER tesz említést a balatoni dévérkeszeg növekedéséről; WOYNAROVICH néhány adatot is közöl. WUNDER feltételezése szerint azért növekszik ebben a vízben lassan a dévérkeszeg (a németországi tavakéhoz viszonyítva), mert az itt élő populáció egyedszáma — minden más fajhoz viszonyítva — rendkívül nagy, melynek következtében a táplálékkonkurrencia is számottevő (SEBESTYÉN 1943, WOYNAROVICH 1958, WUNDER 1930a, b). BERG, BAUCH, LASKAR és NIKOLSKI számos adatot közöl német, orosz folyók és tavak dévérkeszeg állományának értékmérő tulajdonságairól (BAUCH 1953, BERG 1949, LASKAR 1941, NIKOLSKI 1957.) Ha összehasonlítást teszünk, úgy megállapítható, hogy a balatoni dévérkeszegek az első években jobb fejlődést érnek el, mint pl. a németországi populációk példányai. Ez avval magyarázható, hogy a Balaton viszonylag sekély, könnyen felmelegszik- így a tenyészidőszak is hosszabb. Mivel azonban a táplálékban szegény víz, az idősebb egyedek már kevés táplálékot találnak — kivéve a Keszthelyi- öblöt, ahol jelentős mennyiségű vándorkagyló *(Dreissena polymorpha)* él, — így fejlődésük is lelassul és messze elmarad a Duna-, a Don deltájának, a SzU új víztárolóinak, a félsós vízű (brak) tengerparti öblök- (Haffok)nek dévérkeszegjeitől, ahol nem ritka a 4—5 kg súlyú példány sem (ENTZ GÉZA és SEBESTYÉN 1942).

A vizsgálat leírása

Vizsgálatomban összesen 881 db dévérkeszeg szerepelt. Az anyagban másodéves és annál idősebb halak szerepeltek, vagyis azok a példányok, melyek a 4 cm szembőségű hálóban fennakadtak. Valamennyi a Balaton északi medencéjéből származott. Az anyagot a Balatoni Halászati Vállalat bocsátotta

rendelkezésemre, melyért ezúton is köszönetet mondok. Az adatok felvételének ideje: 1963. december 9.

Az egyedek életkorának meghatározásához szükséges pikkelyeket — halanként kb. 8—10 db-ot — a hátúszó és az oldalvonal közti részről gyűjtöttem. Az állatról eltávolított pikkelyeket külön erre a célra készített, kemény lapú és fényes felületű papírból készült füzet lapjai közé helyeztem. Mindig ugyanerre a lapra került az illető hal egyéb adata is.

A testméreteket — a testhosszt (L_c) és a teljes hosszt (L_t) — VÁMOSI-féle mérőládával végeztem. Az értékeket 0,5 cm-nyi pontossággal állapítottam meg.

A súlyt — ha a hal 100 g-on felül volt — tizedes beosztású mérleggel — ha 100 g-on aluli súly volt — levélmérleggel állapítottam meg.

Az egyedek életkorának megállapítását pikkelyévgyűrűk alapján, az állategészségügyi gyakorlatban alkalmazott trichinoszkóppal végeztem. (Tudomásom szerint még senki sem alkalmazta ezt a műszert halpikkelyek vizsgálatára, életkor meghatározására. Minthogy rendkívül egyszerű és gyors eljárás, javaslom, mint új módszer alkalmazását.) Minden halról 4—4 db pikkelyt helyeztem a trichinoszkópba, s azokat egyenként kinagyítva válogattam ki a legépebb példányt, melynél az évgyűrűket tisztán, világosan lehetett értékelni.

A testhossz, a teljes hossz és a súly adataiból számítottam ki az egyes korcsoportok értékeinek számtani átlagát ($\bar{x}$), a szóródás nagyságát (a $\pm$ s értéket; s = standard deviáció) és a v %-ot) ez utóbbi azt fejezi ki, hogy hány %-ban tér el az s az $\bar{x}$tól).

Eredmények

Az I. táblázat feltünteti, hogy a balatoni dévérkeszeg egyes korcsoportjai (II., III., IV., V., VI., és VII. nyaras halak) milyen átlagos testméreteket értek el, és, hogy ezekkel kapcsolatban milyen szóródási értékeket tapasztaltam. A II. táblázat a korcsoportok határértékeit, vagyis a legkisebb és legnagyobb egyedek testméreteit és súlyát mutatja.

A nyert adatokból megállapítható, hogy a Balatonból kifogott dévérkeszeg zöme a három-, négy- és ötnyaras egyedek közül kerül ki. A vizsgálatban szerepelt anyagból — darabszám tekintetében — a kétévesek 12, a háromévesek 30, a négyévesek 29, az ötévesek 24, a hatévesek 4 és a hétévesek 1%-ban szerepeltek (az egyes %-os értékek kerekített számok !). Súly vonatkozásában 6,7 kg-t (3%) a kétnyarasok: 40,0 kg-t (16%) a háromnyarasok; 80,9 kg-t (32%)a négynyarasok; 94, 6 kg-t (38%) az ötnyarasok; 21,8 kg-t (8%) a hatnyarasok és 7,7 kg-t (3%) a hétnyarasok csoportja adta az összmennyiségből.

Összefoglalás

A szerző 881 db balatoni dévérkeszegről *(Abramis brama L.)* vett fel testhossz (L_c), teljes hossz (L_t) és súlyméreteket ill. értékeket. Az I. és II. táblázatban korcsoportonként, biometriai feldolgozásban ismerteti a kapott adatokat.

A nyert értékekből megállapítja, hogy a Balatonból kifogott dévérkeszeg nagy része a harmadik, negyedik és ötödik évjárat egyedei közül kerül ki (30, 29 és 24%-os mennyiségben). Súly tekintetében 154, 277 és 390 g-ot érnek el átlagosan a három-, négy- és ötéves balatoni dévérkeszegek.

Table 1.

Wachstumdaten der aus dem Nordbecken des Balaton stammenden Individuen der Bleie-Population an Hand der Messungen des Jahres 1963

1. táblázat

A Balaton északi medencéjének dévérkeszeg-populációjából származó példányok növekedési adatai 1963. évi mérések alapján

Jahrgang Évjárat	Zahl d. untersuchten Ind.-Stück A vizsgált egyedek száma, db	Körperlänge (Lc) Testhossz (Lc)			Gesamtlänge (Lt) Teljes hossz (Lt)			Gewicht — Súly		
		x cm	± s cm	v%	x cm	±s cm	v%	x dkg	± s dkg	v%
II.	102	13.9	1,8	12,9	17,9	2,3	13,5	6,6	2,4	36,4
III.	260	17,8	2,0	11,7	22,9	2,6	11,7	15,4	3,9	29,1
IV.	256	22,7	2,9	12,9	29,0	2,4	8,2	27,7	6,7	26,6
V.	217	25,8	2,0	8,0	32,9	2,4	7,4	39,0	8,0	21,6
VI.	37	29,8	2,8	9,4	37,5	3,2	8,6	59,2	17,0	29,7
VII.	9	33,3	2,2	6,6	41,3	3,0	7,4	77,8	17,1	22,5

Table 2.

Extremwerte einzelner Jahrgänge der untersuchten Bleie-Population im Balaton-See

2. táblázat

A vizsgált balatoni dévérkeszeg-populáció egyes évjáratainál talált szélső értékek

Jahrgang Évjárat	Zahl d. untersuchten Ind.-Stück A vizsgált egyedek száma, db	Körperlänge (Lc) cm Testhossz (Lc) cm		Gesamtlänge (Lt) Teljes hossz (Lt)		Gewicht dkg Súly, dkg	
		Min.	Max.	Min.	Max.	Min.	Max
II.	102	10,0	17,0	13,0	22,0	4,0	13,0
III.	260	11,5	23,0	14,5	29,5	5,0	27,0
IV.	256	16,5	27,5	21,5	33,0	13,0	38,0
V.	217	22,0	31,0	29,0	38,5	25,0	62,0
VI.	37	23,0	37,0	29,0	45,0	28,0	130,0
VII.	9	29,0	36,0	37,0	44,0	52,0	94,0

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BEITRÄGE ZUM WACHSTUM DER BLEIE *(ABRAMIS BRAMA L.)* IM BALATON-SEE

Bethen Pénzes

Zusammenfassung

Autor nahm von 881 Stück Bleien vom Balaton-See *(Abramis brama L.)* Maass beziehungsweise Werte über Körperlänge (L_0), volle Länge (L_t) sowie Gewicht auf. In den Tabellen Nr. 1. und 2. sind die erhaltenen Angaben je nach Altersgruppen geschieden, in biometrischer Aufarbeitung ersichtlich.

Aus den gewonnenen Werten stellt er fest, dass ein grosser Teil der aus dem Balaton gefischten Bleien sich aus ihrem dritten, vierten und fünften Jahrgang ergibt (30, 29 und 24 perzentuelle Mengen). Bezüglich Gewicht erreichen die zwei-, vier- und fünfjährigen Bleien des Balaton durchschnittlich ein Gewicht von 154, 277 und 390 Grammen.

ДАННЫЕ О РОСТЕ БАЛАТОНСКОГО ЛЕЩА *(ABRAMIS BRAMA L.)*

Бетхен Пензэш

Измерен 881 лещ из Балатона в отношении длины тела (L_0), полной длины (L_t) и веса. Биометрически обработанные данные представлены на таблицах 1 и 2.

Большинство выловленных лещей представлено 3-, 4- и 5-летними особями. Они составляют 30, 29 и 24 улова соответственно. Вес 3-, 4- и 5-летних рыб достигает 154, 277 и 390 г. соответственно.

ANNAL. BIOL. TIHANY	33	177—192	HUNGARIA 1966

TÁJÉKOZÓDÓ VIZSGÁLATOK A BALATON NYÍLTVIZE ISZAPLAKÓ RÁKJAINAK MINŐSÉGI ÉS MENNYISÉGI VISZONYAIRÓL

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Érkezett: 1966 március 22-én

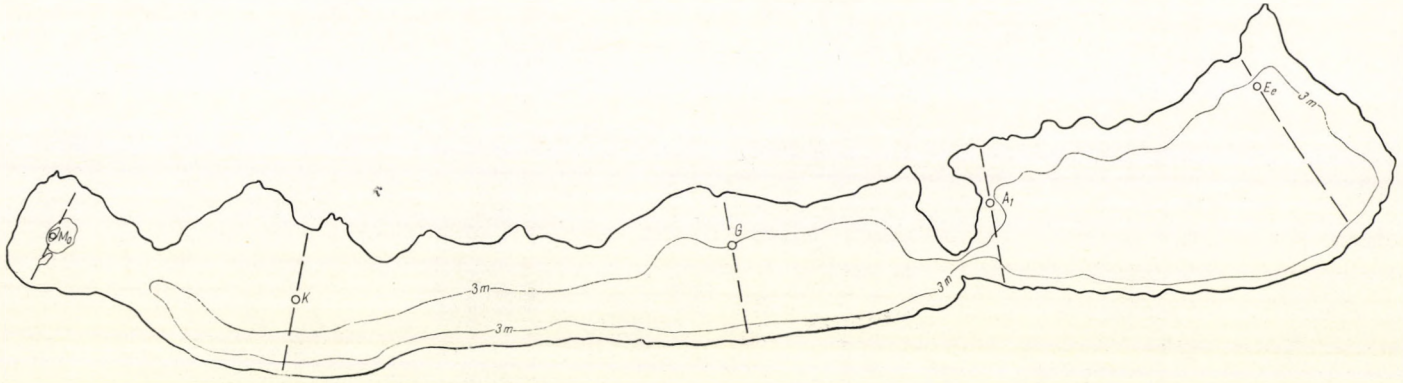
A Balaton nyíltvíz iszapjára (eprofundal) vonatkozó adatok a Chironomidákat kivéve, nagyon korlátozottak. A Balaton kutatása több, mint félévszázados múltra tekint vissza, ennek ellenére a kutatási eredmények jórészt a Tihanyi-félsziget közelében levő vízterületekre vonatkoznak. Feltűnően szegényes adatokat találunk a nyíltvíz iszaplakó rákjai vonatkozásában. A legelső adatok DADAY (1897) nevéhez fűződnek, aki 7 fenéklakó rákot *(1 Cyclopida, 2 Harpacticida, 4 Ostracoda)* említ meg. Hosszú időn keresztül MOON (1934) vizsgálata az egyetlen, mely alapján vélemény alakulhatott ki a Balaton fenéklakó állatairól annak ellenére, hogy SEBESTYÉN (1948) több ízben felhívta a figyelmet e fontos biotop vizsgálatára. MOON adatai a tó egyetlen szelvénye (Balatonfüred—Zamárdi), nyári időszakára vonatkoztak. A kutatások csak a mikrószkópos nagyságrendű állatokra terjedtek ki, mivel abban az időben limnológiai és halászatbiológiai kutatásokban az iszapvizsgálatok céljaira 1 mm lyukbőségű üledékrostát használtak. Rákok vonatkozásában így 2 *Amphipodát* [*Gammarus (Rivulogammarus) roeseli, Corophium curvispinum f. devium*] említ partközelből. Újabb adatokat SEBESTYÉN (1947) közöl az Intézet előtti nyílt vízből. Később ENTZ (1954) a „Mikrocrustacea-kra" vonatkozó egyedszám % és súly % adatokat ismertet. Az első részletesebb — de nem a Tihanyi-félsziget környékéről származó — iszaplakó rák-adat a Keszthelyi-öbölből származik. (ENTZ, PONYI, TAMÁS 1963). Újabban SEBESTYÉN (1965) Tihany, Balatonfüred, Akali és Keszthely előtti vizek iszapjából ismertetett adatokat.

Vizsgálataink célkitűzése az volt, hogy további megfigyelések alapján adatokat gyűjtsünk a fenéklakó állatokkal kapcsolatban.

Gyűjtések helye, ideje és egyéb adatok

Az iszapmintavételek a tó DNy-i részének 3, az ÉK-i részének pedig 2 pontján, a tótükör hossztengelyére merőleges harántszelvényeken történtek (v.ö. SEBESTYÉN 1960, 118—119), melyek a következők *(1. ábra)*:

1. Gyenesdiás—Zala folyó torkolata, gyenesdiási parttól kb. 2800 m („M_0").
2. Szigliget—Balatonmária, az északi parttól kb. 400 m („K").



1. ábra. Gyűjtőhelyek a Balatonon. Magyarázat a módszertani részben.

Abb. 1. Sammelstellen im Balaton-See. Erklärung s. »Methodik«.

3. Ságpuszta—Balatonszemes, az északi parttól kb. 2500 m („G").
4. Balatonfüred, Fenékfürdő—Zamárdi alsó, Tihany Biológiai Kutatóintézet előtt kb. 500 m („A_1").
5. Balatonalmádi—Balatonvilágos, északi parttól kb. 2000 m („E_e").

A gyűjtések körülményeivel kapcsolatos adatokat (vízmélység, vízhőmérséklet, átlászóság, valamint a pontos gyűjtési időt) és a megjegyzéseket az *1. táblázat* foglalja össze.

A keresztszelvények kiválasztását ill. számát a korábbi vizsgálatok (Sebestyén 1960, Entz 1965 stb.), valamint az Intézetünk Hidrobiológiai Osztályának kapacitása szabta meg. Az iszapvizsgálatok mellett egyidőben más kutatások is folytak (fito- és zooplankton mennyiségi és minőségi vizsgálatok, stb.). A gyűjtések ideje: 1965. VI. 9—10; VII. 1—2; VIII. 3—4; IX. 7—8; X. 13—14.

Mintavétel és feldolgozás

A korábbi vizsgálatok kapcsán megállapítottuk (Entz, Ponyi, Tamás 1963), hogy a rákok zömmel az iszap felső rétegének 1 cm-ben találhatók. Ezért az Ekman-Birge iszapmarkolóval vett minták esetében a felső 1 cm-es rétegből azonos felszínű iszapot szedtünk le, hogy a különböző helyekről származó állatanyag — minőségi összehasonlításán túlmenően — mennyiségi viszonyairól is adatokat nyerhessünk. Az iszapmarkolóval kiemelt minták közül csak azokat használtuk fel, melyeknek felső rétege, szemmel láthatóan bolygatástól mentesnek látszott. Minden gyűjtőhelyről megfelelő nagyságú műanyagkanállal, $\sim$ 114 cm^2 felszínű iszapot mertünk le, és az állatokat a korábban kidolgozott módszer szerint (Entz, Ponyi, Tamás 1963, 114. o.), laboratóriumban válogattuk ki.

Áttekintés és néhány megjegyzés a vizsgálatok során talált nem pelágikus fajokról

A vizsgálati periódus alatt 28000 cm^2 felszínű iszapot és kb. 1100 db iszaplakó rákot dolgoztunk fel. Ennek során figyelmen kívül hagytuk azon nagyszámban előforduló rákokat, melyek elsősorban a planktonra jellemzőek, így: *Diaphanosoma brachyurum* Lievin, *Daphnia hyalina* var. *lacustris* G. O. Sars, *D. hyalina* var. *galeata* G. O. Sars, *Bosmina longirostris f. pellucida* Stingelin, *Leptodora kindtii* Focke, *Eudipatomus gracilis* G. O. Sars, *Cyclops vicinus* Uljan., *Mesocyclops* (s. str.) *leuckarti* (Claus).

Cladocera

1. *Latona setifera* (O. E. Müller) 1875

Magyarország faunájára új. Az elterjedésre vonatkozó irodalmi adatok (Lilljeborg 1900, Mauilova 1965, Scourfield és Harding 1958, Flössner 1964 stb.) arra utalnak, hogy elsősorban Európa és Amerika északi vidéke tavaiból és nagyobb vizeiből ismert. Úgy látszik, hogy Európa déli területeiről hiányzik. Ezt bizonyítaná pl. az a tény is, hogy *Cladocera* szempontból alaposan átkutatott kaukázusi területről sem ismert (Behning 1941). Thienemann

(1950) az állat elterjedési területéül Grönlandot, Európát és Észak-Amerikát jelölte meg.

Az irodalmi adatok alapján inkább a tavak parti régióiban él. WAGLER (1937) szerint: „Ist wahrscheinlich nicht selten, wird aber wegen der geringen Individuendichte u. oder versteckteren Lebensweise weniger beobachtet" (23. o). „On the bottom near shore and to moderate depths in lakes" írja életmódjáról SCOURFIELD és HARDING (1958, 12. o.). Biológiájával kapcsolatosan (MANUILOVA 1964, p. 115) még tudjuk, hogy lassú folyású folyókból is ismeretes. Monociklikus. A partenogenetikus peték száma 10-ig terjedhet. A kétivarú szaporodás okt.—márc. idejére esik.

Először dr. SEBESTYÉN OLGA által gyűjtött anyagban (Intézet előtti nyílt víz 1948. V. 11. 2 db), később saját gyűjtéseinkben is megtaláltuk (M_0, G, A_1, E_2 pontokon). Adataink azt mutatják, hogy az egész Balaton nyíltvizének iszap-felszínén meglelhető. Iszapmarkolóval vett mintákban egy, a dredge-mintákban szinte minden alkalommal találtunk néhány példányt.

2. *Macrothrix laticornis* (JURINE) 1820.

M_0 A_1, E_e gyűjtőhelyeken találtuk. Vizsgálati periódusban az utóbbi helyen fordult elő legnagyobb számban. Iszapkedvelő faj.

3. *Ilyocryptus agilis* KRUZ 1874.

Korábbi vizsgálatainkhoz hasonlóan (ENTZ, PONYI, TAMÁS 1963) nagyon szórványosan előkerülő faj. Egyedül G gyűjtőhelyen találtuk. Iszapkedvelő faj.

4. *Alona quadrangularis* (O. F. MÜLLER) 1785.

M_0, K, A_1 és E_e pontokon gyűjtöttük. Az említett gyűjtőhelyek közül A_1 volt egyedszámban a leggazdagabb. Az iszapkedvelő Cladocerák egyik leggyakrabban előforduló képviselője (SEBESTYÉN 1965).

5. *Alona affinis* LEYDIG 1860.

A következő gyűjtőhelyeken találtuk: M_0, K, E_e. Az előbbi fajhoz mérten sokkal kisebb egyedszámban. A balatoni nádasokban a bolyhos bevonat leggyakoribb Cladocerája (MESCHKAT 1934, PONYI 1962, SEBESTYÉN 1965). Detrituskedvelő forma.

6. *Alonella rostrata* (KOCH) 1841. (Syn. *Rhynchotalona rostrata* (KOCH).

FREY (1959) alapos vizsgálatokat végzett a Chydoridae fejpajzs porusain.

Ennek alapján — meggyőzően — azt állítja, hogy: „The European species *Rhynchotalona rostrata* is quite obviously placed incorrectly in this genus, because the pore arrangement is of the *Alonella* type, and it is herewith trasferred to the genus *Alonella* (u. o. p. 36). Egyes kutatók (MANUILOVA 1964) azonban továbbra is a *Rhynchotalona genusba* sorolják, mások (SEBESTYÉN 1965 és FLÖSSNER 1964) azonban már az *Alonella* genusba helyezik át. A gyűjtések során — egy kivételével (G) — az összes vizsgálati helyen megtaláltuk. A legnagyobb egyedszámban az A_1 helyen. Iszapkedvelő forma.

7. *Leydigia leydigii* (LEYDIG) 1860.

Ezt a fajt először DADAY (1888) említi a siófoki partokról, hosszú évek után a Keszthelyi-öböl nyiltvíz iszapjából került elő (ENTZ, PONYI,

TAMÁS 1963). 1965. évi vizsgálatok során ugyancsak ott gyűjtöttük (M_0). Másutt nem találtuk. A Balaton iszapján úgy látszik ritka. Iszapkedvelő állat.

8. *Leydigia acanthocercoides* (FISCHER) 1854.

A Balaton egyik legközönségesebb fenéklakó állata (SEBESTYÉN 1965). Gyűjtéseink során, G gyűjtőhely kivételével, mindenütt megtaláltuk. Legnagyobb egyedszámban a Keszthelyi-öbölben (M_0).

9. *Pleuroxus uncinatus* BAIRD 1850. (Syn. *Pleuroxus balatonicus* DADAY).

DADAY (1885, 1888) néhány jelentéktelen morfológiai különbség alapján új fajként írta le. Később SEBESTYÉN (1947) megerősíti e faj önálló balatoni létezését. A leírás alapján legfontosabb bélyegnek tartható, hogy „Post-abdomen of the male differs definitely from any other species of the genus, being very similar to that of the female" (u. o. 12. o.). Erről azonban kiderült (FREY 1965), hogy a Chydoridanál előforduló gynandromorphismus-jelenségével állunk szemben. FREY szerint a *Pleuroxus balatonicus* DADAY 1885, taxon „it is a small subspecies of *Pleuroxus uncinatus* BAIRD, 1850, in which the males are usually gynandromorphic but may occasionally be normal" (u. o. 33.). Az 5 gyűjtőhely közül 3-ban megtaláltuk (M_0, A_1, E_e). Legnagyobb számban a Tihany előtti nyíltvízből (A_1).

10. *Monospilus dispar* SARS.

A Balaton közönséges fenéklakó szervezete (SEBESTYÉN 1965). A mintavételi helyeken egy kivételével (A_1) mindenütt gyűjtöttük.

Ostracoda

11. *Candona balatonica* DADAY, 1894.

Kevés egyedszámban az M_0, G, A_1 gyűjtőhelyekről.

12. *Candona* sp. *juv.*

Minden gyűjtőhelyen előfordul. A korábbi vizsgálatokhoz hasonlóan (ENTZ, PONYI, TAMÁS 1963) a faji hovátartozását most sem lehetett megállapítani. A legnagyobb egyedszámban G gyűjtőhelyen találtuk.

13. *Ilyocypris gibba* (RAMDOHR) 1808.

Csak a Füzfői-sarok közelében (E_e), néhány példány.

14. *Darwinula stevensoni* (BRADY et ROBERTSON) 1870.

A *Candona* sp.-hez hasonlóan a gyűjtőhelyek mindegyikén megtaláltuk. A Balaton fenékiszapjának jellegzetes szervezete (ENTZ, PONYI, TAMÁS 1963). Legtöbb egyedet a G-ponton gyűjtöttük.

Copepoda

15. *Paracyclops fimbriatus* (FISCHER) 1853.

Csupán két helyről, szórványosan került elő (M_0, A_1). Feltűnő, hogy korábbi években a Keszthelyi-öbölben ugyanazon helyen (M_0) és időben

jelentős mennyiségben gyűjtöttük (ENTZ, PONYI, TAMÁS 1963). Balatoni adatok arra utalnak, hogy inkább iszaplakó faj.

