A MAGYAR TUDOMÁNYOS AKADÉMIA
TIHANYI BIOLÓGIAI KUTATÓINTÉZETÉNEK ÉVKÖNYVE
(1969)
(VOL. XXXVI.)

ANNALES INSTITUTI BIOLOGICI (TIHANY) HUNGARICAE ACADEMIAE SCIENTIARUM

TIHANY, 1969

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Szerkesztő:

S.-Rózsa Katalin

ANNAL. BIOL. TIHANY	36	3—10	HUNGARIA 1969

IDENTIFICATION OF CENTRAL NEURONS INNERVATING THE HEART OF *LYMNAEA STAGNALIS* L. (GASTROPODA)

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Received: January 30th, 1969

The question whether only the abdominal ganglion or other ganglia are also involved in the regulation of heart activity of Gastropods has long been discussed. According to VAN TIEL (1942) the nervous centres inhibiting and stimulating heart activity are located in the abdominal ganglion, while KOSHTOYANTZ, SMIRNOVA and POPKOVA (1956) believe the abdominal ganglion to be the centre of inhibition and the cerebral ganglion the site of initiation of both inhibitory and excitatory effects.

For the identification of central neurons from which direct fibres run to the heart, the method of COHEN and JACKLET (1965), which proved to be useful with Molluscs (SALÁNKI and GUBICZA (1967) seemed to be suitable. The method is based on the observation that axon regeneration is associated with accumulation of RNA in the central neurons which can be visualized with malachite green-pyronine staining.

In the present work those central neurons of *Lymnaea stagnalis* are described in which RNA accumulation were noted after the cutting through of the intestinal nerve.

Material and method

Experiments were performed on medium-sized specimens of *Lymnaea stagnalis* L. anaesthesized in 0,05—0,08 % solution of nembuthal until complete relaxation was achieved. Then the central nervous system was cut through the branch of the intestinal nerve innervating the heart was sectioned. After this surgical intervention the animals were placed in aquaria containing oxygenized circulating Balaton water for a period of 1—3 days. Only the specimens that survived operation were used for histological preparations.

The central nervous system was fixed in CARNOY's solution for 45 minutes. After the orientation of the ganglia, the material was embedded in paraffin and serially sectioned (thickness of sections : 7μ). Sectioning was always made in a horizontal plane in relation to the animal's horizontal posture. The sections were stained with mixture of malachite green (Edward Gurr, No 315) and pyronine y (PMaG) (GT Gurr, England) according to the method of BAKER and WILLIAMS (1965) modified by SALÁNKI and GUBICZA (1967).

The sections were examined under the light microscope. Comparative measurements were made on the size of neurons showing signs of regeneration and on their localization.

According to their size the nerve cells were classified in three groups:
large (120 μ or larger)
medium-sized (from 50 to 120 μ)
small (under 50 μ)

To facilitate localization of the cells the sections prepared from the ganglia were divided into three zones:

Zone I = ventral third of the ganglion
Zone II = median third of the ganglion
Zone III = dorsal third of the ganglion

By this division localization in the three zones of the neurons showing signs of regeneration was made possible *(Fig. 9)* on the basis of sections belonging to the given zone.

Parallel with the histological preparation of the material obtained from the operated animals, similar preparations were made from the nervous system of intact animals. Classification of cells according to size was performed in the control group, too. This method proved to be useful in evaluating the size and localization of nerve cells showing signs of regeneration. The percentage ratio of pyroninophilic cells was expressed by the method described by SALÁNKI and GUBICZA (1967) on *Anodonta cygnea*.

In order to see whether an increase of RNA occurs owing to operative trauma, additional control examinations were performed on the central nervous system of animals in which a skin section similar to that of the operated animals was made. The experiments were conducted in May and June on specimens of *Lymnaea stagnalis* collected from the Balaton 1—2 days prior to operation.

In the present work the results obtained on 8 animals in which nerve section was made, 8 controls and 4 animals with skin section are reported.

Results

1) *PMaG staining of central neurons in control animals*

Staining with PMaG yielded a faint homogeneous reaction in the cytoplasm of central neurons of the control animals. The so-called perinuclear ring localized around the nuclei of certain neurons in the central nervous system of *Lymnaea stagnalis* stained intensively with pyronine. The neurons possessing a perinuclear ring have small nucleoli and no RNA granulesare present in their nuclei *(Fig. 1)*. The number of such cells was found to vary in the experimental animals and their presence was not always demonstrable. They are usually scattered in various areas of the nervous system displaying no regular localization pattern.

The cytoplasm of some cells in the central nervous system of *Lymnaea stagnalis* contains RNA granules of 1—5 μ diameter *(Fig. 2)*. Neurons containing such RNA particles are usually arranged in groups and are most frequently found in the cerebral ganglion. In other ganglia they are seldom encountered. No regularity was observed in the localization of nerve cells rich

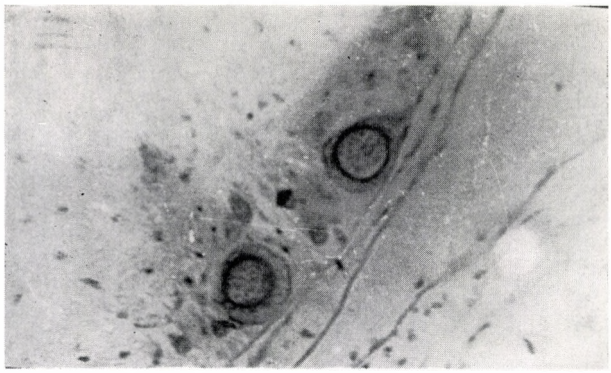

Fig. 1. Small pyroninophilic granular nerve cell in the cerebral ganglion of control *Lymnaea stagnalis.* ×320

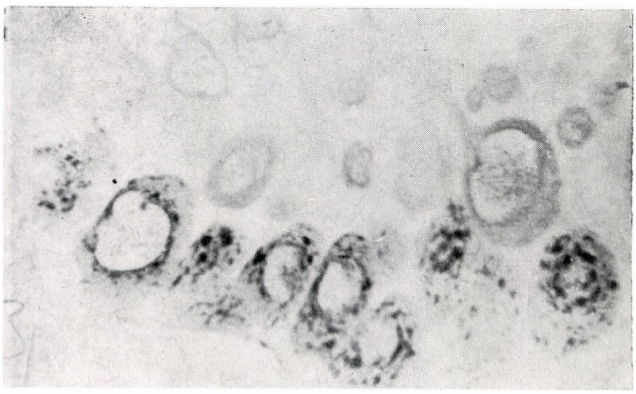

Fig. 2. RNA granules in the cytoplasm of neurons in the cerebral ganglion of control *Lymnaea stagnalis* ×320

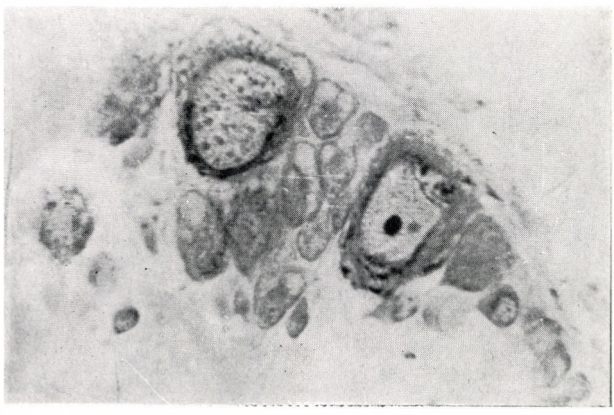

Fig. 3. Characteristic large nucleolus in the nerve cell of the abdominal ganglion of control *Lymnaea stagnalis* ×320

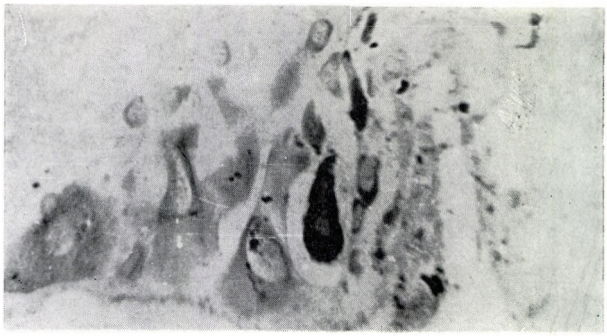

Fig. 4. Small (25 × 50 μ) pyroninophilic nerve cell in the right cerebral ganglion ×400

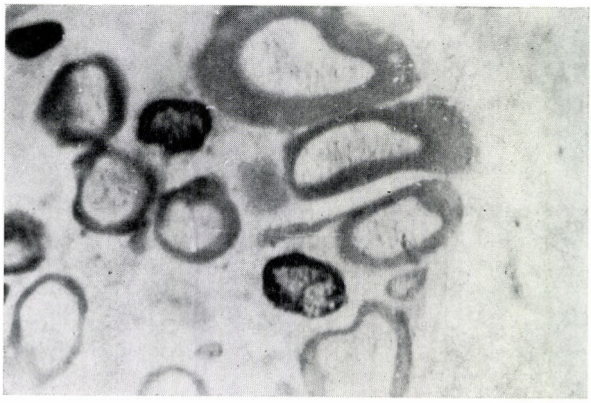

Fig. 5. Small pyroninophilic granular neuron among the cells of the frontal lobe of the cerebral ganglion. ×320

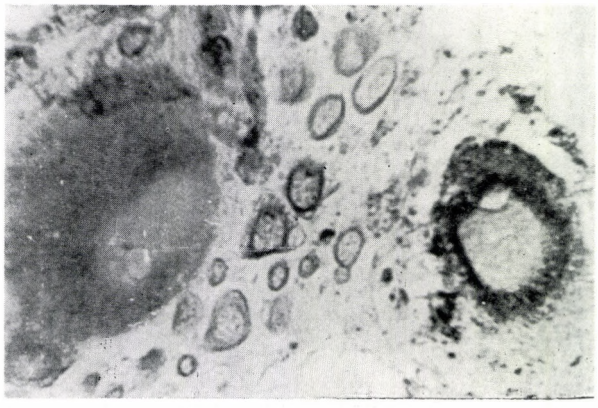

Fig. 6. Small (40 μ) pyroninophilic granular neuron in the right parietal ganglion. ×450

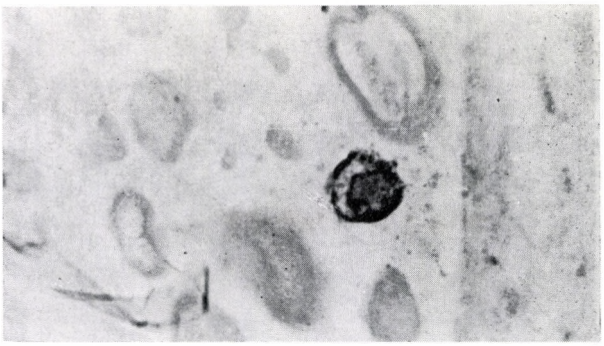

Fig. 7. Small (40 μ) pyroninophilic neuron in the right pedal ganglion. $\times 450$

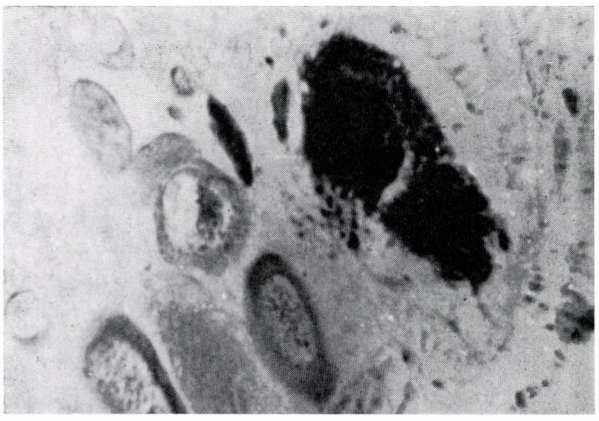

Fig. 8. Large pyroninophilic granular nerve cell in the abdominal ganglion $\times 320$

in RNA, their presence is sometimes undemonstrable. In the control animals the neurons with no perinuclear pyroninophilic ring were found to contain large nucleoli (3—11 μ), as shown in *Fig. 3*.

The amount of RNA granules in the nervous system of animals in which a similar skin section was performed as in animals submitted to nerve injury, corresponded to the amount of RNA found in the intact controls. In their neurons the perinuclear ring, RNA granules appearing irregularly in the cytoplasm and large nucleoli were demonstrable. No changes in the cytoplasmic RNA could be observed in the central neurons in this group of animals.

2) *Pyroninophilia and localization of axon-damaged neurons*

After axon injury the cytoplasm of some nerve cells was found to contain a granular substance staining well with pyronine y *(Fig. 4)*. The nuclei of these cells exhibiting granular pyroninophilia stained more intensively with malachite green than the nuclei of control cells. The nucleoli were inferior in size compared with the large nucleoli characteristic of the intact cells. No pyroninophilic cells possessing the characteristics described above were encountered in any of the neurons of the control animals. Aggregation of pyroninophilic material observed soon after nerve section was absent in the cytoplasm of the neurons if the sections were incubated with ribonuclease. Removal by the enzyme of the pyroninophilic material confirms its RNA nature.

On the basis of these observations granular pyroninophilia of the cytoplasm was accepted as sign of regeneration in the central nervous system of *Lymnaea*. As early as after 24 hours following nerve section the typical pattern of granular pyroninophilia could be observed and 56 hours after operation these cells were in a state of disintegration *(Fig. 5)*.

Localization of neurons exhibiting granular pyroninophilia after the intersection of the intestinal nerve is summarized in *Fig. 9*. The typical granular pyroninophilia was found to appear always in identic cells of the animals submitted to nerve injury. Our data have verified that the right and left pleural ganglia do not contain neurons exhibiting granular pyroninophilia. The cells containing pyroninophilic granules have an assymetrical localization in the central nervous system. In the left ganglia — except the pedal ganglion — there were fewer granular cells and pyroninophilic granulation was visible only in the giant cells of the right ganglia *(Fig.* 6, 7, 8).

The lowest number of pyroninophilic neurons was observed in the ventral part (zone I) of the ganglia. In this area positive reaction with pyronine y was obtained only in the right and left cerebral, and pedal ganglia. In the middle part of the ganglion (zone II) the highest number of neurons showing signs of regeneration was observed. In the basal part (zone III) pyroninophilic neurons were absent in the pleural and pedal ganglia. Concentration of RNA granules were demonstrated only in both cerebral and parietal ganglia, as well as in the abdominal ganglion *(Fig. 9)*.

In addition to the distribution in depth, localization of neurons showing signs of regeneration can be facilitated by the observation that in the abdominal ganglion, at the site of origin of the intestinal nerve, 4—5 pyroninophilic cells are always visible, while another characteristic group of cells is found on

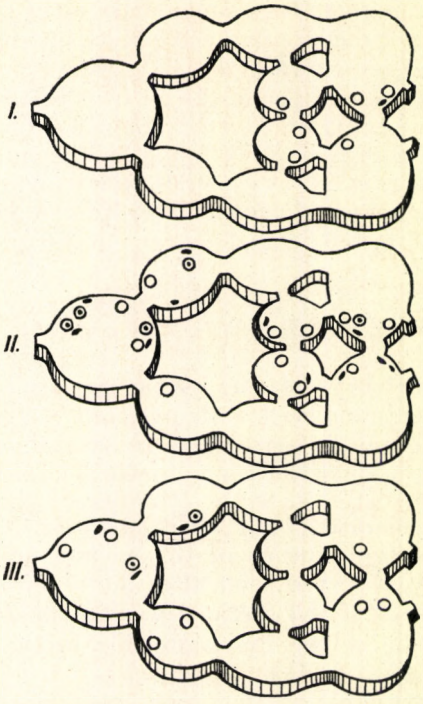

Fig. 9. Scheme of localization in the ganglia of neurons showing signs of retrograde regeneration in sections prepared from material varying in depth.
I = ventral zone
II = median zone
III = dorsal zone
⊙ = large neurons (over 120 μ)
O = medium-sized neurons (50—120 μ)
— = small neurons (under 50 μ)

the opposite side of the ganglion, at the issue of the parieto-abdominal connective. In the basal part (zone III) of the left parietal ganglion a giant cell showing granular pyroninophilia was found in each animal examined *(Fig. 6)*. In the cerebral ganglia most of the pyroninophilic cells were found around the site of origin of the commissura cerebro-cerebralis and in the area of procerebrum. *Table 1* shows the numerical distribution of neurons sending axons to the intestinal nerve.

According to the results of our examinations the number of neurons sending direct fibres to the heart of *Lymnaea* is 46. The percentage ratio of medium-sized, small and large neurons is 52,2, 32,6 and 15,2, respectively. In the right and left cerebral ganglion the total number of neurons involved (15) is superior to the number of reacting cells in the abdominal ganglion (13), though the nerve branch running to the heart takes origin from the latter ganglion. In the parietal and pedal ganglia identic number of neurons (9) are involved.

The fibres of the intestinal nerves consist mostly of small and medium-sized cells. Considering, however, that in the nervous system of *Lymnaea*

Table 1

Numerical distribution of nerve cells showing signs of regeneration, in the central nervous system of *Lymnaea*

Ganglion		Number of neurons			Total:
		large	medium-sized	small	
Cerebral	left	1	5	2	15
	right	—	4	3	
Pleural	left	—	—	—	
	right	—	—	—	
Parietal	left	2	1	3	9
	right	—	3	—	
Pedal	left	—	3	1	9
	right	—	4	1	
Abdominal		4	4	5	13

1,5—10% of the total neurons is composed of giant cells, 22—37% of medium-sized and 72% of small cells, a more important role must be attributed to giant cells than that shown by this percentage ratio in the regulation of heart activity because nearly one third of the giant cells send direct fibres to the intestinal nerve.

The percentage of giant cells involved in the regulation of heart activity is the highest in the abdominal ganglion directly innervating the heart. Compared ot their total number, the participation of small cells in the composition of the intestinal nerve is of the slightest degree.

Discussion

Central neurons of *Lymnaea stagnalis* following axon injury can be identified by staining with malachite green pyronine, similarly to *Anodonta* (Salánki and Gubicza, 1967). In the *Lymnaea* the appearance of the perinuclear ring of RNA is, however, not the sign of axon regeneration but it is believed to be a symptom concomitant with normal physiological processes of the animal. This assumption is supported by the data of Boer (1965) who described different types of RNA aggregates in the nervous system of *Lymnaea*, correlating them with the secretory activity of the nerve cells. According to Boer the perinuclear ring consisting of fine granules of RNA is characteristic of Gomori-positive cells (Boer, 1965). In a terrestrial snail *(Zachryssia guanensis)* the RNA ring was found to disappear during hibernation. This finding seems to lay stress on the correlation of RNA concentration and activity of the animal (Urbá-Holmgreen and Holmgreen, 1968).

The sign of regeneration in the neurons of *Lymnaea* is the considerable increase of RNA granules in the cytoplasm. This kind of RNA concentration

differs distinctly from that noted in normal ganglia *(Fig.s 1,2* and *4)*. Digestion of this material by ribonuclease undoubtedly indicates the presence of a specific RNA accumulation.

On the basis of our experimental data it may be concluded that the two cerebral, the two parietal, the abdominal and the two pedal ganglia send direct fibres to the intestinal nerve branch innervating the heart. Considering the number of cells involved the cerebral ganglia seem to have the largest share (15 cells), then the abdominal ganglion (13 cells) and finally the parietal and pedal ganglia (9 cells each).

Our results show that the theory of the exclusive role of the abdominal ganglion in the regulation of heart activity in Gastropods (VAN TIEL, 1942) is untenable. We have demonstrated that in addition to the abdominal and cerebral ganglia, the parietal and pedal ganglia are also involved in this regulation *(Fig. 9)*. Our examinations confirm the statement of WILLOWS (1968) on the participation of giant cells in the control of peripheral organs. We have also found that the percentage of giant cells involved in the regulation of heart activity was the highest compared to their total number.

According to our data there is a morphological basis for the formation inhibitory and excitatory centres also in the pedal and parietal ganglia. The inhibitory of excitatory nature of neurons identified morphologically can, however, be clarified only after further pysiological investigations.

Summary

1. Axon-injured neurons in the central nervous system of *Lymnaea stagnalis* can be identified on the basis of granular RNA accumulation demonstrable in their cytoplasm. This granular RNA can be fairly distinguished from the homogeneous fine RNA granules present in normal animals.

2. Following intersection of the intestinal nerve specific RNA accumulation were found in the neurons of cerebral, parietal, abdominal and pedal ganglia. In all, 46 neurons emit direct fibres to the intestinal nerve.

3. Appearance of cytoplasmic concentration of RNA following intersection of the intestinal nerve is an evidence that in the regulation of heart activity the giant cells have the largest share in proportion to their total number.

4. In addition to the cerebral and abdominal ganglia, parietal and pedal ganglia are also involved in the regulation of heart activity in Gastropods.

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GASTROPODÁK SZÍVÉT BEIDEGZŐ KÖZPONTI NEURONOK AZONOSÍTÁSA

Gubicza András és *S.-Rózsa Katalin*

Összefoglalás

1. *Lymnaea stagnalis* központi idegrendszerében az axonkárosított neuronok identifikálhatók a citoplazmájukban kimutatható szemcsés RNS felhalmozódás alapján. E szemcsés RNS felhalmozódás jól elkülöníthető a normál állatokban is megtalálható homogén, apróbb szemcsés RNS képződményektől.

2. A szívet innerváló intestinális ideg átmetszése után a cerebrális, parietális, abdominális és pedális ganglionokban találhatók specifikus RNS felhalmozódást mutató idegsejtek. Összesen 46 neuron küld direkt rostot az intestinális idegbe.

3. Az intestinális ideg átmetszése után megfigyelt RNS felhalmozódás azt bizonyítja, hogy a szívműködés regulálásában össz-számukhoz viszonyítva az óriássejtek vannak legnagyobb arányban képviselve.

4. A cerebrális és abdominális ganglionokon kívül a parietális és pedális ganglionok is részt vesznek a szívműködés szabályozásában Gastropodákon.

ЛОКАЛИЗАЦИЯ ЦЕНТРАЛЬНЫХ НЕЙРОНОВ ИННЕРВИРУЮЩИХ СЕРДЦЕ БОЛЬШОГО ПРУДОВИКА (БЮХОНОГИЙ)

А. Губица и *К. Ш.-Рожа*

1. В центральной нервной системе большого прудовика можно идентифицировать нейроны после перерезки их аксонов на основе накопления зерен РНК в их цитоплазме. Это зернистое накоплене РНК резко отличается от мелькозернистого, гомогенного скопления РНК, характерного для контрольных животных.

2. После перерезки интестинального нерва иннервирующего сердце клетки указывающие специфическое скопление РНК обнаруживаются в церебральных, париетальных, педальных и абдоминальном ганглиях. Всего 46 клеток посылают аксоны в интестинальный нерв.

3. Полученные результаты указывают на то, что так называемые гигантские клетки участвуют в наивысшем проценте в регуляции сердца по сравнению их общим числом.

4. Кроме абдоминального и церебральных ганглиев париетальные и педальные ганглии тоже участвуют в регуляции сердечной деятельности Брюхоногих.

ANNAL. BIOL. TIHANY	36	11—17	HUNGARIA 1969

COMPARATIVE STUDY ON THE NISSL SUBSTANCE AND RNA CONTENT IN THE CENTRAL NERVOUS SYSTEM OF *ANODONTA CYGNEA* L.

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Received: February 21st, 1969

It has been known for many years that a close connection exists between the basophilic material (Nissl substance) and the ribonucleic acid (RNA) content of nerve cells (CASPERSSON, 1941). Numerous authors have described that the changes in RNA content of neurons are correlated with the function and regeneration of these cells (HYDEN, 1943; VOGT and VOGT, 1946; BRODSKY, 1956; PEVZNER, 1964; COHEN and JACKLET, 1965; KUPFER and DOWNER, 1967). It was also found that parallel with the changes in RNA content the nuclei and nucleoli of neurons change in size. Modification in the amount of Nissl substance and RNA content of nerve cells are known to be connected with the periodic activity of the animal, as demonstrated on freshwater mussel (SALÁNKI et al. 1965; ZS.-NAGY et al. 1966). Specific localization of RNA concentrations was found to occur during regeneration (SALÁNKI and GUBICZA, 1969).

As the stains used for the demonstration of the Nissl substance colour all the basophilic materials of the cell, a difference may arise between the amount of basophilic substance and RNA content owing to rapid RNA changes, i.e. to intense metabolic activity of the cell. Therefore, our experiments have been conducted on freshwater mussels as in the nerve cells of these animals relatively rapid functional changes can be evoked (SALÁNKI et al. 1968). The purpose of our investigations was to study whether identic results can be obtained under different conditions with the two staining procedures used for the demonstration of RNA content.

Material and method

Examinations were performed on the cerebral, visceral and pedal ganglia of freshwater mussel (*Anodonta cygnea* L.). The ganglia were removed surrounded by connective tissue and fixed in a mixture of formalin, alcohol and acetic acid (3 : 1 : 3) (BRODSKY, 1960) for 25—40 minutes, depending on size. Fixation was followed by oriented embedding. Serial sectioning of the cerebral ganglion was always made sagittally, while the visceral and pedal ganglia were cut in a horizontal plane. Every second section was mounted on the same slide. One series of sections was stained with cresyl violet according to FRENTROM (KISZELY and PÓSALAKY, 1964) and the other series with a

mixture of pyronine y (GT Gurr, England) and malachite green (Edward Gurr, No 315), as described by BAKER and WILLIAMS (1965). Digestion with ribonuclease was performed according to BRACHET's (1965) method using a preparation of Koch Light Lab. (42 K unit/mg). The control material was removed from the mussels at the beginning of the active and quiescent periods (SALÁNKI et al. 1965), while the experimental material was removed after the animals were kept for a certain period of time under the following experimental conditions or submitted to nerve section:

1) Keeping the animals in an O_2 deficient environment for 2—6 days.

12—16 cm long specimens of *Anodonta* were placed in separate containers each containing 2 litres Balaton water. The surface of the water was covered with paraffin oil. The activity of mussels kept under such conditions was registered with an actograph (SALÁNKI and BALLA, 1964). The ganglia were removed 2, 4, and 6 days after the beginning of the experiment.

2) Nerve section.

The cerebro-visceral connective (CVc) on the left side was cut in 4 animals and two days later, the left cerebral and the visceral ganglia were removed and the material was processed as usual.

Results

In the ganglia of the mussels examined the cytoplasm of nerve cells exhibited a similar staining whether cresyl violet or pyronine-malachite green (PMaG) technique was employed, as demonstrated by the identification of sections of large cells, stained in different staining series. In fact, comparison of successive sections of the same ganglion, one stained with cresyl violet and the other with PMaG revealed a similar percentage distribution of cells exhibiting stronger or weaker affinity for the dyes *(Fig. 1)*. The amount of basophilic material, i.e. RNA content of the cells in the different ganglia was found to change depending on the periodic activity of the animal *(Table 1,* control).