16. *Acanthocyclops viridis* (JURINE) 1820.

A tó parti övében (nádas, hínáros) közönséges és nagy tömegben előforduló szervezet (PONYI 1957, 1962).

17. *Acanthocyclops vernalis vernalis* (FISCHER) 1853.

Balatoni előfordulására eddig nem volt biztos adatunk. DADAY (1897) a „*Cyclops pulchellus* KOCH" és „*Cyclops diaphanus* SARS" fajokat említi a tóból, melyeket azonban nem lehet a *vernalis*-al egyeztetni. (Lásd még DADAY 1885, p. 220. és 246). Egyedül a Keszthelyi-öbölben (M_0) gyűjtöttük kis egyedszámban. A Balaton faunájára új.

18. *Acanthocyclops vernalis robostus* (SARS) 1863.

Ugyancsak a Keszthelyi-öbölből (M_0), azonban sokkal nagyobb egyedszámban, mint a törzsalak. A Balaton faunájára új. A törzsalak és az alfaj a legkülönbözőbb vizekben megtalálható. Ökológiailag igen érdekes, hogy az alfaj hazai folyók (Duna, Tisza) hyporeáljának is jellemző, nagy egyedszámban előforduló képviselője (J. E. PONYI, L. PONYI 1961; PONYI 1966, kézirat).

19. *Ectinosoma abrau* (KRITSCHAGIN) 1873.

A Balaton fenékfaunájának legnagyobb egyedszánban előforduló rákja. Iszaplakó.

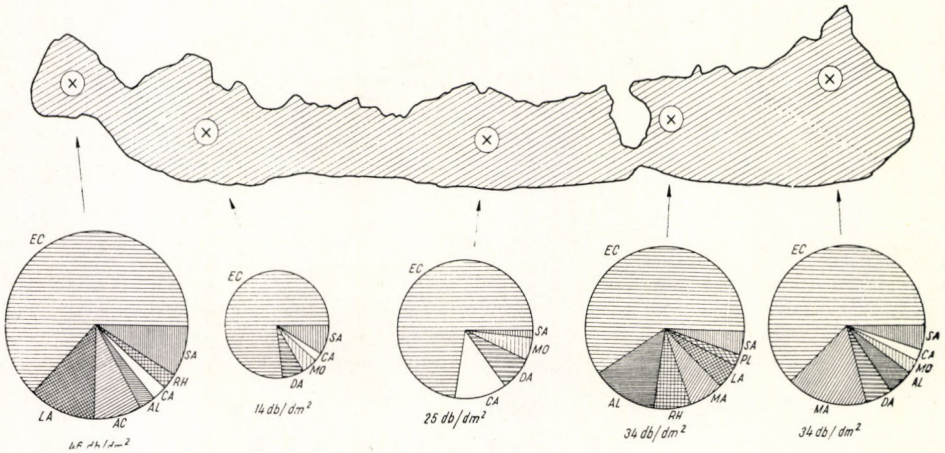
20. *Nannopus palustris* BRADY 1880.

Vizsgálatok során 3 gyűjtőhelyről (G, A_1, E_e) kevés egyedszámban került elő. Az eddigi adatok alapján (PONYI 1960, 1965) jellegzetes (sohasem nagy egyedszámban előforduló) iszaplakó szervezetnek tekinthető.

A nem pelágikus fajok mennyiségi és minőségi megoszlása a különböző gyűjtőhelyeken

A fajok mennyiségi megoszlása a vizsgálati idő alatt (1965. VI. 9. —X. 1.) egy-egy gyűjtőhelyre vonatkozóan igen változó. Ez az egyedszám-ingadozás a metodikai hiányosságokon túlmenően a fajok népességdinamikai viszonyaiból, az egyenetlen eloszlásból stb. adódik (HANKÓ 1926, SEBESTYÉN 1965). Ezek a tények mind arra hívják fel a figyelmet, hogy a különböző rákfajok számszerű adataiból csak óvatosan vonhatunk le következtetéseket. A rákfajok mennyiségi változásának megfigyelése és leírása az egyes gyűjtőhelyeken — a fentebb említetteken kívül — a kevés egyedszám miatt sem lehetséges (kivételt képvisel az *Ectinosoma abrau*, melyre később visszatérünk). Ezért az 5 hónapra vonatkozó átlagértékeket adhatjuk meg, melyek némi képet nyújtanak a rákok mennyiségi viszonyairól. Ezt megtehetjük, mivel a vizsgált fajok többsége életpályájának aktív szakasza a vizsgálati időszakon belül esett (SEBESTYÉN 1947, 1948 és saját megfigyelések). 5 alkalommal végzett gyűjtés során minden gyűjtőhelyre vonatkoztatva közel 6 dm^2 felszínű iszapot válogattunk ki és az erre eső rákmennyiséget számoltuk át 1 dm^2-re. Ebből az adódott, hogy a Keszthelyi-öböl iszapja (M_0) leggazdagabb rákokban

(46 db/dm²), legszegényebb a Szigligeti-öböl (K), ahol számuk 14db/dm²-re csökken, a következő gyűjtési helyen (G) már ismét emelkedik (25 db/dm²). Meglepő, hogy az ÉK-i medence két pontján ($A_1 = 34$ db/dm², $E_e = 34$ db/dm²) az összmennyiség megegyezett (2. ábra). Úgylátszik, hogy az iszaplakó rákok mennyisége szempontjából az ÉK-i medence egységesebb, valamint a Keszthelyi-öböl kivételével gazdagabb a DNY-i-nál.



2. ábra. Rákegyüttesek alakulása a vizsgált időszakon belül a Balaton fenékiszapján. EC = *Ectinosoma abrau* DA = *Darwinula stevensoni* CA = *Candona* sp. *juv.* AL = *Alona quadrangularis* LA = *Leydigia acanthocercoides* RH = *Alonella rostrata* MO = *Monospilus dispar* MA = *Macrothrix laticornis* AC = *Acanthocyclops* PL = *Pleuroxus uncinatus* SA = egyéb fajok (sonstige Arten).

Abb. 2. Gestaltung der Krebsgemeinschaften im Bodenschlamm des Balaton-Sees innerhalb der untersuchten Periode.

A 2. ábra alapján látható, hogy az *Ectinosoma abrau* egyedszámban jelentősen meghaladja a *Cladocera* és *Ostracoda* mennyiségét. Igen érdekesen alakult az *Ectinosoma*-k száma az egyes gyűjtőhelyeken, valamint a két medencére vonatkoztatva. Úgy látszik, hogy az *Ectinosoma* népességdinamikai viszonyai, a tó két medencéjében különbözőképpen alakulnak. Az ÉK-i medence mindkét part közeli gyűjtőhelyen (A_1, E_e) a maximális egyedszámot IX. 7-én, a DNy-i medence 3 pontján (M_o, G, K) pedig X. 13-án találtuk. Érdemes megjegyezni, hogy a mikrocrustaceák mennyiségi megoszlása éppen fordított tendenciát mutat, mint a Chironomidáké (Entz 1965). Míg az utóbbi legnagyobb tömegben a tó középső részein és legkisebb mennyiségben a Keszthelyi-öböl, ill. az ÉK-i medencében található, addig a rákok esetében éppen fordítva van. Lehetséges, hogy ez a jelenség is az üledékviszonyokkal magyarázható (uo. 133. o.).

A minőségi viszonyok alakulása lényegében megegyezik a fentebb mondottakéval. Fajban legszegényebb vizsgálati terület a tó középső szakasza, míg a Keszthelyi-öböl, valamint a tó ÉK-i medencéje lényegesen gazdagabb (lásd 2. táblázat). Az egyes gyűjtőterületeken a rákfajok %-os megoszlása különböző. Egyedüli kivétel úgy látszik az *Ectinosoma*, mely a rákegyütteseken belül eléggé egyenletesen található meg. A 2. táblázatot szemlélve,

Tabelle 1.

Einige Daten über die Umständen der zwischen den 9. Juni—14. Oktober 1965 stattgefundenen Sammlungen

Zeitpunkt des Sammelns		Sammelstelle*	Stunde des Sammelns	Wassertiefe in m	Wassertemperatur im Bodenschlamm °C	Durchsichtigkeit (Secchi-Scheibe) cm	Bemerkungen**
Juni	9.	M_0	7	3,0	18,0	45	
		K	12	4,2	19,5	36	
		G	17	4,2	18,5	92	
	10.	A_1	16	3,5	19,0	50	
		E_e	18	3,2	19,0	64	
Juli	1.	M_0	7	2,9	22,0	80	
		K	9	3,8	24,0	40	
		G	16	4,0	27,0	92	
	2.	A_1	15	3,5	24,0	92	
		E	17	3,3	23,0	66	
August	3.	M_0	7	3,0	20,0	39	*Melosira*-Wasserfärbung. Wasserfarbe: bräunlich
		K	9	3,6	20,0	35	Wasserblüte verursacht durch das massenhafte Auftretens von *Melosira*. Wasserfarbe: gelb
		G	16	3,9	21,0	56	*Melosira*-Wasserfärbung. Wasserfarbe: gelblich
	4.	A_1	15	3,3	22,0	51	
		E_e	18	3,7	21,0	64	
September	7.	M_0	7	2,7	18,0	62	Vereinzelte Wasserblüte von *Microcystis-Aphanizomenon*.
		K	10	3,6	19,0	51	Vereinzelte Wasserblüte imfolge des massenhaften Auftretens von *Microcystis-Aphanizomenon*.
		G	16	4,0	19,0	50	
	8.	A_1	15	3,7	20,0	86	
		E	19	2,8	20,0	—	
Oktober	15.	M_0	7	2,5	13,0	114	
		K	11	3,3	13,0	66	
		G	16	3,7	15,0	95	
	14.	A_1	15	3,7	14,0	120	
		E_e	18	3,2	14,0	—	

* Erklärung im Text.
** Die algologischen Daten stellte Dr. Gizella Tamás zur Verfügung.

1. táblázat

Néhány adat az 1965. jún. 9—okt. 14-e között végzett gyűjtések körülményeiről

Gyűjtés ideje		Gyűjtés* helye	Gyűjtés órája	Vízmélység (m-ben)	Vízhőfok a fenékiszapján C°	Átlátszóság (Secchi-korong) cm	Megjegyzés**
Június	9.	M_0	7	3,0	18,0	45	
		K	12	4,2	19,5	36	
		G	17	4,2	18,5	92	
	10.	A_1	16	3,5	19,0	50	
		E_e	18	3,2	19,0	64	
Július	1.	M_0	7	2,9	22,0	80	
		K	9	3,8	24,0	40	
		G	16	4,0	27,0	92	
	2.	A_1	15	3,5	24,0	92	
		E_e	17	3,3	23,0	66	
Augusztus	3.	M_0	7	3,0	20,0	39	*Melosira* vízszíneződés, a víz színe barnás árnyalatú
		K	9	3,6	20,0	35	*Melosira* tömeges fellépése okozta vízvirágzás. A víz színe sárga árnyalatú
		G	16	3,9	21,0	56	*Melosira* vízszíneződés, a víz színe sárgás árnyalatú
	4.	A_1	15	3,3	22,0	51	
		E_e	18	3,7	21,0	64	
Szeptember	7.	M_0	7	2,7	18,0	62	*Microcystis-Aphanizomenon* szórt vízvirágzás
		K	10	3,6	19,0	51	*Microcystis-Aphanizomenon* tömeges fellépése következtében szórt vízvirágzás
		G	16	4,0	19,0	50	
		A_1	15	3,7	20,0	86	
		E_e	19	2,8	20,0	—	
Október	15.	M_0	7	2,5	13,0	114	
		K	11	3,3	13,0	66	
		G	16	3,7	15,0	95	
	14.	A_1	15	3,7	14,0	120	
		E_e	18	3,2	14,0	—	

* Magyarázat a szövegben.

** Az algológiai adatokat dr. *Tamás Gizella* bocsátotta rendelkezésünkre.

úgylátszik, hogy az iszaplakó rákfajok alapján a Balaton eprofundálja 3 részre tagolódik:

1. A Balaton középső, fajszegény területe, melyet a *Darwinula stevensoni*, *Candona* sp. és *Monospilus dispar* együttessel jellemezhetünk.

2. A tó ÉK-i medencéje fajban már gazdagabb. Jellemző együttese: *Alona quadrangularis*, *Macrothrix laticornis*, *Nannopus palustris*.

3. Keszthelyi-öböl, mely a *Leydigia acanthocercoides* és az *Acanthocyclops* genus képviselőivel jellemezhető legjobban.

Tabelle 2.

Prozentuelle Verteilung der Krebse an Hand der Durchschnittswerte der 5 Sammelstellen

2. táblázat

A rákok %-os megoszlása az 5 gyűjtőhely átlagos adatai alapján

Fajok Arten	M_0 Keszthely	K Szigliget	G Ságpuszta	A_1 Tihany	E_e Füzfő
1. *Ectinosoma abrau*	63,1	76,8	72,5	59,1	61,8
2. *Darwinula stevensoni*	1,9	6,1	7,7	1,1	5,1
3. *Candona* sp. *juv.*	2,4	2,6	12,0	1,0	2,1
4. *Alona quadrangularis*	3,4	2,5	—	13,2	5,2
5. *Leydigia acanthocercoides*	11,4	2,3	—	4,7	1,6
6. *Alonella rostrata*	2,3	1,2	—	7,3	0,5
7. *Monospilus dispar*	0,8	4,9	4,9	—	2,6
8. *Macrothrix laticornis*	0,4	—	—	6,8	16,5
9. *Acanthocyclops* sp. *juv.*	3,0	—	—	1,6	0,6
10. *Alona affinis*	1,5	1,2	—	—	1,5
11. *Pleuroxus uncinatus*	0,5	—	—	2,1	0,5
12. *Nannopus palustris*	—	—	1,4	1,6	0,5
13. *Candona balatonica*	0,7	—	0,8	0,5	—
14. *Acanthocyclops viridis*	1,1	2,4	—	—	—
15. *Paracyclops fimbriatus*	0,7	—	—	1,0	—
16. *Acanthocyclops ver. rob.*	4,9	—	—	—	—
17. *Iliocypris gibba*	—	—	—	—	1,5
18. *Acanthocyclops ver. ver.*	1,1	—	—	—	—
19. *Iliocryptus agilis*	—	—	0,7	—	—
20. *Leydigia leydigii*	0,4	—	—	—	—
21. *Latona setifera*	0,4	—	—	—	—

Néhány megjegyzés a Balaton nyíltvízi iszapjában élő rákegyüttesek változásairól

A változások összehasonlítására csupán Daday (1897), Sebestyén (1947), Entz, Ponyi, Tamás (1963) adatai állnak rendelkezésünkre. Az adatok többsége csak minőségi összevetésre alkalmas. Daday (1897. 177. o.) megállapítása szerint a nyíltvíz iszapfaunájához szorosabban csak 7 rákfaj tartozik:

1. *Cyclops bathybius* Dad. (= *Paracyclops fimbriatus* (Fischer)).
2. *Canthocamptus tentaculatus* Dad. (= *Nitocrella hibernica* (Brady)).
3. *Ectinosoma Edwardsii* Rich. (= *Ectinosoma abrau* (Kritsch.)).

4. *Candona fabaeformis* FISCHER.
5. *Iliocypris gibba* (RAMDOHR).
6. *Darwinula Stewensonii* BR. (= *Darwinula stevensoni* (BRADY et ROBERTSON)).
7. *Limnicythere inopinata* BR. (= *Limnocythere inopinata* (BAIRD)).

A 7 faj közül a *Limnocythere inopinata* és a *Nitocrella hibernica* nem került elő a jelen vizsgálatok során sem. Az utóbbi rák a korábbi kutatások szerint (PONYI 1962) a vízinövények bevonatlakója, így hiánya megmagyarázható.

A *Limnocythere* hiánya az iszapfaunában azonban elgondolkoztató, mivel DADAY (uo. 165. o.) igen gyakorinak mondja a *Darwinula* társaságában. A *Limnocythere* genus fajai külső habitusra is olyan jellemző bélyegekkel rendelkeznek, hogy tévedésről aligha lehet szó.

A *3. táblázatban* foglaltuk össze azokat az adatokat, amelyek a Tihany előtti nyíltvíz iszapjára vonatkoznak. Ezek közül összehasonlító értékkel

Tabelle 3.

Die aus dem vor dem Institut befindlichen Bodenschlamm (A_1) des offenen Wassers notierten Krebsarten

3. táblázat

A MTA Biológiai Kutatóintézete (Tihany) előtti nyíltvíz iszapjából (A_1) feljegyzett rákfajok

Arten Fajok	SEBESTYÉN 1947*, Zeitpunkt des Sammelns: VI. 1945—X. 1946 SEBESTYÉN 1947* gyűjtési idő 1945 V.—1946 X.	ENTZ, PONYI, TAMÁS 1963 Zeitpunkt des Sammelns: VII. 1962 ENTZ, PONYI, TAMÁS 1963 gyűjt. idő 1962. VII.	PONYI 1966 Zeitpunkt des Sammelns: VI.—X—1965 PONYI 1966 gyűjt. idő 1965 VI.—X.
Cladocera			
Macrothrix laticornis	+	–	+
Alona quadrangularis	+	+	+
Alona affinis	+	–	–
Alonella rostrata	+	–	+
Leydigia acanthocercoides	+	–	+
Pleuroxus uncinatus	+	–	+
Monospilus dispar	+	–	–
Ostracoda			
Candona balatonica	–	–	+
Candona sp. *juv.* (nem balatonica)	–	–	+
Ilyocypris gibba	–	+	–
Darwinula stevensoni	–	+	+
Copepoda			
Paracyclops fimbriatus	–	+	+
Acanthocyclops sp.	–	–	+
Ectinosoma abrau	+	+	+
Nannopus palustris	–	–	+

* Sebestyén untersuchte nur *Cladocera*, an *Ectinosoma* wurde nur hingewiesen.
* SEBESTYÉN csak a *Cladocera-t* vizsgálta, az *Ectinosoma-ra* csak utal.

SEBESTYÉN (1947) és a mi jelenlegi adataink rendelkeznek, mivel az előbbi több mint egy éves, az utóbbi féléves gyűjtés eredménye. Értékelés szempontjából a *Cladocera*-k jöhetnek számításba. Azt látjuk, hogy 1945—46-hoz képest jelenleg csupán 2 faj *(Alona affinis, Monospilus dispar)* „hiányzik". A *Monospilus*-ról ismert, hogy egy és ugyanazon helyen mennyire változó a népsűrűsége (SEBESTYÉN 1965), így ezt a „hiányt" bizonyos fenntartással kell fogadni, míg újabb eredmények ismételten meg nem erősítik. Hasonlóképpen kell kezelni az *Alona affinis* „eltűnését" is, mivel csak újabban ismert azon rendszertani bélyeg, mely alapján az *affinis* biztosan elkülöníthető a *quadrangularis*-tól, továbbá, hogy a két formát biztosan külön fajként kell számon tartani (FREY 1959).

Tabelle 4.

Änderungen in der prozentuellen Zusammensetzung der Krebse am M_0 Punkt des Keszthelyer-Buchtes.

4. táblázat

A rákok összetételének %-os változása a Keszthelyi-öböl M_0 pontján

Ordo	1962		1965	
	máj. 9.	júl. 19.	jún. 9.	júl. 1.
Cladocera.....	0,0	6,2	24,0	24,7
Ostracoda	28,6	68,8	6,8	0,0
Copepoda.....	71,4	25,0	69,2	75,3

Ez ideig, a legrészletesebb adatok a Keszthelyi-öbölre (M_0) vonatkoznak. A fajok listáján kívül ismerjük azok %-os megoszlását is. 1962-ben a Cladocera csak néhány %-ban fordult elő, azonban 1965 hasonló időszakában lényegesen emelkedett a számuk. Ugyanakkor az *Ostracoda* viszonylagos mennyisége csökkent (4. táblázat). A *Copepoda*-k közül a *Paracyclops fimbriatus* 1962. máj.—júl.-ban jelentős szerepet töltött be a rákegyüttesben, 1965. jún.—júl.-ban jelentéktelen a száma, viszont az *Ectinosoma* egyedszáma nagymértékben megnőtt (vö. ENTZ, PONYI, TAMÁS 1963. p. 118—119). Ezek a tények, továbbá az *Acanthocyclops vernalis* — β-mesoszaprób szervezet — (RYLOW 1948) megjelenése a Balatonban, bizonyos változást jelez ugyan, mely valószínűleg a tó külső szennyeződésével függhet össze (SEBESTYÉN 1953, PONYI 1965). Annyi bizonyos, hogy jelentősebb minőségi változást nem tudtunk kimutatni, a mennyiségi változások észlelésére pedig nincsenek megfelelő összehasonlító adataink.

Összefoglalás

A szerző 1965. VI. 9. és X. 14. között a Balaton 5 különböző pontjáról gyűjtött nyíltvízi iszapmintákat vizsgálta meg abból a célból, hogy a fenéklakó rákokkal kapcsolatosan további adatokat szolgáltasson.

A vizsgálatok során 20, nem pelágikus fajt, ill. alfajt mutatott ki, melyek közül a *Latona setifera* (O. E. MÜLLER) Magyarország, az *Acanthocyclops vernalis vernalis* (FISCHER) és alfaja az *Acanthocyclops vernalis robostus* (SARS) pedig a Balaton faunájára újak.

A fajok mennyiségi megoszlása a vizsgálati idő alatt egy-egy gyűjtőhelyre vonatkozóan igen változó. Ez az egyedszám ingadozás a metodikai

hiányosságokon túlmenően a fajok népességdinamikai viszonyaiból, az egyenetlen eloszlásból, stb. adódik. Időbeli változások megfigyelése és leírása az egyes gyűjtőhelyeken a kis egyedszám miatt — az *Ectinosoma abrau* kivételével — nem lehetséges. Az átlagos értékek azonban rávilágítanak a Balaton iszapjában élő rákok heterogén horizontális megoszlására.

Leggazdagabbnak tűnik rákokban a Keszthelyi-öböl iszapja, legszegényebb a Szigligeti-öböl. Úgy látszik, hogy az iszaplakó rákok mennyisége szempontjából az ÉK-i medence egységesebb, mint a DNy-i.

A rákok zömét az *Ectinosoma* alkotja, mely egyedszámban jelentősen meghaladja a többit. A népességdinamikai viszonyai a tó különböző területein úgy látszik nem egyformák. Az ÉK-i medencében az egyedszám maximuma szeptemberben, a másik medencében októberben van.

Az *Entomostraca* mennyiségi megoszlása éppen fordított, mint a *Chironomida-ké* (Entz 1965). Míg az utóbbiak legnagyobb tömegben a Balaton középső részén, addig a rákok inkább a tó két végéhez közel eső területen találhatók. Fajgazdagság szempontjából is az előbbihez hasonló a helyzet. Míg a tó két végső szakaszán a fajok száma 12—17, addig a középső részen 7—9.

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ORIENTIERENDE UNTERSUCHUNGEN ÜBER DIE QUALITATIVEN UND QUANTITATIVEN VERHÄLTNISSE DER SCHLAMMBEWOHNENDEN KREBSE IM OFFENEN WASSER DES BALATON

Jenő E. Ponyi

Zusammenfassung

Autor untersuchte die zwischen dem 9. Juni und 14. Oktober 1965 aus fünf verschiedenen Punkten des Balaton gesammelte Schlammproben des offenen Wassers zu dem Zwecke, um weitere Angaben bezüglich der grundbewohnenden Krebse sammeln zu können.

Im Laufe seiner Untersuchungen bestimmte er 20, nicht pelagische Arten beziehungsweise Unterarten, von denen *Latona setifera* (O. E. Müller) für die Fauna Ungarns, *Acanthocyclops vernalis vernalis* (Fischer), sowie deren Unterart, *Acanthocyclops vernalis robustus* (Sars) für die Fauna des Balaton-Sees neu sind. —

Die quantitative Verteilung der Arten war während der Zeit der Untersuchungen (9. Juni—1. Oktober 1965) hinsichtlich je einer Sammelstelle recht verschieden. Diese Schwankung der Individuenzahl ergibt sich über die metodischen Mängel hinaus, aus den populationsdynamischen Verhältnissen, der unregelmässigen Verteilung u. s. w.