As regards nuclear staining a difference was noted between the results of the two methods: some nuclei stained intensively with cresyl violet,while others hardly exhibited any coloration. Generally, nuclear staining was rather faint with PMaG. On the other hand, intensive coloration was observed in the nucleoli both with cresyl violet and with pyronine malachite green staining.

A) *Animals kept in an O_2 deficient environment*

The longer the mussels were deprived of oxygen, the lower was the number of cells containing much basophilic substance, i.e. RNA in their cytoplasm *(Fig. 2)*. After the second day this seemed to be independent of the period of activity of the animal *(Table 1)*.

There was a consistent parallelism between reduction of Nissl substance and that of RNA content.

In the mussels kept under hypoxic conditions there was a considerable increase in the number of cells with nuclei staining intensively with cresyl violet, while no increase in staining was noted with the pyronine-malachite green technique. The nuclei showing a strong coloration were relatively small in size *(Fig. 3)*. The longer the animal was in O_2 deficient milieu, the higher the number of nerve cells possessing relatively small and well stainable nuclei *(Fig. 2)*.

Table 1

Percentage of nerve cells exhibiting different degrees of basophilic material content (filled, half-filled or empty) in the central nervous system of mussels kept in an anaerobic environment, on the basis of data obtained from 8 animals in each group (Staining with cresyl violet)

Duration	Beginning of active period				End of active period			
	Number of cells	filled %	half-filled %	empty %	Number of cells	filled %	half-filled %	empty %
2 days	12 746	42,6	37,2	17,2	12 232	6,2	18,4	75,4
4 days	13 125	4,8	7,3	87,8	12 614	4,5	6,9	88,6
6 days	12 207	4,1	7,6	88,3	13 403	3,3	7,4	89,3
Control	8 650	71,5	16,4	12,1	8 141	7,8	21,3	70,9

Nuclear staining seems to be independent of the amount of Nissl substance present in the cytoplasm. The nuclei of cells filled or half-filled with basophilic material or being completely empty may stain well with cresyl violet. Within the ganglion these cells are usually grouped.

Table 2

Percentage of nerve cells containing small nuclei showing intensive staining with cresyl violet, in the central nervous system of mussels kept in an oxygen deficient milieu

Days	Number of cells examined	Intensive nuclear staining (%)
2	13 216	14,7
4	12 431	37,3
6	12 814	86,5
Control	7 941	6,4

Both in the controls and in the mussels kept in an environment deficient in oxygen, the nuclear membrane of many cells exhibited intensive staining with cresyl violet. The nucleoli always showed a lively coloration. Usually, the cytoplasms of these cells were slightly basophilic, though occasional strong cytoplasmic staining was also noted *(Fig. 4)*. Such increase in basophilic material localized on the nuclear membrane was found to be more frequent in summer than in winter *(Table 3)*. With PMaG such patterns were seldom seen except in a few large nerve cells.

Table 3

Incidence of cells with basophilic substance localized on the nuclear-cytoplasmic border

	Number of cells examined	Cresyl violet staining %	Number of cells examined	PMaG staining %
In summer	8 432	11,4	8 141	3,7
In winter	7 685	1,7	8 624	0,2
In spring and autumn	12 436	3,1	12 139	0,8

B) *Appearance of RNA ring in the period of regeneration following nerve section*

As has been described in a previous work (SALÁNKI and GUBICZA, 1969) a perinuclear ring consisting of RNA appears around the nuclei of some neurons of the *Anodonta* after their axons have been cut. Evaluation of sections exposed to alternate staining revealed that the perinuclear ring stained well also with cresyl violet. After digestion with ribonuclease the basophilic substance (cresyl violet staining) remained intact *(Fig. 5)* while the PMaG technique became negative.

Discussion

By employing cresyl violet (a stain for basophilic substances) and pyronine-malachite green (a specific stain for RNA) we have demonstrated that in agreement with data in the literature, there is a close connection as to localization between the amount of Nissl substance and RNA content of the cytoplasm of neurons in the central nervous system of mussels. The changes ensuing in consequence of experimental conditions were demonstrable with both methods not only as regards the Nissl substance but also the perinuclear RNA ring appearing as a cytoplasmic regeneration phenomenon after nerve section. It was also demonstrated that the appearance of a perinuclear RNA ring, as a sign of regeneration, described on some invertebrates (COHEN and JACKLET, 1965; SALÁNKI and GUBICZA, 1969) is not only a concentration of RNA, but also accumulation of a basophilic material associated with increased protein synthesis which is not removed by digestion of ribonuclease.

Similar pattern, though only in part referring to RNA, was noted in cells showing an increase of basophilic material in the nuclei and sometimes around the nuclear membrane which showed a strong coloration with cresyl violet but — except in a few cases — no pyroninophilia.

That the nucleus does not stain intensively with pyronine is easy to understand if we consider the large amounts of DNA and small RNA present in it. The nucleolus in which transfer RNA is probably taking place (SIRLIN et al. 1961) always shows intensive staining with PMaG and cresyl violet alike. Most likely, the strong cresyl violet staining of the nucleus noted in certain cases indicates increased intensity of processes connected with DNA. Such staining was noted almost exclusively in cells with nuclei smaller than the average size, however, it can hardly be attributed to a concentration due to the reduction of the nuclei.

The sharp contoured basophilic substance concentrated on the border of the nucleus and cytoplasm seems also to be indicative of an increased nuclear activity. This basophilic substance was not stainable, or only very rarely, with PMaG. Such cells were more frequently encountered in summer than in winter which seems to indicate a correlation between their appearance and certain seasonal changes in the metabolic activity of the animal. BARANYI (1966) and ZS.-NAGY (1967) described seasonal changes of certain components in the nerve cells of mussels. Our data are comparable with those of URBA-HOLMGREEN and HOLMGREEN (1968) who reported on the disappearance of perinuclear RNA in snail neurons during experimental hibernation. The simultaneous appearance of several such cells seems to indicate that within the nervous system there may be functionally differing cell groups.

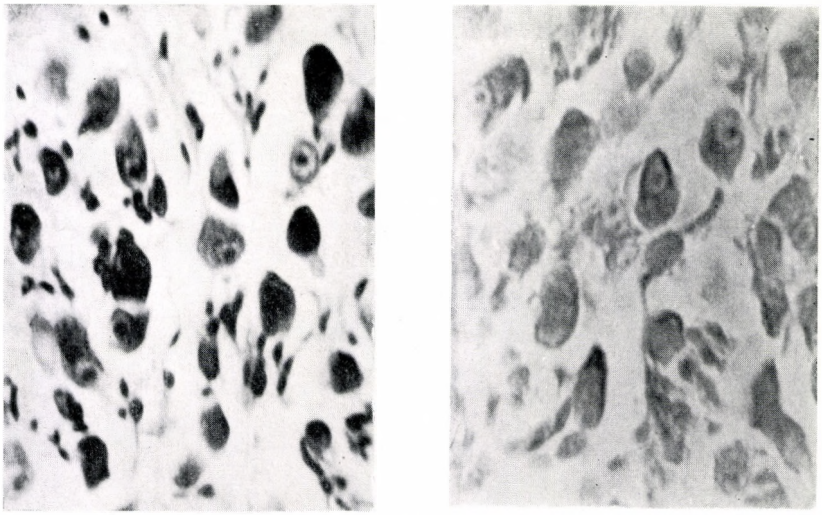

Fig. 1. Cresyl violet (A) and pyronine-malachite green (B) staining in cells showing intensive basophilia (cerebral ganglion, at the beginning of the period of activity). ×420

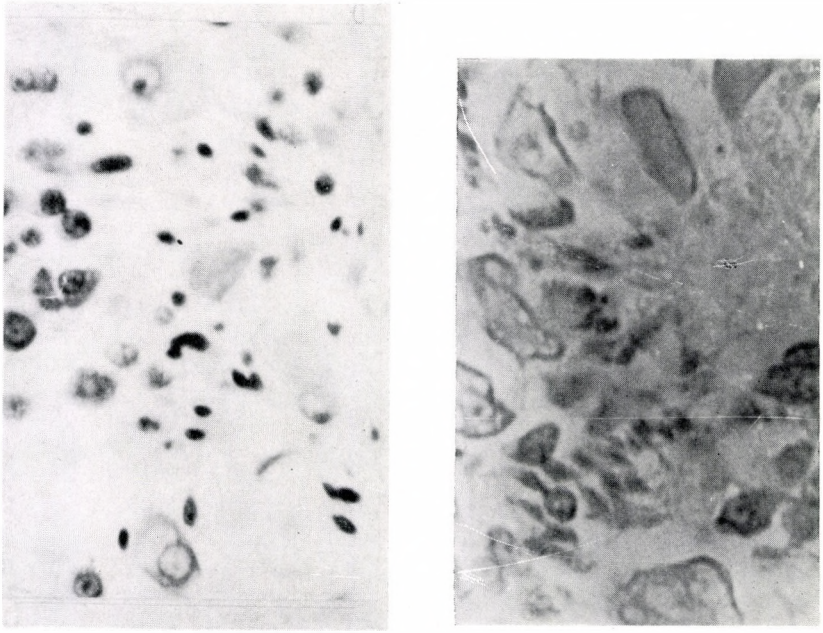

Fig. 2. Cresyl violet (A) and PMaG (B) staining in cells showing slight basophilia (cerebral ganglion, at the beginning of the rest period). ×540

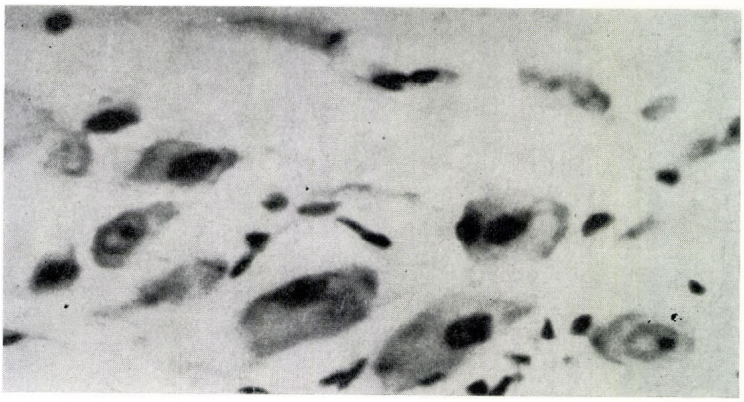

Fig. 3. Nerve cells with small nuclei showing strong staining with cresyl violet (visceral ganglion, on the 6th day of O_2-deficiency). ×540

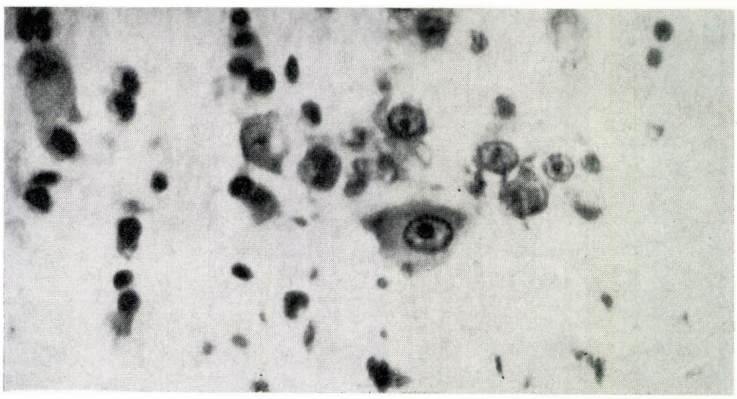

Fig. 4. Concentration of basophilic substance localized on the nuclear membrane (cell group in the visceral ganglion) Summer period. ×420

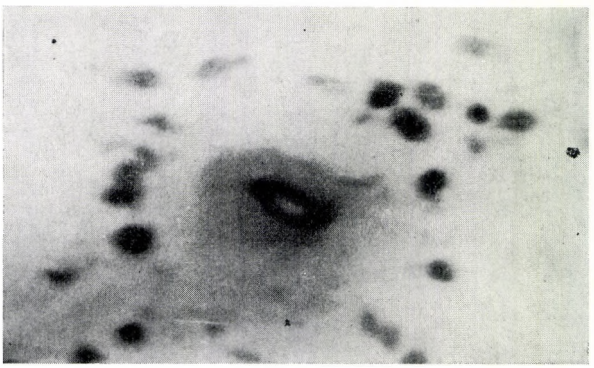

Fig. 5. Nerve cells showing a perinuclear ring formed after nerve section, digested with ribonuclease and stained with cresyl violet (cerebral ganglion). ×650

The correlation between the amount of RNA and periodical activity of the animal was fairly demonstrable in the controls and even on the second day of oxygen deficiency of the experimental animals (SALÁNKI et al. 1965; ZS.-NAGY et al. 1966). Beginning from the second day a rapid decrease was noted in the basophilic substance of the cells and a considerable reduction occurred in the duration of active periods (SALÁNKI, 1965). Presumably, oxygen deficiency has a depressing effect on the entire metabolism of nerve cells which is then reflected in the RNA and protein synthesis. The diminution in size observed in large cells seems likewise to be connected with this effect. Consequently, the majority of the cells contain constantly small amounts of basophilic substance which is manifested in the regulation of periodic activity by producing unusually long rest periods alternating with short periods of activity. In empty cells, however, which are present in large numbers due to slow RNA synthesis this correlation between the basophilic substance of the cytoplasm and change in periodic activity of the animal could not be demonstrated with the method employed.

Summary

From the results obtained with cresyl violet and pyronine-malachite green staining of neurons of the central nervous system in *Anodonta cygnea* the following observations were made:

1. In the cytoplasm of nerve cells, even with various amounts of basophilic substance, a close parallelism was noted in the localization and amount of the substance stained with cresyl violet and PMaG.

2. The relatively small nuclei of certain cells stained intensively only with cresyl violet. In O_2 deficiency the nuclei exhibited a decrease in size and an increase in basophilia. Similarly, staining of the basophilic material localized on the nucleus-cytoplasm border was characteristic of the Nissl substance.

3. After nerve section the perinuclear ring appearing as a sign of regeneration, contained not only RNA but also a concentration of basophilic material that remained unaffected by digestion with ribonuclease.

4. O_2 deficiency lasting for several days caused a radical decrease in the amount of basophilic substance of nerve cells. In such cases the pattern of the periodic activity of the animal also changes, and the relationship between the quantity of RNA content in the active and rest periods was not demonstrable with the method employed.

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NISSL-ANYAG ÉS RNS ÖSSZEHASONLÍTÓ VIZSGÁLATA *ANODONTA CYGNEA* L. KÖZPONTI IDEGRENDSZERÉBEN

Gubicza András és *Salánki János*

Összefoglalás

Anodonta cygnea központi idegrendszere sejtjeinek krezilibolya és pyronin-malachitzöld festődését vizsgálva megállapítottuk:

1. A sejtek citoplazmájában változó mennyiségű bazofil állomány esetén is szoros egyezés van a kétféle eljárással festődő anyag lokalizációjában és mennyiségi előfordulásában. Ugyanez érvényes a magvacska festődésére is.

2. Egyes sejtekben előforduló, viszonylag kisméretű mag csak krezilibolyával festődik, O_2-hiányban a sejtmagok megkisebbedése és bazofiliájuk növekedése észlelhető. Ugyancsak a Nissl-anyagra jellemzően festődik a mag-plazma határra lokalizált bazofil anyagfelhalmozódás is.

3. Idegátmetszés utáni perinucleáris regenerációs gyűrű nemcsak RNS-t tartalmaz, hanem egyben olyan anyagtömörülés is, mely bazofiliáját megtartja ribonucleázzal történt emésztés után is.

4. Többnapos oxigénelégtelenségben az idegsejtek bazofil állománya radikálisan csökken. Ilyenkor az aktivitási minta is megváltozik, s a RNS tartalomnak az aktív és nyugalmi szakaszokkal összefüggő mennyiségi változása nem mutatható ki.

СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ МАТЕРИАЛА НИССЛА И РНК В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ БЕЗЗУБКИ

А. Губица и *Я. Шаланки*

Изучая окрашиваемость клеток центральной нервной системы беззубки с методом крезилвиолета и пиронин-малахитовым зеленым было установлено, что:

1. Даже в тех случаях когда количество базофильного компонента цитоплазмы изменяется, обнаруживается тесная взаимосвязь в локализации и количественном распределении, вещества окрашиваемого этими двумя способами. То же самое характерно и для ядрышка.

2. Ядро малого размера, находящегося в определенных клетках, окрашивается только крезилвиолетом. В отсутствии кислорода размер ядра клеток уменьшается и его базофилия повышается. Базофильное вещество локализованное на границе ядра и цитоплазмы окрашивается таке как и материал Ниссля.

3. Околоядерное регенерационное кольцо, образующееся после перерезки нерва, содержит не только РНК, но и скопление такого типа материала, который сохраняет свою базофилию и после обработки клетки рибонуклеазой.

4. После продолжительного недостатка кислорода базофильный компонент нервных клеток значительно снижается. В таких условиях меняется и тип активности, и нельзя выявить больше те количественные изменения в содержании РНК, которые сопровождали периода активности и покоя животного в нормальных условиях.

ANNAL. BIOL. TIHANY	36	19—24	HUNGARIA 1969

5HTP—DOPA DECARBOXYLASE IN THE NERVOUS SYSTEM AND OTHER TISSUES OF *ANODONTA CYGNEA* L. (PELECYPODA)

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Received: 13th February, 1969

A considerable amount of serotonin (5HT) and dopamine (DA) have been found in the nervous system and other tissues of molluscs and many data refer to their role in the neural regulation (WELSH, 1957; KOSHTOYANTS and RÓZSA, 1961; GERSCHENFELD and STEFANI, 1962; SWEENEY, 1960; SALÁNKI, 1963; DAHL et al. 1966; KERKUT and WALKER, 1962; ZS.-NAGY, 1968).

The synthesis of 5HT and dopamine from 5-hydroxytryptophan (5HTP) and 3,4-dihydroxyphenylalanine (DOPA) respectively, necessitating the participation of 5HTP and/or DOPA decarboxylase has been extensively studied in vertebrates. However, in invertebrates, particularly in molluscs our information is rather incomplete concerning the occurrence and distribution of this enzyme (WELSH and MOORHEAD, 1959; KERKUT and COTTRELL, 1963, CARDOT, 1963a, b). Using homogenizates of different tissues it was found that serotonin and dopamine are synthetized by the same enzyme, decarboxylizing also other amino acids both in vertebrates (HAGEN and COHEN, 1966) and in the nervous system of *Helix pomatia* (CARDOT, 1966).

In the present study the 5HTP and DOPA decarboxylase activity of the nervous system and of other tissues were investigated in *Anodonta cygnea* L. Our aim was to obtain data partly about the quantitative distribution partly about the identity or difference of the 5HT and the dopamine synthetizing enzymes.

Material and methods

During the experiments the homogenizates of cerebral, visceral and pedal ganglia, cerebro-visceral connective (CVc), heart, gills and mantle of *Anodonta cygnea* L. as well as the lymph were used.

The tissues were collected in ice-cold physiological saline, measured after drying on filter paper and homogenized in physiological saline with Potter-Elvehjem homogenizers. The lymph was drained from the heart and was diluted with the incubation solution in proportion 1 : 2,5.

Incubation was carried out at 25 $\pm$ 0.1 °C, in the presence of iproniazid. The pH was adjusted to 8 for the examination of 5HTP decarboxylaze and to 7 for that of the DOPA decarboxylase.

The mixture was agitated throughout the incubation period.

In the incubation mixture the concentrations of the tissue homogenizates were as follows: ganglia 20 mg/ml, CVc 10 mg/ml, muscle 25 mg/ml, other tissues 50 mg/ml. In the medium, the concentration of pyridoxal 5-phosphate, iproniazid and phosphate-buffer were 20 μg/ml, 200 μg/ml and 0,1 M respectively. As substrates DL-5HTP in concentration $4{,}54 \cdot 10^{-4}$ M, and DL-DOPA in concentration $2{,}54 \cdot 10^{-3}$ M were used.

After a 15 min preincubation period the incubations were carried out for 2 hours in the case of 5HTP and for 1 hour in the case of DOPA.

The serotonin concentration was determined by the method of BOGDANSKI (KUNTZMAN et al. 1961). Dopamine was separated from DOPA with ion exchange resin (Amberlite IRC-50) according to the description of LOVENBERG et al. (1962) and the concentration was read directly in an Aminco-Bowman spectrophotofluorimeter. The uncorrected extinction and fluorescent wave lengths (mμ) were as follows: serotonin, 300 and 540; dopamine 282 and 330.

K_M values were estimated with the Lineweaver-Burk plot. Taking into consideration that we used DL-5HTP and DL-DOPA, where the L and D forms are present in equimolar concentrations, however the enzyme acts only on the L-form, the K_M values given in *Table III* were calculated by reducing the values estimated with the Lineweaver-Burk plot to 50 per cent. The enzyme activities are expressed in μg amine/g wet weight/hour.

Results

Working with the homogenate of *Busycon* ganglia WELSH and MOORHEAD (1958) and MIROLLI (1968) found that pyridoxal 5-phosphate is unnecessary as cofactor for the 5HTP decarboxylase assay. According to our results in the presence of the cofactor the 5HTP decarboxylase activity increased in the homogenates of Anodonta ganglia and of the CVc by 100 and 170 per cent respectively. Therefore, in our experiments we used in every case also pyridoxal 5-phosphate.

It was found that the central nervous system of the fresh water mussel is capable to synthetize serotonin from 5HTP and dopamine from DOPA in a considerable degree, referring to the presence of 5HTP and DOPA decarboxylase *(Table I)*. Remarkably high activities were measured in the CVc, connecting cerebral and visceral ganglia. 5HTP and DOPA decarboxylase were found also in the homogenizates of the heart, gills and mantle, but the activities of these tissues were rather low as compared to that of the nervous system. Activity was not detectable either in the muscle or in the lymph.

The production of dopamine proved to be more intensive in all tissues than that of the serotonin. The values and the rate of the synthetized dopamine and serotonin are given in *Table I*.

The K_M values were estimated in the homogenizates of the ganglia and CVc for both substrates. Values are presented in *Table II*.

Both values of activities and values of K_M show that the affinity of the substrate to the enzime is greater in the case of DOPA than in the case of 5 HTP.

It is well known that decarboxylation of DOPA could be inhibited by 5HTP and vice versa, and further the decarboxylation of both substrates

Table I

Values of 5HT and dopamine synthesis in different tissues

Tissue	Synthetized amine (μg/g wet weight/hour)		Dopamine/5HT
	5HT	dopamine	
All the ganglia together	53	345	6,5
Cerebral ganglia	56	376	6,6
Visceral ganglia	45	277	6,1
Pedal ganglia	60	383	6,3
CVc	102	610	6,0
Heart	1—2	2—3	~2
Gills	2—3	10—11	~4
Mantle	2—3	8—10	~4
Adductor muscle	0	0	
Lymph	0	0	

Table II

K_M values of 5HTP-DOPA decarboxylase in the homogenizates of nervous tissues

Substrate	All the ganglia together	CVc
DL—5HTP	$1{,}4 \cdot 10^{-5}$	$9{,}0 \cdot 10^{-6}$
DL—DOPA	$7{,}8 \cdot 10^{-5}$	$2{,}94 \cdot 10^{-4}$

could be blocked by α-methyl DOPA (PLETSCHER et al. 1966). During the present study we found that the necessary concentration of DOPA for a 50 per cent blocking of the serotonin synthesis is lower than vice versa. The synthesis of serotonin and dopamine were inhibited by α-methyl DOPA in identical degree. Data are summarized in *Table III*.

Table III

Concentrations of 5HTP, DOPA and α-methyl DOPA necessary for 50 per cent inhibition of the decarboxylase activity

Tissue	Substrate	5HTP	DOPA	α-methyl DOPA
Ganglia	5HTP		$6{,}2 \cdot 10^{-4}$M	$1{,}8 \cdot 10^{-4}$M
	DOPA	$2{,}2 \cdot 10^{-3}$M		$4{,}0 \cdot 10^{-4}$M
CVc	5HTP		$6{,}0 \cdot 10^{-4}$M	$2{,}0 \cdot 10^{-4}$M
	DOPA	$2{,}0 \cdot 10^{-3}$M		$3{,}0 \cdot 10^{-4}$M

(Concentration of the substrates: 5HTP — $4{,}54 \cdot 10^{-4}$M
DOPA — $2{,}54 \cdot 10^{-3}$M)

Discussion

Our results show that the capability for dopamine synthesis is higher than for serotonin synthesis in the various tissues of *Anodonta cygnea* L. This is valid especially for the ganglia and CVc, however the differences are well

expressed also in the cases of the gills and mantle. For the explanation of this phenomenon there is no need to suppose the presence of two different enzymes. Similarly different values were obtained for 5HTP and DOPA decarboxylization in vertebrates, where the identity of the 5HTP and DOPA decarboxylase is proved in various ways (HAGEN and COHEN, 1966).

Our experiments concerning enzyme inhibition refer to the presence of one and the same enzyme. There are numerous data showing that a mutual inhibition of decarboxylation exists between 5HTP and DOPA (PLETSCHER et al. 1966). This was found also in our case. The inhibitory effects caused by α-methyl DOPA prove also the identity of the 5HTP and DOPA decarboxylase enzymes (PLETSCHER et al. 1966). The K_M values obtained for different tissues are nearly the same both in the case of 5HTP and DOPA, and correspond to that found in other cases (HAGEN and COHEN, 1966).

Comparing the present data with the concentration of serotonin in the tissues subjected to examination (HIRIPI, 1966) it can be concluded that it is the nervous tissue where both decarboxylase activity and 5HT concentration reach the highest level. However, there is a difference between the ganglia and the CVc. Namely, the concentration of 5HT was 40 per cent lower in the CVc as compared to that of the ganglia, while the decarboxylase activity of the CVc proved to be twice as high as in the ganglia. Another incongruity occurs in the adductor muscles, where a definite 5HT concentration but no decarboxylase activity was demonstrable. It is remarkable that the case is just the opposite in the heart muscle. These differences in the ganglia, CVc and adductors may be connected with transportation of 5HT along nerves, a phenomenon, which is well known for some neurotransmitters (DAHLSTRÖM and HOGGENDAL, 1966). It may be supposed, that the 5HT content of the CVc is low because this nerve stores the synthetized serotonin in a less degree than ganglia do which may serve as storage organ for 5HT. On the other hand, there is probably no 5HT synthesis in the adductors, but the 5HT being transported into the nerve endings from the ganglia is well measurable. It is supposed that the low decarboxylase activities of the gills and mantle originate from nerve elements present in these tissues. Fluorescent microscopical investigations could possibly give further informations in this respect.