(HANKÓ 1926, SEBESTYÉN 1965). Diese Tatsachen lenken alle die Aufmerksamkeit darauf hin, dass man aus den zahlenmässigen Angaben der verschiedenen Krebsarten nur sehr vorsichtig Schlüsse ableiten dürfe. Eine Beobachtung und Beschreibung der quantitativen Veränderung in den einzelnen Sammelstellen — ausser den obenerwähnten — ist auch wegen der geringen Individuenzahl nicht möglich; eine Ausnahme bildet bloss *Ectinosoma abrau*, worauf wir später noch zurückkommen wollen. Aus diesem Grunde können wir bloss die auf fünf Monate bezogenen Durchschnittswerte angeben, welche einigermaassen ein Bild der quantitativen Verhältnisse der Krebse bieten. Die können wir umso eher tun, als der aktive Lebensabschnitt der Mehrzahl der untersuchten Arten innerhalb des erwähnten Zeitraumes fällt (SEBESTYÉN 1947, 1948, und eigene Beobachtungen). Im Laufe der fünf vorgenommenen Sammlungen haben wir für jede Sammelstelle etwa 6 dm^2 Schlammoberfläche gewählt und die darauf entfallende Krebsenmenge für je 1 dm^2 umgerechnet. Daraus ergab sich, dass der Schlamm der Bucht von Keszthely (M_0) am meisten reich an Krebsen ist (46 Stück pro dm^2), dagegen die Bucht von Szigliget am ärmsten erscheint (K), wo deren Anzahl auf 14 Stück je dm^2, herabsinkt; an der nächsten Sammelstelle (G) steigt diese Anzahl wieder auf 25 Stück je dm^2 an. Auffallend ist es, dass die Gesamtanzahl an zwei Punkten des Nordostbeckens (in A_1 = 34 Stück je dm^2, in E_e = 34 Stück je dm^2) übereinstimmte *(Abb. 2.)*. Wie es scheint, ist das Nordost-Becken hinsichtlich der schlammbewohnenden Krebse einheitlicher und, — ausgenommen die Bucht von Keszthely, — auch reicher, als das Südwest-Becken.

Aus der *Abbildung Nr. 2* ist zu entnehmen, dass *Ectinosoma abrau* quantitativ die Menge der Cladoceren und Ostracoden an Individuenzahl bedeutend übertrifft. Recht interessant gestaltet sich die Anzahl von *Ectinosoma* an den einzelnen Sammelstellen, sowie hinsichtlich der beiden Becken. Es scheint, dass die populationsdynamischen Verhältnisse von *Ectinosoma* sich im den beiden Becken des Sees verschiedentlich gestalten. An beiden ufernahen Sammelstellen des Nordost-Ufers (A_1, E*e*) war die maximale Individuenzahl am 7. September, während wir dieses Maximum am 13. Oktober an 3 Punkten des Südwest-Beckens (M_0, G, K) feststellen. Interessant ist es auch, dass die quantitative Verteilung der Mikrocrustaceen gerade eine entgegengesetzte Tendenz aufweist, als die der Chironomiden (ENTZ 1965). Während die letzteren in grössten Mengen in den Mittelteilen des Sees und in kleinsten Mengen in der Bucht von Keszthely beziehungsweise im Nordost-Becken vorkommen, gestaltet sich das Verhältnis der Krebse gerade umgekehrt. Es ist möglich, dass sich diese Erscheinung aus den Bodensediments-Verhältnissen erklären lässt (ibid. Seite 133).

Die Gestaltung der qualitativen Verhältnisse stimmt im Wesentlichen mit den obigen Ausführungen überein. An Arten erweist sich der mittlere Untersuchungsabschnitt des Sees am ärmsten, wogegen die Bucht von Keszthely und das nordöstliche Seebecken daran wesentlich reicher sind *(siehe Tabelle 2.)*. Die perzentuelle Verteilung der Krebsarten ist in den einzelnen Sammelgebieten verschieden. Als einzige Ausnahme ist *Ectinosoma* zu nennen, welche innerhalb der Krebsgemeinschaften genügend einheitlich anzutreffen ist.

Bei Betrachtung der Tabelle 2. scheint es, dass das Eprofundal des Balaton hinsichtlich der grundbewohnenden Krebsarten in drei Teile zerfällt, und zwar:

1. in das mittlere, artenarme Gebiet des Balaton, welches wir durch *Darwinula stevensoni*, *Candona* sp. und die Gemeinschaft von *Monospilus dispar* charakterisieren können;

2. das Nordost-Becken, welches bereits reicher an Arten ist. Charakteristische Gemeinschaften sind: *Alona quadrangularis*, *Macrothrix laticornis*, *Nannopus palustris*;

3. die Bucht von Keszthely, welche am besten durch *Leydigia acanthocercoides* und Vertreter des genus *Acanthocyclops* gekennzeichnet werden kann.

Bisher beziehen sich die am meisten detaillierten Angaben auf die Bucht von Keszthely (M_0). Ausser der Liste der Arten ist uns auch deren perzentuelle Verbreitung bekannt. Im Jahre 1962 fand sich Cladocera bloss zu einigen Perzenten, doch stieg ihre Anzahl in demselben Zeitraum des Jahres 1965 wesentlich an. Die verhältnismässige Anzahl von Ostracoden verminderte sich in derselben Zeit *(Tabelle 4.)*. Unter den Copepoden spielte *Paracyclops fimbriatus* im Mai — Juni 1962 eine bedeutende Rolle in der Krebsgemeinschaft; im Juni—Juli 1965 ist ihre Anzahl unbedeutend, dafür stieg die Individuenzahl von *Ectinosoma* stark an (Vgl. ENTZ, PONYI, TAMÁS 1963, p. 118—119). Diese Tatsachen, sodann das Erscheinen von *Acanthocyclops vernalis* (RYLOW 1948) — einem ß-mesosaproben Organismus — im Balaton bedeuten zwar eine gewisse Veränderung, die jedoch aller Wahrscheinlichkeit nach (SEBESTYÉN 1953, PONYI 1965). Aus der Umwelt stammenden Verunreinigung des Sees zurückzuführen ist.

КАЧЕСТВЕННЫЕ И КОЛИЧЕСТВЕННЫЕ ОРИЕНТИРОВОЧНЫЕ ИССЛЕДОВАНИЯ РАЧКОВ, ЖИВУЩИХ В ИЛЕ ОТКРЫТОЙ ЧАСТИ БАЛАТОНА

Йенэ Поньи

Образцы ила, собранные в пяти разных точках открытой части Балатона в период с 9 июня по 14 октября 1965 г, обрабатывались для получения дальнейших данных о рачках, обитающих в иле.

Обнаружено 20 непелагических видов либо разновидностей, среди которых *Latona setifera* (O. F. Müller) — новая форма для фауны Венгрии и *Acanthocyclops vernalis vernalis* (Fischer) *Acanthocyclops vernalis robustus* (Sars) — новая форма для Балатона.

Количество рачков в течение периода изучения сильно менялось для каждой из точек. Малая численность собранных рачков не позволяет количественно описать изменения во времени для всех видов, кроме *Ectinosoma abrau*. Все же полученные материалы свидетельствуют о значительной неоднородности горизонтального распределения рачков.

Наиболее богат рачками ил Кестхейского залива и наиболее беден Сиглигетский залив. Северо-восточный бассейн представляется более однообразном в видовом отношении, чем юго-западный.

По численности особей *Ectinosoma* значительно превышает остальные виды. Динамика численности в разных точках озера различна. В северо-восточном бассейне численность максимальна в сентябре, а в другом бассейне в октябре.

Количественное распределение рачков *Entomostraca* обратное по сравнению с *Chironomida* (Entz 1965). Последних больше всего в средней части Балатона, в то время как рачков — у обоих концов озера. По видовому разнообразию картина сходная: у концов озера имеется по 12—17 видов, а в средней части лишь 7—9.

Настоящие данные лишь в небольшой части сравнимы с прежними, так как прежде изучалась лишь часть Кестхейского залива. В популяции рачков не найдено количественных изменений, которые можно было бы связать с влиянием пестицидов.

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TÁJÉKOZÓDÓ JELLEGŰ ALGOLÓGIAI VIZSGÁLATOK A BALATON FENÉKISZAPJÁN AZ 1965. ÉVI GYŰJTÉSEK ALAPJÁN

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Érkezett: 1966 március 15-én

Ez a tanulmány csatlakozik azokhoz a korábban megjelent munkákhoz, melyek a Balaton mikrofitobentoszára és zoobentoszára vonatkozó adatokat foglalták magukba (DADAY 1897; PANTOCSEK 1902, 1902a; HANKÓ 1926; MOON 1934; KOL 1938; SEBESTYÉN 1947; ENTZ—TAMÁS 1952; ENTZ 1954, 1965; ENTZ—PONYI—TAMÁS 1963).

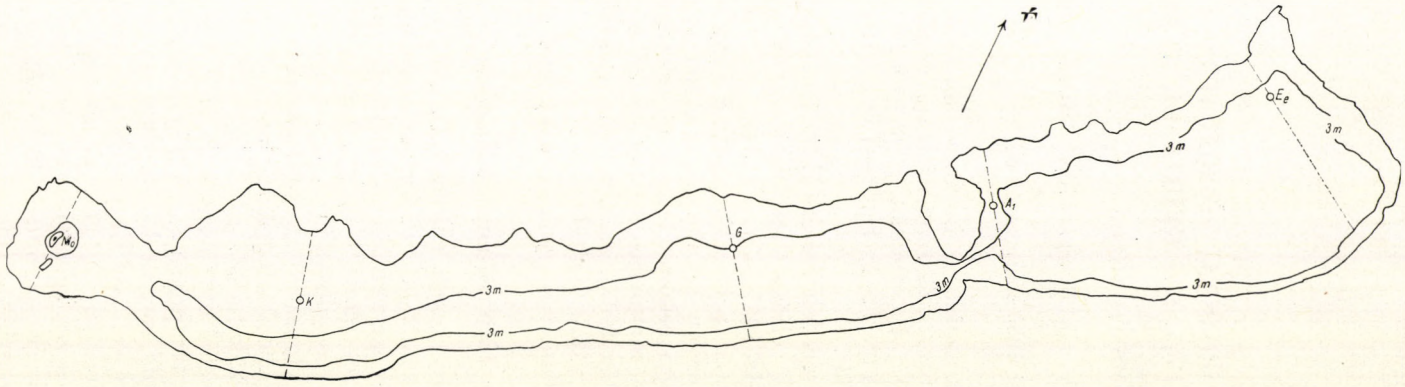
A mikrofitobentosz mennyiségi felmérésére ENTZ BÉLÁval közösen 1952. év első felében igen érdekes megfigyeléseket tettünk a tó több pontjáról gyűjtött iszapmintákon (ENTZ—TAMÁS 1952; ENTZ 1954).

A tó legdélnyugatibb területén, a Keszthelyi-öblöt észak—déli irányban átszelő szelvény mentén 1962. évben munkaközösség keretében vizsgáltuk az üledék szemcsenagyságát, vegyi összetételét, az üledék felső 40 cm-ében levő kagylóhéjak megoszlását, a microcrustaceakat és az epipelikus kovamoszatokat (ENTZ—PONYI—TAMÁS 1963). Az algológiai vizsgálatok minőségi jellegűek voltak, az előfordulás gyakoriságát betűkkel (e = előfordul egy-két példányban; k = kevés példányban; s = gyakori, sok) jelöltük.

Gyűjtések helye, ideje és anyagfeldolgozás

Az 1965. év tavaszán bekövetkezett nagyméretű balatoni halpusztulás nyomán a pusztulás körülményeinek és biológiai hatásainak kutatására májusban a Hidrobiológiai Osztály szervezésében több iszapmintát gyűtöttünk a Tihany, Biológiai Kutatóintézet előtti (A_1) gyűjtőhelyről (kb. 500 m). A korábbi évek iszapmintáival összehasonlítva azt tapasztaltuk, hogy a jelenlegi minták sokkal több elpusztult kovamoszatvázat tartalmaztak. Éppen ezért az eddigi minőségi jellegű vizsgálatok helyett, most már mennyiségi meghatározások váltak szükségessé.

Az iszapmintavételek júniustól októberig a tó DNy-i részének három pontján, az ÉK-i részének pedig két pontján, a tükör hossztengelyére merőleges harántszelvényeken *(1. ábra)* történtek (vö. SEBESTYÉN 1960). A vízmélység mindig legalább 3 m-es volt. Az EKMAN iszapmarkolóval felszinre hozott anyag vékony felületi rétegéből $\sim$ 114 cm^2, mindig egyforma mennyiséget emeltünk ki algológiai és zoológiai (PONYI 1966) meghatározás céljából. Az állatok kiválogatása után az iszapmintát 48 órán át ülepedni hagytuk. A korábbi mikrofitobentosz mennyiségi vizsgálatoknál bevált módszert ENTZ—



1. ábra. A Balaton vázlatos térképe a mintavételi helyek megjelölésével M_0 = Gyenesdiás — Zala folyó torkolata, Gyenesdiás parttól kb 2800 m. K = Szigliget — Balatonmária, az északi parttól kb 4000 m. G = Ságpuszta — Balatonszemes, az északi parttól kb 2500 m. A_1 = Balatonfüred, Fenékfürdő — Zamárdi alsó, Tihany, Biológiai Kutatóintézet előtt kb. 500 m. E_e = Balatonalmádi — Balatonvilágos, északi parttól kb 2000 m.

Abb. 1. Skizzenhafte See-Karte des Balaton, mit Bezeichnung der Probenentnahme- Stellen. M_0 = zwischen Gyenesdiás und der Mündung des Zala-Flusses, cca 2800 m vom Nordufer; K = zwischen Szigliget und Balatonmária, cca 4000 m vom Nordufer; G = zwischen Ságpuszta und Balatonszemes, cca 2500 m vom Nordufer; A_1 = zwischen Balatonfüred—Fenékfürdő und Zamárdi, cca 500 m vor dem Biologischen Forschungsinstitut; E_e = zwischen Balatonalmádi und Balatonvilágos, cca 2000 m vom Nordufer; É = Nord

Tamás 1952; Entz 1954) alkalmaztuk kisebb módosítással. A 48 órán át ülepedett mintákból ötször 1 cm² iszapfelület valamennyi (élő és elpusztult) algáját feljegyeztük és az így kapott adatot számítottuk át 1 dm² felületre.

A harántszelvények helyzetét és egyéb gyűjtési adatokat az *1. táblázat* tartalmazza.

Vizsgálati eredmények

Az 5 gyűjtőhely 25 iszapmintájából meghatározott algafajok a gombával együtt az alábbi 6 nagy rendszertani törzsbe tartoznak, gyakorisági sorrendben:

	faj	*változat*
Chrysophyta	59	3
Chlorophyta	18	4
Cyanophyta	8	—
Euglenophyta	6	—
Pyrrophyta	2	—
Mycophyta	1	—
Összesen	94	7

A Chrysophyta törzs két osztályának (*Xanthophyceae* 1, *Bacillariophyceae* 61) fajai voltak képviselve. A mintákban a *Bacillariophyceae* osztály iszaplakó fajai közül néhány helyenként igen nagy népességet ért el: *Fragilaria construens, Amphora ovalis, Fragilaria pinnata, Diploneis elliptica, Nitzschia amphibia,* stb. A tó nyiltvizéből jól ismert pelagikus fajok közül a *Melosira granulata, M. granulata* var. *angustissima, Cyclotella bodanica, C. ocellata, Nitzschia acicularis, Cymatopleura elliptica, C. solea, Asterionella formosa, Stenopterobia pelagica, Synedra acus* var. *angustissima* helyenként nagy népességben fordult elő az iszapmintákban.

A tó parti kövein (Felföldy 1958; Tamás 1958a; Tamás—Gellért 1958, 1959), nádon és hínárleveleken (Entz 1943; Tamás 1963, 1964) nagyobb népességben előforduló fajok közül a *Cocconeis placentula, Cymbellak, Diatomak, Epithemiak, Gomphonema olivaceum, Melosira varians, Rhoicosphenia curvata* szórványosan fordult elő a mintákban.

A *Synedra parasitica* leggyakrabban a Balaton nyiltvizében csaknem állandóan jelen levő nagy termetű *Cymatopleurákat* és a *Nitzschia sigmoideakat* használja fel megtelepedésre (Tamás 1965, 232o). Az epifita *Synedra parasitica* elpusztult vázai szórványosan szerepeltek.

Az iszapminták adatai alapján a *Chrysophyta* törzs helyenkénti százalékos előfordulása 34—50% között változott. Az összalgafajszámnak 61%-át teszi ki a törzs.

Gyakoriság szempontjából a *Chlorophyta* törzs foglalja el a második helyet. A fajok túlnyomó többsége a *Chlorococcales* rendbe tartozik. Csaknem kivétel nélkül valamennyi a nyiltvíz lakója. Az iszapmintákban helyenként nagyobb népességben csupán a *Crucigenia, Dictyosphaerium,* az *Ankistrodesmusok* és a *Scenedesmus quadricauda* szerepelt. Néhány mintában a *Pediastrum clathratum* elpusztult sejtjeinek száma igen jelentős volt.

Az adatok alapján a *Chlorophyta* törzs helyenkénti százalékos előfordulása 8—12% között változott. Az összalgafajszámnak 22%-át teszi ki a törzs.

A *Cyanophyta* törzs 8 faja közül a pelagikus *Lyngbyak* csaknem valamennyi mintában, helyenként jelentős népességben fordultak elő (l. 2. táblázat adatait).

A *Cyanophyta* törzs helyenkénti százalékos előfordulása az iszapminták adatai alapján 4—6% között változott. Az összalgafajszámnak 8%-át teszi ki a törzs.

Az *Euglenophyta* törzset csupán 6 faj képviselte a mintákban. Ezek előfordulása szórványos, népessége pedig igen alacsony volt. A törzs helyenkénti százalékos előfordulása 0—3% között változott. Az összalgafajszámnak 6%-át teszi ki a törzs.

A *Pyrrophyta* törzs *Dinophyceae* osztályának 2 képviselője csupán a Ságpuszta — Balatonszemes (G) júniusi iszapmintában fordult elő. A törzs az összalgafajszámnak 2%-át teszi ki.

A *Mycophyta* törzs egyetlen képviselője a *Dactylosporium* sp. szórványosan fordult elő. Ez a faj gyakoribb a tó nyiltvizében (TAMÁS 1965) és a parti öv detrituszturzásaiban. Az összalgafajszámnak 1%-át teszi ki a *Mycophyta* törzs.

Az 5 gyűjtőhely közül algafajokban a leggazdagabb a tihanyi Biológiai Kutatóintézet (A_1) előtti (72), a legszegényebb pedig (52) a Ságpuszta — Balatonszemes közötti (G) iszapminta volt.

Az 5 gyűjtőhely 25 iszapmintájából meghatározott 101 féle moszat közül élő állapotban csupán egy-egy gyűjtőhely mintájában igen alacsony egyedszámban fordultak elő az alábbi fajok:

Anabaena spiroides KLEBS, Ee IX. 150/dm²;
Aphanothece clathrata var. *brevis* BACHM., K VI, 6200/dm², A_1 VII, 21700/dm²;
Gomphosphaeria lacustris CHOD., K VII, 12400/dm², G VII, 3100/dm²;
Euglena ehrenbergii KLEBS, K VIII, 150/dm²;
E. oxyuris SCHMARDA, A_1 VI, 150/dm², E_e VII, 310/dm²;
Euglena sp., A_1 VII, 460/dm²;
Phacus sp., A_1 IX, 150/dm²;
Ceratium hirundinella (O. F. MÜLL.) SCHRANK, G VI, 460/dm²;
Peridinium inconspicuum LEMM., G VI, 150/dm²;
Ankistrodesmus falcatus var. *aciculare* (A. BRAUN) G. S. WEST, K VI, 310/dm²;
A. falcatus var. *mirabile* W. & G. S. WEST, G VI, 150/dm², E_e IX, 150/dm²;
A. lacustris (CHOD.) OSTENF., M_0 VI, 770/dm², E_e VI, 150/dm²;
Chodatella balatonica SCHERFFEL, K VI, 150/dm², G VI, 150/dm²;
C. quadriseta LEMM., M_0 VI, 150/dm²;
Kirchneriella lunaris (KIRCHN.) MOEBIUS, K VI, 150/dm²;
K. obesa (W. WEST) SCHMIDLE, M_0 VI, 150/dm², A_1 IX, 150/dm²;
Oocystis solitaria WITTR., A_1 VI, 310/dm²;
O. submarina LAGERH., A_1 VII, 310/dm²;
Scenedesmus intermedius var. *acaudatus* HORTOB., A_1 VI, 310/dm²;
S. quadricauda var. *quadrispina* (CHOD.) G. M. SMITH, E_e VI, 310/dm²;
S. tenuispina CHOD., M_0 VI, 310/dm²;
Planktonema lauterbornii SCHMIDLE, G VII, X, 150/dm², A_1 IX, 150/dm²;
Campylodiscus noricus var. *hibernica* (EHR.) GRUN., A_1 VI, X, 150/dm², E_e VIII, 150/dm²;
Gyrosigma prolongatum (W. SMITH) CLEVE, M_0 VIII, X, 150/dm², K IX, 150/dm²;
Nitzschia hungarica GRUN., M_0 X, 1080/dm²;
N. tryblionella var. *levidensis* (W. SMITH) GRUN., K VI, 310/dm², G VI, 150/dm²;
Nitzschia sp., E_e VI, 150/dm²;
Dactylosporium sp., K VIII, 150/dm², G IX, 150/dm², E_e VI, 150/dm²;
Rajzó spórák, M_0 X, 460/dm², K X, 310/dm².

Az iszapminták meghatározása során az alábbi néhány algafajnak csupán az elpusztult vázát tudtuk feljegyezni:

Amphora ovalis var. *pediculus* KÜTZ., M_0 VIII, 150/dm², G VI, 310/dm²;
Diatoma elongatum var. *tenuis* (AG.) V. HEURCK, G VI, 150/dm², A_1 VI, 150/dm²;
Epithemia hyndmanni W. SMITH, M_0 VIII, 150/dm², A_1 VI, 150/dm²;
Gomphonema olivaceum (LYNGB.) KÜTZ., M_0 IX, 150/dm², K VI, 150/dm², A_1 VIII, 150/dm²;
Gyrosigma acuminatum (KÜTZ.) RABENH., A_1 VII, X, 150/dm²;
Melosira varians C. A. AG., M_0 X, 310/dm²;
Navicula pupula KÜTZ., A_1 VII, 150/dm², E_e VI, 150/dm²;
N. scutelloides W. SMITH, A_1 IX, 310/dm²;
N. tuscula (EHR.) GRUN., K VIII, 150/dm², A_1 IX, 150/dm²;
Rhoicosphenia curvata (KÜTZ.) GRUN., K VII, 150/dm²;
Surirella elegans EHR., A_1 VIII, 150/dm²;
S. tenera GREG., E_e VII, 310/dm²;
Synedra capitata EHR., M_0 VI, 150/dm²;
Pediastrum boryanum (TURP.) MENEGH., G VI, 150/dm², VII. 2480/dm².

A *Melosira granulata* és *M. granulata* var. *angustissima* a Keszthelyi-öböl, Szigliget — Balatonmária és Ságpuszta — Balatonszemes között az augusztusi nyiltvízben tömeges fellépése következtében vízszíneződést idézett elő (v. ö. SEBESTYÉN 1949). Ez a jelenség az iszap felületi rétegére is hatással volt. Ebben az időben a Keszthelyi-öböl, és Szigliget — Balatonmária iszapmintáiban az összes élő kovamoszatszámnak mintegy háromnegyede *Melosira*. A gyűjtés időpontjában (augusztus 3-án) a vízszíneződés a Keszthelyi-öbölben feltehetően már túlhaladta a maximumot, míg a másik két gyűjtőhelyen (K és G) éppen a csúcsponton volt. Igazolja a feltevést az egyes gyűjtőhelyek iszapfelületén talált élő és elpusztult *Melosirák* számaránya (M_0 az elpusztult vázak fele *Melosira*, K és G, mintáiban az összes elpusztult vázaknak csupán a negyede *Melosira*).