Our results are contradictory to those of WELSH and MOORHEAD (1959) and MIROLLI (1958) claiming that the activity of 5HTP-DOPA decarboxylase is not influenced by the presence in the incubation medium of pyridoxal 5-phosphate. The apparent contradiction may originate from the different degree of dilution of the cofactor during homogenization. It occurs also in vertebrates that because of similar reasons in one case pyridoxal 5-phosphate increases, while in other cases (e.g. brain or liver of mouse) does not influence the decarboxylase activity of the homogenizate (HAGEN and COHEN, 1966)

Summary

1. 5HTP-DOPA decarboxylase activity was found in the homogenizates of the cerebral, visceral and pedal ganglia, cerebro-visceral connective, heart, gill and mantle of *Anodonta cygnea* L. The synthesis proved to be 4—6 times faster for dopamine than for serotonin.

2. In the nervous tissue the enzyme activity expressed in μg amine/g wet weight/hour was 50—100 for serotonin and 300—600 for dopamine while in other tissues these values remain under 10. The rates of the enzyme activity correspond more or less to the serotonin content of these tissues. Neither in the muscle nor in lymph was 5HTP-DOPA decarboxylase demonstratable.

3. 5HTP and DOPA are decarboxylated by one and the same enzyme, but the affinity of the DOPA is greater to the enzyme than that of the 5HTP.

4. Addition of pyridoxal phosphate to the homogenate increases the enzyme activity in a considerable degree.

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5HTP-DEKARBOXILÁZ (DOPA-DEKARBOXILÁZ) VIZSGÁLATA *ANODONTA CYGNEA* L. IDEGRENDSZERÉBEN ÉS EGYÉB SZÖVETEIBEN

Hiripi László és *Salánki János*

Összefoglalás

1. *Anodonta cygnea* cerebrális, viscerális és pedális ganglionja, cerebroviscerális konnektívuma, szív, kopoltyú és köpenyszövetének homogenizátuma rendelkezik 5HTP-DOPA dekarboxiláz aktivitással. Az enzim a dopamint 4—6-szor gyorsabban szintetizálja, mint az 5HT-t.

2. Az aktivitás értéke az idegszövetben 5HT-re nézve 50—100, dopaminra 300—600 μg/g nedves súly/gram. Egyéb szövetekben ezek az értékek 2—10 között vannak. Az arányok nagyjából megfelelnek az 5HT tartalomnak. A záróizomban és limfában 5HTP-DOPA dekarboxiláz aktivitást nem sikerült kimutatni.

3. Az 5HTP és DOPA dekarboxilálást ugyanazon enzim végzi, de a DOPA-nak nagyobb az affinitása az enzimhez.

4. A homogenizátum enzimaktivitását piridoxal-5-foszfát hozzáadása jelentősen fokozta.

ИЗУЧЕНИЕ 5-ОКСИТРИПТОФАН-ДЕКАРБОКСИЛАЗЫ (ДОФА-ДЕКАРБОКСИЛАЗЫ) В НЕРВНОЙ СИСТЕМЕ И ДРУГИХ ТКАНЯХ БЕЗЗУБКИ

Л. Хирипи и *Я. Шаланки*

1. Гомогенат церебрального, висцерального и педального ганглиев, цереброви-сцерального коннектива, сердца, жабер и мантии обладает 5—ОТФ-дофа-декарбоксилазной активностью. Этот фермент синтезирует дофамин в 4—6 раз быстрее, чем серотонин.

2. Величина активности в нервной ткани для 5—ОТФ 50—100 цг/г свежей ткани, а для дофамина 300—600 цг/г. В остальных тканях это значение 2—10. Это соотношение согласуется с содержанием серотонина. В запирательной мышце и лимфе не удалось найти 5—ОТФ и ДОФА-декарбоксилазной активности.

3. Декарбоксилирование 5—ОТФ и ДОФА осуществляется одним и тем же ферментом, но реактивность ДОФА к этому ферменту выше.

4. Ферментная активность гомогената значительно увеличивается под влиянием пиридоксаль-5-фосфата.

ANNAL. BIOL. TIHANY	36	25—39	HUNGARIA 1969

MAKE AND BREAK RESPONSES OF *ANODONTA* NERVE EVOKED BY DIRECT CURRENT STIMULATION

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Received: 27 January, 1969.

The cathodal origin of the nerve-impulse and the failure to appear or nevertheless sometimes the emergence of an action potential with a decreased amplitude when the anode is proximal to the recording electrodes, are known as the basic principles of electrophysiology (PFLÜGER; 1859; BURES, 1960). This latter phenomenon, that can be observed mainly on isolated nerves at special conditions of the stimulation has been explained in the old literature (BIEDERMANN, 1895) by the supposition of the so called virtual cathode claiming that it would be responsible for the anode-make and cathode-break excitations. LORENTE de NO (1947) explained such virtual cathode-phenomena on isolated nerves by the irregularities of the interpolar nerve-segment. LORENTE de NO has concluded that the anode-excitation is not originating from a virtual cathode yet exists when the stimulating current is very low and/or the nerve is near to its unstable state.

The aim of our experiments was to describe and interpret the laws of direct current excitation for the cerebrovisceral connectives (CVc) of *Anodonta cygnea* L., at a constant arrangement of the electrodes. It will be pointed out that the PFLÜGER's laws, the WALLER's formula and the supposition of the virtual cathode represent a simplification, can not explain the facts and are not describing unequivocally the behaviour of CVc.

Methods

The isolated cerebrovisceral connectives (CVc) of *Anodonta cygnea* L. were kept in physiological solution of MARCZYNSKY (1959) and later during the leading off, the nerve was lifted into paraffin oil (SALÁNKI et al. 1964). Only such samples of paraffin oil were suitable which do not contain more volatile hydrocarbons (LÁBOS, unpublished). Four silver-electrodes were applied. Among them the stimulatory electrode situated proximally to the recording ones, was an earthed silver plate of 10 mm, the others were made from silver wire of 0,2 mm diameter *(Fig. 1)*. The action potential of some mV was amplified by a symmetric RC-amplifier (DISA, 14COO2, 200 MΩ, 24 pF; 1,6—500 cps). For stimulation the constant voltage output of a DISA Multistim equipment was applied.

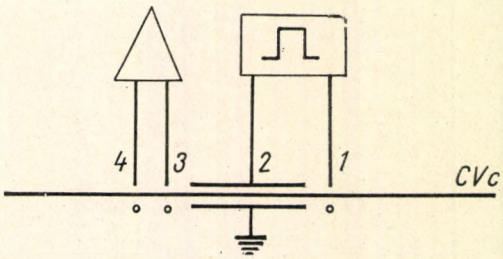

Fig. 1. Experimental arrangement. The 1st electrode is 1 mm away from the proximal edge of the 2nd electrode of 10 mm. Distance between the 2nd and 3rd electrodes is also 1 mm. Between the 3rd and 4th electrodes it is 10 mm.

Passive components (LÁBOS and VARANKA, 1966) owing to the special leading off and amplification have not been observed up to 1,6 V of stimulatory voltage. Between 1,6 and 2,0 V, it increased the amplitude by a value representing not more than 10 per cent. As it is a systematic error, it did not trouble the comparisons. The amplitudes of action potentials were measured peak to peak at the maximal component. The designations of the responses are the following:

1. cathode-make response — CC
2. cathode-break response — CO
3. anode-make response — AC
4. anode-break response — AO

In all cases the marking of the polarity had reference to the larger, earthed and stimulatory electrode, proximal to the leading off pair.

The temperature was 25—28 °C.

Results

1. *Polarity-dependence of the amplitude-voltage characteristics at short pulses*

Fig. 2 shows that in general the amplitude of the response is higher, if the electrode proximal to the recording ones, — otherwise always earthed — is used as anode. Thus, the voltages necessary to activate to 50 per cent, using pulses of 3 msec, are 0,8 and 1,2V, stimulating by pulses of 10 msec are 0,42 and 0.82V, respectively, according to polarity whether it is earth-positive or earth-negative. Therefore it can be seen, that the anode-proximal excitation is dominating.

2. *Amplitude-voltage diagrams when applying pulses of 1 sec*

In general the on- and off-responses begin to separate above 100 msec duration of the stimulatory pulses. Already during a stimulus of 1 sec, this separation takes place entirely. As one can notice in *Fig. 3* both make and break responses can originate under the influence of cathode (earth-negative) or anode (earth-positive) stimulation. Furthermore, the amplitude of the responses can be arranged in diverse sequences depending on the stimulatory voltage. Almost all possible sequences exist well reproducibly.

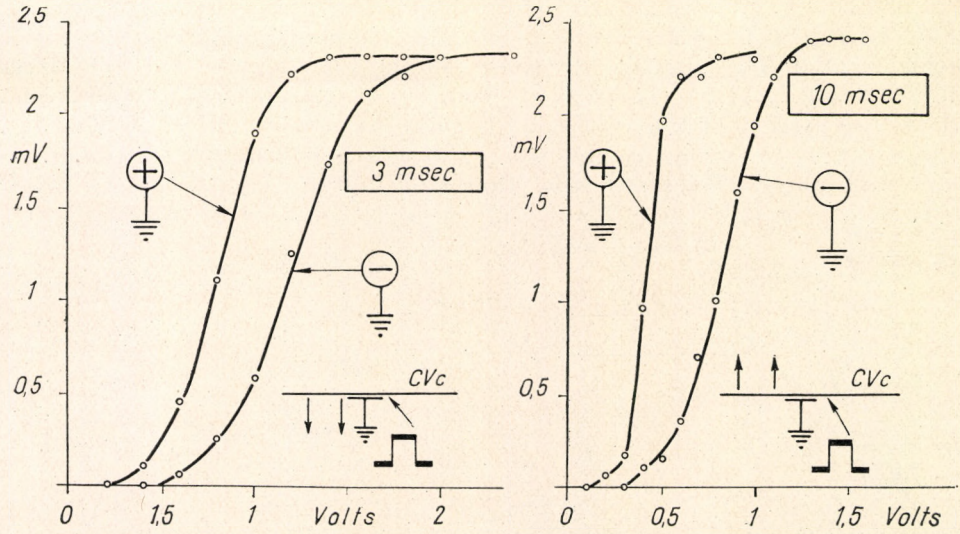

Fig. 2. Amplitude-voltage diagrams at 3 and 10 msec pulses. Earth-positive and earth-negative stimulations

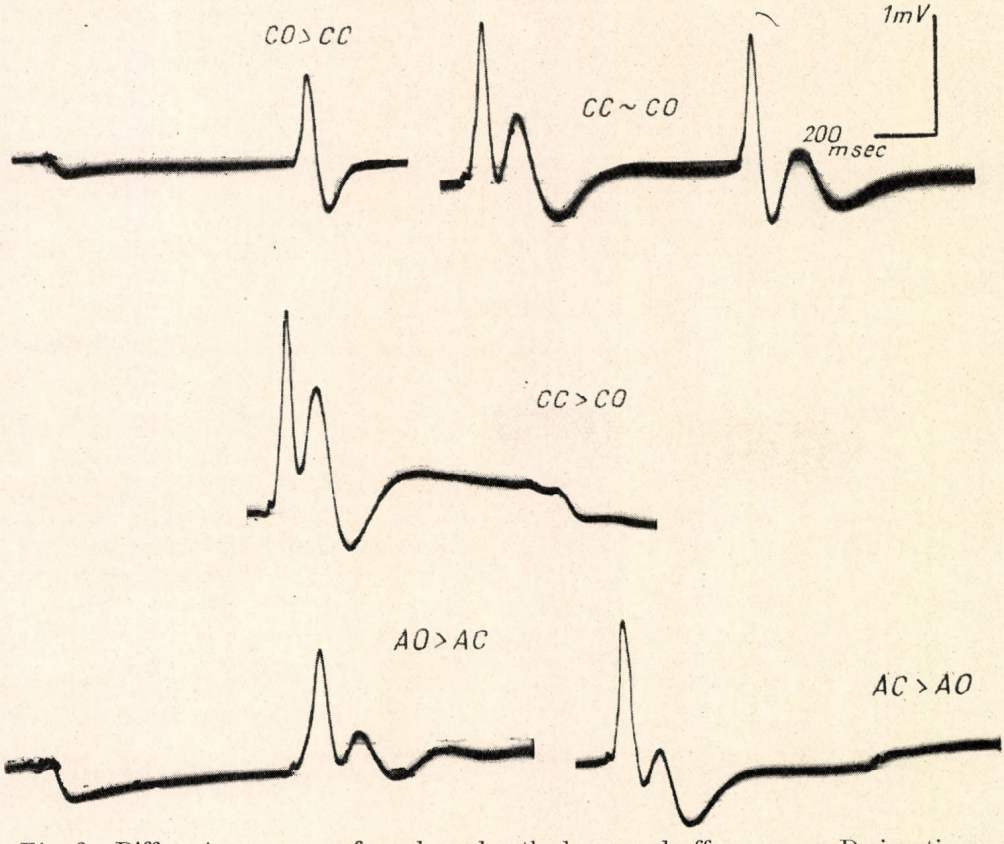

Fig. 3. Different sequences of anode and cathode on and off responses. Designations see in the text

In *Fig. 4/A* the average of amplitude voltage diagrams of 15 nerves is demonstrated. The standard deviations of the CC, AC, CO and AO curves are 12,5 ± 9,7; 21,2 ± 12,2; 25,4 ± 9,6; 25,4 ± 15,2 per cent respectively. It is observable that the values of CC curve have the minimal uncertainty. The standard deviations of the remaining ones is higher by 1,5—2. The differences are statistically significant. Thus $0{,}05 > P > 0{,}02$ ($f = 38$; $t = 2{,}14$) is valid for the differences of the coefficients of variation in the cases of CC

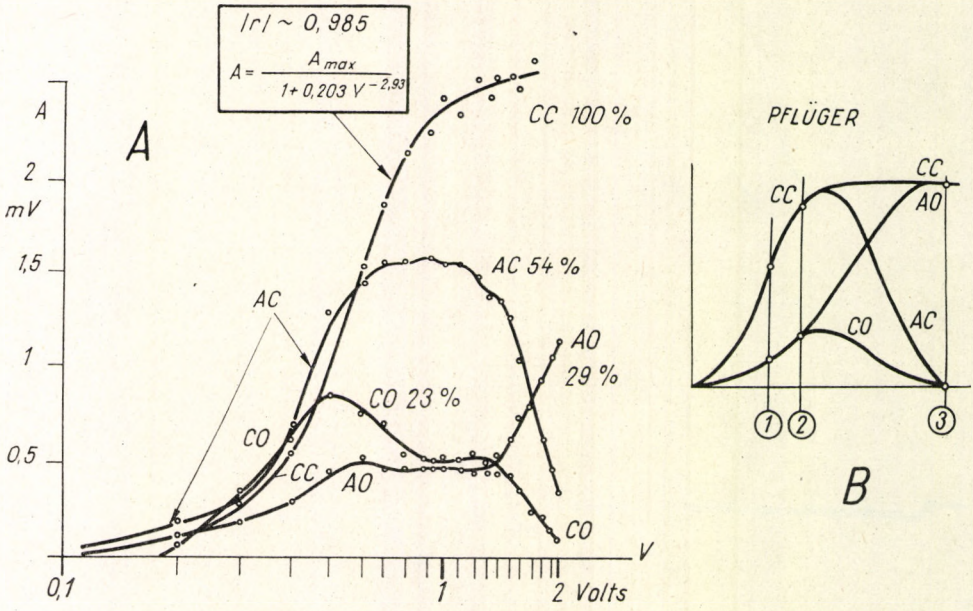

Fig. 4. A — Amplitude-voltage curves of 15 nerves at the 4 kinds of excitation. B — The ensemble of curves expected according to PFLÜGER's laws. Data in per cent design the ratios of surfaces up to 2 Volts. Empiric equation for CC is also demonstrated. Duration is 1 sec.

and AC curves. It is noticeable that the dispersions of the low amplitudes are higher and the standard deviation in a sense follows the course of the averages *(Fig. 5)*.

In *Fig. 4* a deviation of the four curves can be well observed. The CC-response has always an S-shaped curve. The value of saturation is 2,53 ± 0,66 mV. The voltage evoking the half-maximum is 0,45 ± 0,49V. The AC-curve starts a little more steeply than the CC, later by a lower amplitude (1.56 ± 0,82 mV) it reaches to a maximum of plateau and finally at about 2—2.5 V V of stimulatory voltage disappears. The CO one has two maxima at 0.5 and 1,2V. Their values are 0,85 ± 0,89 mV and 0,54 ± 0,39 mV resp. The CO diagram also starts more steeply than the CC one, and it vanishes at 2V. The AO-curve starts least abruptly of all and culminates broadly at a low value (0,54 ± 0,64 mV), but from 1,25 V it begins newly to increase. Its steady-state value is about 1,5 mV. The ratios of the surfaces under the curves represent a typical sequence, if the summation is carried out to the steady-state values:

$CC > AC > AO > CO$ and finally
$CC > AO > AC > CO$

The four curves form a complicated system of intersection points. The number of intersections of not random type is in average 4—5, but in extrem-

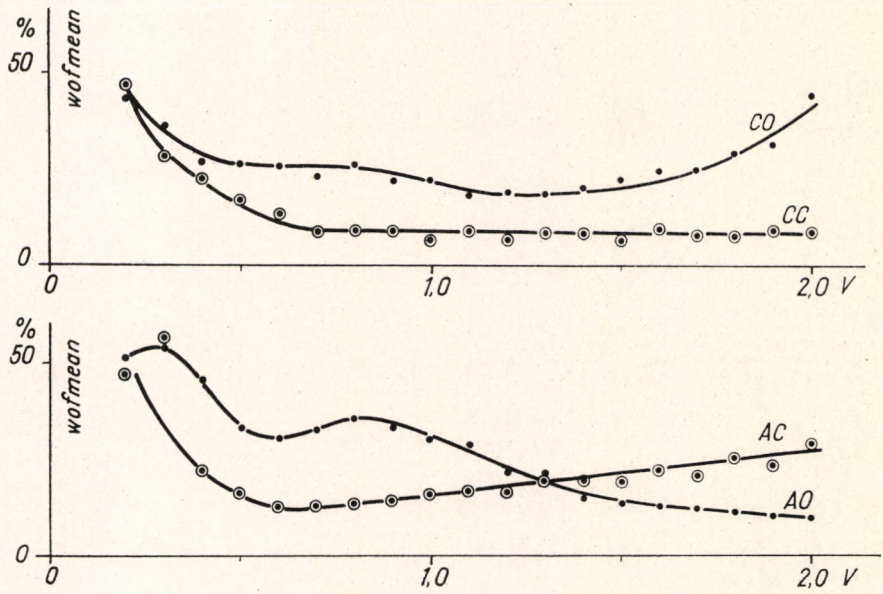

Fig. 5. Variation coefficients of averages plotted against voltage for CC—AC—AO—CO responses (belong to the data of *Fig. 4)*

ities is 0—10. The 4—5 observed intersections imply the same number of changes in the sequences of amplitude. In the demonstrated case the sequences are the following:

1. $AC > CO > CC > AO$	if $0 < V <$	0,45 V
2. $AC > CC > CO > AO$	if $0{,}45 < V <$	0,65
3. $CC > AC > CO > AO$	if $0{,}65 < V <$	1,35
4. $CC > AC > AO > CO$	if $1{,}35 < V <$	1,68
5. $CC > AO > AC > CO$	if $1{,}68 < V <$	

Among these, the 4th corresponds to the physiological contraction-sequence of Waller. Referring to the lst (different) stimulatory electrode the 5 sequences are

1. $CC_1 > AO_1 > AC_1 > CO_1$
2. $CC_1 > AC_1 > AO_1 > CO_1$
3. $AC_1 > CC_1 > AO_1 > CO_1$
4. $AC_1 > CC_1 > CO_1 > AO_1$
5. $AC_1 > CO_1 > CC_1 > AO_1$

Also in this case, the Waller-formula is not valid but for a very short interval.

The statistical significance of the differences in amplitudes of the 5 given sequences have to be tested only at low voltage values, as the changes of the 3rd-5th sequences correspond to very high differences. For the average-diagrams the significance of the deviations does not subsist. The cause of this lies in the fact that the range of AC $>$ CC occurs at different intervals duty increasing the dispersion. But the normalization of the curves is problematic. After all, the range of AC $>$ CC has been found in fact in 11 cases out of 15 and in these cases the CC-response may be 5 times higher than AC *(Fig. 6)*. In 4 cases CC $>$ AC is valid. The relation of CO $>$ CC can be observable in half of the cases, of similar dispersing range.

3. *Types of responses*

The differences among some characteristic types of nerve get lost in the course of averaging. These types merit a special discussion. Here it must be stressed that the pecularities in the sequences may not issue from the conditions of the measurement when speaking about CC—CO or AC—AO response-pairs, because they are separated just by the direct current of 1 sec, the effect of which is examined. But the AC—CC, AO—CO, AC—CO, AO—CO relations must be compared to each other more carefully because they were not evoked by the same pulses. Applying a suitable set of measurements and controls, we can eliminate this difficulty.

One of the types is when the CO response is entirely absent. In this case the system of intersections is simple. In other cases, when the CO-response is not too high and the CC-dominance is absolute, the relations of curves are also simple *(Figs. 6A* and *6B)*.

On the contrary, if both peaks of both on-response curves (AO and CO) are explicit *(Fig. 7A)*, the intersections are numerous. In such a case one can observe that the first maxima run together and the breaking down of the 2nd maximum in the CO-curve, the 2nd increase of AO at the saturation manifest themselves at about the same voltages as the breaking down of AC which possibly is of two-phase *(Figs. 7A* and *7B)*.

It occurs accidentally, that the AO response is dominating, mainly when AC is of low value and its maximum is not wide *(Fig. 8.)*.

20 out of 24 possible orders have been observed. However, some of these show themselves only when activating certain nerves. In the table, the numbers of intervals are listed of which a given sequence was found to be characteristic.

Data obtained for the site of intersections are the following (examined on 15 nerves):

1. CC · CO	7	intersections	0,54 $\pm$ 0,17	Volt
2. CC · AO	6		0,57 $\pm$ 0,22	
3. CC · AC	11		0,55 $\pm$ 0,29	
4. AC · AO	15		1,72 $\pm$ 0,73	
5. AC · CO	13		1,05 $\pm$ 0,57	
6. AO · CO	17		1,19 $\pm$ 0,52	(average $\pm$ s.d.)

It can be seen that the 3 first points of intersections between 0.5 and 0.6 Volt coincide, forming an initial and unstable system of intersections. They occur in a half of the cases and are found near to the half-saturation value of the CC curve. The found interaction do not represent a system so far uniform.

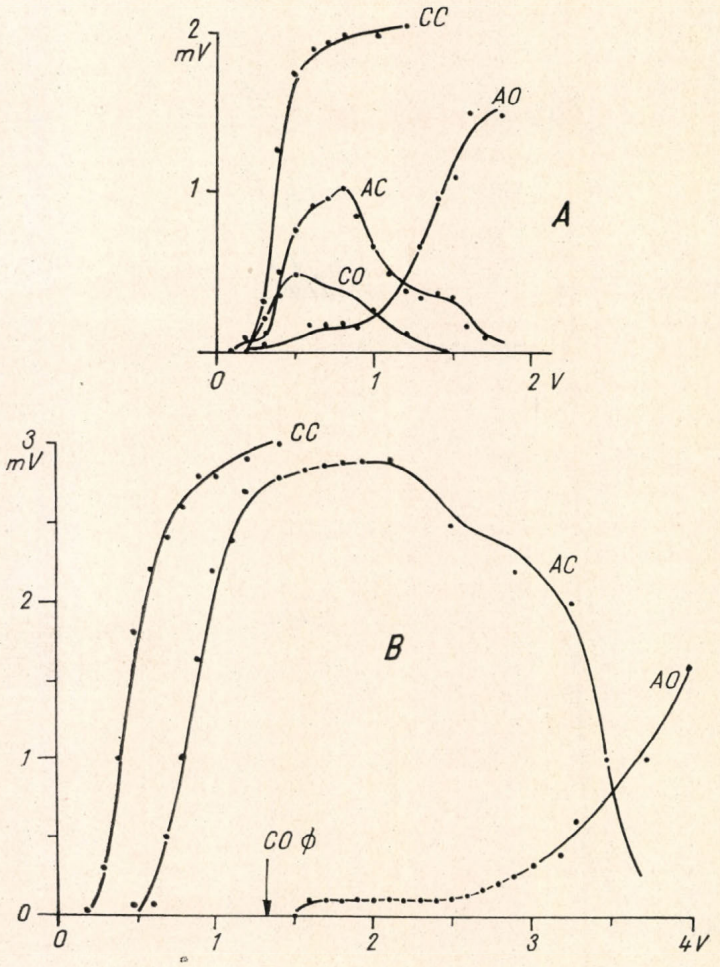

Fig. 6. Extreme types of response, when CO response is low or absent

The sites of AC × AO and AC × CO points deviate significantly ($f = 26$; $t = 2{,}74$; $0{,}02 > P > 0{,}01$). The difference between the AC × AO and AO ×. AO × CO points is significant as well ($f = 30$; $t = 2{,}41$; $0{,}05 > P > 0{,}02$) But the deviation between AC × CO and AO × CO points is not significant statistically ($P > 0{,}05$).

Thus the 6 possible intersections group in the vicinity of 3 values of stimulus-strength.

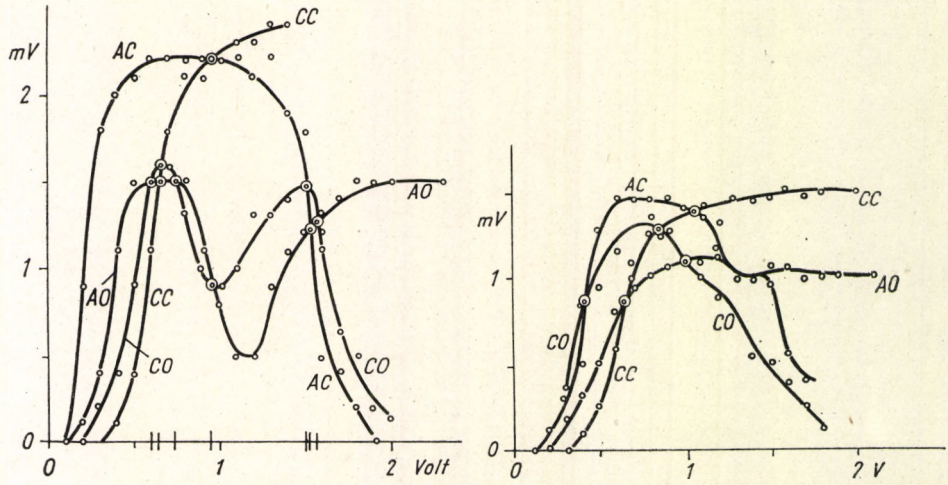

Fig. 7. Extreme types of response, when amplitudes of AC, CO and AO are high

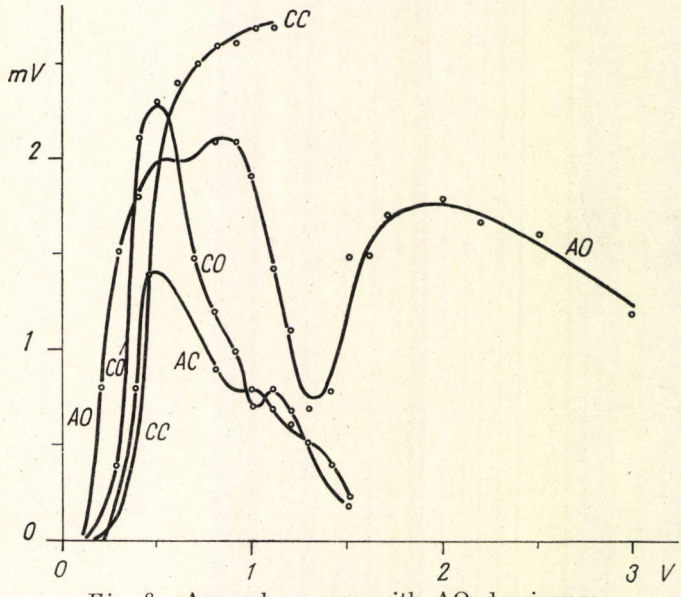

Fig. 8. Anomalous case with AO dominance

4. *The slope of CC-curves at different d durations of pulses*

Only the CC out of the four curves could be approached simply. The approximation was carried out by the following way. The quantities $\log \frac{A}{A_{max}-A}$ was calculated from the maximal and actual values of amplitude. These were plotted against log V, when we obtained a line *(Fig. 9)*.