A szeptemberi és októberi adatoknál az összes kovamoszatok (élők és elpusztultak is) háromnegyedét *Melosira* adja. A tó ÉK-i részére a *Melosira* vízszíneződés nem terjedt ki. Ugyancsak szeptemberben a Keszthelyi-öböl és Szigliget közötti részén a tónak *Microcystis flos-aquae — Aphanizomenon flos-aquae* var. *klebahnii* szórt vízvirágzás (sensu HORTOBÁGYI 1962) zajlott le (v. ö. TAMÁS 1965a). Az iszapmintákban ekkor az élő kovamoszatokon kívül csak nagyon csekély számban jegyezhettünk fel más élő algákat *(Oscillatoria, Lyngbya)*. Az elpusztult algákra vonatkozó adatok között pedig *Microcystis flos-aque* és *Aphanizomenon flos-aquae* var. *klebahnii* szerepelt kizárólag. A már előbbiekben említett *Melosira* vízszíneződéshez hasonlóan, a *Microcystis-Aphanizomenon* vízvirágzás is csupán a tó DNy-i részének Keszthelyi-öblére és az ahhoz legközelebb eső területre terjedt ki.

Helyenként egy-egy mintában nagy számban fordult elő a *Fragilaria construens* (Keszthelyi-öböl VI; Szigliget VII. feltűnő sok volt a Tihanyi Intézet előtti VIII, IX, X mintákban). Az *Amphora ovalis* is helyenként meglehetősen nagy népességben szerepelt *(l. 2. táblázat* adatait). Ez utóbbi faj tihanyi május-júniusi adatairól meg kell jegyezni, hogy az élő példányok számának csaknem fele nagyon halvány sárgaszínű, igen lassan mozgó, másik része pedig élénk sötét, szinte barnás árnyalatú és jól mozgó volt.

Az összesített *(3. táblázat)* adatokból jól látható, hogy az élő kovamoszatok száma a Keszthelyi-öböl szeptemberi iszapmintájában a legmagasabb (116400/dm²), míg a legalacsonyabb értéket a Balatonalmádi — Balatonvilágos októberi (2910/dm²) mintában találtuk. Helyenként és időnként a mintákban jelenlevő élő kovamoszatok és elpusztult kovavázak aránya a korábbi évek vizsgálati adtaihoz (ezideig még nem közölt eredmények) képest eltérő. A pár

évvel ezelőtti (Keszthelyi-öböl, stb.) felvételek adataiból és az újabb vizsgálatokból kitűnt, hogy a régebbi mintákban az élők száma többszörösen meghaladta az elpusztultak számát. Az 1965. évben végzett vizsgálatokból látható, hogy helyenként és időnként a számarány az élő és elpusztult között néha 1 : 1, de előfordult 1 : 2 is. A legszembetűnőbb valamennyi között a Balatonalmádi — Balatonvilágos közötti októberi minta volt (1 : 10,8).

A más algatörzsekhez tartozó élő és elpusztult számarányok között is találunk néhány nagyon meglepő adatot *(l. 3. táblázat)*.

Összefoglalás

Szerző az 1965 év tavaszán bekövetkezett nagyméretű balatoni halpusztulás nyomán a pusztulás körülményeinek és biológiai hatásainak kutatására júniustól — októberig a Hidrobiológiai Osztály szervezésében a tó DNy-i részének három pontján, az ÉK-i részének pedig két pontján a tükör hossztengelyére merőleges harántszelvényeken *(l. 1 ábra)* gyűjtött iszapmintákat (gyűjtési adatokat l. *az 1. táblázaton)*. Az Ekman iszapmarkolóval felszínre hozott anyagból, mindig a vékony felületi rétegből ~ 114 cm^2. mennyiséget emeltek ki algológiai és zoológiai (Ponyi 1966) meghatározásra. Az állatok kiválogatása után a mintát 48 órai ülepedés után a korábbi mikrofitobentosz vizsgálatoknál bevált módszer (Entz—Tamás 1952; Entz 1954) kisebb módosításával alkalmazta. A 48 órán át ülepedett mintákból ötször 1 cm^2 iszapfelület valamennyi (élő és elpusztult) algáját feljegyezte és az így kapott adatokat számította át 1 dm^2 iszapfelületre.

Az 5 gyűjtőhely 25 iszapmintájából meghatározott 94 faj és 7 változat a gombával együtt 6 nagy rendszertani törzsbe tartozik, gyakorisági sorrendben: *Chrysophyta* 62, *Chlorophyta* 22, *Cyanophyta* 8, *Euglenophyta* 6, *Pyrrophyta* 2, *Mycophyta* 1.

Az 5 gyűjtőhely közül algafajokban a leggazdagabb a tihanyi (A_1) Biológiai Kutatóintézet előtti (72), a legszegényebb pedig (52) a Ságpuszta — Balatonszemes közötti (G) minta volt.

Az augusztusi iszapminták gyűjtésekor a nyiltvízi hálós- és merített mintákban a *Melosira granulata* és a *M. granulata* var. *angustissima* tömeges fellépése vízszíneződést idézett elő. Ez a jelenség az iszap felületi rétegére is kihatott. Ebben az időben a Keszthelyi-öböl és Szigliget — Balatonmária iszapmintáiban az összes élő kovamoszatszámnak mintegy háromnegyede a *Melosira*. Szerző feltételezi, hogy az augusztusi gyűjtés időpontjában a *Melosira* vízszíneződés a Keszthelyi-öbölben már túlhaladta a maximumot, míg a másik két gyűjtőhelyen (Szigliget — Balatonmária és Ságpuszta — Balatonszemes között) éppen a csúcsponton volt. Feltevését igazolja az egyes gyűjtőhelyek iszapfelületéről meghatározott élő és elpusztult *Melosirák* számaránya *(l. 2. táblázat élő és váz adatait)*. A szeptemberi és októberi adatoknál az összes kovamoszatok (élő és elpusztult is) háromnegyede *Melosira*.

A Keszthelyi -öböl és Szigliget között a szeptemberi gyűjtések idején a nyiltvízben *Microcystis flos-aquae* és *Aphanizomenon flos-aquae* var. *klebahnii* szórt vízvirágzás jelenségét figyelték meg. Az iszapmintákban ekkor az élő kovamoszatokon kívül más élő alga nagyon csekély számban fordult elő *(Oscillatoria, Lyngbya)*, az elpusztult adatok között pedig a *Microcystis flos-aquae* és az *Aphanizomenon flos-aquae* var. *klebahnii* szerepelt kizárólag.

1. Táblázat — Tabelle 1

Az 1965. évi gyűjtések helye, ideje és egyéb adatai

Ort und Zeitpunkt der Probenentnahme in 1965 usw.

Gyűjtőhelyek	Időpont	Vízhőfok C°	Levegőhőfok C°	Vízmélység, cm	*Átlátszóság, cm	Megjegyzés
M_0	VI. 9. 7 h	18	17,8	300	45	
	VII. 1. 7 „	22	23	290	80	
	**VIII. 3. 7 „	20	21	300	39	A víz színe barnás árnyalatú volt
	+ IX. 7. 8 „	18	19	276	62	
	X. 13. 7 „	13	6	250	114	
K	VI. 9. 12 h	19,5	18	420	36	
	VII. 1. 9 „	24	25	380	40	
	** VIII. 3. 9 „	20	23	367	35	A víz színe sárgás árnyalatú volt
	+ IX. 7. 10 „	19	21,5	365	51	
	X. 13. 11 „	13	16	330	66	
G	VI. 9. 16 h	18,5	20	420	42	
	VII. 1. 16 „	27	32	400	92	
	** VIII. 3. 16 „	21	25	396	56	A víz színe sárgás árnyalatú volt
	IX. 7. 16 „	19	24	400	50	
	X. 13. 16 „	15	15	370	95	
A_1	VI. 10. 16 h	19	18,5	350	50	
	VII. 2. 15 „	24	24	355	92	
	VIII. 4. 15 „	22	26	335	51	
	IX. 8. 15 „	20	24	371	86	
	X. 14. 15 „	14	18	370	120	
E_e	VI. 10. 18 h	19	18,5	327	64	
	VII. 2. 18 „	23	25	330	66	
	VIII. 4. 18 „	21	26	373	64	
	IX. 8. 19 „	20	20	280	—	
	X. 14. 18 „	14	16	320	—	

Rövidítések:

Gyűjtőhelyek = Sammelstellen. Időpont = Zeitpunkt. Vízhőfok, C° = Wassertemperatur. Levegőhőfok, C° = Lufttemperatur. Vízmélység, cm = Wassertiefe. *Átlátszóság, cm = Durchsichtigkeit. Megjegyzés = Bemerkungen. A víz színe barnás árnyalatú volt = Die Farbe des Wassers war braunlich. A víz színe sárgás árnyalatú volt = Die Farbe des Wassers war gelblich. Jelmagyarázat = Zeichenerklärung.

* Mérések SECCHI-koronggal = Die Werte beziehen sich auf Messungen mit der Secchischeibe.

** Merített és hálóplanktonmintákban a *Melosira granulata* és a *M. granulata* var. *angustissima* tömeges fellépése következtében vízszíneződés = Das massenhafte Auftreten von *Melosira granulata* und *Melosira granulata* var. *angustissima* verursachte im offenen Wasser entnommenen Schöpf- und Netzfilterproben eine Vegetationsfärbung.

+ Merített és hálóplanktonmintákban *Microcystis flos-aquae* és *Aphanizomenon flos-aquae* var. *klebahnii* szórt vízvirágzás = Das massenhafte Auftreten von *Microcystis flos-aquae* und *Aphanizomenon flos-aquae* var. *klebahnii* verursachte im offenen Wasser das Erscheinen zerstreuter Wasserblüte.

2. Táblázat — Tabelle 2

A Balaton nyíltvízi fenékiszapmintáinak mennyiségi algológiai adatai az 1965. évi gyűjtések alapján (i/dm² = egyedszám/dm² × 1000) = Quantitative Mikrophytobentosuntersuchungen aus dem Eprofundal des Balaton-Sees auf Grund der Sammlungen des Jahres 1965 (i/dm² = Individuenzahl pro dm² × 1000)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
CYANOPHYTA											
Aphanizomenon flos-aquae var. *klebahnii* Elenk.	VI.	0,1	0,7	0,1	1,0	0,3	—	0,4	0,6	0,1	0,7
	VII.	—	1,2	—	0,3	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	0,7	—	—
	IX.	—	0,4	—	0,1	—	0,1	—	0,1	—	0,1
	X.	—	0,3	—	—	—	—	—	—	—	—
Coelosphaerium kützingianum Naeg.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	3,1	—	—	—	12,4	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,3	—	0,3	—
	X.	—	—	—	—	—	—	—	—	0,1	—
Lyngbya circumreta G. S. West	VI.	0,3	0,3	0,1	0,1	0,1	0,3	0,3	0,3	0,6	1,5
	VII.	0,6	—	0,3	—	0,4	0,3	0,3	—	0,6	0,6
	VIII.	0,1	0,1	—	0,3	0,7	0,9	0,6	0,9	0,4	1,0
	IX.	—	—	0,3	0,3	0,3	0,3	1,0	1,5	0,1	1,8
	X.	—	—	0,1	—	0,3	—	0,6	1,0	1,5	2,3
Lyngbya limnetica Lemm.	VI.	0,1	—	0,1	—	0,4	—	—	—	0,7	—
	VII.	—	—	0,1	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	0,3	—	—	—	0,3	—	0,3	0,1
	X.	—	—	0,4	—	0,1	—	—	0,4	—	0,1
Oscillatoria amphibia Ag.	VI.	0,1	—	—	—	0,4	—	0,1	—	—	—
	VII.	—	—	—	—	0,1	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	0,1	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	0,1	—
EUGLENOPHYTA											
Euglena limnophila var. *minor* Drez.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	0,6	0,4
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—
Trachelomonas volvocina Ehr.	VI.	0,1	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	0,1	0,4	—	—	—	—	—	—	—	—
CHRYSOPHYTA											
Amphora ovalis Kütz.	VI.	0,9	0,3	7,7	2,3	0,3	0,6	18,5	1,8	1,2	0,7
	VII.	0,7	3,1	1,2	3,4	0,6	1,0	7,7	3,4	1,5	3,8
	VIII.	1,8	0,3	1,2	1,8	0,3	0,3	1,8	3,1	1,0	0,3
	IX.	1,5	0,6	1,5	0,6	4,0	1,5	8,5	3,5	2,0	1,0
	X.	2,6	2,3	5,4	2,0	0,3	0,4	5,1	5,5	0,1	1,8

2. Táblázat (folytatás) — Tabelle 2 (Fortsetzung)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
Asterionella formosa	VI.	—	—	—	—	—	—	—	—	—	—
Hassal	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	0,7	0,9	0,4	—	—	—	0,1	—	—	—
	IX.	—	0,1	—	0,1	—	—	—	—	—	—
	X.	—	0,9	—	—	—	—	—	—	—	—
Caloneis schumanniana	VI.	—	—	0,3	—	—	0,3	0,4	0,1	—	—
var. *biconstricta*	VII.	—	0,3	—	—	—	—	—	—	—	—
Grun.	VIII.	—	—	—	—	—	—	0,1	0,3	0,1	0,1
	IX.	0,1	—	—	—	—	—	—	0,6	0,3	0,4
	X.	—	—	—	—	—	—	—	0,1	—	0,9
Cocconeis placentula	VI.	—	—	—	—	—	—	—	—	—	—
Ehr.	VII.	1,0	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	0,4	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	0,1	—	—	—	—	—	—	—	—
Cyclotella bodanica	VI.	—	0,7	—	0,7	—	2,4	—	1,0	0,3	1,5
Eulenst.	VII.	—	3,8	—	2,3	0,3	3,1	0,1	0,7	—	1,2
	VIII.	0,3	1,5	0,6	0,4	0,1	1,7	—	1,8	—	0,3
	IX.	—	1,7	—	0,3	—	0,6	1,0	1,8	0,9	0,7
	X.	0,6	3,1	0,1	0,6	—	0,9	—	1,5	0,1	1,5
Cyclotella ocellata	VI.	—	1,0	0,4	0,9	—	1,5	—	1,5	—	2,3
Pant.	VII.	—	0,9	2,3	5,4	0,6	4,6	—	3,8	—	4,6
	VIII.	—	2,4	1,2	4,6	—	3,1	—	3,5	—	1,5
	IX.	—	0,4	—	2,3	—	1,5	2,1	3,8	0,7	1,2
	X.	—	—	—	2,1	0,4	1,2	—	1,8	—	8,5
Cymatopleura elliptica	VI.	—	—	—	—	—	—	0,1	—	0,3	—
(Bréb.) W. Smith	VII.	0,4	—	0,9	0,1	0,3	—	0,3	0,6	0,6	1,2
	VIII.	0,3	0,1	0,7	0,1	—	0,1	0,3	—	0,1	0,1
	IX.	0,1	—	—	0,1	—	0,1	—	0,1	0,4	1,2
	X.	—	0,1	0,1	—	—	—	0,1	0,7	—	0,4
Cymatopleura solea	VI.	—	—	—	—	—	—	0,1	0,1	—	0,1
(Bréb.) W. Smith	VII.	—	—	—	—	—	—	—	0,3	—	—
	VIII.	—	—	—	—	—	—	—	—	0,3	—
	IX.	—	—	—	—	—	0,1	—	0,1	—	—
	X.	—	—	—	—	—	—	—	0,6	—	0,6
Cymbella affinis	VI.	—	—	—	—	—	—	—	—	—	—
Kütz.	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	0,1	—	—	0,3	—	—	—	0,3	—	—
	IX.	—	—	—	—	—	—	—	—	1,5	—
	X.	—	—	—	—	—	—	—	—	—	0,1
Cymbella cymbiformis	VI.	0,1	0,3	—	—	—	—	—	—	—	—
(Kütz.) V. Heurck	VII.	—	—	—	—	—	—	—	0,1	—	—
	VIII.	—	—	—	0,4	—	—	—	0,6	—	—
	IX.	0,3	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—

2. Táblázat (folytatás) — Tabelle 2 (Fortsetzung)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
Diatoma vulgare BORY	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	0,1	—	0,1	—	—
	X.	—	—	—	—	—	0,1	0,1	—	—	—
Diploneis domblittensis (GRUN.) CLEVE	VI.	—	0,3	0,1	—	0,1	0,3	0,9	0,3	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	0,4	—	0,1	—
	IX.	—	—	—	—	—	—	—	0,6	—	0,1
	X.	—	—	—	—	—	—	—	—	—	—
Diploneis elliptica (KÜTZ.) CLEVE	VI.	0,1	0,4	0,1	—	—	0,9	3,8	1,8	0,1	0,9
	VII.	—	0,3	—	—	—	1,0	0,9	1,0	0,9	1,5
	VIII.	—	—	0,3	—	0,3	0,3	0,7	1,7	0,6	0,3
	IX.	0,4	0,1	—	—	0,1	0,1	0,6	3,4	1,0	0,4
	X.	—	—	—	—	0,4	0,1	0,6	1,2	0,1	2,6
Diploneis puella (SCHUM.) CLEVE	VI.	—	—	—	0,3	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	0,4	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	0,4	—	—	—
Epithemia sorex KÜTZ.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	0,1	0,1	—	—
	IX.	—	—	—	—	—	—	—	0,1	—	0,3
	X.	—	—	—	—	—	—	—	—	—	0,1
Fragilaria construens (EHR.) GRUN.	VI.	4,9	10,8	—	3,1	1,5	3,1	3,1	3,1	6,2	—
	VII.	3,8	8,0	6,2	4,9	1,2	—	3,5	1,8	5,2	—
	VIII.	4,9	0,7	—	4,6	—	—	66,3	4,9	—	—
	IX.	11,0	—	3,2	2,1	—	2,4	28,6	14,1	—	—
	X.	2,7	0,7	—	1,8	—	—	9,3	15,5	0,7	—
Fragilaria pinnata EHR.	VI.	—	—	—	1,7	2,7	0,7	—	—	—	—
	VII.	—	5,2	2,1	—	—	—	3,1	—	—	—
	VIII.	—	—	—	3,4	—	1,5	15,5	3,1	0,7	—
	IX.	—	—	—	—	2,3	—	9,3	3,1	2,3	—
	X.	—	—	1,5	—	—	—	4,6	3,8	—	0,6
Gyrosigma distortum var. *parkeri* HARRIS	VI.	—	—	0,3	—	—	0,1	—	—	0,1	0,4
	VII.	—	—	—	—	—	—	—	—	1,2	0,6
	VIII.	—	—	—	—	—	—	—	—	0,6	—
	IX.	—	—	—	—	—	—	0,1	—	1,5	1,8
	X.	2,7	—	—	—	—	—	—	—	0,1	0,1
Gyrosigma kützingii (GRUN.) CLEVE	VI.	0,3	0,1	0,7	—	—	—	0,6	—	0,3	0,1
	VII.	—	—	—	—	—	—	—	—	—	2,1
	VIII.	0,1	—	—	0,1	—	0,4	—	0,6	0,3	—
	IX.	—	—	—	—	—	—	0,7	—	2,6	—
	X.	0,1	—	—	—	—	—	0,6	0,4	—	0,7

2. Táblázat (folytatás) — Tabelle 2 (Fortsetzung)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
Melosira granulata (Ehr.) Ralfs	VI.	—	0,7	—	0,4	—	1,2	—	3,1	—	1,2
	VII.	—	—	—	1,0	2,0	1,3	—	0,6	—	2,7
	VIII.	29,4	12,4	4,6	4,6	—	1,2	4,1	1,7	—	1,0
	IX.	43,0	30,2	17,3	4,1	0,3	—	—	5,1	—	3,4
	X.	8,0	8,6	—	0,7	—	—	—	12,4	—	6,2
Melosira granulata var. *angustissima* O. Müll.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	0,6	—	1,2	0,3
	VIII.	11,6	4,6	6,2	1,8	3,1	—	—	—	—	—
	IX.	59,3	23,5	11,9	14,1	—	—	—	0,7	—	—
	X.	44,9	44,9	10,8	16,2	1,5	—	—	—	—	—
Navicula costulata Grun.	VI.	—	—	—	—	—	—	0,7	—	—	0,1
	VII.	—	—	—	—	—	—	—	—	—	0,3
	VIII.	—	—	—	—	—	—	0,1	—	0,3	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—
Navicula cryptocephala Kütz.	VI.	—	0,3	0,4	—	0,9	—	0,6	—	—	—
	VII.	1,2	—	0,4	0,4	0,4	—	2,4	—	2,3	—
	VIII.	0,6	—	—	—	0,3	—	0,7	—	2,4	—
	IX.	—	0,1	—	—	0,1	—	1,8	—	1,0	—
	X.	—	—	1,0	—	1,7	—	—	0,3	—	1,5
Navicula dicephala (Ehr.) W. Smith	VI.	—	—	0,1	—	—	—	0,3	—	—	—
	VII.	—	—	—	—	—	—	—	0,1	0,3	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	0,1	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—
Navicula gracilis Ehr.	VI.	—	0,1	0,4	0,7	0,3	0,9	2,1	0,3	0,9	—
	VII.	—	0,7	0,1	—	0,1	—	0,4	—	—	—
	VIII.	0,1	—	0,3	—	0,3	—	—	—	2,0	0,1
	IX.	—	—	0,3	0,4	0,4	—	2,7	0,9	2,1	0,3
	X.	1,2	—	0,9	0,4	0,3	0,1	0,1	0,7	0,3	1,0
Navicula hungarica var. *capitata* (Ehr.) Cleve	VI.	—	—	—	—	—	0,1	—	—	—	—
	VII.	0,7	0,6	—	—	0,1	—	0,4	—	0,3	—
	VIII.	0,1	—	0,1	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,3	0,4	—	—
	X.	—	0,1	—	—	—	—	0,1	—	—	—
Navicula placentula (Ehr.) Grun.	VI.	0,1	0,1	—	0,4	—	—	—	0,6	—	0,6
	VII.	—	0,7	—	—	—	—	0,1	—	—	—
	VIII.	—	—	0,3	—	—	—	—	0,3	—	0,1
	IX.	—	—	—	—	0,3	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—
Neidium dubium (Ehr.) Cleve	VI.	—	—	—	—	—	0,1	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	0,1	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,1	0,1	—	0,1
	X.	—	—	—	—	—	—	—	—	—	—

2. Táblázat (folytatás) — Tabelle 2 (Fortsetzung)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
Nitzschia acicularis W. Smith	VI.	—	—	—	0,1	—	0,3	0,4	—	0,1	0,1
	VII.	—	—	—	—	—	—	0,3	0,3	—	—
	VIII.	—	—	—	—	0,1	—	—	0,6	1,0	0,1
	IX.	0,1	—	—	—	0,4	—	0,1	—	2,1	0,6
	X.	—	—	0,3	—	1,0	0,7	—	—	0,1	—
Nitzschia amphibia Grun.	VI.	—	—	1,5	0,7	0,7	0,3	1,0	0,3	0,6	1,6
	VII.	1,8	—	5,4	0,7	0,6	0,1	1,0	0,3	0,9	—
	VIII.	0,4	—	—	0,3	0,3	0,4	0,7	0,1	1,2	0,1
	IX.	—	0,3	0,4	—	1,0	—	—	—	0,1	—
	X.	0,4	—	—	—	0,9	—	0,3	0,7	0,7	2,6
Nitzschia palea (Kütz.) W. Smith	VI.	0,9	—	3,8	1,2	0,7	0,4	1,5	0,6	—	0,7
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—
Nitzschia sigmoidea (Ehr.) W. Smith	VI.	0,1	0,7	0,3	0,1	—	0,4	—	0,4	—	2,3
	VII.	2,3	1,5	3,8	1,7	0,1	—	3,8	0,4	3,1	1,2
	VIII.	0,1	—	0,4	0,4	—	0,6	1,0	0,3	1,2	0,1
	IX.	—	—	—	—	0,3	0,1	0,9	0,3	1,2	0,7
	X.	0,1	—	0,6	—	1,2	—	1,0	0,7	0,3	0,4
Nitzschia tryblionella var. *debilis* (Arnott) A. Mayer	VI.	—	0,1	—	—	—	0,1	—	0,1	—	—
	VII.	—	0,7	—	—	—	0,1	—	0,1	—	—
	VIII.	—	—	—	—	—	—	—	0,1	0,4	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	0,1	—	—
Opephora martyi Héribaud	VI.	—	0,1	—	—	—	0,4	—	0,3	—	—
	VII.	—	—	—	0,1	—	—	—	0,1	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	0,3	—	—	—	—
	X.	—	—	—	—	—	—	—	0,1	—	0,3
Pinnularia sp.	VI.	—	—	0,1	—	—	—	—	—	—	—
	VII.	—	—	—	0,6	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	0,1	—	—	—	—	—	0,1	—
	X.	—	—	—	—	—	—	—	—	—	—
Rhopalodia gibba (Ehr.) O. Müll.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	0,1	—	—	—	—	—	0,4	—	—
	IX.	—	—	—	—	—	0,1	—	0,3	—	—
	X.	0,1	—	—	—	—	—	—	—	—	—
Stauroneis phoenicenteron Ehr.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	0,3	—	—	0,6
	VIII.	—	—	—	—	—	—	—	—	—	0,1
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—

2. Táblázat (folytatás) — Tabelle 2 (Fortsetzung)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
Stenopterobia pelagica Hust.	VI.	—	—	—	—	—	—	—	—	—	0,1
	VII.	0,4	—	0,4	—	—	—	0,1	—	—	0,3
	VIII.	0,1	—	—	0,1	—	0,1	—	0,1	—	—
	IX.	—	—	—	—	—	—	—	0,1	—	—
	X.	—	—	—	—	0,1	—	—	—	—	—
Surirella robusta var. *splendida* (Ehr.) V. Heurck	VI.	0,1	—	—	—	—	—	0,3	0,1	0,4	0,1
	VII.	0,1	—	0,4	—	—	—	0,3	—	0,9	0,6
	VIII.	—	—	0,1	—	—	—	0,1	—	1,5	0,1
	IX.	—	—	—	—	0,4	0,4	—	0,4	0,4	1,5
	X.	—	0,1	—	—	—	0,1	—	1,0	—	0,9
Surirella turgida W. Smith	VI.	—	0,1	0,1	—	—	—	—	0,1	—	—
	VII.	—	—	—	—	—	—	0,1	—	—	—
	VIII.	—	—	—	—	—	—	—	—	0,1	—
	IX.	—	—	—	—	—	—	—	—	0,1	—
	X.	—	—	—	—	—	—	—	—	—	0,1
Synedra acus var. *angustissima* Grun.	VI.	—	0,1	—	0,1	0,1	0,7	—	2,3	0,3	4,0
	VII.	—	—	—	—	—	—	—	0,1	—	1,5
	VIII.	—	0,1	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,1	0,7	—	0,3
	X.	—	—	—	—	—	—	—	—	—	—
Synedra parasitica (W. Smith) Hust.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	0,1	—	—	—	—	—	—
	VIII.	—	—	—	0,1	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	—	0,1	—	—
	X.	—	0,1	—	—	—	—	—	—	—	—
Synedra ulna (Nitzsch.) Ehr.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	0,1	—	—	—
	VIII.	—	—	—	—	—	—	0,4	0,1	—	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	—
CHLOROPHYTA											
Ankistrodesmus falcatus (Corda) Ralfs	VI.	0,4	—	0,3	—	0,1	—	—	—	—	—
	VII.	—	—	—	—	0,1	—	0,3	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,4	—	0,4	—
	X.	—	—	0,4	—	—	—	—	—	0,1	—
Ankistrodesmus falcatus var. *spirilliformis* G. S. West	VI.	0,3	—	—	—	—	—	0,1	—	0,6	—
	VII.	—	—	—	—	0,1	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,3	—	0,7	—
	X.	—	—	—	—	—	—	—	—	—	—
Closterium aciculare West	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	0,3	—
	VIII.	—	0,1	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	0,1	—	—	—
	X.	—	—	—	—	—	—	—	0,1	—	—

2. Táblázat (folytatás) — Tabelle 2 (Fortsetzung)

Gyűjtőhelyek, időpont		Mo		K		G		A_1		Ee	
Fajok felsorolása		élő	váz	élő	váz	élő	váz	élő	váz	élő	váz
Cosmarium sp.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	0,1	—	—	—	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	—	—	—	—	0,1
Crucigenia quadrata var. *octogona* Schmidle	VI.	—	—	2,4	—	—	—	2,4	—	—	—
	VII.	—	—	—	—	—	—	2,4	—	4,9	—
	VIII.	—	—	—	—	—	—	2,4	—	—	—
	IX.	—	—	—	—	—	—	2,4	—	4,9	—
	X.	—	—	—	—	—	—	—	—	—	—
Dictyosphaerium pulchellum Wood.	VI.	—	—	—	—	—	—	—	—	—	—
	VII.	—	—	2,4	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	2,4	—	—	—
	IX.	—	—	—	—	—	—	0,3	—	0,4	—
	X.	—	—	—	—	—	—	—	—	—	—
Pediastrum clathratum (Schroet.) Lemm.	VI.	—	—	—	—	—	—	—	—	—	2,4
	VII.	—	—	—	—	—	2,4	—	2,4	—	9,9
	VIII.	—	2,4	—	2,4	—	—	—	—	—	—
	IX.	—	—	—	—	—	—	—	—	—	—
	X.	—	—	—	—	—	2,4	—	—	—	2,4
Scenedesmus quadricauda (Turp.) Bréb.	VI.	0,3	0,6	0,3	0,3	0,3	—	0,6	—	0,9	—
	VII.	—	—	—	—	0,9	—	0,9	—	0,6	—
	VIII.	—	—	—	—	—	—	—	—	0,6	—
	IX.	—	—	—	—	—	—	—	—	0,3	—
	X.	—	—	—	—	—	—	—	—	—	—
Staurastrum gracile Ralfs	VI.	—	—	—	—	—	0,1	—	—	—	—
	VII.	—	—	—	—	—	—	—	—	—	—
	VIII.	—	—	—	—	—	—	—	—	—	0,1
	IX.	—	—	—	—	—	—	—	0,1	0,1	—
	X.	—	—	—	—	—	—	—	0,1	—	0,3

Gyűjtőhelyek, időpont = Sammelstellen und Zeitpunkt des Vorkommens
Fajok felsorolása = Artenverzeichnis; élő = lebenden Algen; váz = abgestorbenen Algen

A *Melosira* vízszíneződés és a *Microcystis — Aphanizomenon* szórt vízvirágzás is csupán a tó DNy-i részének Keszthelyi-öblére és az ahhoz közeli területekre terjedt ki.

Az összesített *(l. 3. táblázat)* adatokból kitűnik, hogy az élő kovamoszatok száma a Keszthelyi-öböl szeptemberi iszapmintájában a legmagasabb (116400 dm^2), míg a legalacsonyabb a Balatonalmádi — Balatonvilágos októberi (2910/dm^2) adat.

A pár évvel ezelőtti iszapminta felvételek adatait (ez ideig még nem közölt eredmények) összevetve az újabb adatokkal, feltűnő az, hogy régebben a mintákban az élők száma többszörösen meghaladta az elpusztultak számát. Az 1965. évi adatok helyenként és időnként a régebbi adatoktól eltérnek, azok fordítottját mutatják. Helyenként és időnként az élő és elpusztultak közötti számarány 1:1, de előfordult 1:2 is *(l. 3. táblázat)*.

3. Táblázat — Tabelle 3

A Balaton nyíltvízi fenékiszapmintáinak összesített mennyiségi algológiai adatai az 1965. évi gyűjtések alapján — i/dm² = egyedszám/dm² = Zusammenfassende quantitative Mikrophytobentosuntersuchungen aus dem Eprofundal des Balaton-Sees auf Grund der Sammlungen des Jahres 1965 — i/dm² = Individuenzahl pro dm²

Gyűjtési hely, idő		Élő kovamoszat	Váz kovamoszat	Élő : váz kovamoszat	Élő moszat	Váz moszat	Élő : vázmoszat
M_0	VI.	7 880	17 090	1 : 2,1	3 990	1 700	2,3 : 1
	VII.	12 980	26 330	1 : 2	620	1 240	1 : 2
	VIII.	51 410	23 990	2,1 : 1	300	2 780	1 : 9,2
	IX.	116 400	57 470	2 : 1	150	460	1 : 3
	X.	65 220	61 810	1 : 1	610	770	1 : 1,2
K	VI.	17 210	13 430	1,2 : 1	10 360	1 540	6,7 : 1
	VII.	23 680	21 490	1 : 1	15 340	310	49,4 : 1
	VIII.	16 870	23 830	1 : 1,4	300	2 790	1 : 9
	IX.	35 160	24 460	1,4 : 1	620	460	1,3 : 1
	X.	21 060	24 150	1 : 1,1	1 380	—	1 : 0
G	VI.	7 830	16 020	1 : 2	2 750	610	4,5 : 1
	VII.	6 640	11 550	1 : 1,7	8 500	5 420	1,5 : 1
	VIII.	4 950	10 050	1 : 2	770	930	1 : 1,2
	IX.	10 040	7 870	1,2 : 1	460	460	1 : 1
	X.	8 190	4 000	2 : 1	610	2 480	1 : 4
A_1	VI.	34 780	18 810	1,8 : 1	4 940	930	5,3 : 1
	VII.	26 250	15 130	1,7 : 1	26 500	2 480	10,6 : 1
	VIII.	93 350	24 590	3,7 : 1	5 580	1 700	3,2 : 1
	IX.	57 620	41 770	1,3 : 1	5 860	2 000	2,9 : 1
	X.	22 900	48 300	1 : 2,1	620	1 990	1 : 3,2
E_e	VI.	11 290	17 660	1 : 1,5	3 700	5 060	1 : 1,3
	VII.	18 750	23 400	1 : 1,2	19 220	10 540	1,8 : 1
	VIII.	15 290	4 910	3,1 : 1	1 700	1 690	1 : 1
	IX.	21 180	14 670	1,4 : 1	8 180	2 160	3,7 : 1
	X.	2 910	31 720	1 : 10,9	2 000	5 560	1 : 2,7

Gyűjtőhelyek, időpont = Sammelstellen und Zeitpunkt des Vorkommens
élő kovamoszat = lebenden Kieselalgen; váz kovamoszat = abgestorbenen Kieselalgen; élő moszat = lebenden Algen; váz moszat = abgestorbenen Algen; élő kovamoszat: váz kovamoszat = Das Zahlenverhältnis zwischen lebenden und abgestorbenen Kieselalgen; élő moszat: váz moszat = Das Zahlenverhältnis zwischen lebenden und abgestorbenen Algen

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ORIENTIERENDE ALGOLOGISCHE UNTERSUCHUNGEN IM BODENSCHLAMM DES BALATON-SEES AUF GRUND DER SAMMLUNGEN DES JAHRES 1965.

Gizella Tamás

Autor sammelte in Verfolg der im Frühjahr 1965 eingetretenen bedeutenden Fischverluste zur Untersuchung der Umstände und der biologischen Auswirkung der Verluste vom Juni bis Oktober, in der Organisation der Hydrobiologischen Abteilung an drei Stellen des Südwestteiles des Balaton, sodann an zwei Stellen des Nordostteiles

in, auf die Längsachse des Seespiegels senkrechten Querprofilen (siehe *Abbildung 1.)* Schlammproben (siehe die Sammeldaten in *Tabelle 1.)*. Aus der mittels des EKMANschen Schlammgreifers gehobenen Materie wurden stets aus der dünnen oberen Schichte cca 114 cm^2 zur algologischen Bestimmung entnommen (PONYI 1966). Nach Auswahl des zoologischen Materials wurde die Probe nach einer etwa 48 stündigen Sedimentation mittels einer geringfügigen Modifizierung der, bei früheren Mikrophytobentos-Untersuchungen bewährten Methode (ENTZ—TAMÁS 1952; ENTZ 1954) untersucht. Aus den durch 48 Stunden hindurch sedimentierten Proben wurden von einer 5 × 1 cm^2 Schlammoberfläche sämtliche (— lebenden und abgestorbenen —) Algen aufgezeichnet und die also gewonnenen Daten auf 1 dm^2 Schlammoberfläche umgerechnet. —

Aus den von 5 Sammelstellen entnommenen 25 Schlammproben bestimmten 94 Arten und 7 Variationen gehören, mit den Pilzen zusammen, zu 6 grossen Stämmen und zwar nach Häufigkeit geordnet folgendermassen: *Chrysophyten* 62, *Chlorophyten* 22, *Cyanophyten* 8, *Euglenophyten* 6, *Pyrrophyten* 2, *Mycophyten* 1.

Unter den 5 Sammelstellen ist die in Tihany vor dem Biologischen Forschungsinstitut (A_1) entnommene (72) an Algenarten am reichsten und die zwischen Ságpuszta und Balatonszemes (G) entnommene Probe (52) am ärmsten.

Gelegentlich der im August vorgenommenen Sammlung der Schlammproben verursachte das massenhafte Auftreten von *Melosira granulata* und *Melosira granulata* var. *angustissima* im offenen Wasser entnommenen Schöpf- und Netzfilterproben eine Vegetationsfärbung. Diese Erscheinung wirkte sich auch auf die obere Schlammschichte aus. In den, in der Bucht von Keszthely sowie zwischen Szigliget und Balatonmária in diesem Zeitpunkt entnommenen Schlammproben machte *Melosira* etwa drei Dritteile der Anzahl sämtlicher lebenden Kieselalgen aus. Autor nimmt an, dass im Zeitpunkt der Augustsammlung die durch *Melosira* verursachte Vegetationsfärbung in der Bucht von Keszthely ihr Maximum bereits überschritten hatte, wogegen diese in den beiden anderen Sammelstellen (Szigliget—Balatonmária und Ságpuszta—Balatonszemes) gerade ihr Maximum erreichte. Ihre Annahme wird auch durch das Anzahlverhältnis der in den einzelnen Sammelstellen entnommenen Schlammoberflächen festgestellten lebenden und abgestorbenen *Melosiren* bestätigt (siehe: die Daten der lebenden und abgestorbenen *Melosiren* in *Tabelle Nr. 2.)*. Auch bei den Angaben vom September und Oktober bilden (— lebende und abgestorbene —) *Melosiren* ein Drittel sämtlicher Kieselalgen.

Zwischen der Bucht von Keszthely und Szigliget wurden gelegentlich der September-Sammlungen im offenen Wasser das Erscheinen zerstreuter Wasserblüte von *Microcystis flos-aquae* und *Aphanizomenon flos aquae* var. *klebahnii* beobachtet. Zu dieser Zeit wurden ausser den lebenden Kieselalgen sonstige Algen bloss in sehr geringer Menge vorgefunden *(Oscillatoria, Lyngbya);* unter den abgestorbenen Daten kamen ausschliesslich *Microcystis flos-aquae* und *Aphanizomenon flos-aquae* var. *klebahnii.*

Die *Melosira*-Vegetationsfärbung sowie die zerstreute Wasserblüte von *Microcystis-Aphanizomenon* war auch bloss auf die im südwestlichen Teil des Sees befindliche Bucht von Keszthely und auf die benachbarten Gebiete beschränkt.

Aus den zusammenfassenden Daten der *Tabelle Nr. 3.* erhellt, dass die Anzahl der lebenden Kieselalgen in der September-Schlammprobe aus der Bucht von Keszthely am höchsten (— 116.400 dm^2 —) und in der im Oktober zwischen Balatonalmádi—Balatonvilágos entnommenen Probe am niedrigsten (— 2.910 dm^2 —) war.

Wenn man die Angaben der vor einigen Jahren entnommenen Schlammproben (— bisher nicht mitgeteilte Ergebnisse ! —) mit den neueren Daten vergleicht, erscheint es auffallend, dass früher die Anzahl der lebenden jene der abgestorbenen in den Proben mehrfach übersteigt. Die Daten aus dem Jahre 1965 weichen stellenweise und zeitweise von den älteren Daten ab und zeigen ein umgekehrtes Verhältnis. Stellenweise und zeitweise beträgt das Zahlenverhältnis zwischen lebenden und abgestorbenen 1 : 1, doch kommt auch ein Verhältnis von 1 : 2 vor (siehe: *Tabelle Nr. 3.)*.

ОРИЕНТИРОВОЧНЫЕ ИССЛЕДОВАНИЕ ВОДОРОСЛЕЙ ИЛА БАЛАТОНА НА ОСНОВЕ МАТЕРИАЛА, СОБРАННОГО В 1965 ГОДУ

Гизелла Тамаш

Была проведена работа для выяснения причин и условий массовый гибели рыб в Балатоне весной 1965-ого года. В трех пунктах юго-западной части и во двух пунктах северо-восточной части озера были собрана образцы ила перпендикулярно на разной

глубине залегания *(1.1. рис.)* (данные приведены *а таблице № 1)*. Материал поднимали на поверхность по методу Ekman, затем брали всегда одинаковое количество ила на альгологическое и зоологическое исследование Ponyi, 1966.). После удаления животных и 48 часового осождения был применен метод исследования микрофитобентоса Entz, Tamás, 1952, Entz, 1954) из 5×1 cm² образцов, осажденных в течении 48 часов, были описаны все водоросли (и живущие и погибшие) и затем был проведен пересчет на 1 dn² поверхности ила.

В 25 образцах, собранных в 5 местях, было найдено 94 видов и 7 разновидностей, которые относились к 6 большим систематическим группам: *Chrysophyta* 62, *Chlorophyta* 22, *Cyanophyta* 8, *Euglenophyta* 6, *Pyrrophyta* 2, *Mycophyta* 1.
Самым богатым образцом по водорослым является образец перед Биологическом Институтом в Тихани (A_1) а самым бедным — образец между Шагпуста—Балатонсемеш.

Во время сборов образцов ила в августе образцах сачка открытой воды можно было найти в массовом маштабе *Melosira granulata* и *M. granulata* var. *angustissima*, которые окрашивали воду. Это обстоятельство влияло и на поверхность ила. В это время в образцах ила Кестхейского залива, Сиглигета и Балатонмарии 3/4 всех живущих кремневых водорослей представлено родо *Melosira*. Автор предполагает, что во время сбора материала в августе окрашивание воды родом *Melosira* в Кестхейском заливе уже было после максимума, а в остальных двух пунктах (Сиглигет—Балатонмария и Шагпуста—Балатонсемеш) как раз находилось в максимуме. Это предположение оправдывается численностью живых и мертвых *Melosira* на поверхности ила. (см. *таблицу 1 и 2)*. В сентебре и октябре 3/4 всех кремневых водорослей представлены родом *Melosira*.

Описано окрашивание воды водорослями *Microcystis flos-aquae* и *Aphanizomenon flos-aque* var. *klebahnii* в сентябре в открытой воде Кестхейского залива и Сиглигета. В образцах ила в этот период число живущих водорослей было низким *(Oscillatoria, Lyngbya)* и из погибщих нашли только *Microcystis flos-aquae* и *Aphanizomenon flos-aquae* var. *klebahnii*.

Окрашение воды водорослями *Melosira Microcystis-Aphanizomenon* обнаруживалось только в юго-западной части озера, а именно в Кестхейском заливе и близких к нему местах.

Из *таблиц № 1 и 3* видно, что число живых кремневых водорослей ила самое высокое в Кестхейском заливе в сентябре, (116400) dm² а самое низкое — в Балатоналмади — Балатонвилагош в октябре (2910/dm²).

Сравнение данные образцов ила с прежними показывает, что раньше число живых водорослей превышало во много раз число погибших водорослей. Данные, полученные в 1965 году, дают обратную пропорцию. Местами и временами эта пропорция меняется, но обычно характерно отношение 1:1 или 1:2 в пользу погибших (см. *таблицу 1 и 3)*.

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BEITRÄGE ZUR ALGENFLORA DES BALATON-SEES. IV. VORKOMMEN DER EPIPLANKTONISCHEN ORGANISMEN *COLACIUM CYCLOPICOLA* (GICKLH.) BOURR. UND *C. SIMPLEX* HUBER-PESTALOZZI

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Die im Jahre 1958 begonnene Serie (TAMÁS 1958, 353—358; 1962, 267—273; 1964, 255—272:) enthält aus den verschiedenen Lebensräumen des Sees gesammelte und bestimmte algologische Angaben.

Vorliegende Studie behaltet teils aus dem offenen Wasser (Sammelstelle A_1) vor dem Biologischen Forschungsinstitut in Tihany mehrere Jahre hindurch — möglichst wöchentlich — systematisch gesammelte, teilweise an 5 Stellen des Sees im Laufe des Jahres 1965 vom Juni bis Oktober monatlich systematisch gesammelte Netzfilterproben (Netze Nr. 6 und Nr. 25) beziehungsweise einige recht interessante Angaben aus denselben.*) Diese letztgenannte Probensammlungs-Serie wurde in Ansehung der ausserordentlichen Fischverluste im Balaton des Jahres 1965 zur Erforschung der Umstände der Seuche und deren biologischen Auswirkung seitens der Hidrobiologischen Abteilung organisiert (Vgl. PONYI 1966; TAMÁS 1966) durchgeführt.

Epiplankton

An den im offenen Wasser unseres Sees lebenden Schwebekrebsen und pflanzlichen Organismen finden sich häufig Epibionten. Aus den bisherigen im Balaton angestellten Untersuchungen erhellt, dass zwischen den Verhältnissen der Umwelt und der Art und gewisser formeller Eigenheiten der Epibionten ein Zusammenhang besteht (STILLER, 1954). Mit den, an Planktonkrebsen vorkommenden Epibionten befassten sich mehrere Studien (STILLER 1935; 1941; 1949—1950; 1953; SEBESTYÉN 1951; FOTT 1958).

Die balatoner Epiphyten des Phytoplanktons und die bezügliche Literatur haben wir bereits in einer früheren Studie eingehend besprochen (TAMÁS 1962). Aus dem Balaton sind uns bisher systematisch zu mehreren Algenstämmen gehörige epibionte Algenarten bekannt, von welchen wir im Folgenden uns mit einigen Arten des genus *Colacium* des Euglenophyten-Stammes eingehender beschäftigen wollen.

* Detaillierte Angaben bezüglich der Sammelstellen und Sammlungen siehe: TAMÁS 1966, *Tabelle 1.* sowie *Abbildung Nr. 1.*

Aus der Algenliteratur des Balaton (KOL 1938; SZEMES 1957; TAMÁS 1959; 1963) waren lange Zeit hindurch von den im offenen Wasser lebenden Schwebekrebsen bloss *Colacium arbuscula* ST. und *C. vesiculosum* EHR. bekannt. In den, aus verschiedenen Punkten des Sees gesammelten Netzfilterproben findet sich auch die letztgenannte Art bedeutsam häufiger (Vgl. TAMÁS 1965, 232—234). Aus dem offenen Wasser vor dem Biologischen Forschungsinstitut von Tihany am 22. Mai 1962 gesammelten Netzfilterproben haben wir einige Exemplare von *Colacium simplex* HUBER—PESTALOZZI an *Keratella* aufgezeichnet (TAMÁS 1964, 248). Diese Art bewies im letzten Jahre sowohl hinsichtlich ihrer Verbreitung, als auch hinsichtlich der Häufigkeit ihres Vorkommens bedeutende Veränderungen.

Ebenfalls in den, vor der Biologischen Forschungsinstitut von Tihany am 6. März gesammelten Netzfilterproben haben wir in einer *Brachionus*-Gruppe Exemplare von *Colacium cyclopicola* (GICKLH.) BOURR beobachtet. Mit dieser zusammen finden sich im offenen Wasser unseres Sees vier *Colacium*-Arten.

Colacium cyclopicola (GICKLH.) BOURR

(Syn.: *Euglena cyclopicola* GICKLH.)

HUBER-PESTALOZZI in THIENEMANN 16, 4, p. 126, Abb. 109, 1955.

Die Zellen sind verkehrt eiförmig oder haben Birnenform. Sie sind im allgemeinen 14—20 μ lang. Chromatophoren 5—7, scheibenförmig, ohne Pyrenoide. Die Zellen haften unmittelbar am Wirtstier an, ohne Gallertstiele.

Wie bereits im Vorhergehenden erwähnt, fanden sich als erste Angaben in der Netztfilterprobe vor dem Biologischen Forschungsinstitut von Tihany am 6. März 1958 einige an *Brachionus* haftende Exemplare. Die Länge dieser beträgt 17,5 μ, die Breite am freien Ende 12,5 μ. In den folgenden, am 30. Juni in der Bucht von Keszthely (M_0) Abends und am 1. Juli in der Frühe gesammelten Netzfilterproben fanden sich diese auf *Cyclopsen* und auch frei in auffallend grosser Individuenzahl. Von der Keszthely-er Bucht gegen die Nordseite des Sees fortschreitend fanden wir sie zwischen Szigliget—Balatonmária (K) und zwischen Ságpuszta—Balatonszemes (G) gleichfalls am 1. Juli in bedeutender Anzahl an *Cyclopsen*. In der tihanyer Sammelstelle (A_1) trafen wir sie ebenfalls an *Cyclopsen* an. In den, am 13.—14. Oktober gesammelten Proben fanden sich auch den, in den aufgezählten Sommersammelstellen gefundenen ähnliche Stücke vor, zu denen auch die in Ufernähe bei Balatonaliga gefundenen Proben zugereiht werden können.