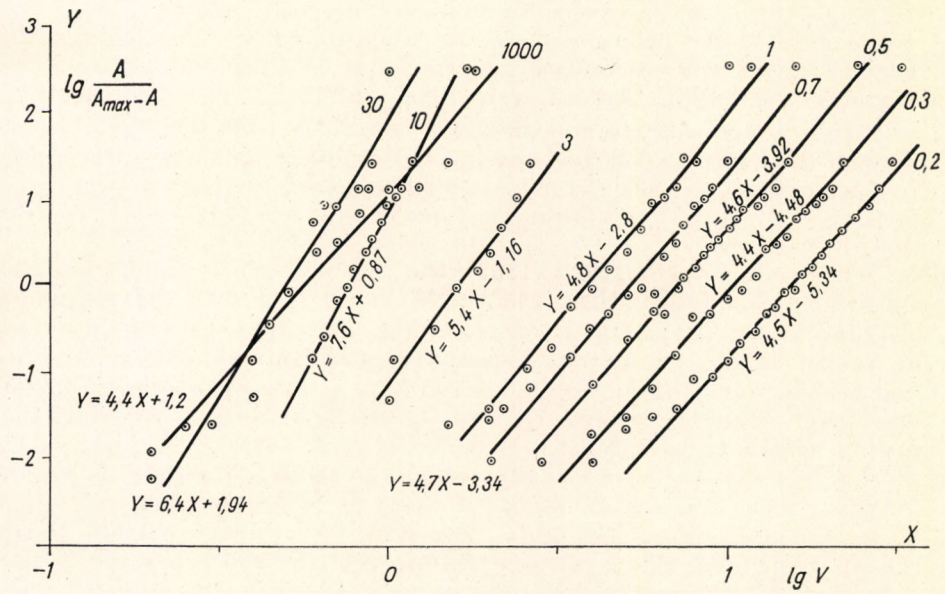

Fig. 9. Linearization of the amplitude-voltage curves. Duration was changed between 0,2 and 1000 msec. Explanation in the text.

From this

$$\log \frac{A}{A_{max}-A} = \alpha \log V + \delta$$

and

$$A = \frac{A_{max}}{1+\beta V_Z^{-\alpha}} \sim \frac{A_{max}}{1 + (V_{0,5}/V_Z)^{\alpha}}$$

where $-\log\beta = \delta$. The α-constant is about 4,5 for short pulses. At medium values (3—30 msec) it is 5,5—7,5 and finally for d = 1 sec, the slope decreases newly, $\alpha = 2,9 - 4,5$. The value of β is much more dependent on d. If for $Z = \frac{A}{A_{max}}$ degree of the activation the strength-duration relation is $V_Z(d)$, then $\beta = \left(\frac{1}{Z} - 1\right) V_Z^{\alpha}(d)$ or $\beta^{-1/\alpha} = V_{0,5}(d)$.

Discussion

For an acceptable interpretation of the observed phenomena it is necessary to take into account the following factors:

1. the character of excitation
2. the site of origin of the responses
3. the condition of the zone influencing the excitation during its propagation (electrotonus, accomodation, refracterity)
4. the different character of stimulatory electrode
5. the fact that the CVc is a complex nerve (Zhukov, 1946; Lábos et al. 1963; Gupta et al. 1969).

Talking of these we have some preliminary suppositions.

ad 1 and 2. — Both opening and closing excitations exist. Threshold of the latter is higher when stimulating by pulses of long duration (HILL, 1936; WERIGO, 1883, 1901). According to PFLÜGER (1859) the make-excitation originates at the cathode, and the break one comes from the anode. In his opinion the make- and cathode-excitations are more intensive. According to the old literature the virtual cathode-phenomena contradict PFLÜGER's laws only apparently, according to LORENTE de NO (1947) they contradict them in reality.

ad 3. — According to PFLÜGER (1859), the cathode-zone is facilitatory the anode-zone is inhibitory. HILL (1936) and WERIGO (1189, 1901) have claimed just the opposite. The attention to this seeming contradiction has been directed by NASZONOV (1958). Both opinions are well explained by the accomodation and the dynamics of subthreshold-excitation. But, for example, the cathode-effect is inhibitory and that of the anode is facilitatory when the nerve has been treated by KCl (WORONZOV, 1924, 1925).

ad 4. — In our case the electrode (2) proximal to the recording pair is larger, for this reason it is less different.

ad 5. — Measurements of the amplitudes have been carried out always at the lst component, this is why the influence of the 2nd one is negligible.

In *Fig. 4B*, the postulates of PFLÜGER's laws and the contraction-sequence of WALLER are schematically demonstrated. In our case the earth-negative stimulus is descending, the earth-positive is ascending relating to the indicator (leading off electrodes). *Fig. 4B* was drawn after a usual description of the phenomena in recent text-books (LISSÁK, 1961). We like to underline that the decrease of CO excitation is thought to be an unstable phenomenon and WERIGO's depressive cathode-effect is supposed to be elicite by the decrease.

Summing up our experimental results it can be claimed unequivocally that PFLÜGER-WALLER laws are highly simplified. One of the causes explaining this situation may be that the laws in question are laws for contraction. In this study the excitation is indicated by an action potential and this is why the details are more finely reflected.

The classical interpretation (PFLÜGER, 1859), that the make-excitation is dominating and excitation generally is evoked by cathode-make (CC) or by anode-break (AO), gives the following explanation. The CC and AC-responses come from the earth electrode and the AC and CO ones originate at the different electrode. The CC response spreads without disturbs to the leading off, but the AC one travels coming from a farther point troubled by the intermediate anode-zone which goes from strength to strength. Therefore, its value at saturation must be lower and depressed at higher voltages.

The threshold of the off-responses is higher, this is why AO $<$ CC (both come from the earth-electrode). The CO excitation is the anode-off response of the different electrode, therefore, CO $<$ AC. The disappearance of the CO phenomenon is due to the depressive cathode-effect (WERIGO, 1889, 1901). The intermediate cathode-zone is namely accomodated. The breaking down of CO is an obligatory phenomenon, contrary to common knowledge.

In our opinion, the AC response stands higher than CC after a short pulse and often even following a pulse of 1 sec, because the effect of higher current-density at the different electrode cannot be surpassed by the intermediate and yet weak anode-zone. In the given case the condition indicated

by LORENTE de No (1947), i.e. the weak stimulus cannot show clearly the cause. At the AC × CC intersection the inhibitory effect of the anode zone just excels this effect of the different electrode. But after short pulses *(Fig. 2)* an anode-zone capable to inhibit the response could not have been formed yet. Such a short stimulus which can evoke a maximal response does not represent a weak stimulus.

The sequences of average-curve *(Fig. 4A)* referring to the adequate electrodes are the following:

1.	$CC_1 > AO_1 > CO_2 > AO_2$	different electrode is dominating
2.	$CC_1 > CC_2 > AO_1 > AO_2$	
3.	$CC_2 > CC_1 > AO_1 > AO_2$	closing-dominance
4.	$CC_2 > CC_1 > AO_2 > AO_1$	
5.	$CC_2 > AO_2 > CC_1 > AO_1$	closing and proximal dominance

This explanation, in fact, corresponds to the PFLÜGER's laws and takes into account the accomodation as well. The cathode-depressive-effect is consequent. WALLER' formul is not valid steadily referring to either of the electrodes.

Therefore the CC or AO responses, which become saturated, originate proximally and the CO or AC ones are of distal origin troubled during their propagation. This is supported by the following observation. In *Fig. 3*, in the case of the response, where both CO and CC-species are visible, there is a delay of about 60 msec between the two comopnents of both responses. This may correspond to a real difference of travelling way. The supposition of a virtual cathode is not required.

However, the explicit two maxima of CO, AC and AO curves require further explanation. We think that this is just the phenomenon expressing mostly the complicated determination of the response-ensemble. Essentially the problem consists in a decision. Whether the sites of origin for the two maxima are same or not, i.e. whether true CO and AC exist excitations or not? Whether the 6 parts of the AC, AO and CO curves and 1 or perhaps 2 sections of CC correspond to the theoretically possible 8 kinds of excitation (CC, AC, AO, CO or distal and proximal)?

A contribution of the two components cannot be led to the two maxima because of the way of measurement. The saturation of the 2nd maximum of AO also speaks against such a supposition. Furthermore, it would be contradictory that the maxima and the points of inflexion are intimately connected, e.g. the 2nd increase of AO is correlated to the breaking down of CO and AC. When the first maximum is reached, both components increase and after both decrease, and finally, both increase again.

We do not think it likely — contrary to LORENTE de No's opinion (1947) — that true cathode-off and anode-on excitations exist under physiological conditions, therefore, we search the cause of curves with two maxima and inflections (CO, AC, AO) in other factors. It may be supposed that the process itself under the intermediate electrodes disturbing the spreading is of two-phase and this shows itself in the two maxima of the amplitude-voltage curves. The different determinations of the curves are shown clearly by the uncertainty *(Fig. 5)* of the CC-curve which is not the same as that of the remaining three (CO, AC, AO). When several rivalling factors are responsible for a given value

Table 1

Sequences

Sequence >				Number of observed intervals	Remarks
CC	AC	AO	CO	10	At high voltage; it is not at the saturation (between 1—2 Volt)
CC	AC	CO	AO	14	Mediocre voltage (1 Volt)
CC	AO	AC	CO	15	At saturation is regular (above 2 Volt)
CC	AO	CO	AC	6	
CC	CO	AC	AO	6	
CC	CO	AO	AC	5	
AC	CC	AO	CO	5	
AC	CC	CO	AO	6	
AC	AO	CC	CO	2	At long duration and low voltage transient or at high voltage
AC	AO	CO	CC	2	At short pulses is consequent
AC	CO	CC	AO	3	*(Fig. 2)*
AC	CO	AO	CC	3	
AO	CC	AC	CO	Ø	Were not observed
AO	CC	CO	AC	Ø	
AO	AC	CC	CO	Ø	
AO	AC	CO	CC	Ø	
AO	CO	CC	AC	1	Anomalous *(Fig. 8)*
AO	CO	AC	CC	1	Anomalous *(Fig. 8)*
CO	CC	AO	AO	1	
CO	CC	AO	AC	2	More seldom at low voltage (below 0.6 V)
CO	AC	CC	AO	2	
CO	AC	AO	CC	1	
CO	AO	CC	AC	1	
CO	AO	AC	CC	1	

of the amplitude, the standard deviation could be significant. This is just the case of the CO, AO, AC curves, having break and/or distal origin.

Concerning the examples of response-types extremely deviating from each other we think of the variability of accomodation to be important. Some values of accomodation-constant may represent an increase of excitability (LÁBOS and FAZEKAS, unpublished). Probably LORENTE de NO's comment (1947) on the connection existing between the dominance of anode- or off-responses and the instability of the nerve is related to this phenomenon mentioned before.

The duration-dependence of the slope of CC-curves — in our opinion — is related to the progressive separation of the on- and off-responses. When stimulating by short pulses (0,2—1 msec), only one response contributes to the amplitude. But between 3 and 30 msec, where the curves are steeper *(Fig. 9)*, both the make- and break-responses participate in the amplitude. The slope is newly decreased ($\alpha = 3-4{,}5$) at a stimulus of 1 sec, where the separation is entire and only the CC response is measured. The on or off character of the responses to short stimuli (d $<$ msec) is questionable, though their usual interpretation is of on-type (HILL, 1936). This is supported also by the similarity of α-values for the short and 1 sec stimuli.

The behaviour of the single-axon preparations (TASAKI, 1951; HODGKIN, 1965) under the effect of direct current can not be compared directly with the responses of CVc, mainly because of their ,,all or nothing'' character. For this reason a discussion of the related literature may not be absolutely necessary. Considering the response of an axon, it can be stated that there are not similar amplitude-voltage characteristics or they are of incomparably steeper. In spite of the complexity of CVc, its similar diagrams, already contain very high exponents ($\alpha = 3-7$). Such high exponents can issue not only because of the narrow band of fibre-histogram (LÁBOS et al. 1963), but they can be related to the high exponents for Na^+ and K^+ conductance in the HODGKIN-HUXLEY equations as well (HODGKIN and HUXLEY, 1952; HODGKIN, 1965; MOORE, 1968).

Summary

The laws of direct current stimulation were studied on the cerebro-visceral connectives of *Anodonta cygnea* L. at a given arrangement of the electrodes. The indicator of the excitation was the action potential.

It has been established, that all possible responses (cathode, anode, closing, opening) and almost all of their possible sequences exist. The voltage-dependences of the responses are different. All of these diagrams but the cathode-on curve, are of two-phase. The cathode-on and anode-off responses show a saturation.

The 4 curves intersect one another at 4—5 points in an average. The intersections form a regular system. They are grouping near to 3—4 different values of stimulus-strength.

The order of the responses depends on the voltage. At least 5 regular sequences are observable. The steady-state sequence is not identical with the Waller's formula. It is (designation see in the text):

$$CC > AO > AC > CO \quad \text{that is} \quad CC_2 > AO_2 > CC_1 > AO_1$$

Depending on the condition of the nerve the off-responses may be very high or they may be entirely absent.

CC-curves were approached at several durations of pulses. Their formul is $A = A_{max}[1 + \beta V^{-\alpha}]^{-1}$, where α and β are dependent on the pulse-duration; $\alpha \sim 3-7$ and $\beta^{-1/\alpha} = V_{0,5}(d)$.

The empiric equation expressing the whole (V,d)-function is

$$A_Z = \frac{A_{max}}{1 + [V_{0,5}(d)/V_Z(d)]^{\alpha}}$$

where the $V_Z(d)$ curves represent strength-duration relations belonging to Z degree of activation ($0 < Z < 1$).

Stimulated by short pulses, at the given electrodes, the anode-proximal excitation is always higher.

In our opinion both on and off excitations take place at both of the stimulating electrodes. Their relation is determined by their ab ovo different thresholds, by accomodation, spreading, size and arrangement of electrodes. The existence of true cathode-off and anode-on excitations are not probable. A supposition of the virtual cathode is not required.

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ANODONTA IDEG ZÁRÁSI ÉS NYITÁSI INGERÜLETE EGYENÁRAMÚ INGERLÉS HATÁSÁRA

Lábos Elemér

Összefoglalás

Anodonta cygnea L. cerebroviscerális connectivumán vizsgáltuk adott elektródarendszer mellett az egyenáramú ingerlés törvényszerűségeit. Az ingerület indikátora akciós potenciál volt.

Megállapítottuk, hogy minden lehetséges válasz (katód, anód, zárási, nyitási) és közöttük csaknem minden amplitúdó sorrend létezik. A válaszok ingerfeszültségfüggése eltér. A görbék a katódzárási válasz kivételével kétfázisúak. A katódzárási és anódnyitási válasz telítési jellegű.

A 4 görbe átlagosan 4—5 helyen metszi egymást. A metszéspontok törvényszerű rendszert képeznek. 3—4 eltérő ingerintenzitás körül csoportosulnak.

A válaszok sorrendje feszültségfüggő. Legalább 5 törvényszerű sorrend létezik. Az egyensúlyi sorrend nem azonos a Waller-formulával, hanem

$$CC > AO > AC > CO, \text{ azaz } CC_2 > AO_2 > CC_1 > AO_1$$

Az ideg állapotától függően a nyitási válaszok igen nagyok lehetnek vagy teljesen hiányozhatnak.

A CC-görbéket különböző impulzusszélességeknél közelítettük. Ennek alakja $A = A_{max} (1 + \beta V^{-\alpha})^{-1}$, ahol α és β impulzusszélességfüggő; $\alpha \sim 3—7$.

Az amplitúdó teljes (V, d)-függését kifejező empirikus egyenlet

$$A_Z = \frac{A_{max}}{1 + [V_{0,5}(d)/V_Z(d)]^{\alpha}}$$

ahol a $V_Z(d)$ görbék a Z aktiválásához ($0 < Z < 1$) tartozó ingerintenzitás-időtartam-összefüggések.

Rövid időtartamú ingereknél az adott elektródák mellett mindig az anód-proximális ingerület nagyobb.

Véleményünk szerint mindkét ingerlő elektródnál keletkezik zárási és nyitási ingerület. Ezek viszonyát ab ovo eltérő küszöbük, az akkomodáció, a terjedés és az elektródméret ill. elrendezés szabja meg. Valódi katódnyitási és anódzárási ingerület valószínűtlen. Ezek a folyamatok a másik elektródnál kezdődnek. Virtuális katód feltételezésére nincs szükség.

ВОЗБУЖДЕНИЯ ЗАМЫКАНИЯ И РАЗМЫКАНИЯ НЕРВА БЕЗЗУБКИ ПОД ВЛИЯНИЕМ ПОСТОЯННОГО ТОКА

Э. Лабош

Были изучены закономерности возбуждения при помощи постоянного тока применением данной системы электродов к церебровисцервальному коннективу беззубки.

Было установлено, что существуют все возможные реакции: (реакция на катод анод, размыкания и замыкания) и между ними обнаруживаются все возможные порядки амплитуд. Зависимость ответов от напряжения раздражения разная. Кривые, за изключением реакции на размыкание катода, являются двухфазными. Реакции размыкания катода и замыкания анода носят насыщнный характер.

В среднем 4 кривых пересекают друг друга в 4—5 пунктах. Места пересечений создают закономерную систему. Они появляются при 3—4 различной интенсивности возбуждения.

Порядок ответа зависит от напряжения. Существуют по крайней мере 5 закономерных порядков. Порядок равновесия не соответствует формуле Валлера, а $CC > A_0 > AC > CO$ т. е. $CC_2 > AO_2 > CC_1 > AO_1$. В зависимости от состояния нерва реакции на, размыкание могут быть огромными или полностью отсутсвуют.

Приближение кривых *C* осуществлялось при помощи импульсов разной продолжительности. Это имело следующий вид:

$$A = A_{max}(1 + \beta V^{-\alpha})^{-1}$$

где α и β зависят от продолжительности импульса: $\alpha \sim 3—7$. Полная зависимость амплитуды (V, d) выражается по следующему эмпирическому уравнению:

$$A_z = \frac{A_{max}}{1 + [V_{0,5}(d)V_z(d)]^{\alpha}}$$

где кривые $V_z(d)$ относятся к активности $z(0 < z < 1)$ и выражают зависимость интенсивности и продольжительности возбуждения. При данной системе электродов после кратковременного раздражения проксимальное возбуждение анода всегда выше.

По нашему мнению, возбуждение замыкания и размыкания возникает под обоим раздражающим электродом. Их отношения определяются различным порогом аккомодации, распространения а также размером и расположением электродов. Подлинного возбуждения размыкания катода и замыкания анода не существует. Эти процессы берут свое начало под другим электродом. Нет надобности предположения виртуального катода.

ANNAL. BIOL. TIHANY	36	41—56	HUNGARIA 1969

FREQUENCY-DEPENDENCE OF ACTION POTENTIAL ON NONMYELINATED NERVE OF *ANODONTA CYGNEA* L. (PELECYPODA)

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Received: 6th February 1969

Contraction or relaxation of anterior and posterior adductors in *Anodonta cygnea* can be evoked when repetitively stimulating the cerebro-visceral connective (CVc) running between the cerebral and visceral ganglia. The type of response depends on the stimulus-parameters (voltage, frequency, duration of pulses and of the train; PAVLOV, 1885; SALÁNKI and LÁBOS, 1963; SALÁNKI et al. 1968). According to suppositions the observed parameter-dependence is due to fibre-composition of CVc containing both excitatory and inhibitory fibres.

The heterogeneous fibre-composition of CVc is proved also by the complex action potential which can be recorded (SVERDLOV, 1956; ZHUKOV, 1946; SALÁNKI et al. 1964) and by the analysis of fibre-diameters (LÁBOS et al. 1963; GUPTA et al. 1969). However, examining the electrophysiological properties of CVc (SALÁNKI et al. 1963) it has become clear too that (1) the duration of the electrically evoked action potential is about 0,4—0,6 sec, questioning the interpretation of stimulation by frequencies above 1,5—2,5 cps; (2) the amplitude of action potential decreases when the frequency is increased.

To answer the emerging problems, a thorough knowledge about the frequency-dependence of the nerve-response could be of help. This is why the purpose of our experiments was to examine the CVc-responses to a train-stimulation of different duration and frequency. In the interpretation of the responses some questions concerning to the electric structure of the examined nerve were necessarily raised.

Methods

The experiments were carried out on isolated CVc-s of fresh-water clam (*Anodonta cygnea* L.). The nerves were kept preceding the experiment in physiological solution (MARCZYNSKI, 1959), later for leading off they were lifted into paraffin oil.

For stimulation a DISA Multistim equipment was used. Voltage (V) of square pulses was changed between 0,1 and 5 volts, durations (d) were 0,1—100 msec. and frequencies (ω) of 0,5—1000 cps were chosen.

Earth-negative stimuli (LÁBOS, 1965) were applied. 100 per cent activation means the situation when the maximal amplitude was evoked by a single pulse. Generally the degree of activation was lower.

For leading off silver wires of 0,2 mm diameter, for ground-electrode a silver plate were utilized according to the arrangement demonstrated in *Fig. 1.*

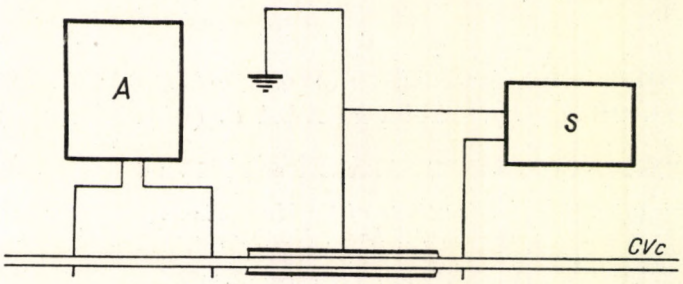

Fig. 1. Experimental arrangement.
A = amplifier, S = stimulator, CVc = nerve bundle. Distances of electrodes: 1—10 (Length of earth-electrode) — 1—10 mm.

The signals were amplified by a DISA EMG symmetric pre-amplifier (200 M Ω, 24 pF) and by a DISA 51BO1 end-amplifier (1,6—500 cps).

Owing to the symmetric recording the slow, passive potential of polarization (LÁBOS and VARANKA, 1966) was not recorded even after stimuli of 1 sec. The experiments were carried out at room temperature (20—26 °C).

Experimental results

As the systematically repeated trains may lead to a remaining effect (fatigue or potentiation), it has been examined whether identical trains of 5 sec repeated several times in 30 or 60 sec lead to a constant effect or not.

The nerve was stimulated in each minute by trains of 5 V, 3 msec, 3,75 cps, 5 sec parameters ($\sim$ 100 per cent activation). It has been found after 10 stimulations that the first amplitude decreased by 60 per cent. However, the amplitude measured at the 5th second — which was originally 40 per cent of the lst amplitude — remained practically unchanged. Thus, the a_5/a_0 ratio of amplitude increased from the original 40 per cent up to 100 per cent. At the same time it has been observed, that under the effect of repeated stimulations a maximum of the response-amplitude in the first 5 sec has appeared which after some repetitions has shifted to a later period of time. The results might be distorted by this cause therefore a pause of 10 min was allowed between the succesive trains to elapse.

When stimulating a fresh preparation by trains, the amplitudes of responses evoked by the consecutive pulses are not the same. This is observable even when the stimulus-frequency is low (1 cps) and during the time between two stimuli the whole cycle of the action potential can pass off, and also when the interval between two stimuli is shorter.

Considerable differences were furthermore observed in the responses given to trains at the beginning of stimulation or later and also after fatigue. For this reason it is practical to draw distinction between the behaviours of fresh and previously more or less stimulated CVc.