Hinsichtlich der geographischen Verbreitung der Art stehen uns bloss einige Angaben zur Verfügung: in Frankreich in dem kleinen Teich neben dem Walde bei Sénart an *Copepoden* (BOURELLY 1947); in der Czechoslowakei in den kleinen Teichen um Prag und Marienbad an *Cyclopsen* (CICKLHORN 1925); in Nepal, im Fischteich nebst Katmandu am 6. März 1956 (HIRANO 1963) wurden welche angetroffen.

Ihr Vorkommen im Balaton ist auch für Ungarn neu. —

Colacium simplex HUBER—PESTALOZZI

HUBER—PESTALOZZI in THIENEMANN 16, 4, p. 123, Abb. 107, 1955.

Die Zellen sind verkehrt eiförmig, elliptisch oder kugelförmig. Chromatophoren 7—10 scheibenförmig, ohne Pyrenoide. An den frei beweglichen, kugelförmigen ist auch ein Stigma zu sehen, die Geissel beträgt das anderthalbfache

der Körperlänge. Die angehefteten Zellen haften gewöhnlich ohne Stiel in kleineren Büscheln am Wirtstier an.

Wie oben bereits erwähnt, haben wir die erste Angabe aus dem offenen Wasser vor dem Biologischen Forschungsinstitut von Tihany (A_1) am 22. Mai 1962 in der Netzfilterprobe an *Keratella* aufgezeichnet. In den Sammlungen vom Jahre 1965 kam diese Art in der Bucht von Keszthely (M_0), zwischen Szigliget—Balatonmária (K), sodann zwischen Ságpuszta—Balatonszemes an der Sammelstelle vor Tihany (am 30. Juni, 1., 2., 30. Juli, 1., 2. August und 7, 8. September) in auffallend hoher Individuenzahl vor. Als Wirtstiere galten *Keratella, Cyclops, Daphnia* und *Nauplius*. Ausser den aufgezählten Proben haben wir sie aus der tihanyer Sammelstelle in den, — möglichst wöchentlich vorgenommenen — regelmässig gesammelten Netzfilterproben (23. Juni; 14., 20. Juli, 16., 30. August. 14., 22., 27. September und 5., 19. Oktober 1965) ebenfalls aufgezeichnet. Zwischen Balatomalmádi—Balatonvilágos fanden wir sie bloss am 8. September, an der ufernahen Sammelstelle von Balatonaliga im Material vom 14. Oktober.

Laut unseren Beobachtungen erreichte dieser Epibiont gleichfalls in der Bucht von Keszthely die höchste Individuenzahl. An einem Wirtstier zählten wir 30—40 Stück und kam sie auch in bedeutender Anzahl frei vor. In der tihanyer Sammelstelle wurde sie auch im Laufe der Jahre in mehreren Netzfilterproben vorgefunden, doch war ihre Anzahl selbst zur Zeit des warmen Wassers nur 5—10 Exemplare pro Wirtstier.

Hinsichtlich ihrer geographischen Verbreitung besitzen wir nur wenige Angaben: Schweiz: Jochsee (Canton Unterwalden) in dem, 2222 m über dem Meeresspiegel liegenden stark eutrophen Bergsee in 0,5—1,5 m Tiefe sind zwei Formen ihres Vorkommens bekannt, die eine als frei schwebender Planktonorganismus, der in grossen Mengen vorkommend sogar Wasserblüte verursachen konnte und sich als Epibiont auch an *Rotatorien-* und *Crustazeen-Arten*, ja sogar an Fadenalgen anheftete (Huber—Pestalozzi 1955, 123). In der Nähe von Zürich trat sie in zwei kleineren Teichen massenhaft auf; in Ungarn: im Zsomboer Wald in der Nähe von Szeged kam sie im Morastwasser vor (Uherkovich, 1962).

Die Einbürgerung neuerer Epibionten, deren stellenweise plötzlich hoch angestiegene Individuenzahl in der Zeit des warmen Wassers, die in den letzten Jahren wiederkehrenden Wasserblüten und Vegetationsfärbungen [Hortobágyi 1962; Tamás 1965/a] lenken unsere Aufmerksamkeit auf die im offenen Wasser des Sees stufenweise fortschreitenden Veränderungen.

Zusammenfassung

In der vorliegenden Studie berichtet Autor teils in dem offenen Wasser vor dem Biologischen Forscherinstitut von Tihany [Sammelstelle A_1] durch Jahre hindurch gesammelten, teils aus den, zur Untersuchung der Ursache und der biologischen Auswirkung der im Frühjahr 1965 entstandenen ausserordentlichen Fischverluste im Rahmen der durch die Biologische Abteilung organisierten Untersuchungen vom Juni bis Oktober an fünf Stellen des Sees monatlich systematisch gesammelten Netzfilterproben (Nr. 6 und 25) gewonnene interessante Angaben. (Siehe Fussnote auf Seite 1.*)

Aus der Algenliteratur des Balaton sind bisher systemgemäss zu mehreren Algenstämmen gehörige epibionte Algenarten bekannt (Sebestyén 1951; Fott

1958; TAMÁS 1962). Vom Euglenophyten-Stamm waren lange Zeit hindurch bloss *Colacium arbuscula* ST. und *C. vesiculosum* EHR. als Epibionten von Krebsen des offenen Wassers bekannt.

Autor beobachtete an der Sammelstelle von Tihany (A_1) in der Netzfilterprobe vom 6. März 1958 als erste mehrere Exemplare der Art *Colacium cyclopicola* an *Brachionus*. In den, im Südwestlichen und Nordöstlichen Teile des Sees im Juli des Jahres 1965 gesammelten Netzfilterproben fand sich sowohl die schwebende, als die an *Cyclops* angeheftete Form in bedeutender Individuenzahl vor. Von der geographischen Verbreitung dieser Art ist uns nur wenig bekannt. (Siehe S. 212)

Die ersten Exemplare von *Colacium simplex* wurden gleichfalls in den Netzfilterproben der Tihanyer Sammelstelle (A_1) vom 22. Mai 1962 durch den Autor an *Keratella* bestimmt. In den Sammlungen des Jahres 1965 fand Autor in beiden Teilen des Sees solche in auffallend grosser Individuenzahl. Als Wirtstiere figurierten *Keratella, Cyclops, Daphnia* und auch *Nauplius*. Es konnte festgestellt werden, dass die grösste Individuenzahl in der Bucht von Keszthely (M_0) zur Warmwasserzeit erreicht wurde, welche pro Wirtstier 30—40 Stück betrug. Dabei waren auch frei schwebende Exemplare zahlreich anzutreffen.

Von der geographischen Verbreitung stehen uns nur wenige Angaben zur Verfügung (Siehe S. 213)

Aus den Untersuchungen ist zu entnehmen, dass *Colacium cyclopicola* und *Colacium simplex* im Jahre 1965 zur Warmwasserzeit in den Proben aus der Bucht von Keszthely die höchste Individuenzahl erreichte.

Die Einbürgerung neuerer Epibionten, deren plötzlich ansteigende Individuenzahl zur Warmwasserzeit, die jährlich sich wiederholenden Wasserblüten und Vegetationsfärbungen [HORTOBÁGYI 1962; TAMÁS 1965) a] lenken die Aufmerksamkeit auf die im offenen Wasser des Balatonsees stufenweise fortschreitenden Veränderungen.

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ADATOK A BALATON MOSZATFLÓRÁJÁHOZ IV. A *COLACIUM CYCLOPICOLA* (GICKL.) BOURR. ÉS A *C. SIMPLEX* HUBER—PESTALOZZI EPIPLANKTONIKUS SZERVEZETEK ELŐFORDULÁSA

Tamás Gizella

Összefoglalás

Szerző ebben a tanulmányban részben a tihanyi Biológiai Kutatóintézet előtti (A_1 gyh) nyíltvízből éveken át, részben pedig az 1965. év tavaszán bekövetkezett nagymértékű halpusztulás körülményeinek és biológiai hatásainak kutatására a Hidrobiológiai Osztály szervezésében júniustól —októberig a tó 5 pontján* havonta rendszeresen gyűjtött hálószüredék minták (No 6 és No 25) néhány igen érdekes adatát ismerteti.

A balatoni algairodalomból ezideig rendszertanilag több algatörzsbe tartozó epibiont algafaj ismert (SEBESYÉN 1951; FOTT 1958; TAMÁS 1962). Az Euglenophyta törzsből hosszú ideig csupán a *Colacium arbuscula* ST. és a *C. vesiculosum* EHR. szerepelt nyíltvízi rákok epibiontjaként.

Szerző a tihanyi (A_1) gyűjtőhely 1958 március 6-i hálószüredékében *Brachionus*on figyelte meg először a *Colacium cyclopicola* faj több példányát. Az 1965. évi gyűjtések során a tó DNy-i és ÉK-i részének júliusi hálószüredék mintáiban jelentős példányszámban szerepelt a szabadon lebegő és a *Cyclops*okra rögzült alakja is. E faj földrajzi elterjedéséről csak keveset tudunk (l. 212 old.).

A *Colacium simplex* első példányait ugyancsak a tihanyi (A_1) gyűjtőhely 1962. május 22-i hálószüredék mintából, *Keratella*ról határozta meg. Az 1965. évi gyűjtésekben

a tó mindkét részében feltűnő magas egyedszámban találta. Gazdaállatként *Keratella*, *Cyclops*, *Daphnia* és Nauplius is szerepelt. Megállapítható volt, hogy a melegvíz idején a Keszthelyi-öbölben (M_0) érte el az eddigi legmagasabb egyedszámot, mely gazdaállatonként 30—40 db-ot tett ki. Ugyanekkor a szabadon lebegő példányok száma is jelentős volt. Földrajzi elterjedéséről csak kevés adat áll rendelkezésre (l. 213 old.).

A vizsgálati adatokból kitűnt, az, hogy a *Colacium cyclopicola* és a *C. simplex* 1965. évben a melegvíz idején a Keszthelyi-öböl mintáiban érte el a legmagasabb egyedszámot.

Újabb epibiontok betelepülése, azok helyenkénti hirtelen magas egyedszáma a melegvíz időszakában, az évről-évre megismétlődő vízvirágzások és vízszíneződések (Hortobágyi 1962; Tamás 1965a) jelenségével együttesen felhívják a figyelmet a tó nyíltvizében fokozatosan előrehaladó változásra.

ДАННЫЕ К ФЛОРЕ ВОДОРОСЛЕЙ БАЛАТОНА. IV. РАСПРОСТРАНЕНИЕ ЭПИПЛАНКТОННЫХ *COLACIUM CYCLOPICOLA* (Gicken.) Bourr. Й *C. Simplex* Huber-Pestalozzi

Гизелла Тамаш

Автор приводит данные об анализе образцов сачка (№ 6 и № 25) собранных в открытой воде (A_1) Балатона перед Биологическим Институтом в течение многих лет и также в 5 разных пунктах Балатона раз в месяце с июля по октябрь 1965 года с целью выяснения массовой гибели рыб весной этого же года.

Из литературы балатонских водорослей известно несколько видов водорослей, относящихся систематически к эпибионтам (Sebestyén, 1951, Fott, Tamás, 1962). Долгое время из типа Euglennphyta *Colacium arbuscula* St. только *C. vesiculosum* Ehr были известны как эпибионты рачков открытой воды.

Автор впервые обнаружил несколько экземпляров вида *Colacium cyclopicola* на *Brachinos*, собранных в тиханьском местообитании (A_1) 6-ого марта 1958 года. В образцах, собранных в 1965 году в юго-западной и северо-восточной части озера, обнаруживалось значительное число этих видов, привязанных к cyclop-у и в свободноплавающей форме. Мало известно о географическом распределении этого вида.

Первый экземпляр *Colacium Simplex* был обнаружен на Keratell в местообитании A_1 в образце сачка от 22-ого мая 1962-ого года. В образцах, собранных в 1965 году было найдено высокое число этого вида в обеих частях озера. Хозяевами его являлись *Keratella*, *Daphnia*, и *Nauplius*,

Было устанавлено, что во время теплой воды обнаруживается максимальное число особей в Кестхейском заливе, где на одном хозяине насчитывается до 30—40 экземпляров. Тут же было найдено высокое число и свободноплавающих форм. О географическом распределении этого вида тоже мало известно.

Из данных видно, что *Colacium cyclopicola* и *C. simplex* достигали максимальной численности в 1965 году во время нагревания воды в Кестхейском заливе.

Появление новых эпибионтов и повышение их числа во время нагревания воды, а также окрашивание воды, повторяющеся ежегодно (Hortobágyi, 1962, Tamás, 1965 a.) обращают на себя внимание в связи с изменениями происходящими в открытой воде озера.

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ON pH CONDITIONS OF THE ALIMENTARY CANALS OF SOME CRUSTACEANS

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Except for some practically most important groups of Insects very few literary data are found concerning the pH of the gastric juice of animals of 1 cm or lower order of magnitude. Particularly few are the literary data on Crustaceans of small size. The pH of the gastric juice of *Daphnia magna* and that of the various parts of the digestive tract were examined by KRÜGER (1925), von DEHN (1930) and HASLER (1935) but rather differing results have been obtained. RANKIN (1929) found in *Simocephalus vetulus* at the beginning of the alimentary canal 6.8, at its end 8.0 pH. BOND (1934) investigating *Calanus finmarchicus* could establish only slightly alkaline pH without a definite value. NICHOLLS (1931) using the method of WIGGLESWORTH obtained for the pH of the alimentary canal of *Ligia oceanica* the following results: foregut 6.3; hepatopancreas 6.0; intestine 6.5; rectum 6. 2. From the literary data of recent years the works of DE GIUSTI et al. (1962) and AGRAWAL (1963) are worth mentioning. Both authors examine the pH conditions of the juices of the alimentary canal of Amphipoda with various methods and different results.

In a previous work (PONYI AND P. ZÁNKAI 1966) the pH-optimum of the proteolytic enzymes of the digestive system of some domestic Crustaceans *(Astacus leptodactylus, Asellus aquaticus, Gammarus roeseli, Dicerogammarus haematobaphes balatonicus, Limnomysis benedeni)* was examined. In the present examinations the pH conditions of the juices in the alimentary canal of these animals were studied also under natural conditions and compared with our data obtained up to now. The importance of the examinations of similar character is stressed by KRISHNA and SAXENA (1963) who investigating the pH conditions of *Tribolium castaneum* found that the pH of the intestinal content agrees with the pH optimum of the various enzymes contained.

Material and method

I. Animals examined and places of collection:

Limnomysis benedeni CZERN, *Dicerogammarus haematobaphes balatonicus* PONYI and *Dicerogammarus villosus bispinosus* MART. were collected from various sites of the Balaton, Gammarus *(Rivulogammarus roeseli* var. *triacanthus*

SCHÄFERNA and *Asellus aquaticus L.)* from the Aszófő creek. For comparison and to reproduce the literary data also the species *Astacus leptodactylus* ESCH. was examined.

II. *The pH conditions of the gastric juices are generally examined with the following methods:*

1. Feeding method (DAY and POWNING (1949), SINHA (1959), SRIVASTAVA (1960), DE GIUSTI and co-workers (1962), KRISHNA and SAXENA (1963).

2. pH determination with indicator paper SINHA (1959), RASTOGI and DATTA GUPTA (1962), AGRAWAL (1963).

3. So-called dilution method RASTOGI and DATTA GUPTA (1962).

In our experiments we endeavoured to determine the pH of the gastric juice of the animals as far as possible without lesion of the alimentary canal, under natural conditions, therefore we used in the first place the feeding method supplemented with two control methods (modified WIGGLESWORTH method and determination with BECKMAN'S pH meter).

1. The feeding method was employed as follows: animals starved for 5—24—48 hours were placed in a solution containing proper feed and indicator or only indicator respectively, then the change of colour in the intestinal canal after lifting out the digestive tract *(Amphipoda, Asellus)* or without *(Limnomysis)* obwserved after 5—24—48—72 hours under the binocular microscope at a white light. In each experimental series 25—30 animals were used up, as far as possible of larger body, distributed in groups of 5. In the various groups 2—3 animals gave evaluable results on the average.

After a number of preliminary experiments we succeeded in finding for *Amphipoda* such readily consumed feed which well binds the indicator and does not release it even in water. This stuff is agar from which we measured out 1 g in dry condition, soaked for 24 hours in distilled water and adding 10 mg indicator powder boiled down in 5 ccm of distilled water. After cooling down this was supplied as food cut into small cubes. We tried also casein (HAMMARSTEIN) which, however, was not willingly consumed by the animals. With yeast recommended by DE GIUSTI (1962) we could not obtain good results either, because the cells of the yeast used by us did not bind the indicator at all and on the other hand they have their own colour which makes reading off of the values difficult, beside this was not readily consumed by the animals either.

As an indicator the 0.1 per cent aqueous solution of the indicators of the sulphonphtalein series was used because in these the pH deviation caused by salt and proteins is relatively low and they are not toxic (DAY and POWNING, 1949). The series consisting of bromphenol blue (pK = 3.8), chlorphenol red (pK = 6.0), bromcresol red (pK = 6.12), bromthymol blue (pK = 7.1), neutral red (pK = 7.4), phenol red (pK = 7.8), cresol red (pK = 6.25) reaching from the 4.8 to 8.8 pH domain was completed in given cases with methyl red (pK = 5.0).

For *Asellus* and *Limnomysis* no such stuff was found which were readily consumed by these so from some indicators and water of the Lake Balaton a 0.1 per cent solution was made and the animals were kept in this solution.

2. To control this method we used the methodics of WIGGLESWORTH (1927) with some modification. We adopted the use of the slide with paraffin so that we made such small holes in the still tepid paraffin in which about 0.002

ml of the solution found place. Then we brought together from a pH series of M/15 KH_2PO_4-Na_2HPO_4 buffer and the corresponding indicator 0.001 ml in these little holes and subsequently covered the whole with a slide. These were the comparative solutions of known pH. In another hole instead of the buffer of known pH the material to be examined was placed. Determination of pH was supplied by the agreement of colour shades. This method was only used in *Amphipoda*. The juice to be examined which originated in most cases from the stomach and the end of the medium intestine was sucked out by a micro-buretta. As the neutral red indicator does not dissolve readily in water, here we dispensed with its use. The great many data obtained from the use of this method were statistically evaluated.

3. pH conditions of the hepatopancreas of *Amphipoda* and *Asellus* and in many cases also those of the gastric juice are difficult to determine as the colour of the indicators which may enter into consideration is yellow-red or yellowish-blue which shades may be covered by the colour of the juices. Therefore we attempted to dilute these juices and to determine their pH with BECKMAN'S pH-meter. Since here small quantities of liquids have to be measured it was necessary to control these. For this purpose from the above described phosphate buffer of known pH 0.3—0.5 ml were dropped on a slide then drawn away in a narrow stripe and the pH repeatedly determined. Subsequently the hepatopancreas or sucked out gastric juice of 15—27 animals was collected on a cooled slide, then 2 × distilled water on glass added and similarly prepared for the measurement as described above. When more than 0.5 ml distilled water was added the gastric juices were often no more able to buffer the solution. In some cases when a comparatively greater amount of juice was available we measured the pH without adding distilled water. The difference between the two types of measurement was not greater than the deviation of the pH values between the groups.

Before measurement the small tubes of the *Amphipoda* and *Asellus* hepatopancreas were dissected out and cut off before the entry of the intestine. After placing on the slide we cut them through once more to facilitate the flow out of the juices. The gastric juice was sucked out according to the method described in point 2.

At every measurement the pH of the juice from 20—25—30 animals was determined.

Of *Astacus* the pH of the hepatopancreas and gastric juice of 5 animals was measured, the result obtained is the mean value of these measurements.

Results

1. Limnomysis benedeni

The animal is quite transparent and although the juice of the hepatopancreas tubes is coloured, the colour is very pale and therefore the pH can be readily determined. On account of the anatomical conditions (5 pairs of hepatopancreas) and small size of the animal only the feeding method could be employed here. The medium intestine can be sometimes broken up in two parts on the basis of the pH conditions. These limits, however, are not constant and not definite *(Fig. 1)*.

Table 1.

The pH conditions of the gastric juices of *Limnomysis benedeni*

Number of measurements	Indicator	Stomach	Hepatopancreas	Beginning of medium intestine	End of medium intestine
13	Chlorphenol red	11 c > 6.0 2 c ~ 6.0	5 c > 6.0 8 c ~ 6.0	> 6.0	> 6.0
16	Bromcresol red	> 6.1	10 c ~ 6.1 6 c < 6.1	> 6.1	> 6.1
9	Bromthymol blue	< 7.1	< 7.1	> 7.1	> 7.1
11	Neutral red	< 7.4	< 7.4	7 c > 7.4 4 c ~ 7.4	> 7.4
15	Phenol red	< 7.8	< 7.8	< 7.8	6 c > 7.8 9 c ~ 7.8
14	Cresol red	< 8.2	< 8.2	< 8.2	6 c ~ 8.2 8 c < 8.2

c = number of cases

1. táblázat

Limnomysis benedeni emésztőnedveinek pH-viszonyai

Mérések száma	Indikátor	Gyomor	Hepatopancreas	Középbél eleje	Középbél vége
13	klórfenol vörös	11 e > 6.0 2 e ~ 6,0	5 e > 6,0 8 e ~ 6,0	> 6,0	> 6,0
16	Brómkresol vörös	> 6,1	10 e ~ 6,1 6 e < 6,1	> 6,1	> 6,1
9	Brómtimolkék	< 7,1	< 7,1	< 7,1	< 7,1
11	Neutrál vörös	< 7,4	< 7,4	7 e > 7,4 4 e ~ 7,4	> 7,4
15	Fenol vörös	< 7,8	< 7,8	< 7,8	6 e > 7,8 9 e ~ 7,8
14	Kresol vörös	< 8,2	< 8,2	< 8,2	6 e ~ 8,2 8 e < 8,2

e = esetek száma.

As it appears from the *Table 1* the pH of the gastric juice is >6.0 or 6.1 and <7.1; in the case of the hepatopancreas the pH falls for the most part between 6.0—6.1. Although from these measurements no precise values can be read off, it may be established that the pH of the hepatopancreas falls between 6.0—6.2 since the transition points of 2 indicators are just near these values.

2. In *Amphipoda*, as it appears from *Fig. 2*, the 4 hepatopancreas tubes and the stomach as well as the anterior dorsal coecum and the posterior dorsal coeca are anatomical units readily separating from the intestine. The medium intestine extending from the stomach to the entry of the posterior coeca is anatomically uniform, but can be divided on the basis of pH values established with feeding experiments into 2 or 3 parts.

The pH conditions of part 1 perfectly agree with the pH of the hepatopancreas (therefore not indicated on the *Tables*), the pH conditions of the short-

er or longer part of sector 2 lasting about to the end of the posterior dorsal coeca and often readily recognizable, in other cases disappearing are under the influence of the hepatopancreas juice present even there sometimes neutral

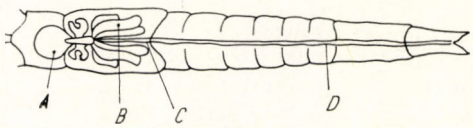

Fig. 1. Schematic drawing of the alimentary tract of *Limnomysis benedeni* CZERN. A = stomach, B = hepatopancreas, C = beginning of medium intestine, D = end of medium intestine. The pH at the site C is frequently lower than at D.

1. ábra: Limnomysis benedeni CZERN. emésztőtraktusának vázlatos rajza. A = gyomor, B = hepatopancreas, C = középbél eleje, D = középbél vége, A pH C helyen gyakran alacsonyabb, mint D -n

sometimes more alkaline, in conformity with the pH conditions prevailing in the last third of the intestine. In the last third of the intestine the pH is always alkaline *(Tables 2.* and *3.)*.

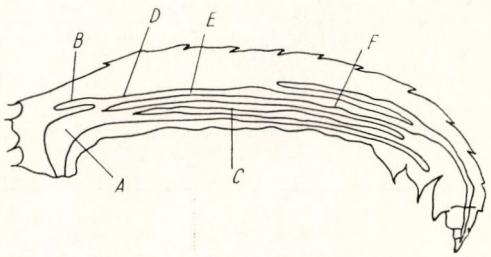

Fig. 2. Schematic drawing of the alimentary canal of *Amphipoda* A = stomach, B = anterior dorsal coeca, C = hepatopancreas, D = beginning of medium intestine, E = median, from the viewpoint of pH transitory part of medium intestine, F = end of medium intestine, posterior dorsal coeca

2. ábra: Amphipoda emésztőcsatorna vázlatos rajza. A = gyomor, B = elülső dorsalis coeca, C = hepatopancreas, D = középbél eleje, E = = középbél középső, pH szempontból átmeneti része, F = középbél vége, hátulsó dorsalis coeca

Between *Dicerogammarus villosus bispinosus* and *Dicerogammarus haematobaphes balatonicus* no essential difference exists either as regards the mode of nutrition or the quality of the feed consumed or in the pH values obtained; so on the Table the two species were drawn together.

Gammarus roeseli were continuously examined over the whole year but no seasonal differences found.

On the basis of the results from the feeding experiments we established that there is no essential difference between the pH conditions of the gastric juices of the 3 *Amphipoda* species. The results in *Tables 2.* and *3.* were controlled with WIGGLESWORTH'S method *(Table 4.)*.

Table 2.

The pH conditions of the gastric juices of *Dicerogammarus villosus bispinosus* and *Dicerogammarus haematobaphes balatonicus*

Number of measurements	Indicator	Stomach	Hepatopancreas	Med. intest.* 2nd third	Med. intest. 3rd. third
17	Chlorphenol red	13 c > 6.0 4 c < 6.0	5 c > 6.0 5 c ~ 6.0 7 c < 6.0	> 6.0	> 6.0
29	Bromcresol red	18 c > 6.1 3 c ~ 6.1 8 c < 6.1	10 c ~ 6.1 19 c < 6.1	> 6.1	> 6.1
26	Bromthymol blue	1 c ~ 7.1 25 c < 7.1	< 7.1	> 7.1	> 7.1
17	Neutral red	< 7.4	< 7.4	4 c > 7.4 6 c ~ 7.4 7 c < 7.4	> 7.4
21	Phenol red	< 7.8	< 7.8	< 7.8	12 c > 7.8 6 c ~ 7.8 3 c < 7.8
14	Cresol red	< 8.2	< 8.2	< 8.2	10 c < 8.2 4 c ~ 8.2

c = number of cases
Med. intest. = medium intestine

2. táblázat

Dicerogammarus villosus bispinosus és *Dicerogammarus haematobaphes* balatonicus emésztőnedveinek pH-viszonyai

Mérések száma	Indikátor	Gyomor	Hepatopancreas	Középbél 2. harmad	Középbél 3. harmad
17	Klórfenol vörös	13 e > 6,0 4 e < 6,0	5 e > 6,0 5 e ~ 6,0 7 e < 6,0	> 6,0	> 6,0
29	Brómkresolvörös	18 e > 6,1 3 e ~ 6,1 8 e < 6,1	10 e ~ 6,1 19 e < 6,1	> 6,1	> 6,1
26	Brómtimolkék	1 e ~ 7,1 25 e < 7,1	< 7,1	> 7,1	> 7,1
17	Neutrálvörös	< 7,4	< 7,4	4 e > 7,4 6 e > 7,4 7 e < 7,4	> 7,4
21	Fenolvörös	< 7,8	< 7,8	< 7,8	12 e > 7,8 6 e ~ 7,8 3 e < 7,8
14	Kresolvörös	< 8.2	< 8,2	< 8,2	10 e < 8,2 4 e ~ 8,2

Accordingly, the pH of the gastric juice of *Dicerogammarus villosus bispinosus* varies between 5.88—6.37; that of *Dicerogammarus haematobaphes balatonicus* between 5.82—6.37 and of *Gammarus roeseli* between the limits of 5.97 and 6.63, while the pH of the intestinal juice in the last third of the

Table 3

The pH conditions of the gastric juices of *Gammarus roeseli*

Number of measurements	Indicator	Stomach	Hepatopancreas	Med. intest. 2nd third	Med. intest 3rd third
30	Chlorphenol red	19 c > 6.0 6 c ~ 6.0 5 c < 6.0	19 c > 6.0 11 c < 6.0	> 6.0	> 6.0
25	Bromcresol red	19 c > 6.1 4 c ~ 6.1 2 c < 6.1	10 c > 6.1 4 c ~ 6.1 11 c < 6.1	> 6.1	> 6.1
37	Bromthymol blue	2 c ~ 7.1 35 c < 7.1	< 7.1	> 7.1	> 7.1
36	Neutral red	< 7.4	< 7.4	19 c > 7.4 9 c ~ 7.4 8 c < 7.4	> 7.4
54	Phenol red	< 7.8	< 7.8	18 c ~ 7.8 36 c < 7.8	15 c > 7.8 9 c ~ 7.8 30 c < 7.8
43	Cresol red	< 8.2	< 8.2	8.2	25 c ~ 8.2 18 c < 8.2

3 táblázat

Gammarus roeseli emésztőnedveinek pH-viszonyai

Mérések száma	Indikátor	Gyomor	Hepatopancreas	Középbél 2. harmad	Középbél 3. harmad
30	Klórfenolvörös	19 e > 6,0 6 e ~ 6,0 5 e < 6,0	19 e > 6,0 11 e < 6,0	> 6,0	> 6,0
25	Brómkresolvörös	19 e > 6,1 4 e ~ 6,1 2 e < 6,1	10 e > 6,1 4 e ~ 6,1 11 e < 6,1	> 6,1	> 6,1
37	Brómtimolkék	2 e ~ 7,1 35 e < 7,1	< 7,1	> 7,1	> 7,1
36	Neutrál vörös	< 7,4	< 7,4	19 e > 7,4 9 e ~ 7,4 8 e < 7,4	> 7,4
54	Fenolvörös	< 7,8	< 7,8	18 e ~ 7,8 36 e < 7,8	15 e > 7,8 9 e ~ 7,8 30 e < 7,8
43	Kresolvörös	< 8,2	< 8,2	< 8,2	25 e ~ 8,2 18 e < 8,2

medium intestine fell in the case of *villosus* between 7.72—8.06 in *haematobaphes* between 7.59—8.07 and in *roeseli* between 7.61—7.99.

The pH of the hepatopancreas or gastric juice respectively of 322 *Amphipoda* was measured with BECKMAN'S pH-meter. The pH of the hepatopancreas juice of *Gammarus roeseli* was 6.16 on the average; in the case of *Dicerogammarus villosus bispinosus* it was 6.12 while in *Dicerogammarus haematobaphes*

Table 4.

Determination of the pH of the digestive systems of *Amphipoda* by WIGGLESWORTH's method

Animal	Juice	Indicator	Measurements	$\bar{x}$	S ±
Dicerogammarus villosus bispinosus	Stomach	Chlorphenol red	51	6.18	0.18
	,,	Bromcresol red	60	6.18	0.19
	,,	Bromthymol blue	44	6.19	0.21
	Medium intestine 3rd third	Phenol red	32	7.89	0.17
		Cresol red	27	7.95	0.11
Dicerogammarus haematobaphes balatonicus	Stomach	Chlorphenol red	40	6.19	0.18
	,,	Bromcresol red	24	6.0	0.18
	,,	Bromthymol blue	13	6.1	0.16
	Medium intestine 3rd third	Phenol red	37	7.83	0.24
		Cresol red	14	7.80	0.18
Gammarus roeseli	Stomach	Chlorphenol red	20	6.17	0.20
		Bromcresol red	17	6.19	0.21
		Bromthymol blue	55	6.32	0.31
	Medium intestine 3rd third	Phenol red	17	7.80	0.19
		Cresol red	39	7.79	0.12

4. táblázat

Az *Amphipodák* emésztőrendszerei pH-jának meghatározása Wigglesworth-módszerrel

Állat neve	Nedv	Indikátor	Mérések	$\bar{x}$	S ±
Dicerogammarus villosus bispinosus	gyomor	klórfenolvörös	51	6,18	0,18
	,,	brómkresolvörös	60	6,18	0,19
	,,	brómtimolkék	44	6,19	0,21
	középbél 3. harmad	fenolvörös	32	7,89	0,17
	középbél 3. harmad	kresolvörös	27	7,95	0,11
Dicerogammarus haematobaphes balatonicus	gyomor	klórfenolvörös	40	6,19	0,18
	,,	brómkresolvörös	24	6,0	0,18
	,,	brómtimolkék	13	6,1	0,16
	középbél 3. harmad	fenolvörös	37	7,83	0,24
	középbél 3. harmad	kresolvörös	14	7,80	0,18
Gammarus roeseli	gyomor	klórfenolvörös	20	6,17	0,20
	,,	brómkresolvörös	17	6,19	0,21
	,,	brómtimolkék	55	6,32	0,31
	középbél 3. harmad	fenolvörös	17	7,80	0,19
	középbél 3. harmad	kresolvörös	39	7,72	0,12

balatonicus 6.10. The gastric juice gave for all three species higher values i. e. in the above order of species 6.37:, 6.31 and 6.25.

3. The digestive canal of *Asellus (Fig. 3)* was divided in feeding experiments on the basis of the pH of the juices found in them into 2 parts *(Table 5)*. To the first part belong the hepatopancreas tubes while the second part is determined by the pH of the juices in the gastric and intestinal tract. The 4 hepatopancreas tubes under the influence of various indicators (mainly chlorphenol red and bromcresol red) often stained in two ways: the secretion at the end of the tubes proved to be more acid than in the vicinity of the entry.

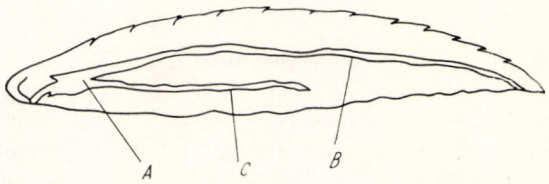

Fig. 3. Schematic drawing of the alimentary canal of *Asellus aquaticus*
A = stomach, B = medium intestine, C = hepatopancreas
3. ábra: Asellus aquaticus emésztőcsatorna vázlatos rajza.
A = gyomor, B = középbél, C = hepatopancreas

Table 5.

The pH conditions of the digestive juices of *Asellus aquaticus*

Number of measurements	Indicator	Hepatopancreas	Stomach + intestine
15	Chlorphenol red	~ 6.0	> 6.0
9	Bromcresol red	3 c ~ 6.1	
		6 c < 6.1	> 6.1
11	Bromthymol blue	< 7.1	3 c > 7.1
			8 c ~ 7.1
12	Neutral red	< 7.4	< 7.4
12	Phenol red	< 7.8	< 7.8
10	Cresol red	< 8.2	< 8.2

5. táblázat

Asellus a uqaticus emésztőnedveinek pH-viszonyai

Mérések száma	Indikátor	Hepatopancreas	Gyomor + bél
15	Klórfenolvörös ..	$\sim 6{,}0$	$> 6{,}0$
9	Brómkresolvörös .	3 e $\sim 6{,}1$	
		6 e $< 6{,}1$	$> 6{,}1$
11	Brómtimolkék ...	$< 7{,}1$	3 e $> 7{,}1$
			8 e $\sim 7{,}1$
12	Neutrálvörös	$< 7{,}4$	$< 7{,}4$
12	Fenolvörös	$< 7{,}8$	$< 7{,}8$
10	Kresolvörös	$< 8{,}2$	$< 8{,}2$

The pH values of the hepatopancreas of *Asellus aquaticus*, after having been prepared according to the methodics described were measured also with BECKMAN'S pH meter. In the case of 96 animals a mean value of 6.08 was found.

Of the indicators enumerated in the methodical part we did not use bromphenol blue and methyl red because we found in the course of preliminary experiments that their pK falls outside the area of the pH of the digestive juices of the animals examined.

In no species did we determine the pH of the rectum because this is covered by chitin cuticle and, except for *Asellus*, it never showed staining.

Discussion

The Crustaceans examined can be ranged taxonomically in 4 groups (ordo): *Mysidacea (Limnomysis* b) *Amphipoda (Dicerogammarus and Gammarus* r.*)*, *Isopoda (Asellus* a.) and *Decapoda (Astacus l.)*. Our results were compared with the data of other authors in the case of 3 ordines *(Amphipoda, Isopoda, Decapoda)* as in the available literature data were found only for the representatives of these 3 orders.

Our data obtained for the pH conditions of the digestive juices of the species belonging to *Amphipoda* can be well compared with the works of AGRAWAL (1963) and DE GIUSTI and co-workers (1962), although these authors obtained their results with the use of one method (indicator paper or feeding method). Comparison of these results is presented in *Table 6*.

From the Table it appears that our results are nearer to the values found by AGRAWAL and rather differ from the data of DE GIUSTI and co-workers. This is particularly remarkable in the case of the stomach and of the hepatopancreas where regarding the extreme values the deviation is almost 2.0 pH whereas in the 3. third of the medium intestine we obtained values similar to the results of DE GIUSTI.

Table 6.

Comparison of the pH conditions in the digestive tract of the species belonging to the ordo *Amphipoda*

Name of species	Author	Stomach	Hepatopancreas	Medium intestine[1]	End of medium intestine[2]
Corophium volutator	AGRAWAL	6.7	6.2	6.6	—
Hyalella azteca	DE GIUSTI	4.1—4.7	3.8—4.7	6.8—7.2	7.2—7.9
Gammarus limnaeus	DE GIUSTI	4.1—6.3	3.8—4.8	6.4—7.2	7.2—7.9
Dic. vill. bispinosus	P. ZÁNKAI	5.9—6.4	5.8—6.1	7.1—7.4	7.7—8.1
Dic. haem. balatonicus	P. ZÁNKAI	5.8—6.4	5.8—6.1	7.1—7.4	7.6—8.1
Gammarus roeseli	P. ZÁNKAI	6.0—6.6	5.8—6.2	7.1—7.8	7.6—8.0

[1] Medium intestine: results measured in the 2nd third of the medium intestine were ranged here.

[2] End of medium intestine: data of pH conditions found in the 3rd third of the medium intestine were presented here.

6. táblázat

Az *Amphipoda* csoporthoz tartozó fajok emésztőtraktusa pH viszonyainak összehasonlítása

Faj neve	Szerző	Gyomor	Hepatopancreas	Középbél[1]	Középbél vége[2]
Corophium volutator	AGRAWAL	6,7	6,2	6,6	—
Hyalella azteca	DE GIUSTI	4,1—4,7	3,8—4,7	6,8—7,2	7,2—7,9
Gammarus limnaeus	DE GIUSTI	4,1—6,3	3,8—4,8	6,4—7,2	7,2—7,9
Dic. vill. bispinosus	P ZÁNKAI	5,9—6,4	5,8—6,1	7,1—7,4	7,7—8,1
Dic. haem. balatonicus	P. ZÁNKAI	5,8—6,4	5,8—6,1	7,1—7,4	7,6—8,1
Gammarus roeseli	P. ZÁNKAI	6,0—6,6	5,8—6,2	7,1—7,8	7,6—8,0

Középbél[1] = a középbél 2. harmadában mért eredményeket soroltuk ide.
Középbél vége[2] = a középbél 3. harmadában talált pH viszonyok adatait tüntettük itt fel.

These deviations can not be motivated with the difference in the nutrients used in the feeding experiments alone (the above mentioned authors used yeast, rice starch, gelatin etc. as feed). On the basis of a number of authors (SRIVASTAVA, RASTOGI and DATTA GUPTA, WATERHOUSE) and of our own experiments we may state with certainty that the pH of the feed is in no connection with the pH of the digestive tract. It may be assumed that the differences are brought about by specific and geographic dissimilarities, by the different environmental conditions and by the conditions of other types of nutrition together. Both *Gammarus limnaeus* and *Hyalella azteca* are characteristic *Amphipoda* of the American continent, on the nutritional conditions of which PENNAK (1953, p. 436) states: Like the decapods, the scuds are omnivorous, general scavangers. As a contrast, the *Amphipoda* examined by us are in the first place herbivorous *(Dicerogammarus)* and only to a small part carnivorous *(Gammarus* r.).

Between *Asellus aquaticus* and the littoral *Ligia oceanica* pertaining also to this group — inspite of the difference in the methods used for the determination of pH — there is much similarity. The pH of the hepatopancreas is 6.0. in *Ligia*, 6.1 in *Asellus* and in the pH of the intestinal juice there are no such great differences *(Asellus* ~7. 1, *Ligia* 6.5) as could be expected.

We found the pH of the gastric juice of *Astacus leptodactylus* to be 5.2 while VONK (1935) refers to a value of 5.0.

Results obtained from the examination of the pH conditions of the digestive tract of the 6 species point out that between the pH of the gastric juice of *Astacus*, *Amphipoda* and *Asellus* there are great differences whereas the pH conditions of the hepatopancreas of the 6 species examined almost agree (6.1 — 6.3). The last third of the medium intestine *(Amphipoda)* or second part respectively *(Limnomysis)* closely agree while in *Asellus* almost neutral pH was measured from stomach to rectum.

It has been established that between beginning and medial part of the medium intestine of *Limnomysis* and *Amphipoda* and its end substantial differences prevailed from the point of view of pH while at the same time in *Asellus* the whole medium intestine exhibited a uniform value.

The phenomenon that the intestinal tract of the species referred to above could be devided or drawn together *(Asellus)* beside the anatomically separated foregut and medium intestine into further parts, can be explained by the

expansion of the juices impouring into the intestine. The pH of the whole intestinal tract ought to be determined by the juice of the unique gland producing digestive enzyme, the hepatopancreas of about 6 pH. In the development of the somewhat higher pH of the gastric juice — in our opinion — the anterior dorsal coecum may be involved which occurs in all Crustaceans examined and the physiological role and significance of which is not elucidated so far. In the course of our experiments we found that the nutrient particles get into the anterior dorsal coecum the pH of which is nearly neutral. The beginning or first third of the medium intestine in *Amphipoda* agrees with the pH of the hepatopancreas; subsequently the medium intestine readily tracebly gets alkalized to about 8 pH. This alkalinization is brought about by the posterior dorsal coeca. These are indicated as a secretory organ (KAESTNER 1959) which introduces its secretion into the intestine at the limit of medium intestine and rectum.

According to our examinations the pH conditions of the digestive canals are between certain rather constant limits. They are not essentially influenced by the various seasons, the condition of the animal (starving, repletion). As it was shown, between the orders of Crustaceans there are first of all important differences in the pH of the gastric juice. It is very interesting that the greatest differences can be found exactly between the animals most distant from the aspect of phylogenetic relationship e. g. between *Astacus* and *Asellus* $\sim$ 2.0 pH, between *Amphipoda* and *Astacus* $\sim$ 1. 0. pH. In the first case the degree of relationship is more distant than in the latter.

In our earlier work (PONYI and P. ZÁNKAI 1966) the pH — optimum of the endopeptidase in the hepatopancreas digestive juice of the animals enumerated in the Introduction was examined (substrate: denaturated hemoglobin). It has been established that in *Astacus* the optimum is at 5.2 pH which perfectly coincides with the value obtained at the determination of the pH of the gastric juice. In *Amphipoda* we obtained as pH optimum 6.6—7.1. which essentially does not greatly differ from the natural pH conditions. This is particularly valid in the case of *Gammarus roeseli* where the upper limit of the pH of the gastric juice is 6.6. The pH optimum of the hepatopancreas juice of *Asellus* is marked by the values 5.5—6.0 while the natural pH of the hepatopancreas juice is 6. 1. In the case of *Limnomysis* no similar agreement could be demonstrated. These results seem to verify the assumption of KRISHNA and SAXENA (1963) inasmuch as the later examinations corroborate the presence of several enzymes in the digestive juice and the pH optimum of the signle enzymes coincides with the pH optimum of the enzyme-mixtures constituting the gastric juice.

Summary

The pH conditions of the juices in the alimentary canal of 6 Crustaceans *(Limnomysis benedeni, Dicerogammarus haematobaphes balatonicus, Dicerogammarus villosus bispinosus, Gammarus roeseli, Asellus aquaticus, Astacus leptodactylus* were examined with the aid of 3 methods.

In the case of *Limnomysis* the pH of the stomach $>$ 6.0 or 6.1 respectively and $<$7.1, of the hepatopancreas 6.0 — 6.1 The beginning and the end of the medium intestine can often be separated on the basis of the pH differences. The intestinal juice indicates values of 7.4—7.8 pH.

In *Amphipoda* the anatomically unitary medium intestine can be divided according to the pH conditions of its juice in 3 parts: the pH of part 1 coincides with the pH of the hepatopancreas. Section 2 lasts about to the end of the posterior dorsal coeca, it is often readily recognizable, in other cases indistinct, its pH is sometimes acid (hepatopancreatic effect) in other cases neutral (upon the action of the juice of the posterior dorsal coeca). In the last third of the intestine the pH is always alkaline. The pH conditions of the digestive juices of *Dicerogammarus* are as follows: stomach 5.8 — 6.4, hepatopancreas 5.8—6.1, medium intestine 2nd third 7.1—7.4, medium intestine 3rd third 7.6—8.1. In the case of *Gammarus roeseli*: stomach 6.0—6.6, hepatopancreas 5.8—6.2, medium intestine 2nd third 7.1—7.8, 3rd third 7.6—8.0.

In the case of *Asellus aquaticus* the anatomically readily separating stomach and medium intestine on the basis of the pH of their juices must be drawn together, so the pH of the hepatopancreas is 6.1, that of the stomach and medium intestine nearly neutral.

It has been further established that seasons and starving do not essentially influence the pH of the digestive juices.

Author establishes a parallel between the pH optimum obtained with biochemical methods of the gastric juice of some species and the natural pH conditions.

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NÉHÁNY RÁKFAJ EMÉSZTŐCSATORNÁJÁNAK pH-VISZONYAIRÓL

P.-Zánkai Nóra

Összefoglalás

A szerző;6 rákfaj *(Limnomysis benedeni, Dicerogammarus haematobaphes balatonicus Dicerogammarus villosus bispinosus, Gammarus roeseli, Asellus aquaticus, Astacus leptodactylus)* emésztőcsatornájában levő nedvek pH viszonyait vizsgálta 3 módszer segítségével.

A *Limnomysis* esetében a gyomor pH-ja $> 6{,}0$ ill. 6,1 és $< 7{,}1$, a hepatopancreas 6,0—6,1. A középbél eleje és vége gyakran szétválasztható a pH különbségek alapján. A bélnedv 7,4—7,8 pH értékeket mutat.

Az *Amphipodáknál* az anatómiailag egységes középbél a benne levő nedv pH viszonyai alapján 3 részre osztható: 1 rész pH-ja megegyezik a hepatop. pH-jával. A 2. szakasz kb. a hátulsó dorsális coeca-k végéig tart, gyakran jól felismerhető, máskor elmosódik, pH-ja némelykor savas (hepatop. hatás), máskor semleges (hátulsó dorsalis coeca-k nedvének hatására). A bél utolsó harmadában a pH mindig lúgos. A *Dicerogammarusok* emésztőnedveinek pH viszonyai a következőképpen alakulnak: gyomor 5,8—6,4, hepatop. 5,8—6,1, középbél 2. harmad 7,1—7,4, középbél 3. harmad 7,6—8,1. A *Gammarus roeseli* esetében: gyomor 6,0—6,6, hepatop. 5,8—6,2, középbél 2. harmad 7,1—7,8, 3. harmad 7,6—8,0.

Az *Asellus aquat.* esetében az anatómiailag jól elkülönülő gyomrot és középbelet nedveik pH-ja alapján össze kell vonni, így a hepatop. pH-ja 6,1, a gyomor + középbél közel semleges.

Megállapítást nyert továbbá, hogy az évszakok, az éhezés nem befolyásolja jelentősen az emésztőnedvek pH-ját.

A szerző párhuzamba állítja egyes fajok emésztőnedvének biokémiai módszerekkel kapott pH optimumát és a természetes pH viszonyokat.

ОБ УСЛОВИЯХ pH В ПИЩЕВАРИТЕЛЬНОМ ТРАКТЕ НЕКОТОРЫХ ВИДОВ РАЧКОВ

Нора Р.-Занкаи

Изучали pH тремя методами в пищеварительном тракте 6 видов рачков *(Limnosys benedeni, Dicerogammarus haematobaphes balatonicus, Dicerogammarus vielosus bispinosus, Gammarus, roeseli, Asellus aquaticus.*

У *Lymnomysis* pH желудка 6,0 или 6,1 и 7,1, гепатопанкреас 6,0—6,1. По различию pH часто можно выделить в кишке переднюю и заднюю части. pH кишечного сока 7,4—7,8.

У амфипод среднюю кишку, несмотря на ее анатомическую однородность, можно разделить на три части: pH первой части соответствует pH гепатопанкреаса; pH средней части кислый или нейтральный, а задней всегда основной. pH пищеварительных соков *Dicerogammarus:* желудок 5,8—6,4, гепатопанкреас 5,8—6,1, 2-ая часть средней кишки 7,1—7,4, 3-ья часть средней кишки 7,6—8,1. У *Gammarus roeseli:* желудок 6,0—6,6, гепатопанкреас 5,8—6,2, 2-ая часть средней кишки 7,1—7,8, 3-ья часть средней кишки 7,6—8,0.