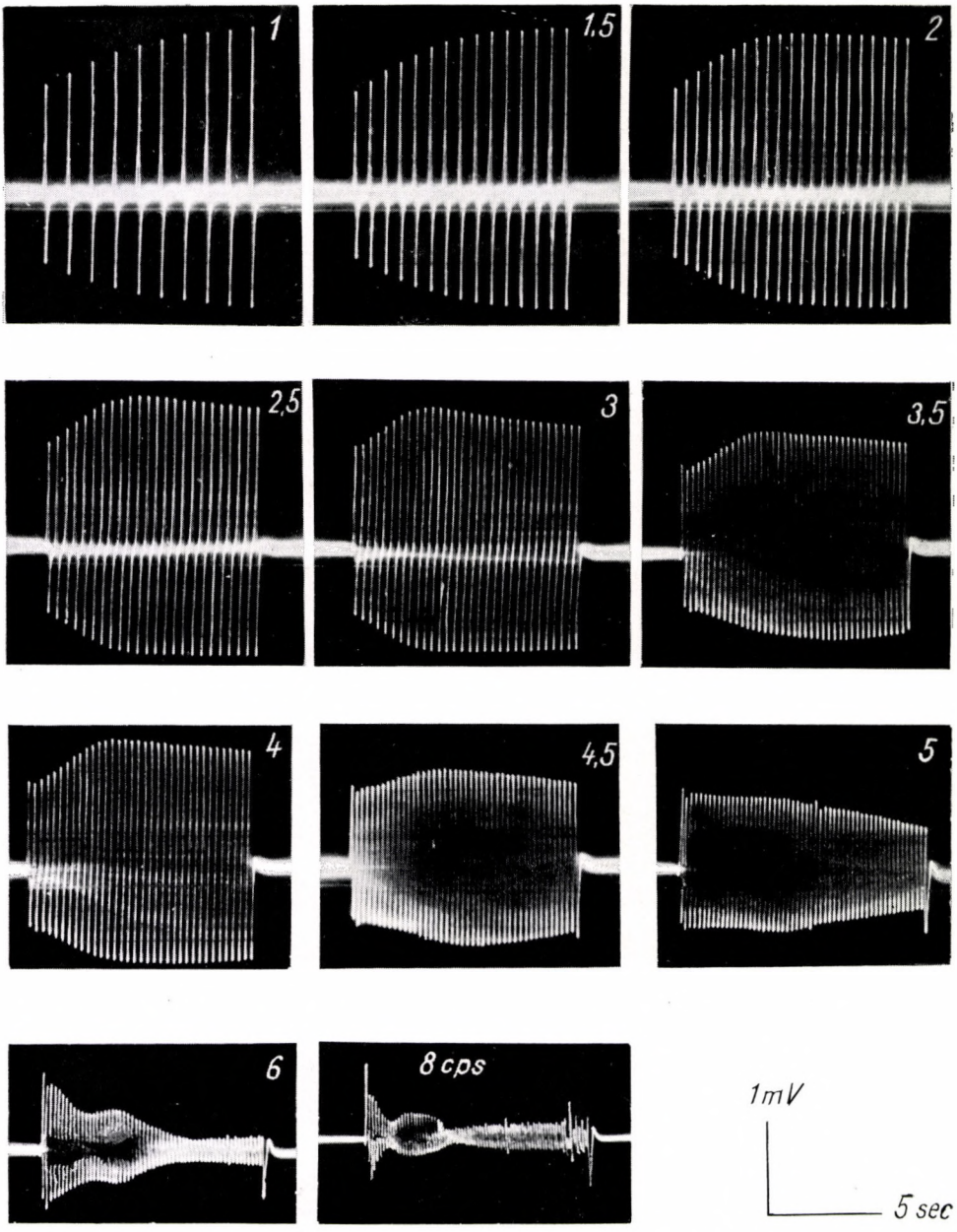

Fig. 2. Frequency-response of a fresh nerve between 1—8 cps in the first 10 sec. Voltage — 5 volt, duration — 3 msec.

1. *Frequency-dependence of the initial amplitudes on fresh nerve*

Stimulating by trains lasting for 10 sec and consisting of pulses of 5V and 3 msec repeated with 1—8 cps frequency, the evoked potentials were recorded *(Fig. 2)*. Later the amplitudes has been measured on the modulation envelope of the responses. Their heights are demonstrated in *Fig. 3*, as expressed in per cent of the lst amplitude in the response-set.

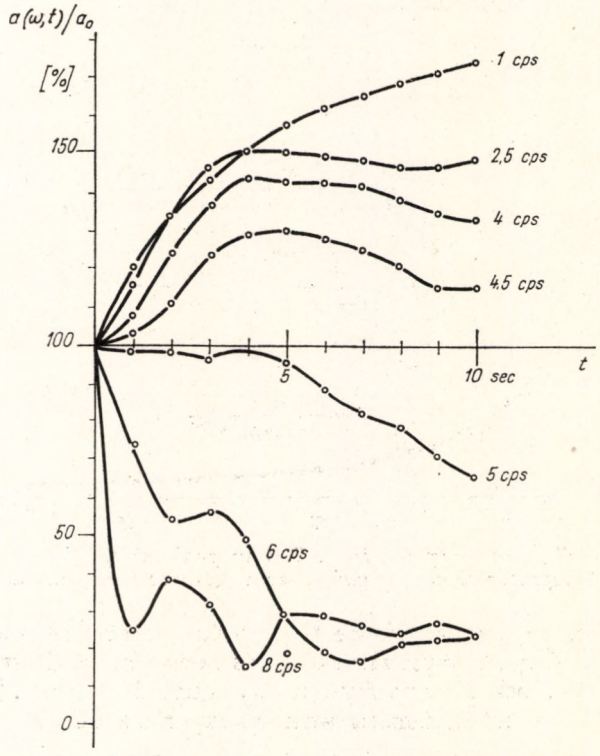

Fig. 3. The modulation-envelopes of the responses in Fig. 2 expressed in per cent of the 1st amplitude

It is observable, that at low frequencies (1—4,5 cps), amplitudes can be measured, which remarkably exceed (up to 120—174 per cent) the initial value within the set. However at high frequencies the amplitude of the 1st action potential is the maximal. An amplitude-oscillation within the sets can be noticed being particularly explicit at 6—8 cps. In certain cases a step-like decrease of amplitude is also detectable. Increasing the frequency a small decrease of the 1st amplitude in the evoked set of potential is noticeable as well. After a long-lasting stimulation a different situation arises.

2. *Variation of the amplitudes on nerves stimulated for a long time*

When the variations of amplitude following a train were examined on nerves stimulated for a long time, then the initial amplitude-increase even at 1 cps frequency, could only be rarely observed. On the contrary, a significant

decrease of amplitude has taken place within 5—12 sec, and later the amplitude of evoked potential remained at a relatively steady level. In *Fig. 4* the averages of data measured on 8 different nerves are demonstrated for stimulations of 0.5—20 cps. The other parameters of stimuli were 2,5—10 V, and 3 msec (roughly 80 per cent activation). The amplitudes were measured at their steady-values and presented in the percentage of the first one in the set. At 3 cps, the amplitude decreases to its half, and at 8-20 cps only 10 per cent of the initial amplitudes is recordable.

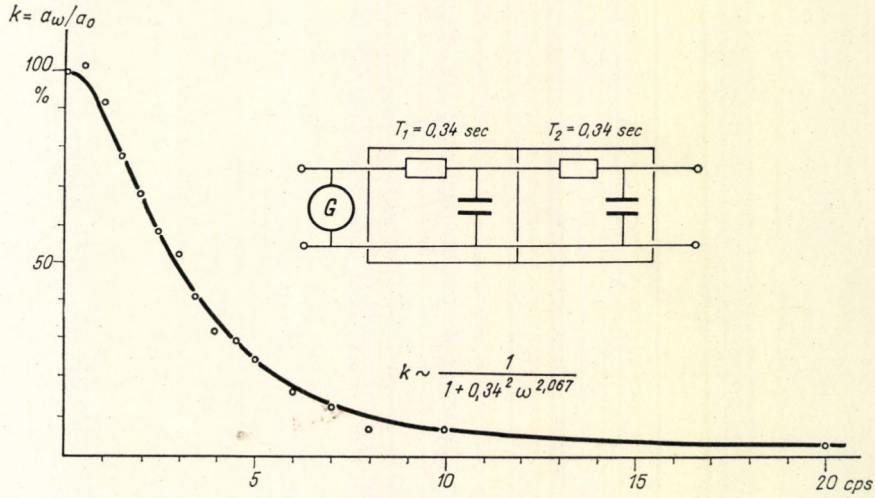

Fig. 4. Steady-state frequency-response. The approximation of the curve is a circuit of two integrator-blocks in series, with 0,34 sec time-constants

The relative values (k) of the steady-state response were approached by several ways. A good approximation has been obtained by a circuit consisting of two RC-block of capacity-output, coupled in series. The frequency response of such a circuit is identical with the experimental values:

$$k = a_\omega/a_0 = [1 + 0.34^2\,\omega^{2,067}]^{-1}$$

In some cases after continuous stimulation of some minutes a definite amplitude-oscillation was observable *(Fig. 10a)*. This oscillation is regular and continuous. Its extreme values may deviate from each other even by 30 per cent. Its frequency falls between 0,01 and 0,1 cps.

3. *Dependence of the train-response on pulse-duration and voltage*

When the frequency in the train is 3,75 cps and d=1 msec, the amplitude of the evoked potentials after 5 sec stimulation becomes steady at a level of 40—50 per cent as compared with the original. A stimulus strength activating up to about 2 mV amplitudes, i.e. near to 100 per cent has been chosen when the duration of the single pulses was 1 msec. To avoid effects which might originate from fatigue, both ascending and descending measurements have been carried out. In the 5th sec (t) of the responses evoked by trains, the amplitudes (a_ω) were measured and compared with a_0 (a_ω/a_0) The obtained ratios,

were plotted against the duration (*Fig. 5*). It was observable, that by increasing d, at the same frequency, the amplitudes of train-responses decrease. Approaching it by an exponential curve we obtained that:

$$\exp\ (a_\omega/a_0) \sim 1{,}512d^{-0{,}235}$$

In *Fig. 6*, applying 1—3 msec and 3,75 cps parameters, the ratios of the amplitude at the 5th sec and at the start of activation (a_ω/a_0) were plotted against the degree of activation. The different degrees of activation (a/a_{max}) were achieved exclusively by changing the voltage. In *Fig. 6* the 1st and the second curves were taken down on fresh and tired nerves at 1 msec, while the 3rd one was obtained on fresh preparation also at 3 msec. All the curves have an explicit maximum between activations of 25—60 per cent. But a potentiation of amplitude could be observed only in fresh nerve at 1 msec, while the tired nerve or by applying a longer pulse a real damping has taken place.

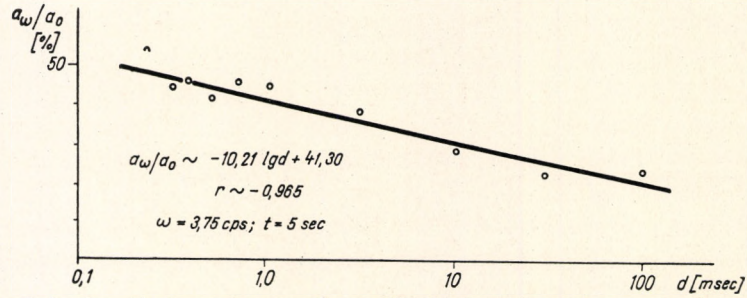

Fig. 5. Pulse length-dependence of amplitude-damping. Average of an ascending and a descending series. Frequency — 3,75 cps; strength — 5 V; duration — 5 sec.

4. *The response of CVc to suprathreshold stimulation of high frequency*

As at in situ experiments of 20—150 cps frequencies are oftenly applied, it seemed to be important to examine the effect of such trains.

It was found that the high-frequency stimulation does not evoke action potentials except at the make and sometimes at the break. The latter is usually lower. During the intermediate time, at most an asynchronous oscillation of the base-line is observable, proceeding often after having finished the train, but it lasts at most for some seconds. The on- and off- potentials are slow, lasting for 1—2 sec. These exceed the duration of potential evoked by single pulses.

5. *The effect of substhreshold, high-frequency stimulation*

By a subthreshold train of proper duration and frequency, a potential can be elicited. Trains of subthreshold pulses with respect to all fibres (1 V, 3 msec) and of 20—150 cps frequency, lasting for 1 sec, evoke on-response (A_{on}) increasing with the frequency. The function is nearly linear between 35—100 cps when it has been plotted against log ω (*Fig. 7*).

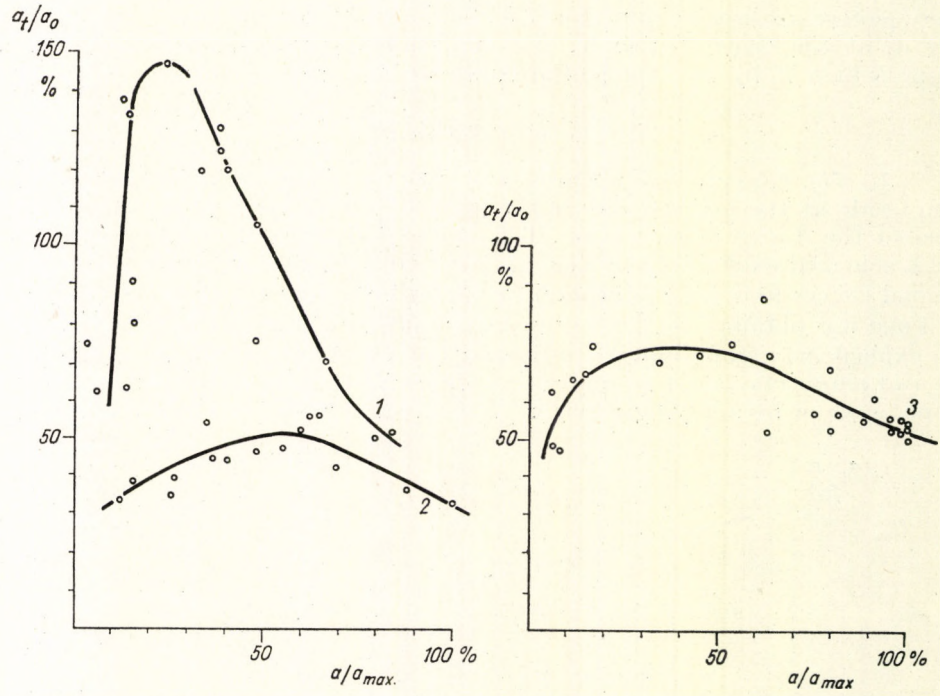

Fig. 6. Strength-dependence of amplitude-damping. Frequency — 3,75 cps; duration — 5 sec.
Abscisse: activation in per cent, *ordinate:* ratio of amplitudes in 5 and 0 sec.
1st and 2nd curves — 1 msec, fresh and tired nerve; 3rd curve — 3 msec

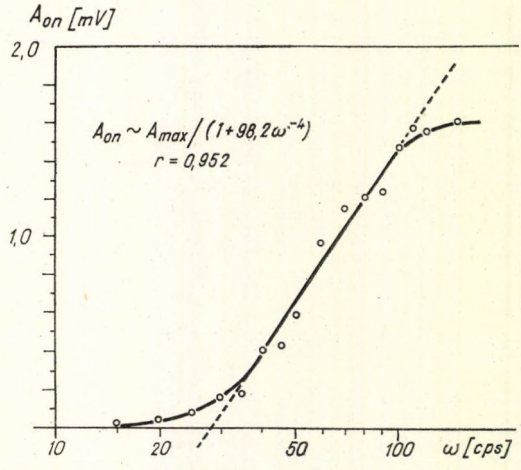

Fig. 7. Frequency-dependence of amplitude (A_{on}). Subthreshold stimuli (1 V, 3 msec) excite after summation

In the given case the amplitudes of action potential evoked by single suprathreshold stimuli are at maximum 2 mV. However, the maximal action potentials evoked by a subthreshold activation are always lower, namely of 1.6—1.7 mV. The dependence of the on-response-amplitude on the frequency can be fitted very well (coefficient of correlation $r \sim 0{,}9520$) by the following way:

$$A_{on}/A_{max} = [1 + 98{,}2\,\omega^{-3{,}983}]^{-1}$$

where the frequency for the half-maximal amplitude is 56,3 cps. Dimension of 98,2 must be a fourth power of frequency. For this reason: $A_{on}/A_{max} \sim [1 + 3{,}15^4\omega^{-4}]^{-1}$. Surprizingly the 3,15 cps value is very near to the half-damping frequency of the diagram in *Fig. 4.*

The number of pulses required to evoke the maximal on-response decreases with the increase of frequency *(Fig. 8)*. This value is about 4,6 or 10

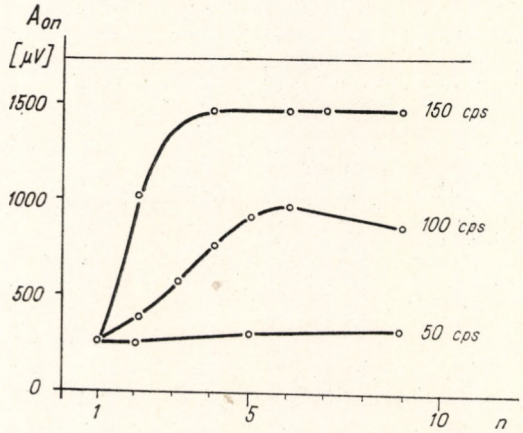

Fig. 8. Amplitude of on-excitation after summation depends on the number of subthreshold stimuli (1 V, 3 msec).

at frequencies of 150, 100, 50 respectively, when the parameters of subthreshold stimuli were chosen as 1 V and 3 msec.

At effective pulsing time of 50 per cent ($\omega = 500(d)$ cps; d in msec), the required voltage to evoke an action potential of a given value was examined, when the duration of train, that is the number of pulses was sufficient for the saturation. The strength-thresholds measured in such a way are generally lower by 1,5—10, than those of the single pulse with the same width.

6. *The behaviour of fatigued nerve*

A nerve has been stimulated for 2 hours with 3 V, 3 msec, 3.75 cps parameters replies by an irregularly oscillating amplitude *(Fig. 9)*. In the given case the amplitude of lastingly excited nerve is 170 ± 27 μV. The distribution is very near to the Gauss-type one, but at the same time its curving can be clearly recognized. It may be noticed that the amplitude of originally 1.5 mV has been decreased by the applied frequency down to its 10 per cent in 2 hours.

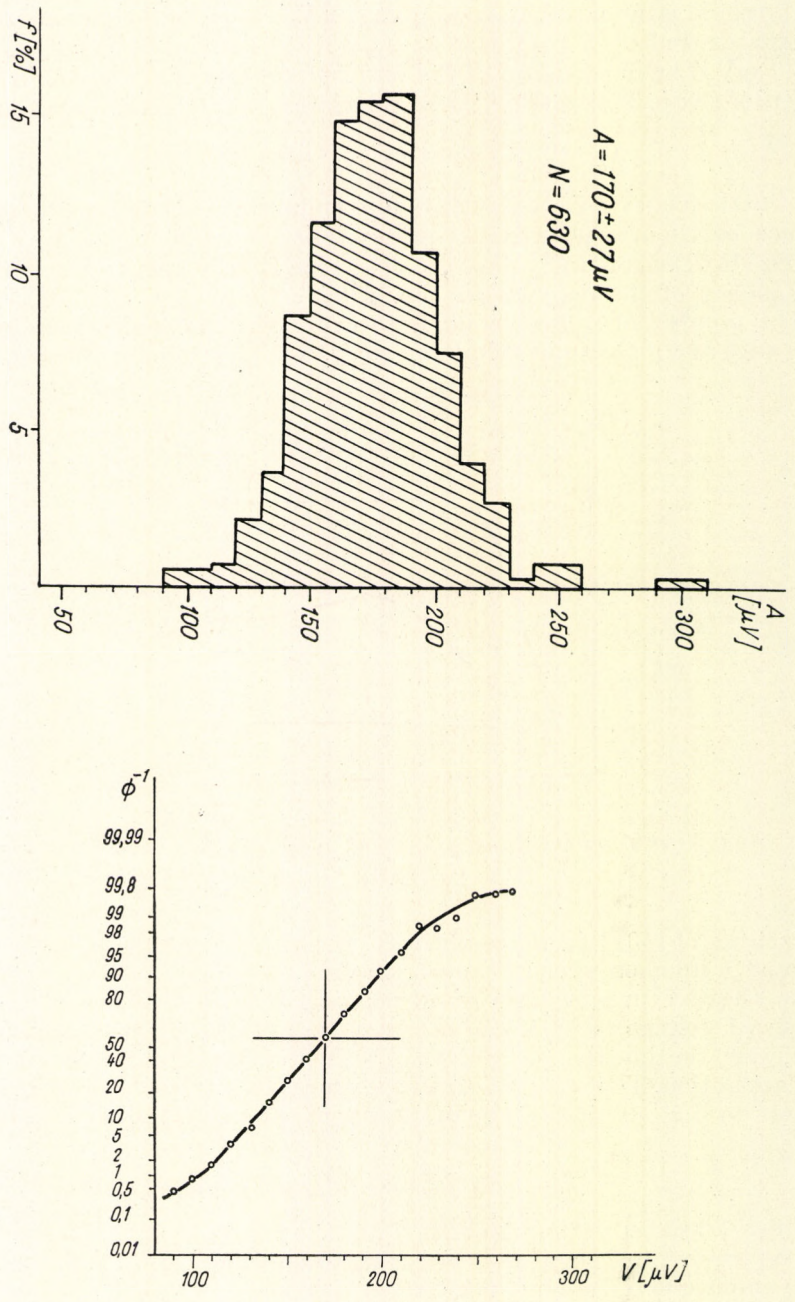

Fig. 9. Random distribution of amplitude.
Abscisse: amplitude in μV; *ordinates:* frequency and Φ^{-1} of Gauss function resp. Number of cases is 630. Average and standard deviation 170 $\pm$ $\pm$ 27 μV

After a period of random amplitude-distribution *(Fig. 10)*, following 2—3 hours stimulation of the nerve, a new phenomenon presents itself. Among the amplitudes regularly recurring extra-potentials appear rising by 10—100 per cent from the background. The phenomenon can be observed on CVc only between 1,3—3,3 cps. Among the extra-pulses, always a given number (N) of identical responses are intermediated. Going towards the extreme frequencies, the extra-amplitudes merge gradually with the background. N depends on the frequency of excitation *(Fig. 10 and 11)*. Distance between

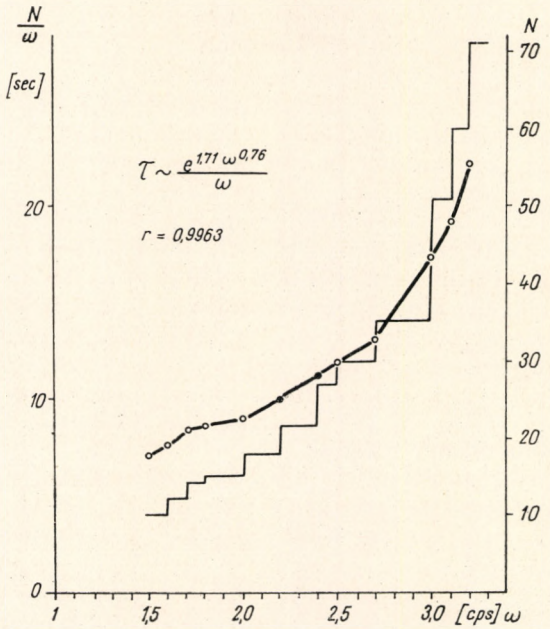

Fig. 10. Discrete modulation of amplitude.

Abscisse: frequency (cps); ordinate: time $\frac{N}{\omega}$ between two extra-amplitude or the number of intermediated normal responses (N)

the extra-potentials increases with the frequency. Dependence of the values of N or $\tau = N/\omega$ interval on frequency can be well fitted ($r \sim 0.99$) by the following empiric equations:

$$N \sim \exp\ (b\ \omega^a) \text{ or } \tau \sim \frac{1}{\omega} \exp\ (b\ \omega^a)$$

where $b \sim 1{,}71$ and $a \sim 0{,}76$ in the demonstrated case. Thus in *Fig. 11* N varies from 10 to 70 between 1,3 and 3,3 cps respectively. The interval changes between 7 and 23 sec.

A compensatory amplitude-oscillation was observed as a satellite-phenomenon of the extra-potentials *(Figs. 11d, f)*. This and the type of response itself is often bimodal *(Fig. 11e)*.

The phenomena are characteristic for the exhaustion, as coming only after a long-lasting stimulation. When the nerve has been driven by a higher

frequency between two measurements, N increases. The following sequence of values was observed:

$$25 \rightarrow 29 \rightarrow 32 \rightarrow 33 \rightarrow 34$$

A thread, examined under identical conditions in physiological solution does not show the phenomenon. The parameters of the stimulator are the same during the occurrence of the phenomenon. A fresh nerve excited by fresh or used electrodes does not answer in such a way.

Discussion

It has been observed earlier on isolated CVc (SALÁNKI et al. 1963) that the amplitude and the number of components of action potentials evoked by single stimulus increases with the intensity and length of the stimulus. This is in good agreement with data (PAVLOV, 1885; SALÁNKI and LÁBOS, 1963) showing that at in situ conditions the type of adductor-response depends on the stimulus-parameters, supporting the supposition according which the inhomogeneity of CVc-fibre-composition (LÁBAS et al. 1963) would be responsible for the phenomena and not the alterations in the condition of muscle as it has been suggested by ZHIRMUNSKAYA (1940) and ZHUKOV (1956).

Different degrees of activation in the nerve can represent states of activation in different groups of fibre. If presuming that the low activation evokes tonus and the increased one involves also the relaxant-fibres, we can easily explain the parameter-dependence.

This interpretation is not so far unequivocal when the muscle response and the action potential amplitudes of CVc are contrasted. Although, at high frequencies ($>$ 10 cps) the action potential disappears except the on- and off-responses, representing a low activation of the CVc, and at the same time the muscle response also becomes newly tonic, nevertheless the two processes do not coincide with each other. A decrease of the 1st component in the action potential presents itself above 2 cps, and its measure is already 90 per cent at 10 cps. But the 2nd component at 2—4 cps frequencies has been significantly damped already. On the other hand, in this domain of frequency the muscle relaxation is yet increasing and the response only above 20 cps begins to be tonic, when the pulse length is 4 msec. One of the causes responsible for the amplitude-decrease with an increase in the frequency is presumably that CVc receives the successive stimuli of frequency above 2—3 cps in the state of refractedress. Probably the more or less polarized state of nerve in the intermediate time also plays a role. Furthermore, a certain degree of fatigue must also be taken into account.

Explaining the mentioned phenomena connected with the muscle-response it may be presumed, that the relaxation centre in the visceral ganglion (SALÁNKI et al. 1963) requires to its triggering a certain degree of activation in one group of fibres and this is at the same time sufficient as well. A continuance of relaxation-control yet or already would not be possible if the fibres were activated by too high or too low frequencies. Although at high frequencies the CVc is not in rest, which can be compared to a direct current polarization, nevertheless, it does not excite the relaxation centre. Such a behaviour of the system can be compared to PAVLOV's (1885) and TAKAHASHI's results

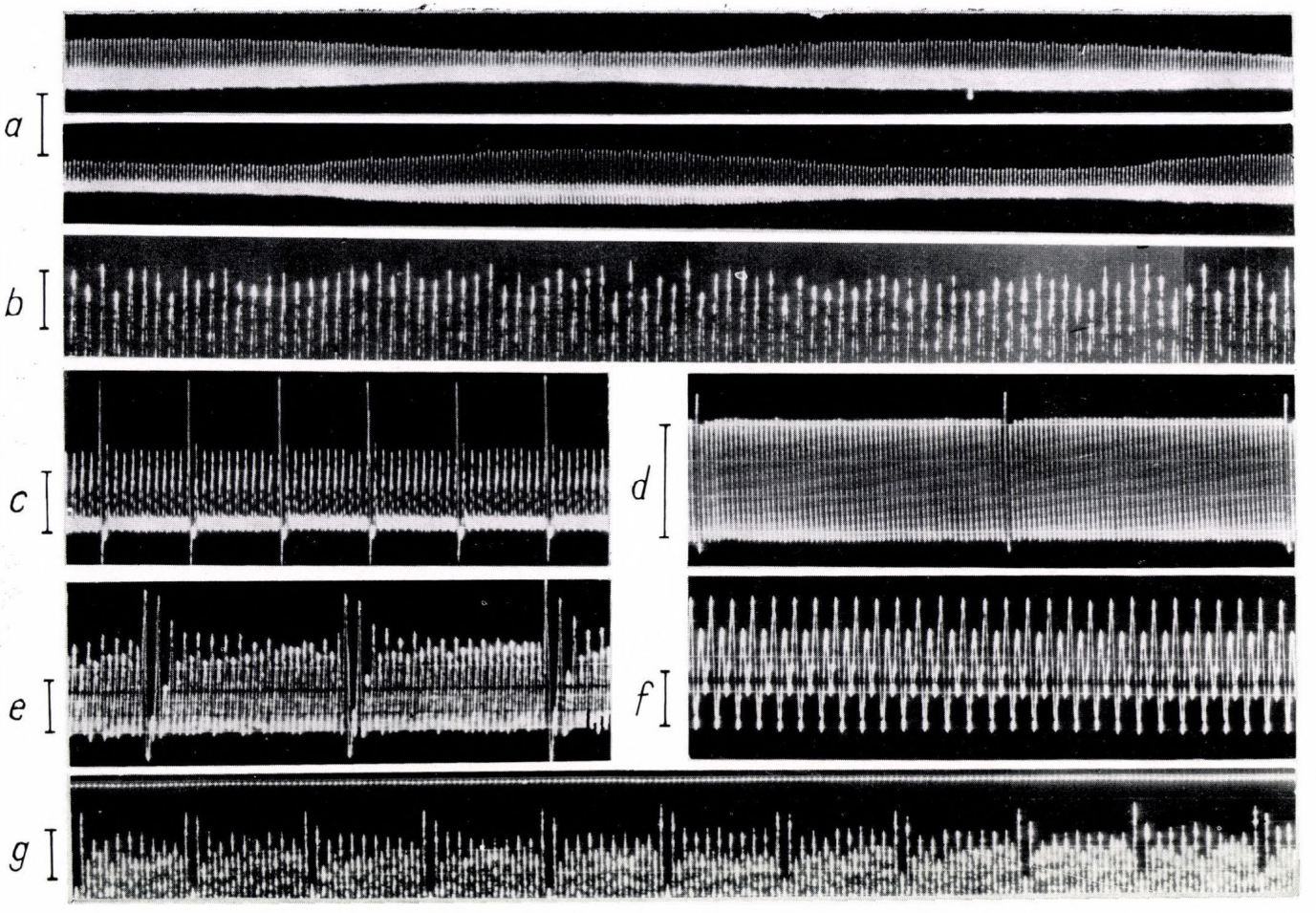

Fig. 11. Types of modulation : a — continuous amplitude-oscillation (ω = 3 cps)b — random oscillation of amplitude (ω = 3.75 cps) b — d — extra-amplitudes at two different frequencies (ω = 2,2 and 3,1 cps) e — compensatory-phenomenon after extra-potential (ω = 2,5 cps) f = bimodal response (ω = 1,5 cps) *g* = extra-amplitudes (upper line: constant stimulator-output) Calibrations: 500, 100, 100, 300, 100, 100 and 100 μV.

describing a tonus evoking effect of d.c. and relaxant influence of a.c. stimulation. A similar observation has been published by WINTON (1937) for direct stimulation of clam-muscle.

The frequency for optimal activation of CVc can be estimated by the following empiric approximation. Increasing the frequency more and more action potential but with a lesser of activating capacity degree arrives at the centre. The resultant of an increasing and a decreasing variable must lead to an optimum. Thus, if a (ω, t) is the frequency-time-fnuction of train-response, the following quantity has a frequency-optimum:

$$E = \sum_{1}^{\omega t} a(\omega,t)$$

This is really an approximation of the surface under the modulation envelope which increases owing to the frequency increase and decreases owing to the more and more frequent summation. The result of such a summation is demonstrated in *Fig. 12.* It was carried out up to 12 sec. It is noticeable, that the

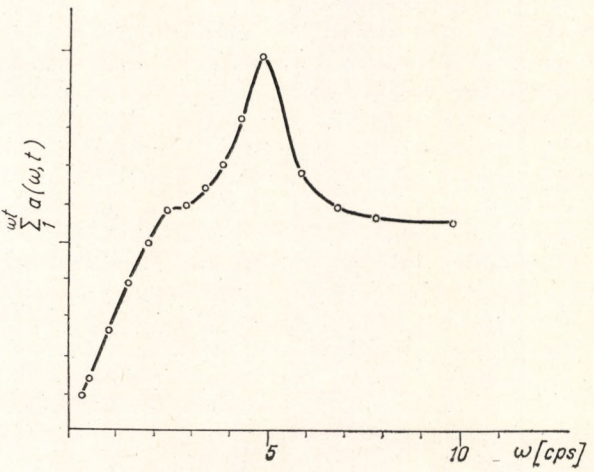

Fig. 12. Explanation see in the text

optimum is at 5 cps. The optimal frequency for the relaxation of adductor posterior, that is 8 cps (SALÁNKI and LÁBOS, 1963) is very near to this value. The muscle-relaxation is maximal when the propagated and accumulated excitation of CVc is maximal. We think that in situ primarily the central factors can explain the difference.

Our results direct attention also to some newer aspects of the cable-like properties of CVc (LÁBOS and VARANKA, 1966). The early increase of amplitude cannot be explained by an interference of the components, because it appears already at 1 cps, when the action potentials are yet separated. We think that it is a manifestation of real inductivity, similar to that demonstrated by COLE and BAKER (1941) on the giant axon of squid. COLE (1941) has mentioned many cases of inductivity not originating from a magnetic source. What we are talking about is simply a storage of kinetics energy. In our case, the maxi-

mum of the potentiated amplitudes is at low frequency (2—4 cps) postulating an improbably high inductance if the connection of the two phenomena were direct. COLE (1941) demonstrates also, that the axon-inductance decreases under the influence of direct current. This is in good agreement with the unstability of the transient amplitude-increase observed by us. This lability is well expressed by the activation-optimum of the amplitude-ratios becoming easily tired *(Fig. 6)*.

Such amplitude-increase was demonstrated by GASSER et al. (1938) on frog n. ischiadicus and it has been thought to be a manifestation of a supernormal phase. UHTOMSKY (1934) called the group of phenomenon appearing after train-stimulation as tetanic-ensemble. ZHUKOV (1946a) attributes a functional importance to this group of phenomena.

A further phenomenon is the uneveness of the transient characteristics probably originating from an interference of the components. Although, always the amplitude of the 1st component was measured, nevertheless, its composition may vary and a fibre-interaction may involve as well. The role of interference among the components can not be neglected either when interpreting the amplitude-modulation.

An early modulation of amplitude similar to that observed by us has been found on human n. ulnaris by UTTAL (1966) who thinks that feed-back loops are important in the realization of such oscillations. The early and also the later amplitude-oscillation of CVc seems to be similar to this. HODGKIN-HUXLEY's (1952) axon-model and its artificial versions (LEWIS, 1966; 1968; HILTZ, 1963) interpret and simulate the real forms of kinetic energy-storage by help of feed-back loops.

The steady-state frequency-response is apparently free of inductive properties. For this reason it may be approached by two RC-blocks of capacity output coupled in series. The empiric circuit-equation for such a block is $a_\omega/a_0 \sim (1 + T^2\,\omega^2)^{-1}$ where $T \sim 0{,}34$ sec. It must be emphasized that this model does not describe well the time-course of modulation-envelope. Therefore it represents only a damped limiting case of a system which is as yet initially inductive. We think the lability of continuous amplitude-modulation to be also a consequence of this high damping.

It may be interesting that the frequency-dependence of on-response elicited by summation has a form of $A/A_{max} = 1/(1 + \omega_0^4\,\omega^{-4})$. This makes likely that the high time-constant determining the suprathreshold frequency-response can play a role in the subthreshold processes as well. $T \sim 1/\omega_0 \sim 0{,}32$ sec is very near to the effective time-constant of the suprathreshold model.

An increase of frequency or pulse length act in the same way. But the effective time- constant is increased considerably slowly by d then by ω. For example, a two-fold increase of d leads to 15 per cent decrease not depending on frequency, while a two fold value of ω causes a damping of 10—75% depending on ω.

The late phenomena point to a fluctuation of the membrane potential and/or the threshold (VERWEEN and DECKSEN, 1965; ten HOOPEN et al. 1963). An amplitude noise of GAUSS-type has been described by VERWEEN and DECKSEN (1965, 1968). The noise is higher than that expected from a thermic source and they interpret it as $K^+ - Na^+$ flux-noise. Our amplitude-dispersion of 15 per cent is higher than the membrane-potential noise of 2 per cent observed by VERWEEN and DECKSEN (1968) and it is difficult to decide for

thermal-, ion- or fibre-origins as the noise is of 10 μV order and the resistance of the nerve is about 100 kΩ/mm. The small curving of the distribution on GAUSS-paper (*Fig. 9b)* is similar to that of the slightly depolarized Ranvier-nodus (VERWEEN and DECKSEN, 1968).

The late extra-amplitudes and the appearance of the bimodal rhythm may be compared to the regular extrasystolia of the heart. MASHIMA and WASHIO (1968) have found that the membrane-potential of frog-sartorius oscillates bimodally when the train of square pulses lasts for a sufficiently long time or has a sufficiently high frequency (150 cps). Such explicitly nonlinear modulations may be due to a discrete variation of threshold or membrane potential. Our control experiments decrease the possible influence of artefacts. It is interesting also, that some weak traces of quantized changes can be detected even in the early phase of the stimulation.

All the phenomena presented here point unequivocally to the fact that the nerve cannot be described by a single linear model of first or second order, but the nerve represents an inductive or active nonlinear cable, the parameters of which can be frequency- and time-dependent.

Summary

Isolated cerebro-visceral connective of *Anodonta cygnea* L. was examined. Its response to a square-pulse train showed the following features

1. At low frequencies ($\omega = 1-3$ cps) it shows transient amplitude-increase, while in the higher range the amplitudes of consecutive potentials decrease. Very often a stable oscillation of amplitude has been observed.
2. Let the nerve be stimulated several times, the amplitude decreases to a relatively steady value without any initial increase. The steady-state value is about 50 per cent of the original when the frequency is 3 cps; at 8—10 cps its value is less than 10 per cent. The frequency response follows the formula of $a_\omega \sim a_0/(1 + T_0^2 \omega^2)$. It is furthermore pulse-duration and strength dependent.
3. The suprathreshold high-frequency stimulation (20—150 cps) elicits only on- and off- responses. On-response is evoked also by subthreshold high-frequency stimulation. Its frequency-dependence is: $A_{on} \sim A_{max}/(1 + \omega_0^4 \omega^{-4})$. The number of pulses required to induce a maximal response decreases with an increase of frequency.
4. A fatigued nerve (after about 2 hours of continous stimulaton responds firstly by a random distribution of amplitudes later extra-responses appear recurring very precisely. The time lapse between two extra-responses is $\tau \sim \omega^{-1} \exp(1{,}71\ \omega^{0{,}76})$; ω in cps.

Because of the above enumarated properties of CVc, the frequency-dependence of the effector (adductor)-tonus can be interpreted only by taking into account the role of the intermediated ganglia. At high frequency stimulation only the on-response represents an excitation. CVc is equivalent with a low frequency, nonlinear cable having active and inductive elements, and being highly damped.

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AKCIÓS POTENCIÁL FREKVENCIA-FÜGGÉSE ANODONTA CYGNEA L. (PELECYPODA) NEM MYELINIZÁLT IDEGÉN

Lábos Elemér, Salánki János és *Varanka István*

Összefoglalás

Anodonta cygnea izolált cerebroviscerális konnektívumának négyszögimpulzus sorozatra adott válaszát vizsgálva azt találtuk, hogy

1. Friss idegen alacsony frekvenciánál ($\omega = 1-3$ cps) kezdeti amplitúdónövekedés van, magasabb frekvenciánál az egymást követő potenciálok amplitúdója csökken. Igen gyakran tartós amplitúdóoszcilláció észlelhető.

2. Többször ingerelt idegen átmeneti növekedés nélkül az amplitúdó csökkent, viszonylag állandó szintre áll be. Az állandósult amplitúdóérték 3 cps esetén az első potenciálnak kb. 50%-a, 8—10 cps esetén kevesebb mint 10%-a. Az amplitúdó/frekvencia karakterisztika $a_\omega \sim a_0/(1 + T_0^2\,\omega^2)$ alakú. A frekvenciaválasz impulzusszélesség és feszültségfüggő.

3. Nagy frekvenciával (20—150 cps) történt ingerlés csak be- és kikapcsolódási effektust vált ki. Küszöbalatti nagyfrekvenciás ingerlés ugyancsak kivált bekapcsolási effektust. Az amplitúdó frekvenciafüggése: $A_{on} = A_{max}/(1 + \omega_0^4\,\omega^{-4})$ alakú. A maximális amplitúdó kiváltásához szükséges impulzusok száma a frekvencia növelésével csökken.

4. Fáradt ideg (közel 2 órás folyamatos ingerlés) válaszát kezdetben véletlenszerű amplitúdóeloszlás, később igen pontosan jelentkező extra válaszok jellemzik. Az extraválaszok közötti idő $T \sim \exp(1{,}71\omega^{0{,}76})/\omega$.

A CVc fenti sajátosságai miatt az effektor (záróizom) tónusának frekvenciafüggése csak a közbeiktatott ganglionok szerepének figyelembevételével értelmezhető. Nagyfrekvenciás ingerlésnél feltehetően csak a bekapcsolási effektus jelent ingert. A CVc alacsony saját-frekvenciájú, erősen csillapított nem lineáris, induktív kábellel ekvivalens.

ЧАСТОТНАЯ ЗАВИСИМОСТЬ ПОТЕНЦИАЛА ДЕЙСТВИЯ В БЕЗМЯКОТНОМ НЕРВЕ БЕЗЗУБКИ

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Изучая реакцию изолированного церебровисцерального коннектива беззубки в ответ на серийное раздражение прямоугольными импульсами было установлено, что:

1. На свежем препарате при применении низких частот ($\omega = 1-3$ цикла/сек) наблюдается начальное увеличение амплитуды, а при более высокой частоте амплитуда потенциалов снижается. Часто наблюдается продолжительное колебание амплитуды.

2. При повторном раздражении нерва амплитуда сохраняется на постоянном, но сниженном уровне. Эта постоянная величина амплитуды в случае 3 цикла/сек соответствует 50% первого потенциала, а при применении 8—10 циклов/сек это значение снижается ниже 10%. Характеристика амплитуды (частоты имеет вид $\omega \sim a_0/(1 + T_0^2\omega^2)$, частота зависит от продолжительности и напряжения импульса.

3. Раздражения с помощью высоких частот (20—150 циклов/сек) вызывают только эффекты замыкания и размыкания. Раздражение подпороговыми частотами вызывает эффект замыкания. Частотная зависимость амплитуды имеет вид: $A = A_{макс}(1 + \omega^4 4\omega^{-4})$. Число импульсов, необходимых для появления максимальной амплитуды, снижается при повышении частоты.

4. Ответ утомленного нерва (после постоянного раздражения в течении 2 часов) вначале характеризуется случайным распределением амплитуд, но позже появляются ответы закономерно. Время между этими ответами $T \sim \exp(1{,}71\omega^{0{,}76}/\omega)$.

Из-за высшеизложенных свойств ЦВК, частотная зависимость эффекторного органа (запирательной мышцы) объяснима только участием промежуточных ганглиев. При раздражении высокой частотой по всей вероятности только эффект замыкания служит раздражителем. ЦВК соответствует сильно утихающемуся, не линейному, индуктивному проводу, обладающему низкой собственной частотой.

ANNAL. BIOL. TIHANY	36	57—62	HUNGARIA 1969

PHARMACOLOGICAL INVESTIGATIONS ON THE 5-HYDROXYTRYPTAMINE AND NORADRENALINE RECEPTORS OF GASTROPODA (*HELIX POMATIA* L.) HEART

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Received: 21st February, 1969

According to previous data both indoalkylamines and catecholamines proved to be stimulatory factors on molluscan hearts (S.-RÓZSA and PÉCSI, 1967). It is the differentiation of the sites of action that could give a basis for the more exact analysis of the effect of various stimulatory factors. As the pharmacology of molluscan hearts has not yet been studied in respect of the sites of action, it is uncertain whether like vertebrate hearts α and β receptors take part in the effect of catecholamines or not. There are, however, some data, referring to the fact that elimination of the inhibitory effect of acetylcholine may be different in vertebrates and molluscan species (SAKHAROV and NISTRATOVA, 1963; S.-RÓZSA, 1966).

In the present paper analyses of the sites of action of 5-hydroxytryptamine (5HT) and noradrenaline (NA) are described on the effect of different pharmacons. The aim of our investigations was also to clear up the differences and similarities in the effects of these agents.

Material and method

Experiments were carried out on isolated hearts of *Helix pomatia* L. Isolation was made as described earlier (S.-RÓZSA and PÉCSI, 1967). MENG's solution (MENG, 1958) was used as a physiological one as well for dissolving the substances.

Experiments were performed between May and October at room temperature (20—24°C) on active snails.

The following agents were used: 5-hydroxytryptamine creatinine sulphate (5HT), Fluka; noradrenaline (NA), Calbiochem; nicotine chloride, Fluka; dichloro-isoproterenole (DCI), Sandoz; iproniazide, Schuchart; imidazole, Calbiochem; α-methyl DOPA, Sandoz; reserpine phosphate, Ciba; azide (NaN_3), BDH.

Results

1. *Effect of different pharmacological agents on the Helix heart*

The effects of nicotine, DCI, iproniazide, imidazole, α-methyl DOPA, reserpine and azide were investigated from the threshold to 10^{-3} M concentrations. Each concentration was tested at least on four hearts. *Table I* summarizes the results obtained in these experiments.

All the applied drugs evoked stimulatory effect. The weakest stimulation was produced by nicotine and DCI, in these cases the increase of the amplitude was long lasting, but never exceeded 20 per cent of the original activity. Inhibition was not observed even 20—30 minutes after the treatment and in high concentrations.

Iproniazide and imidazole proved to be also weak stimulants. They did not produce more than 30 per cent increase in the amplitude even after long lasting treatment, and also failed to produce inhibition.

Table I

Drug	Threshold concentration in M	Type of effect	Maximal increase of the amplitude in per cent
Nicotine	10^{-9}	Stimulation	10—20
DCI	10^{-9}	Stimulation	10—20
Iproniazide	10^{-9}	Stimulation	10—30
Imidazole	10^{-8}	Stimulation	10—30
α-methyl DOPA	10^{-8}	Stimulation	20—40
	10^{-5}	Inhibition	
Reserpine	10^{-9}	Stimulation	30—60
Azide	10^{-9}	Stimulation	100—120

The effects of α-methyl DOPA and reserpine are similar, however the threshold stimulatory concentration of the reserpine is lower (10^{-8} M) than that of the α-methyl DOPA (10^{-7} M). Further difference in the effect of these two agents is that reserpine did not produce inhibition either in high concentrations or after long lasting application, while α-methyl DOPA arrested the heart if 10^{-5} M or higher concentrations were used for longer than 10 minutes. The above effects could be eliminated by repeated washing.

Among the investigated drugs the azide proved to be the most effective stimulating agent, producing a fast increase of the amplitude at 10^{-9} M or in higher concentrations. Using 10^{-5} — 10^{-4} M concentrations the increase of the amplitude reached 100—120 per cent. The effect of the azide could be mitigated by a simple wash out, especially when it was in low concentrations. In no case was any inhibitory effect observed after azide treatment.

It should be noted that all the above mentioned drugs caused only positive inotropic effect and no positive chronotropic effect ever occurred.

2. *Modification of the effects of 5HT and NA by drugs*

After pretreatment of the heart with the drugs mentioned above the effects of 5HT and NA were tested in concentrations by one order higher then their threshold, having been determined earlier (S.-Rózsa and Pécsi, 1967). The effects of 5HT and NA on untreated hearts are demonstrated in *Fig. 1.*

The pretreatment of the heart with drugs lasted for 10—15 minutes. Similar effects occurred also after long-term (30—40 min) pre-incubation.

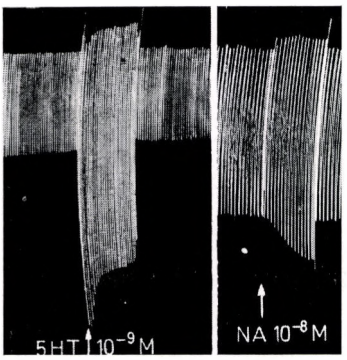

Fig. 1. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noradrenaline on *Helix* heart

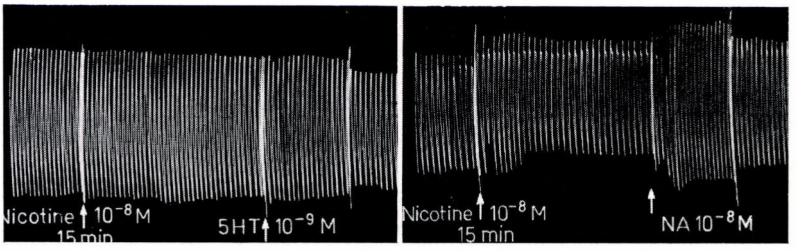

Fig. 2. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noraderenaline after pretreatment the heart with 10^{-8} M nicotine for 15 minutes.

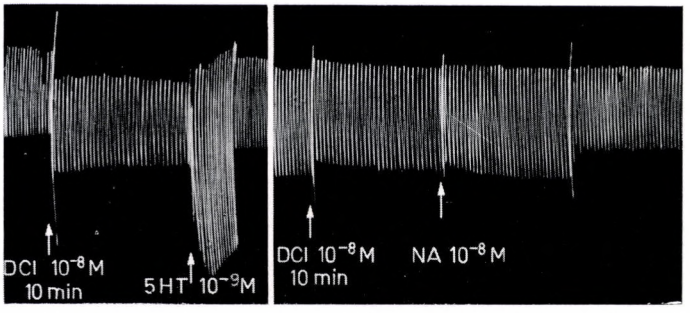

Fig. 3. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noradrenaline after pretreatment with 10^{-8} M DCI lasting for 10 minutes.

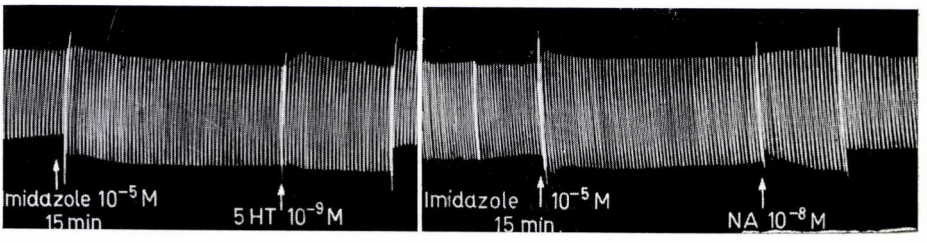

Fig. 4. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noradernaline after pretreatment with 10^{-5} M imidazole.

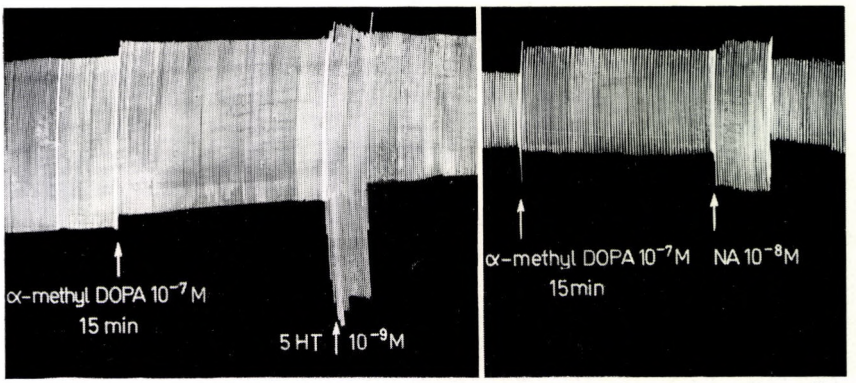

Fig. 5. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noradrenaline after pretreatment with 10^{-7} M α-methyl DOPA

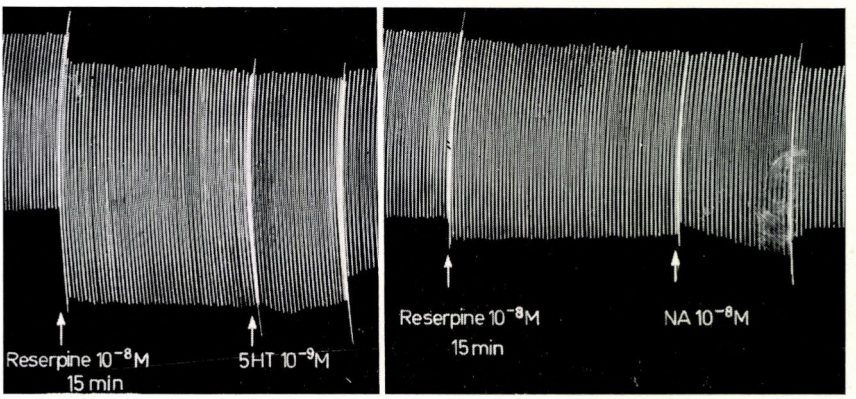

Fig. 6. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noradrenaline after pretreatment with 10^{-8} M reserpine.

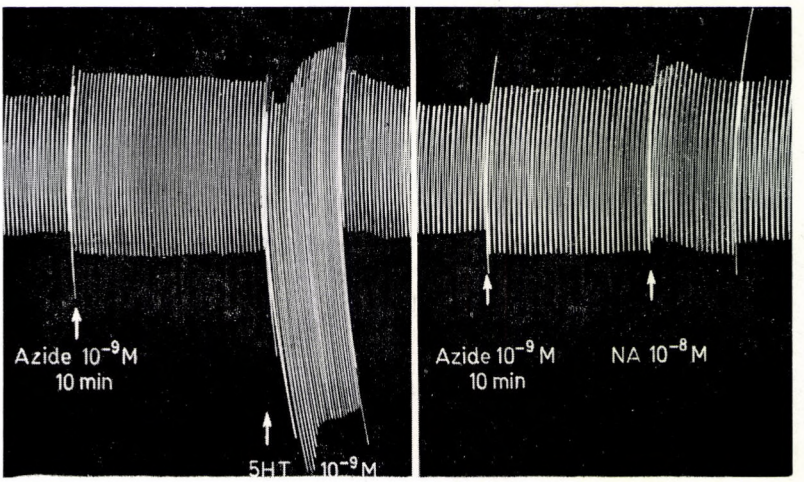

Fig. 7. Effect of 10^{-9} M 5-hydroxytryptamine and 10^{-8} M noradrenaline after pretreatment with 10^{-9} M azide lasting for 10 minutes.

After pretreatment with 10^{-8} M nicotine the heart became insensitive to 10^{-9} M 5HT *(Fig. 2)*. The same pretreatment did not prevent the heart from the stimulatory effect of 10^{-8} M NA only decreased the rate of the amplitude-increase. The stimulatory effect of NA could not be prevented even in higher nicotine concentrations or with longer preincubation.