У *Asellus aquaticus* pH гепатопанкреаса 6,1, а желудка и средней кишки почти нейтрален, хотя анатомически желудок и средняя кишка представляют хорошо различимые отделы.

Сезоны и голодание не влияют на pH пищеварительных соков.

Определенный биохимически оптимум pH для пищеварительных соков сопоставляли с естественным pH соответствующих отделов.

KRÓNIKA

Az Intézetben az 1965-ös évben az előző évekhez hasonlóan három osztály működött.

A *Kísérleti Állattani Osztály* tovább folytatta a gerinctelen állatok idegrendszere szerkezetének, elemi és komplex működésének, valamint neurohumorális szabályozásának vizsgálatát. A kutatásokban fiziológiai, morfológiai és biokémiai módszereket alkalmaztak, a vizsgálati objektumok hazai édesvízi és különféle tengeri Lamellibranchiáták, továbbá Gastropodák voltak.

A *Hidrobiológiai Osztály* tudományos tervét erősen módosította az 1965 tavaszán fellépett nagymértékű balatoni halpusztulás, melynek következtében a munka átmenetileg a pusztulás okainak és körülményeinek vizsgálatára összpontosult. Ennek folytatásaként az év második felében a korábbinál rendszeresebben kezdődött meg a Balaton plankton, bentosz és nektonkutatása. A vizsgálatokba számos más intézmény is bekapcsolódott. A munka koordinálását jelentős mértékben az Intézet végezte és végzi.

A *Kísérleti Növénytani Osztály* a szennyvízbiológia algaélettani problémáival foglalkozott, majd az osztályvezető és beosztottja intézetből való távozása után, november 17-től az Intézet felügyeleti szerve (MTA Biológiai Tudományok Osztálya) a Kísérleti Növénytani Osztály működésének szüneteltetését rendelte el.

Ez év folyamán is aktív tudományos tevékenységet fejtett ki az Intézetben Dr. Sebestyén Olga nyugalmazott osztályvezető. Munkáját a „Tótörténeti tanulmányok balatoni üledéken" c. téma keretében folytatta.

Az Intézet személyi állománya: 1965. január 1-én 48 főből állt, ami a következőképpen oszlott meg: kutató: 16 + 1 tudományos gyakornok, kutatási segéderő: 17, adminisztratív munkaerő: 5, egyéb: 9.

Az Intézet személyi állományában több változás következett be. Szeptember 1-vel megvált a Intézettől Hiripi László vegyész és H.-Vas Éva biológia-kémia szakos tanár. A megüresedett állások egyikére szeptember 15-vel kinevezésre került Varanka István biológia-kémia szakos tanár, aki a Kísérleti Állattani Osztályra nyert beosztást. November 1-vel Dr. Felföldy Lajos, a Kísérleti Növénytani Osztály vezetője és Tóth László tudományos munkatárs távoztak el saját kérésükre a Vízgazdálkodási Tudományos Kutató Intézethez.

Újonnan szervezett állásokra június 1-én PÉCSI TIBOR tudományos gyakornokot, majd szeptember 1-én DR. PONYINÉ ZÁNKAI NÓRÁT nevezték ki tudományos segédmunkatárssá. PÉCSI TIBOR a Kísérleti Állattani Osztályon, DR. PONYINÉ ZÁNKAI NÓRA pedig a Hidrobiológiai Osztályon nyert beosztást. Ezenkívül egy kutatási segéderő került kinevezésre.

Jelentősen növekedett az Intézet műszer-, valamint könyv- és folyóirat-állománya.

Jelentősebb új műszerek:

DISA Multistim, DISA Universal indikátor, fotokimográfok, liofilizátor, kriosztát.

A könyvtárállomány 147 kötettel, a folyóiratállomány 583 kötettel gyarapodott. A könyvtári egységek száma 1965 december 31-én 41.909. Értékes ajándékot kapott Könyvtárunk többek között C. M. TARZWELL professzortól (USA), aki hidrobiológiai különlenyomatgyűjteményét ajándékozta Intézetünknek, valamint UDVARDI MIKLÓS professzortól (USA), aki hat biológiai tárgyú könyvet ajándékozott.

Az 1965. év végén megjelent intézeti Évkönyvünket (Annal. Biol. Tihany, Vol. 32) 555 helyre küldtük el, melynek ellenértékeként mintegy 344 folyóiratot kaptunk cserébe.

Tovább folytatódtak az Intézetben a felújítási munkák. Teljesen befejeződött az intézeti partvédelem korszerűsítése, és elkészült hajó- és csónakkikötőnk is. Teljes külső felújítást kapott a kutatóintézet és megkezdődött az intézeti park rendezése. Az Intézet belső berendezése és bútorzata ugyancsak felújításra került. Az év utolsó negyedében emeletráépítés kezdődött kutatólakások kialakítása céljából. A felújításra és korszerűsítésre költött összeg megközelíti a 2 millió forintot.

Lebontásra került az Intézet korszerűtlen, nem üzemeltethető üvegháza, melynek felhasználható anyaga átadásra került az MTA Vácrátóti Botanikai Kutatóintézetnek.

Az Intézet nemzetközi kapcsolatai tovább fejlődtek. A tudományos munkatársak 12 alkalommal jártak külföldön, s jellemző volt a 2 hónapnál hosszabb kinntartózkodás. Az alábbi kutatók vettek részt külföldi tanulmányutakon:

DR. SALÁNKI JÁNOS intézeti igazgató, 1965 március 1-vel kezdődően 4 hónapot töltött Olaszországban, a Nápolyi Zoológiai Állomáson, majd szeptember 5-től kezdődően 6 hónapot dolgozott Angliában, a Southamptoni Egyetem Biokémiai és Élettani Intézetében. Ez idő alatt részt vett St. Andrewsben a Society for Experimental Biology „Nervous and Hormonal Mechanisms of Integration" szimpoziumán is.

DR. ENTZ BÉLA igazgatóhelyettes, februárban Moszkvában az Össz-szövetségi Hidrobiológiai Konferencián vett részt, majd július 20-tól 6 hetet töltött a Borok-i Hidrobiológiai Intézetben (Szovjetunió), ezt követően részt vett a Nemzetközi Limnológiai Társaság Kongresszusán Varsóban (Lengyelország).

FARKAS TIBOR és HERODEK SÁNDOR tudományos munkatársak szeptemberben két hetet töltöttek a Milànoi Farmakológiai Intézetben, ahol Lipid-Biokémiai Symposiumon és metodikai tanfolyamon vettek részt.

Dr. Zs. Nagy Imre 1964 decemberétől 1965 június elejéig a Szovjetunió Tudományos Akadémiája Szevercovról elnevezett Állatmorfológiai Intézetében (Moszkva) dolgozott, a két intézet között fennálló egyezményes téma keretén belül.

Szabó Ernő június 1-től a Tübingeni Egyetem Kémiai-Növényfiziológiai Intézetében (NSZK) 1 éves szerződéssel dolgozik.

Dr. Ponyi Jenő a Hallei Egyetem Fiziológiai-Kémiai Intézetében (NDK) 3 hónapos tanulmányúton vett részt, szeptembertől kezdődően.

Dr. S.-Rózsa Katalin november 20-tól Angliában, a Southamptoni Egyetem Biokémiai és Élettani Intézetében dolgozott két és fél hónapot.

P. Zánkai Nóra októberben két hetet töltött a Hallei Egyetem Fiziológiai-Kémiai Intézetében (NDK).

Intézetünket is számos kutató kereste fel, akik közül többen hosszabb-rövidebb ideig Tihanyban dolgoztak. Két hétnél több időt töltött az Intézetben:

M. L. Albrecht — Német Tudományos Akadémia Halászatbiológiai Intézete — Berlin (NDK), R. Kilias — Humbold Egyetem, Zoológiai Intézete — Berlin, (NDK), H. A. Hafiez — Kairói Orvosi Egyetem — Egyesült Arab Köztársaság, M. Salah — Alexandriai Egyetem Hidrobiológiai Intézete, Egyesült Arab Köztársaság, J. Paschalsky — Varsói Nencki Intézet — Lengyelország, K. Kuziemsky — Gdanski Orvosi Akadémia Élettani Intézete, Lengyelország, S. N. Nistratova Szovjetunió Tudományos Akadémiájának Szevercovról elnevezett Állatmorfológiai Intézete (Moszkva).

Az említetteken kívül különböző európai és tengerentúli országokból, mintgy 100 szakember tett rövidebb látogatást az Intézetben.

Hazai kutatók közül az alábbiak dolgoztak vendégkutatóként az Intézetben:

A. Bothár Anna — Dunakutató Állomás, Alsógöd
Dr. Meszes Gabriella — ELTE Növényélettani Intézet, Budapest
Dr. Perényi László — Országos Korányi TBC Intézet, Budapest
Tóth János — Dunakutató Állomás, Alsógöd
Dr. Uherkovich Gábor — MTA Tiszakutató Csoportja, Szeged

A nyári hónapokban 11 magyar és 3 német egyetemi hallgató kapcsolódott be az Intézet munkájába 1—1 hónapi időtartammal. Komáromi Zsuzsa és Pinker Ilona diplomamunkájukat is az Intézetben dolgozták ki.

Az Intézet kollaboráció kapcsolatai 1965. év folyamán jelentősen bővültek Tovább folytatódott a korábban kialakult együttműködés az Országos Korányi TBC Intézettel, a Központi Kémiai Kutató Intézettel, a Budapesti Orvostudományi Egyetem Szövettani Intézetével, a Központi Fizikai Kutató Intézettel, az Eötvös Loránd Tudományegyetem Növényélettani Intézetével, az Országos Élelmezés- és Táplálkozástudományi Intézettel. A balatoni halpusztulás kapcsán az Eötvös Loránd Tudományegyetem Állatrendszertani Intézetével, az Országos Közegészségügyi Intézettel, az Országos Mezőgazdasági Minőségvizsgáló Intézettel, a Vízgazdálkodási Tudományos Kutató Intézettel, továbbá az Országos Halászati Felügyelőséggel, Balatoni Halászati Vállalattal, a Veszprémi- és Somogymegyei Közegészségügyi és Járványügyi Állomásokkal és az Állategészségügyi Kutató Intézettel ez évben alakult ki együttműködés.

Augusztus folyamán egynapos programot bonyolított le Intézetünkben az IBRO Budapesten rendezett nemzetközi szemináriuma. Ennek kapcsán a résztvevők elsősorban a Kísérleti Állattani Osztály munkájáról, nevezetesen annak neurobiológiai kutatásairól kaptak részletes tájékoztatást.

1965 október 4—8-ig a Magyar Tudományos Akadémia Biológiai Tudományok Osztály az Intézetben rendezte meg „Az időszerű genetikai kérdések" c. kollokviumot, amelyen nagyrészt fiatal biológus kutatók vettek részt. A kollokviumon 10 előadás hangzott el.

Az Intézet kutatói az év folyamán hazai és nemzetközi rendezvényeken 18 tudományos előadást tartottak.

CHRONICLE

In 1965—as in the previous years — three departments functioned in the Institute.

The *Department of Experimental Zoology* continued investigations on the structure, the elementary and complex functioning of the nervous system and on the neurohumoral regulation of Invertebrates. Inland fresh-water and different marine Lamellibranches and Gastropods were investigated by physiological, morphological and biochemical methods.

The scientific project of the *Department of Hydrobiology* was strongly modified by the heavy fish kill occurring in Lake Balaton in the spring of 1965 as the work was temporarily focussed on the investigations of the causes and circumstances of the fish kill. As consequence in the second half of the year the investigation of the plankton, benthos and nekton of Lake Balaton was carried out more extensively and regularly than earlier. Several institutions joined in this work, coordinated by the Institute.

The *Department of Experimental Botany* investigated the problems of water pollutions connected with the physiology of algae. After the departure of the Head of the Department and his coworker from the Institute, the superintending authority of the Institute (MTA Department of the Biological Sciences) decided the suspension of the activity of this Department from 17 November 1965.

Dr. Olga Sebestyén, retired head of the Hydrobiological Department, continued her work on problems of „Lake history studies on the sediments of Lake Balaton".

Several changes took place in the staff of the Institute. L. HIRIPI chemist and É. H.-VAS biologist left on the 1 September. For one of the vacant posts in the Zoological Department I. VARANKA biologist-chemist was appointed on the 17 September. Dr. L. FELFÖLDY, Head of the Department of Experimental Botany and L. TÓTH scientific research worker left the Institute for VITUKI at their own request on the 1 November. On the 1 of June T. PÉCSI (assitant), on the 1 September. dr. N. P.-ZÁNKAI were appointed as scientific coworkers for the newly organized posts at the Zoological and the Hydrobiological Department resp. Furthermore one technical assitant was also appointed.

Important accessions were made by acquisitions of equipment:

DISA Multistim., DISA Universal Indicator, photokymographs, liophylisator, and kryostate.

The stock of the Library consisted of 41.909 units on the 31 December 1965. The increase of the Library in 1965 showed 147 books and 543 volumes of periodicals. Among other valuable gifts our Library received a collection of hydrobiological reprints from Prof. C. M. TARZWELL (USA) and six biological books from Prof. M. UDVARDY (USA). Our Year Book (Annal. Biol. Tihany Vol. 32.) published at the end of 1965, was forwarded to 555 institutions, in return the Library received 344 periodicals.

Renovations in the Institute were also in progress. The embankment and the pier for ship and boat was finished. The new planning of the park began and outer and inner innovations of the Institute also took place.

At the end of the year work has begun on a new storey of a lodging house for new flats for research workers. The total sum of the renovations and innovations approached 2 million forints. The out-of-date greenhouse was demolished, its usable constituents were delivered to the Botanical Institute of the Hungarian Academy of Sciences at Vácrátót.

Foreign relationships were further extended. The members of the staff visited foreign countries on 12 occasions. The stays abroad in general lasted more than 2 months.

DR. J. SALÁNKI director spent from the 1. March 1965 4 months in Italy at the Zoological Station of Naples, then from the 5. of September he worked in England at the Department of Physiology and Biochemistry of the University of Southampton. He also attended the Symposium of the Society for Experimental Biology on the "Nervous and Hormonal Mechanisms of Integration", held at St. Andrews.

DR. B. ENTZ deputy director attended the Federal Hydrobiological Conference, held in February at Moscow, then spent six weeks in the Hydrobiological Institute Borok (USSR), afterwards attended the SIL Congress in Warsaw (Poland).

T. FARKAS and S. HERODEK spent 2 weeks in September in Milan, where they attended the Symposium on "Drugs Affecting Lipid Metabolism", and the "Course of Methods in Lipid Research".

DR. I.-ZS.-NAGY worked from December 1964 till June 1965 in the Severcov Morphological Institute (Moscow) within the frame of the common projects of the two institutions.

E. SZABÓ worked from the 1 June in the Chemical Plant Physiological Institute of the University of Tübingen on a one-years contract.

DR. J. PONYI spent three months in the Institute of Physiological Chemistry of the University of Halle (East-Germany).

DR. KATALIN S.-RÓZSA worked at the Department of Physiology and Biochemistry of the University of Southampton in England, from the 20 November for 10 weeks.

ELEONÓRA P.-ZÁNKAI spent 2 weeks in October in the Institute of Physiological Chemistry at the University of Halle (East-Germany).

Several investigators were for a shorter or longer time in Tihany. For more than two weeks the following worked in the Institute:

DR. M. R. ALBRECHT, Fishery Biological Institute of the DAW, Berlin (East-Germany),

DR. R. KILIAS, Zoological Institute of the Humbold University, Berlin (East-Germany),

DR. A. A. HAFIZE, Medical Faculty of the University of Cairo (UAR),

Dr. M. Salah, Hydrobiological Faculty of the University of Alexandria (UAR),

Dr. J. Paschalsky, Nencki Institute, Warsaw (Poland),

Dr. K. Kuziemsky, Physiological Institute of the Medical Academy, Gdansk (Poland),

Dr. S. N. Nistratova, Severcov Morphological Institute, Moscow (USSR).

In addition to the above mentioned scientists about a hundred specialists from different European countries and from overseas paid shorter visits to the Institute.

The following Hungarian scientists worked here as guest workers:

Anna A. Bothar, MTA Station for Duna Research, Alsógöd, Dr. Gabriella Meszes, ELTE Institute for Plant Physiology, Budapest, Dr. László Perényi, Korányi TBC Institute, Budapest, Dr. Gábor Uherkovich, MTA Station for Tisza Research, Szeged.

In the summer months 11 Hungarian and 3 German university students participated in the Institute's work for one-one months. Zsuzsa Komáromi and Ilona Pinker prepared their diplom work also in the Institute.

The scientific collaboration of the Institute was significantly enlarged in 1965. The already existing cooperation with the Korányi TBC Institute, Central Chemical Research Institute, BOTE Histological Institute, Central Physical Research Institute, ELTE Institute of Plant Physiology and with the Institute of Nutrition was continued.

New cooperations took place in connection with the fish kill in Lake Balaton with the ELTE Zoosystematical Institute, Institute for Public Health, OMMI, VITUKI, Fishery Advisory Board, Fishery Company of Lake Balaton, KÖJÁL Stations of the counties Veszprém and Somogy and with the Veterinary Research Institute in the course of 1965.

The International Seminary of IBRO spent 1 day in August in Tihany, where they were informed especially on the neurobiological investigations and the work in general of the Department of Experimental Zoology.

In the beginning of October 1965 the Biological Department of the Hungarian Academy of Sciences organized a Colloquium in the Institute on "Actual problems of genetics", attended chiefly by young biologists. On this occasion 18 lectures were delivered by the participants.

Tudományos előadások jegyzéke — List of scientific lectures

Entz B.: Hollandia limnológus szemmel. — Limnológiai Szakosztály 1965. március 8.
Entz B.: Mennyiségi zoobenthosvizsgálatok a Balatonon. — Magyar Biológiai Társaság Állattani Szakosztálya, 1965. március 5.
Entz B.: A Balaton jege. — Magyar Hidrológiai Társaság Középdunántúli Csoportja, 1965. április 27.
Entz B.: A Nemzetközi Hidrológiai Decennium magyar nemzeti munkaterve. Biológia—fizika—kémia. — MTA Vízgazdálkodási, Vízépítési és Hidrológiai Tudományos Bizottság 1965. május 7.
Entz B.: Chironomida tanulmányok a Balatonon. — Moszkva, Össz-szövetségi Hidrobiológiai Konferencia. 1965. február 4.
Entz B.: Benthos investigations in Lake Balaton. — SIL Kongresszus, Varsó 1965. augusztus 26.
Entz B.: Bodenfaunauntersuchungen im Balatonsee. — Borok, Kutatóértekezlet. 1965. augusztus 10.
Entz B.: Die »Berekwässer«, sumpfige Gewässer der Balatonumgebung. — Internationaler Moorkongress, Keszthely, 1965. szeptember 14.
Lábos E. és Turcsányi B.: Xantén festékekkel fényérzékenyített izomkontrakció vizsgálata puhatestű lárva záróizmain. — 2. Biokémiai Vándorgyűlés Budapest 1965. május 27—29.
Lukacsovics F.: Kemo-ökológiai kapcsolatok vizsgálata néhány balatoni halfaj és az Anodonta cygnea L. glochidiuma között. — Állattani Szakosztály ülése Budapest, 1965. június 4.
Zs.-Nagy I.: Fluorescens mikroszkópos vizsgálatok pseudoisocyaninnal az édesvízi kagyló (Anodonta cygnea L.) neuroszekréciós tevékenységén. — Magyar Élettani Társaság 29. Vándorgyűlése Szeged, 1965. július 1—3.
Zs.-Nagy I. és Salánki J.: Cholinesterase histochemiai vizsgálatok néhány Mollusca faj központi idegrendszerében különböző funkcionális állapotokban. — Magyar Élettani Társaság 29. Vándorgyűlése Szeged, 1965. július 1—3.
Pécsi T.: A szív és a záróizomműködés reflexes kapcsolata a tavi kagylónál. — Magyar Biológiai Társaság Állattani Szakosztálya Budapest, 1965. május 7.
Pécsi T., Salánki J. és Lábos E.: Anodonta záróizmok tónusos és fázisos működésének idegi szabályozásáról. — Magyar Élettani Társaság 29. Vándorgyűlése Szeged, 1965. július 1—3.
Ponyi J.: A rákok emésztőrendszerének fehérjebontó enzimeiről. — Állattani Szakosztály, Budapest 1965. február 5.
Salánki J.: Regulation of periodic activity in mussels. — Nápolyi Zoológiai Állomás 1965 május
Salánki J.: Physiological basis of periodic activity in Lamellibranchs. — Southamptoni Egyetem Biokémiai és Élettani Tanszéke, 1965 december.
P.-Zánkai N.: A balatoni nádasok víziatkáiról. — Állattani Szakosztály Budapest. 1965. február 5.

Az intézeti Évkönyv 32. kötetén kívül az 1965. év folyamán megjelent dolgozatok jegyzéke

— List of papers published elsewhere as in Vol. 32 of our Annales

Bombelli, R., T. Farkas, R. Vertua: Investigations on the effect of nicotinic acid on plasma free fatty acid levels. — Med. Pharmacol. Exp. **12,** 8—12.

Entz B.: Übersicht über die ungarische limnologische Literatur. Limnologica (Berlin) **3,** 439—448.

Entz B.: Hydrobiologischen Problemen van de Balaton. — Overdruk uit Vakblad voor Biologen **8.** No.

Földes I., I. Zs.-Nagy, K. Benkő, G. Lévai, P. Ary-Balogh: Elektronmikroskopische Untersuchungen am postembryonalen Epiphysenknorpel der Albinoratte. — Acta Morph. Hung., **13,** 283—299.

Lábos E.: Evidence of complex-formation between chlorpromazine and different xanthene-dyes. — Nature, **209,** 201—202.

Lábos E.: Energetic aspect of the photosensitization of embryonic muscle by xanthene dyes. — Comp. Biochem. Physiol. **17,** 353—362.

Lábos E.: Effect of indolalkylamines, BOL—148 and other compounds on the photosensitized contraction by xanthene-dyes in glochidia of Anodonta. — Comp. Biochem. Physiol., **17,** 1057—1064.

Zs.-Nagy I., K. Benkő: Elektronmikroskopische Untersuchungen über das cerebroviscerale Konnektivum der Muschel Anodonta cygnea, unter Berücksichtigung der Neurosekretenleerung. — Symp. Biol. Hung., **5,** 293—301.

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Zs.-Nagy I., K. S.-Rózsa, J. Salánki, I. Földes, L. Perényi, M. Demeter: Subcellular localization of 5HT in the central nervous system of Lamellibranchiates. — J. Neurochem., **12,** 245—251.

Zs.-Nagy I., J. Salánki: Histochemical investigations of cholinesterase in different molluscs with reference to functional conditions. — Nature, **206,** 842—843.

Paoletti, R., M. Poggi, R. Vertua, T. Farkas: The effect of ionizing radiations on lipid transport. — Progr. Biochem. Pharmacol., **1,** 186—202.

Ponyi J.: Contributions to the problem of the role of proteolytic enzymes im Amphipods. — Symposium on Crustacea. — Mar. Biol. Assoc. India. Abstracts of Papers pp 85.

Sakharov, D. A., V. L. Borovyagin, I. Zs.-Nagy: Light, fluorescence and electron microscopic studies on „neurosecretion" in Tritonia diomedia Bergh (Mollusca, Nudibranchia). — Z. Zellforsch., **68,** 660—673.

Salánki J., E. Lábos: Izom- és idegsejtek elektrofiziológiai vizsgálómódszerei. — MTA Biol. Tud. Oszt. Közl. **8,** 89—118.

Salánki J.: Oxygen level as a specific regulator in the rhythmic activity of fresh-water mussel (Anodonta cygnea L.). — Acta Biol. Hung., **15,** 299—310.

Sári B., I. Zs.-Nagy, T. Fülöp: A hypothalamus laesio hatása a serum cholesterin szintre. — Kísérletes Orvostudomány **17,** 253—257.

Tamás G.: Microcystis tömegvegetáció a Balatonon. — Bot. Közlem. **52.** 97—104.

P.-Zánkai N.: Untersuchungen über die Endopeptidase-Aktivität des Verdauungssystems der höheren Krebse (Malacostraca). — Crustaceana, In press.

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1. ÁLLATTAN — ZOOLOGY — ЗООЛОГИЯ

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A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Merkly László
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