In contrary to nicotine, DCI had no influence on the stimulatory effect of 5HT but protected the heart from the amplitude-increase caused by NA *(Fig. 3)*. Higher than 10^{-8} M concentrations of DCI influenced the effect of NA in the same way.

The imidazole at 10^{-5} M concentration decreased but did not eliminate completely the stimulatory effects of 5HT and NA. Complete block of the 5HT and NA effects could not be achieved even in very high (10^{-4} — 10^{-3} M) concentrations, but lower concentrations were less effective *(Fig. 4)*.

No changes were observable in the effects of 5HT and NA after pretreatment of the heart with iproniazide in concentrations of 10^{-9} — 10^{-3} M. Partly similar results were obtained using α-methyl DOPA in 10^{-7} — 10^{-6} M concentrations, being ineffective or causing a slight potentiation of the effect of 5HT and NA *(Fig. 5)*. Higher concentrations of α-methyl DOPA were not used, as they arrest the heart after 10—15 minutes treatment.

Reserpine producing alone a remarkable increase of the amplitude in 10^{-8} M concentration eliminated nearly completely the stimulatory effect of 5HT and NA *(Fig. 6)*. However some increase in the amplitude to 5HT or NA could be observed even if 10^{-4} M reserpine was used for pretreatment.

Azide itself proved to be a strong stimulatory agent in 10^{-9} M and higher concentrations, but besides this, it potentiated also the stimulatory effects of 5HT in a considerable degree, while nearly completely eliminated the effect of the NA *(Fig. 7)*. The effect of 5HT was potentiated by every concentrations of the azide, and the potentiation increased with increasing 5HT concentrations. At the same time with increasing from 10^{-8} M NA concentrations the inhibitory effect of azide was less and less.

Discussion

All the investigated amines proved to be stimulatory on the *Helix* heart. On the basis of the maximum effect the order of their effectiveness was: nicotine, DCI < iproniazide, imidazole < α-methyl DOPA < reserpine < < azide.

This order does not indicate the participation of similar underlying mechanisms in the effect of different substances, as within the same group there are substances with different mechanisms of action. E. g. nicotine is a cholinergic antagonist, DCI is inhibitor of α-adrenergic receptors, while iproniazide is known as a monoamine oxidase inhibitor and imidazole as inhibitor of β-adrenergic receptors. The effects of the investigated drugs are characterized by the same features as it was described earlier for the effects of amines (S.-Rózsa and Pécsi, 1967): differences were found primarily not in the effective threshold concentrations (10^{-9} — 10^{-8} M) but in the maximal effects they were able to produce *(Table 1.)*.

Up to now except nicotine which produced inhibition on the heart of *Strophocheilos* (Jaeger, 1961) there are no data available about the direct

effects of the above drugs or about their interaction with monoamines on molluscan hearts. The effect of nicotine as we found, also differs from that described by JAEGER (1961).

The effects of all the investigated drugs but imidazole might be explained according to pharmacological mechanisms obtained in vertebrates. The iproniazide could produce the stimulation as an inhibitor of monoamine oxidase (ZELLER and SARKAN, 1962). the reserpine and α-methyl DOPA might evoke stimulation by liberating monoamines or inhibiting of enzymes playing role in the metabolism of monoamines (SHORE, 1962; BRODIE et al. 1967). The stimulatory effect of the azide might occur as a result of inhibition of oxidases (HEWITT and NICHOLAS, 1963) but its influence on ions must also be taken into account. The effect of imidazole can be connected with its α-adrenergic blocking qualities and with its effect on the phosphodiesterase, described on vertebrate hearts (YUNG et al. 1966, BUEDING et al. 1967).

According to our results direct receptor-drug interaction might be taken into account only in the effects of nicotine, and DCI, while in the case of other drugs indirect or mixed effects may be supposed (TRENDELENBURG, 1965). In the former case the similarity between the structure of DCI and noradrenaline fulfils the requirements for supposing the same site of action (ARIENS, 1967).

Among the investigated drugs nicotine, DCI and azide reversed the effects of 5HT and noradrenaline. Nicotine completely eliminated the effect of 5HT but did not alter considerably the effect of the noradrenaline. Responses of opposite sign were obtained by DCI eliminating the stimulatory effect of the noradrenaline but causing no changes in the effect of the 5HT. Data obtained with DCI and nicotine verified that the effect of 5HT has no connection with α-adrenergic reseptors but according to earlier data (JAEGER, 1961; PHILLIS, 1966; S.-RÓZSA and PÉCSI, 1967) it may act on receptor structures which can be blocked with cholinergic inhibitors.

Our results conclude that competitive antagonism may exist between DCI and noradrenaline on *Helix* heart. The elimination of the stimulatory effect of the noradrenaline by DCI showed that the effect of catecholamines on molluscan hearts takes place on the same receptors as on Vertebrates (BLOOM and GOLDMAN, 1966). However, in contrary to the demonstrability of the presence of adrenergic receptors, catecholamines do not play any important part in the stimulatory regulation of molluscan hearts. This is further supported by our earlier data (S. -RÓZSA and PÉCSI, 1967; S.-RÓZSA, 1967). The elimination of the stimulatory effect of 5HT by nicotine can be explained with the ezyme-inhibitory properties of the nicotine but besides this it can play a role as a receptor antagonist too.

Azide (NaN_3) potentiated specifically the stimulatory effect of 5HT and simultaneously decreased the effect of the noradrenaline. At the present we can not interpret the exact mechanisms of this potentiation.

Both reserpine and α-methyl DOPA altered the effects of 5HT and noradrenaline in similar directions: α-methyl DOPA slightly potentiated, while reserpine almost completely eliminated the stimulatory effect of these amines. It is uncertain, whether this phenomenon may be explained with the depletion of monoamines (PLETSCHER, 1968) or not, for the mechanism of the short-term depletion is not yet clear. It is more probable that α-methyl DOPA acts as an inhibitor of different enzymes (PLETSCHER and GEY, 1963).

In spite of the fact that iproniazide is a strong inhibitor of monoamine oxidase this drug did not alter the effect of 5HT and noradrenaline. Presumably iproniazide does not change either the configuration of the receptor or the level of endogenous 5HT and noradrenaline.

The imidazole being an inhibitor of the α-adrenergic receptors and activator of phosphodiesterase decreased the effect of both 5HT and noradrenaline. These effects may be related to the changes of the level of the cyclic 3',5'-AMP taking an important part in the realization of the excitatory effect of 5HT and noradrenaline on the *Helix* heart, as it was shown earlier (S. -Rózsa, 1969).

Summary

The direct effect of nicotine, DCI, iproniazide, imidazole, α-methyl DOPA, reserpine and azide as well as their interaction with 5-hydroxytryptamine and noradrenaline were investigated on the heart of *Helix pomatia* L. It was found that all the drugs used increase heart activity and practically no differences were found in the threshold concentrations but their effect differ in the maximal degree of stimulation.

The nicotine prevented the heart from the stimulatory effect of 5HT, while the effect of the noradrenaline decreased only in a small degree after nicotine pretreatment. On the other hand DCI eliminated the effect of the noradrenaline without changing the effect of 5HT. The effect of 5HT was potentiated by azide but the effect of the noradrenaline was decreased by this agent. Other drugs used altered the effect of 5HT and noradrenaline in the same manner. It seems justified, that in the *Helix* heart the receptors of 5HT and noradrenaline are isolated from each other and the catecholamine receptors do not play important roles in the realization of stimulatory effects. Reserpine was more effective than α-methyl DOPA. The inhibition of monoamine oxidase did not influence the effects caused by 5HT and noradrenaline.

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5-HYDROXYTRYPTAMIN ÉS NORADRENALIN HATÁSÁNAK FARMAKOLÓGIAI ELEMZÉSE *HELIX* SZÍVEN

S.-Rózsa Katalin

Összefoglalás

Vizsgálták nikotin, DCI, iproniazid, imidazol, α-methyl DOPA, reserpin és azid direkt hatását, valamint 5HT és NA effektust befolyásoló tulajdonságát *Helix* szíven. Megállapították, hogy valamennyi vizsgált farmakon serkentő hatású, s lényegesen küszöbkoncentráció tekintetében nem, csak a maximálisan kiváltható serkentés tekintetében különböznek egymástól. A nikotin kivédi az 5HT serkentő hatását, míg a NA hatást jelentéktelen mértékben csökkenti. A DCI viszont az NA effektust szünteti meg az 5HT hatás befolyásolása nélkül. Azid az 5HT effektust potencírozza az NA hatást pedig csökkenti. A többi vizsgált farmakon mindkét amin hatását azonos irányban befolyásolja, Helix szíven az 5HT és NA receptorok izoláltak s az utóbbiak nem jutnak lényeges szerephez a serkentő hatások biztosításában. Monoamin depletálók közül a reserpin hatásosabbnak bizonyult mint az α-methyl DOPA. A MAO gátlásnak nincs lényeges szerepe az aminhatások lefutásában.

ФАРМАКОЛОГИЧЕСКИЙ АНАЛИЗ ДЕЙСТВИЯ СЕРОТОНИНА И НОРАДРЕНАЛИНА НА СЕРДЦЕ ВИНОГРАДНОЙ УЛИТКИ

Каталин Ш.-Рожа

Прямое действие никотина, ДЦИ, ипрониазида, имидазола, α-метил-ДОФА, резерпина и азида, а также их взаимодействие с серотонином и норадреналином были изучены на сердце виноградной улитки. Было установлено, что все изученные фармакологические вещества вызывают стимуляцию и они отличаются друг от друга в отношении максимального эффекта которого они вызывают а не в отношении пороговой концентрации. Никотин снимает стимуляторный эффект серотонина, но действие норадреналина снижает незначительно. Напротив этому, ДЦИ снимает эффект норадреналина без изменения эффекта серотонина. Азид увеличивает эффект серотонина, а эффект норадреналина под его действием снижается. Все остальные фармакологические вещества видоизменяют эффект аминов в одинаковом направлении. Фармакологические рецепторы серотонина и норадреналина разделены в сердце виноградной улитки, и последные не играют существенной роли в реализации стимуляторных воздействий. Резерпин оказался более эффективным, чем α-метил-ДОФА. Торможение моноаминооксидазы не имеет существенного значения в осуществлении эффекта различных моноаминов.

ANNAL. BIOL. TIHANY | 36 | 63—75 | HUNGARIA 1969

IDENTIFIED CELLS IN THE CENTRAL NERVOUS SYSTEM OF *LYMNAEA STAGNALIS* L. (GASTROPODA)

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Received: January 28, 1969

Recent investigations on the giant neurons of Gastropods employing the method of intracellular recording have clarified numerous questions concerning the generation of activity, transmission of impulses, as well as the effect of chemical agents (cit. in: ARVANITAKI and CHALAZONITIS, 1968; CHALAZONITIS, 1968; TAUC, 1967). In addition to descriptions of non-identified cells, numerous authors have described the physiological parameters of some well-defined cells (ARVANITAKI and CHALAZONITIS, 1961; STRUMWASSER, 1965; HUGHES, 1968; KERKUT and MEECH, 1967).

For the better understanding of the intercellular connections and organization of the ganglia, identification of a great number of cells is required. Many attempts were made to achieve the anatomical and functional identification of the most accessible cells in the ganglia of various species of Gastropoda. Cell maps were constructed on *Aplysia* (FRAZIER et al., 1967), *Helix aspersa* (KERKUT and WALKER, 1962; GLAIZNER, 1968) and on *Helix pomatia* (SAKHAROV and SALÁNKI, 1969). As far as we know, no investigations of this kind have been performed on *Lymnaea stagnalis*, though numerous researches have been reported on the nervous system of this snail (JOOSEE, 1964; SOKOLOV et al. 1967; NOLTE, 1968; SALÁNKI, 1968; VEPRINTSEV, 1968; ZEIMAL and VULFIUS, 1968, SAKHAROV and ZS.- NAGY, 1968). Localization on a morphological basis has been attempted in some cells (YARMIZINA et al., 1968).

The purpose of the present experiments was to achieve a visual and functional identification of some cells of the abdominal and right parietal ganglion which are readily accessible and contain the highest number of giant cells in *Lymnaea stagnalis*. We wished to elucidate whether or not the cells having identic localization possess the same type of activity and if they do, to construct a cell map furnishing sufficient data for the precise recognition of certain cells.

Material and Method

The specimens of *Lymnaea stagnalis* L. used for the experiments were collected from lake Balaton or from other lakes near-by. Until use, the animals were kept in streaming Balaton water.

The preparations were made as follows: the entire ganglion ring was

lifted out and fixed in appropriate orientation by means of the surrounding connective tissue and nerves. Care was taken to ensure identic conditions for the visual control of the cells. All the connective tissue was removed from the dorsal surface of the ganglion by which the cells became accessible and fairly visible. The preparations were then placed in a chamber containing saline described by JULLIEN and RIPPLINGER (1948). Membrane and action potentials were recorded by means of glass microelectrodes filled with 2,5 M KCl or NaCl. Resistance of the electrodes ranged between 8 and 25 MΩ. Connection to the amplifier of the electrometer of negative capacity (DISA Type 140 30/3) was made by inserting a bridge circuit (KOKETSU and NISHI, 1957). By means of the bridge the resistance of the microelectrodes could be controlled and it was possible to transmit through the recording electrode a d.c. polarizing current to the preparation under examination. The polarizing current usually caused a shift of 10 mV in the membrane potential. During the experiment the signals were recorded on a magnetic tape and the desired portions were photographed by means of a DISA Universal indicator and photorecorder.

In the course of the experiments we have recorded the activity of a great number of cells and made attempts to identify them in the various preparations. In this study, however, only 18 of them will be described, namely those exhibiting only slight variations in localization and being identifiable with certainty on the basis of electrophysiological indicators *(Fig. 1)*. Identification of each neurons described here was made in more than five preparations.

Experimental results

Identification of the neurons was made on the basis of:

a) visual control,

b) electrophysiological parameters.

Giant cells which could be identified with certainty on the basis of their localization cannot be found in any part of the ganglion. Therefore, neurons exhibiting but minimum variability in size and topography in the various preparations were selected for experimental purposes. Certain individual differences occurring evenly now and then, did not influence the determination of the most frequent localization of the cell, as it appears from the average of about 100 ganglia. A neuron was only considered to be identifiable if in all the preparations it exhibited identic properties according to the following electrophysiological criteria:

a) In case of spontaneously active neurons the time sequence of the potentials. In this regard distinction was made between:
potential series of uniform distribution,
regular and irregular burst activity, and
irregular potential series.

b) In case of synaptically influenced cells the presence of EPSP-s and IPSP-s, and the time sequence of postsynaptic potential series.

c) Form and duration of soma discharge.

d) Effect on spike activity of continuous application of d.c. causing changes in the resting polarity of the membrane.

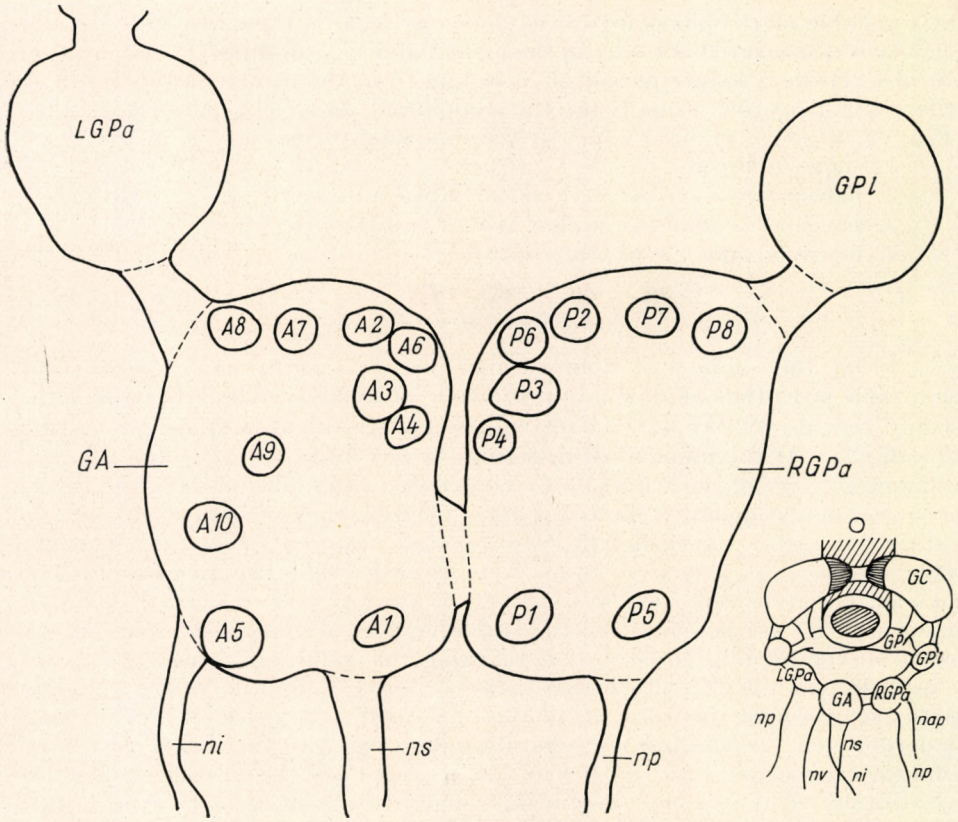

Fig. 1. Scheme of abdominal and right parietal ganglion with identified neurons Right lower part: Schematic drawing of the central nervous system of *Lymaea stagnalis* L. (After DUNCAN, 1961).

GA	=	Ganglion abdominale
RGPa	=	Right ganglion parietale
LGPa	=	Left ganglion parietale
GPl	=	Ganglion pleurale
GP	=	Ganglion pedale
GC	=	Ganglion cerebrale
ni	=	nervus intestinalis
ns	=	nervus splanchnicus
np	=	nervus pallialis
nap	=	nervus anterior pallialis
nv	=	nervus ventropallialis
O	=	Oesophagus

The membrane potentials of neurons exhibited a considerable variability ranging from 30 to 70 mV. In the majority of the cells, however, 40 mV was measured. The amplitude of the action potentials showed great variations, the extreme values being 50 and 114 mV (mean : 68 mV). The impulse duration of the action potential measured at half amplitude ranged from 5 to 34 msec.

It is not always possible to obtain exactly the same activity pattern even from identically numbered neurons identified in different preparations, but certain special details recurring frequently in each cell furnish a suffici-

ently reliable electrophysiological evidence as regards their identity. Variable activity was measured not only in the identifiable cells of different preparations but also during a longer period of recording from the same neuron. In all, 18 cells were identified visually in the abdominal and right parietal ganglion *(Fig. 1)*, which may be divided in the following categories:

1. silent neurons.
2. pacemaker neurons with no synaptic input,
3. pacemaker neurons under synaptic influence, and
4. neurons under synaptic control.

1. *Silent neurons*

From the somata of neurons included in this category, with stable membrane potentials of adequate magnitude no spike activity can be recorded or only very rarely. We have identified two pairs of silent neurons (A6 and P6; A7 and P7). At the moment of inserting the electrode, owing to the injury, a frequent activity of short duration was recorded from both pairs of cell, which however, soon stopped or showed a very low frequency. From time to time, in addition to spikes, marked EPSP-s were also recorded *(Fig. 2/d)*. In a few cases activities of longer duration were recorded but the frequencies were not higher than 0,7 cps (A6), 0,4 cps (P6), 1 cps (A7) and 0,5 cps (P7). Simultaneously with the setting in of the stable level of activity a marked increase occurs in the membrane potential, reaching the value of 70 mV. Such cells, in addition to the identified ones, are scattered along the entire ganglion, particularly among neurons of smaller diameter. They are, however, more numerous near to the line of separation between ganglia. Their membrane potentials are generally over 40 mV, i.e. higher than the average value. Depolarization of appropriate magnitude ought to evoke an activating effect, which indeed occurred in the majority of the cases, but in some cases even a depolarization of 30 mV proved to be insufficient to evoke action potentials.

A common feature of all these identifiable neurons is their response to depolarization which renders possible their activation. Each cell possesses a marked capacity of accomodation. After a very brief period of frequent activity following depolarization, the discharge frequency decreases to a low level (0,2—1 cps). Sometimes the extent of accomodation is so great that the response consists only of one or two potentials *(Fig. 2/a* and *2/b)*. Even when one of the neurons was active prior to depolarization, the frequency of the stabilized response does not exceed 0,2—1 cps.

In the neurons A6 and P6 depolarization evoked in nearly all cases an oscillation of the membrane potential and formation of groups of 2—3 members *(Fig. 2/c)*, while this seldom occurred in the neurons marked A7 and P7.

If the cells were active prior to intervention, a decrease or cessation of frequency occurred to hyperpolarization. In such conditions it became evident that these cells were influenced by synaptic input. If the membrane potential increased, a more or less regular sequence of EPSP, probably originating from one or a few synaptic inputs, were observed. It appears that the seemingly spontaneous soma discharges are preceded by EPSP, i.e. the spikes are evoked by synaptic effect. Owing to high membrane potentials only a few EPSP-s are capable to produce the extent of depolarization needed for the discharge and this is the cause of the low frequency of activity of these cells.

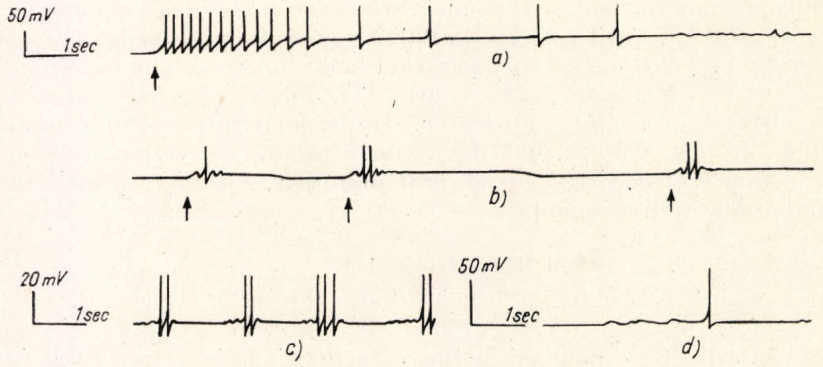

Fig. 2. Different forms of response to depolarization in silent neurons.
a) Continuous depolarization (Cell A7)
b) Repetitive brief depolarization (Cell A7)
c) Oscillation of membrane potential and formation of groups of spikes under the effect of depolarization higher than 10 mV (Cell A6)
d) EPSP series evoking occasional spikes (Cell A6)

2. *Pacemaker neurons without synaptic input*

Two such cells: A3 and P3 were found to belong to this category. Their membrane potential was of medium height ranging from 30 to 48 mV and their action potential of an alongated form exhibited an overshot of 25—35 mV. Of all the neurons examined these cells had the longest impulse duration ranging from 15 to 34 msec *(Fig. 3*/a*)*. Frequency of somatic discharge was

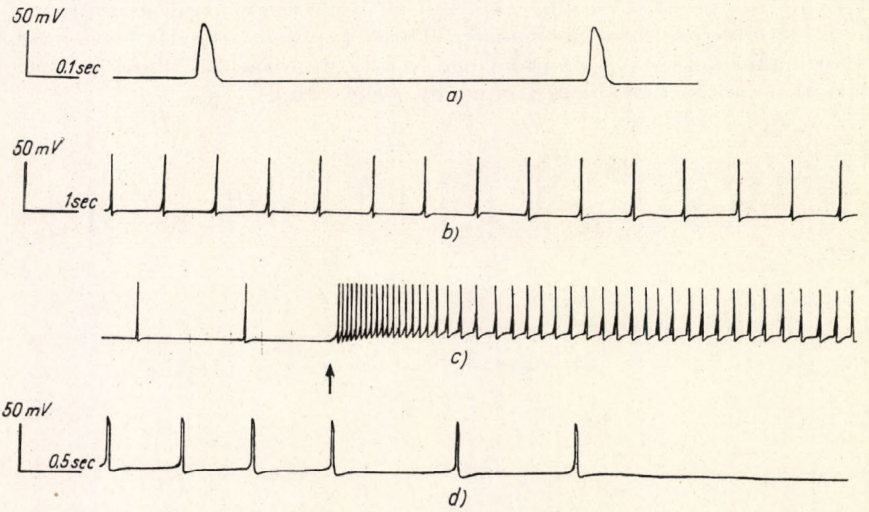

Fig. 3. *a)* Spontaneous action potential exhibiting a characteristic form and duration of impulses (Cell P3).
b) Spontaneous activity with regular continuous series of potentials (Cell P3).
c) Response to depolarization (Cell P3).
d) Activity of cell P3 in hyperpolarized state. Gradual decrease in frequency of potentials followed by inhibition lasting a few minutes (ILD)

uniformly distributed but with some variations in the different preparations (0,7—1,3 cps in A3, and 1—2,3 cps in P3). No synaptic potentials were observed during activity *(Fig. 3/b)*. Depolarization had a marked stimulating effect on frequency which increased to 2—4 cps *(Fig. 3/c)*. Accomodation was absent or was only of an insignificant degree. Hyperpolarization caused a decrease in activity to 0,1—0,5 cps, but the activity usually persisted. In some cases complete blocking occurred for several minutes *(Fig. 3/d)* after which the neuron became active again.

3. *Pacemaker neurons under synaptic influence*

Seven cells were included in this category: The activity of two of them (A4 and P4) was sometimes so similar to that of A3 and P3 that they were hardly distinguishable from each other as regards form, amplitude and the duration of impulse of the spikes *(Fig. 4/a)*. The frequency of A4 ranged from 0,5 to 2 cps, while that of P4 from 0,8 to 3 cps. From time to time a slow oscillation was observed during which a hyperpolarization occurred interrupting the course of activity *(Fig. 4/b)*. Extreme prolongation of the hyperpolarization phase may explain the silence of neurons A4 and P4. In such periods irregular bursts occur due to the effect of EPSP *(Fig. 4/c)* indicating that these cells receive synaptic impulses unlike neurons A3 and P3. Neurons A4 and P4 are also characterized by their lack of accomodation capacity to depolarization.

Neurons marked A1, P1 and A10 showing very similar activity were included in this category. All of them showed pacemaker properties but at the same time they seemed to be under compound synaptic influence. Consequently, they exhibited greatly varying types of activity alternating even during continuous activity of the same neuron. Three types of activity were observed: *a)* Pacemaker activity is dominating. Nearly uniform distribution of activity with occasional oscillation in frequency *(Fig. 5/a)*.

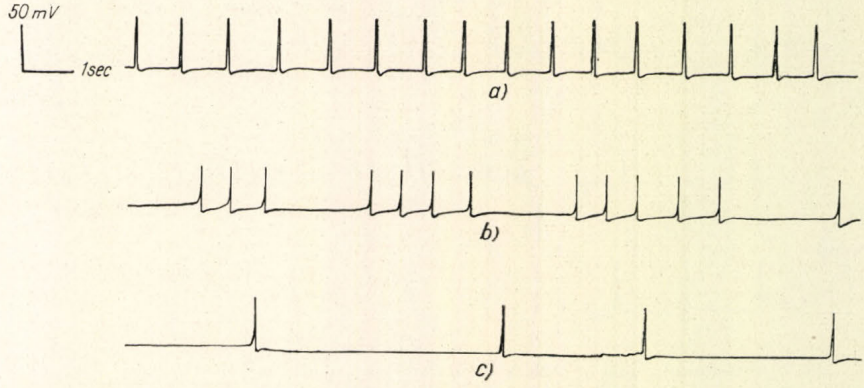

Fig. 4. *a)* Continuous regular series of potentials (Cell A4)
b) Continuous pacemaker activity is occasionally interrupted owing to hyperpolarization of the membrane (Cell A4)
c) Activity of synaptic origin (Cell P4)

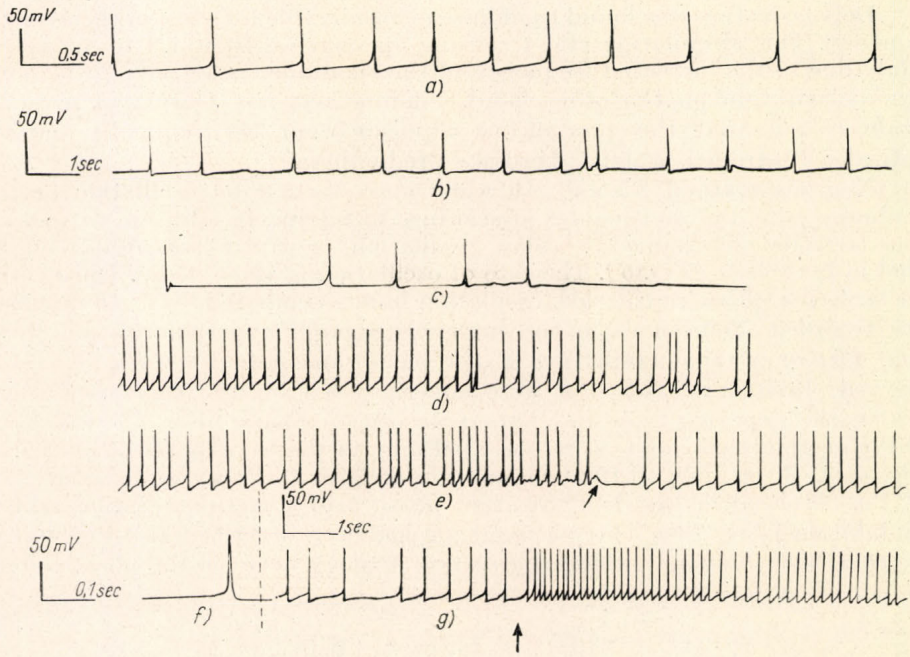

Fig. 5. *a)* Oscillation of frequency in the course of continuous activity (Cell P1)
b) Activity during synaptic control (Cell A1)
c) Activity in hyperpolarized state (Cell A1)
d) Continuous spontaneous activity disturbed from time to time by EPSP input. After cessation of EPSP-s a brief inhibition is recorded (Cell A1).
e) EPSP bursts during continuous activity. After the burst a postexcitatory inhibition appears with a potential similar to „amphoter PSP" in the initial phase (↑).
f) Action potential of short duration from cell A1
g) Effect of depolarization (↑) on frequency (Cell A10)

b) Spontaneous activity is absent, the neuron functions entirely under synaptic control *(Fig. 5/b)*. Frequency is low. In normal state this is seldom seen but usually it can be evoked with the hyperpolarization of the membrane *(Fig. 5/c)*.

c) The cells show spontaneous activity but synaptic influence manifested in the appearance of EPSP-s (single or in series) as shown in *Fig. 5/d* and *5/e*, is also present. Depending on the series of EPSP-s an increase in frequency occurs which is often followed by a long lasting inhibition *(Fig. 5/d, 5/e)*.

The recorded values of average frequency were greatly variable within the domain of 0,1—3 cps. Within the group of potentials an irregular succession of impulses was noted depending on the variations in the summation of arriving EPSP-s.

The impulse duration of action potentials never exceeded 5—15 msec *(Fig. 5/f)*. In addition to EPSP, occasional IPSP-s were also recorded, as well as biphasic postsynaptic potentials followed by inhibition *(Fig. 5/e)*. These potentials are similar to those described by ARVANITAKI and CHALAZONITIS (1965/a) and termed by them as „amphoter postsynaptic potential".

Depolarization was found to induce a considerable increase in the average frequency. This stimulating effect causing an increase from 1.8 to 4,9 cps is mainly due to the increased frequency of the spontaneous activity *(Fig. 5/g)*. Hyperpolarization on the other hand, inhibits activity. During increase of membrane potential, the spontaneous impulses occur less frequently and synaptically controlled activity becomes predominant.

Neurons marked A5 and A9 were characterized by oscillation of the membrane potential and regular appearance of bursts. In addition to spontaneous activity determined by slow oscillation, synaptic potentials can be noted in both cells *(Fig. 6)*. The rate of oscillation is about 1 cps. During the depolarization phase of the slow oscillation bursts composed of 5—15 impulses were recorded. Sometimes in the intervals a large hyperpolarization wave *(Fig. 6/a)* or a rapid oscillation of the membrane potential *(Fig. 6/b)* was observed. Frequently, EPSP-s were superimposed on the slow oscillation, both in the depolarization and hyperpolarization phase *(Fig. 6/c* and *6/d)*. IPSP-s on the other hand, were observed only in the hyperpolarization phase *(Fig. 6/e)*. From time to time summation of both PSPs were observed. The EPSP-s observed in the depolarization phase lead sometimes, rather oddly, to inhibition *(Fig. 6/d)*. This phenomenon has been described also by CHALAZONITIS (1968). Similarly, there is a certain analogy between the phenomenon

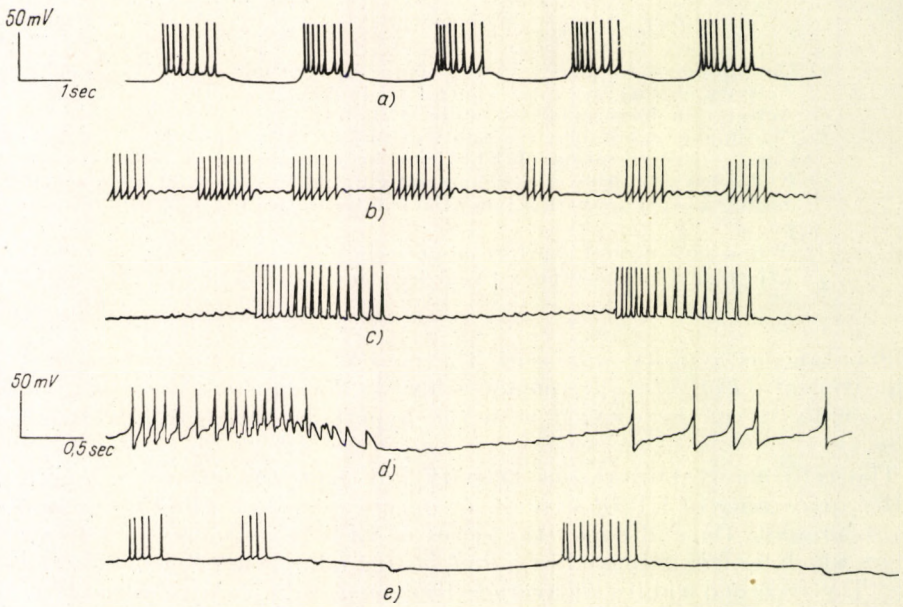

Fig. 6. Activity of bimodal oscillator type (Cell A9).
a) and *b)* Two different types of spontaneous activity related to slow oscillation of the membrane potential.
c) EPSP series and burst activity superimposed on slow oscillation.
d) Activity under synaptic control. Summation of EPSP-s during depolarization phase of membrane potential oscillation. Later on the EPSP increasing in amplitude leads to inhibition.
e) Activity under synaptic control. Giant IPSP-s appear in the hyperpolarization phase.

observed on giant neurons of *Aplysia* (CHALAZONITIS, 1962) and the giant IPSP-s appearing occasionally in the hyperpolarization phase *(Fig. 6/e)*.

Characteristic of the activity of these cells is a gradual increase in the duration of intervals between the spikes during the period of bursts. If the activity is under synaptic influence, the number of burst impulses and the distribution of frequency may be extremely variable owing to the summation of EPSP-s in the depolarization phase. Summation of IPSP-s may increase the duration of the interburst periods.

4. *Synaptically controlled neurons*

Five neurons were included in this category. From neurons marked A8 and P8 a relatively uniform impulse series of medium frequency (0,7—1,2 cps) was recorded. The spikes were evoked by excitatory postsynaptic potentials. Nearly all EPSP-s led to somatic discharges.

Sometimes the activity of the cell was surprisingly regular *(Fig. 7/a)* appearing as if it were of pacemaker origin. By hyperpolarization, however, the synaptic origin of the spikes is fairly demonstrable. At this time only EPSP-s are recorded *(Fig. 7/b)*, while depolarization elicits marked increase in frequency. Now and then IPSP-s also appear causing an interruption of changing duration in the otherwise continuous activity.

Neurons marked A2, P2 and P5, apart from a few cases, showed an activity below 1 cps, probably controlled by a compound synaptic system consisting of excitatory and inhibitory inputs. On the ascending line of the action potentials of medium impulse duration (13—20 msec) local potentials appeared evoked by EPSPs *(Fig. 7/c)*. Sequence of the spikes was more or less irregular with occasional appearance of groups consisting of 2—5 impulses *(Fig. 7/d)*. In some cases the increase of the interval was connected with the appearance of IPSP-s or with the brief hyperpolarization of the membrane seemingly independent of them. Usually the frequency of excitatory synaptic impulses is low, but now and then EPSP bursts of high frequency were also recorded *(Fig. 7/c)*. These bursts do not evoke a high increase in frequency

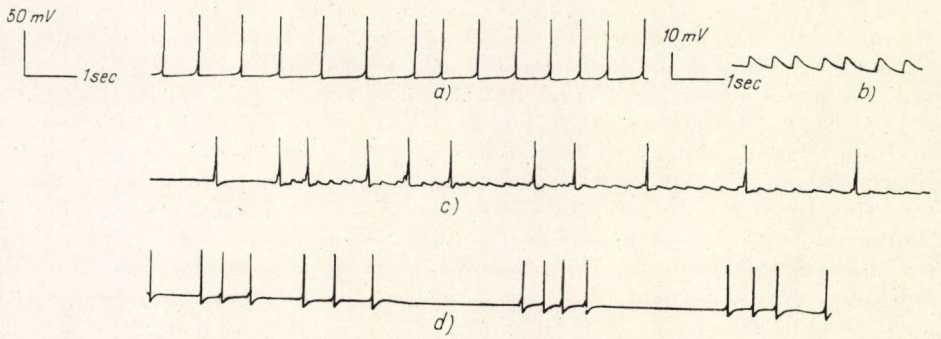

Fig. 7. Activity under synaptic control.
a) Nearly regular, continuous series of potentials (Cell P8)
b) Appearance of EPSP series on hyperpolarization (same cell)
c) EPSP burst with occasionally elicited action potentials (Cell A2)
d) Slow oscillation of membrane potential and spike groups (A2)

of spike activity because the soma cannot follow the increase of frequency of the EPSP-s. Therefore, even in the case of a more frequent synaptic input only a determined number of EPSP-s can evoke somatic discharges.

A characteristic feature of these cells is that in most of them depolarization does not elicit a notable change in frequency. Depolarization often evokes an oscillation in the membrane potential accompanied by cessation of activity and appearance of pseudospikes. Ineffectiveness of depolarization seems to be connected with great capacity of accomodation of the neuron. Activity is always inhibited, often completely abolished by hyperpolarization.

Discussion

The results of our experiments have shown that in the ganglia examined there are numerous cells which can be identified not only visually but also by electrophysiological parameters. As regards their types of activity most of the adjacent cells showed fundamental or partial differences by which differentiation and selection of cells on the localization map was possible *(Fig. 1)*.

Among the cells we could recognize pacemaker and nonpacemaker cells, as well as cells possessing particular properties, such as slow oscillation. This latter type of cells have been described by numerous authors (cit. in: ARVANITAKI and CHALAZONITIS, 1968; FRAZIER et al. 1967) on other gastropods, too. Our results indicate that not all giant cells have the same function, i.e. some of them are activity generators, while others seem to play an integrating role or be central sensory cells. The rich EPSP and frequent IPSP inputs indicate that, in addition to those examined, numerous pacemaker neurons are present in the ganglia which do not belong to giant cells. Sometimes these cells exhibit PSP bursts, at other times irregular and summed potentials which seem to refer to the effect of an oscillator type of cell or to a compound influence. Investigation on the interrelation of identified cells may clarify the question whether the giant cells form an integration system in which several neurons are involved, or their connections are primarily of a different nature.

Some of the particularities of these cells — described also on other gastropods — may be helpful in the identification of cells, e.g. accomodation to depolarization (ALVING, 1968) is a characteristic feature of neurons A6, P6, A7, P7, A2 and P2. The first four of these cells differ from the rest by their low frequency of activity due to the small number of EPSP-s capable of inducing activity because of the high membrane potentials of the cells.

Neurons marked as A5 and A9 have an activity of bimodal oscillator type, similar to that termed as ,,Br type" by ARVANITAKI and CHALAZONITIS (1961) and ,,parabolic burster" by STRUMWASSER (1965). In these two cells we have observed that EPSP-s lead to inhibition, a phenomenon described by CHALAZONITIS (1968) on *Aplysia*. In the initial phase of postexcitatory inhibition following EPSP bursts a potential similar to ,,amphoter PSP" (ARVANITAKI and CHALAZONITIS, 1965a) was observed in A1, P1 and A10. In the same cells the phenomenon termed by TAUC (1958) as ,,long lasting inhibition" (ILD) was also noted.

Cells termed as A6, P6, A7 and P7, included in the group of ,,silent" neurons were differentiated on the basis of their surprisingly high (60—70 mV) membrane potential. This high membrane potential may explain why these cells remain silent in spite of EPSP-s present there.

In synaptically influenced pacemaker neurons modifications of the membrane potentials likewise play an important role in whether pacemaker propriety or synaptic control becomes predominant. The affect of polarization studied in neurons A1, P1 and A10 refer to this role.

The frequency of discharges offers a certain basis for the differentiation of cells but at the same time it exhibits marked varieties within the same cell. This cannot be explained only by the changes of the synaptic input, even in cells under synaptic control, but rather by the sensitivity of most cells to temperature and oxygen (ARVANITAKI and CHALAZONITIS, 1965/b; CARPENTER, 1967). Uniform conditions of temperature and O_2 were not ensured during our experiments. On the other hand, duration of the impulses proved to be a good indicator for the identification of certain cells.

Attention should be called to an interesting phenomenon. We have investigated the abdominal and the right parietal ganglion which are the sites of origin of different nerve branches, but as regards some details they seem to be symmetrical, expecially in certain giant cells. Investigations on such cells revealed that in the majority of the cases they are pairs of cells having identic electrophysiological properties. As it is illustrated on the map the neurons marked with the same number (14 out of 18) are pairs of cell localized symmetrically on the borderline between the ganglia. The embryological basis and functional importance of this finding are unclear so far and it would be interesting to investigate whether these cell pairs are connected in some way and what is their relationship with other neurons in these ganglia.

Summary

Activity characteristics of giant neurons (over 100 μ) identified visually and on the basis of electrophysiological parameters were investigated in the abdominal and right parietal ganglion of *Lymnaea stagnalis* L. by intracellular microelectrodes.

From the experiments it was concluded that

1. The type of activity of neurons having identic topographical localization is identic in each preparation.
2. Different neurons exhibit marked variations in activity.
3. Clear distinction can be made in the preparations between pacemaker and non-pacemaker cells.
4. Most of the cells are under synaptic influence.
5. Some of the neurons examined exhibited activities similar to those observed on other gastropods.
6. The neurons localized identically among the giant cells of the abdominal and right parietal ganglion exhibit a similar or an identic type of activity.

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IDENTIFIKÁLT SEJTEK A *LYMNAEA STAGNALIS* (GASTROPODA) KÖZPONTI IDEGRENDSZERÉBEN

Salánki János és *Kiss István*

Összefoglalás

Vizuálisan és az aktivitás elektrofiziológiai paraméterei alapján jól identifikálható, 100 μ-nál nagyobb átmérőjű sejtek működési sajátosságait vizsgálták intracelluláris elvezetéssel *Lymnaea stagnalis* L. abdominalis, valamint jobb parietális ganglionjában. Az eredmények alapján megállapítható, hogy:

1. A topográfiailag azonos helyzetű neuronok aktivitási típusa preparátumról preparátumra azonos,
2. a különböző neuronok aktivitási típusai jelentősen eltérnek egymástól,
3. a sejtek között jól elkülöníthetők pacemaker és nem pacemaker tulajdonságúak,
4. a vizsgált sejtek többsége sokoldalú szinaptikus befolyás alatt áll,
5. a vizsgált neuronok között felismerhetők olyanok, melyek a más Gastropodákon leírt aktivitási típusokkal megegyezőek,
6. az abdominális és jobb parietális ganglion óriássejtjei között identikusan lokalizáltak aktivitási típusa hasonló ill. azonos.

ИДЕНТИФИЦИРОВАННЫЕ КЛЕТКИ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ БОЛЬШОГО ПРУДОВИКА

Я. Шаланкаи и *И. Киш*

При внутриклеточном отведелении были изучены характерные свойства активности клеток абдоминального и правого париетального ганглиев большого прудовика превосходящие 100 мк по диаметру и идентифицировались на основе их электрофизиологических параметров.

Было установлено, что:

1. В разных препаратах одни и те же клетки показывают одинаковый тип активности.
2. Разные нейроны значительно отличаются друг от друга в отношении типа активности.
3. Клетки отличаются друг от друга и по свойству способности к индукции ритма.
4. Большинство исследованных клеток находится под многочисленнымы синаптическими воздействиями.
5. Среди изученных нейронов были найдены и такие, которые были описаны раньше для других брюхоногих.
6. Тип активности клеток, локализованных паралелльно в правом париетальном и абдоминальном ганглиях, сходный или полностью совпадает.

ANNAL. BIOL. TIHANY	36	77—93	HUNGARIA 1969

ON THE ROLE OF CHOLINERGIC, ADRENERGIC AND TRYPTAMINERGIC MECHANISMS IN THE REGULATION OF A "CATCH" MUSCLE (*ANODONTA CYGNEA* L.)

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Received: 21st February, 1969

The regulation of tonic and phasic activity in the "catch" muscles of Molluscs differs significantly from that found in smooth and sceletal muscles (HOYLE, 1964). Investigations on the chemical basis of regulation existing in this type of muscles being rather specific also in their structure (HANSON and LOWY, 1960; ZS.-NAGY and SALÁNKI, 1965; MILLMANN, 1967) were carried out mainly on the anterior byssus retractor muscle (ABRM) of *Mytilus* (see TWAROG, 1967) and on different Gastropoda preparations (KOSHTOYANTS, 1936; FÄNGE and MATTISSON, 1958; MINKER and KOLTAI, 1961; GREENBERG and JEGLA, 1963; JAEGER, 1964). There are data referring to the role of cholinergic, adrenergic and tryptaminergic mechanisms. In general acetylcholine (ACh) is considered as tonus-increasing, while serotonin (5HT) as tonus-inhibiting agent (FLOREY, 1967). At the same time usually no importance is attributed to the role of adrenergic mechanisms. In cases adrenergic substances were effective, their action was similar mostly to 5HT, in less of the cases to ACh (JAEGER, 1963; HOYLE, 1964; HILL, 1958; GREENBERG and JEGLA, 1963).

In contrary to the fact, that the adductor muscles of *Anodonta cygnea* are characteristic "catch" muscles, up to now direct pharmacological investigations were not carried out on them. The effects of sympathetic and parasympathetic drugs were tested on the whole animal by GEIGER (1929), while the response of the adductor after applying serotonin, adrenaline and ACh to the ganglia were investigated by SALÁNKI (1963) and PUPPI (1963a, b). The pharmacology of the non-definitive adductor of the larvae of *Anodonta* (glochidia) is known better (LÁBOS et al. 1964; LÁBOS, 1966).

The aim of our investigations was to answer the question, which of the main possible chemical mechanisms (cholinergic, adrenergic, tryptaminergic) may exist in the regulation of tonic and/or phasic activity of the adductors at the level of the muscle fibres, or in the neuromuscular transmission.

Material and methods

Investigations were carried out on the posterior adductor muscle of *Anodonta cygnea* L. The adductor was not isolated from the whole of the animal, but we made it mechanically independent of the anterior adductor. The

functioning of the muscle was registered on a kymograph; the upward movement of the lever indicated contracton (closing of the shells).

To render the muscle within reach, above the heart a part of the shell was abolished, and using subcutan needle drugs were injected directly into the middle of the posterior adductor. The volume of the solution varied between 0,2—0,5 ml. The substances were dissolved in physiological saline (MARCZYNSKI, 1959). In each animal also a control was made when only the physiological solution was injected.

In a number of the experiments the spontaneous activity of the adductor was registered and influenced. In other cases the answer of the adductor was evoked by an indirect way: either the cerebro-visceral connectives (CVc) were stimulated by square wave impulse series causing relaxation, or tonic contraction was caused by mechanical stimulation of the mantle as it was described earlier (SALÁNKI and LÁBOS, 1963). The parameters of the electrical stimulation were: 20 volts, 4 msec (duration of the impulse), 8 cps frequency and 30 or 60 sec (duration of the stimulation).

The following drugs were used: acetylcholine chloride (ACh) (Sandoz); nicotine-H-tartarate (BDH); atropine suphate (Fluka); d-tubocurarine-HCl (dTC) (Schuchardt); hexamethonium iodide (Schuchardt); tetraethyl ammonium chloride (TEA) (BDH); tetramethyl ammonium bromide (TMA) (Fluka); eserine salicilate (Merck); neostigmine bromide (Merck); mytolon-HCl (Winthrop Ltd); L-adrenaline-D-H-tartarate (EGA); L-noradrenaline bitartarate (Serva); dopamine-HCl (Sigma); DL-isoproterenole-HCl (IPNA) (Fluka); dibenamine-HCl; dichloro- isoproterenole-HCl (DCI) (Eli et Co. Ltd); ergotamine-H-tartarate (BDH); bromo-d-lysergic acid diethylamide (BOL-148) (Sandoz); serotonin creatinine sulphate (5HT) (Sandoz).

The doses used refer to the whole of the compound (salts).

Each drug was tested at least five times.

Results

In most of the cases the posterior adductor of *Anodonta* relaxes spontaneously after the preparation is ready. This spontaneous relaxation comes about comparatively faster and more frequently in summer than in winter time. We carried out experiments mainly on muscles in relaxed state. A part of the muscles having remained in tonic contraction after preparation relaxed after injection of the physiological saline, others showed relaxation only to the effect of drug-injection. Independent of treatment the muscles performed usually rhythmic contractions. The level of tonus was different taking each individually.

1. *Control*

Intramuscular injection of physiological saline caused the fast contraction of the aductor followed by an immediate relaxation. The length of the whole cycle has taken about 2—5 minutes. As this length of time is not constant, in every case a control was necessary. After the control injection the background activity usually did not change, in several cases, however, a temporary increase or decrease in the frequency was observed. This was taken into account in every case at the evaluation of the experiment.

2. *Effects of ACh and cholinergic pharmacons*

The effects caused by these substances are summarized in *Table I*. Four main effects are emphasized: increase of the tonus level, decrease of the tonus level, increase of the frequency of the phasic contractions, and decrease of the frequency of the phasic contractions. The latter two refer to the frequency of the rhythmic activity.

Table I

The effects of cholinergic drugs

	Dose (μg)	Number of experiments	Increase of tone	Decrease of tone	Increase of rhythm	Decrease of rhythm	No effect
ACh	100	10	5				5
ACh	500	5	5				
Nicotine	100	5	5			5	
TMA	10	6	6			5	
TMA	50	5	5			5	
TMA	100	10	10			10	
TMA	500	5	5			5	
TEA	10	5			2		3
TEA	100	5	1		4		1
Mytolon	100	10	9		3		1
Mytolon	500	12	11		12		
Atropine	100	7			1		6
Atropine	500	10			3		7
dTC	100	5			1		4
dTC	500	5	1		3		1
Hexamethonium	100	10	4		2	1	6
Eserine	100	10	1		1	4	4
Eserine	500	10	3			1	6
Prostigmine	100	10					10
Prostigmine	500	10					10

It was found, that 100—500 μg dose of the ACh cause a temporary tonic response. The degree and duration of this effect increased with the quantity of the ACh. After ACh injection some immediate relaxation occurs, but afterwards the remaining tonic contraction decreases very slowly (at times lingering on for 10 minutes). Repeated ACh injection evoked a less effect *(Fig. 1)*. Simulteneously the decrease of the rhythmic activity was observable.

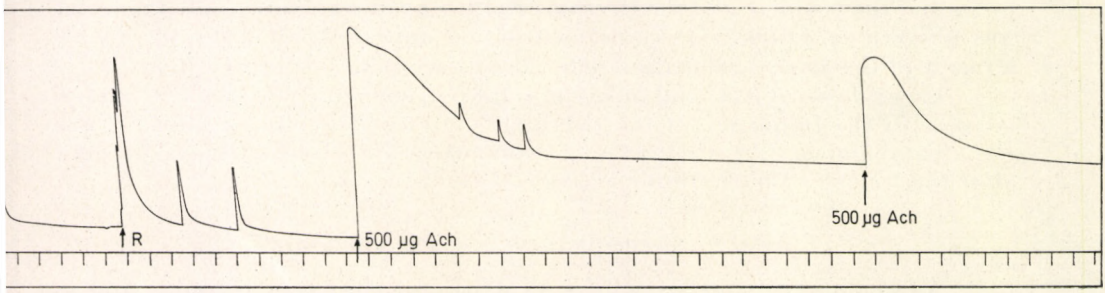

Fig. 1. Effect of ACh on the spontaneous activity of the posterior adductor.
R — injection of physiological solution: ↑ — 500 μg ACh
Time scale — 60 sec

Nicotine caused tonic contraction already in 50—100 μg amount. This effect appears very rapidly and no subsequent relaxation was noticeable. After 5—10 minutes plateau a slow decrease of the tonus occurred, but it remained constant at a new level *(Fig. 2)*. The frequency of the rhythmic activity usually decrease and the amplitudes of the fast contractions in consequence of the high level of tonus are lowered.

TMA increased the tonus very effectively, already in 10 μg dose. The tonic contraction lasted long, only insignificant relaxation could be observed even in 1—2 hours. The previous rhythmic activity may remain untouched *(Fig. 2B)*.

TEA in 100 μg amount caused increase in the rhythmic activity.

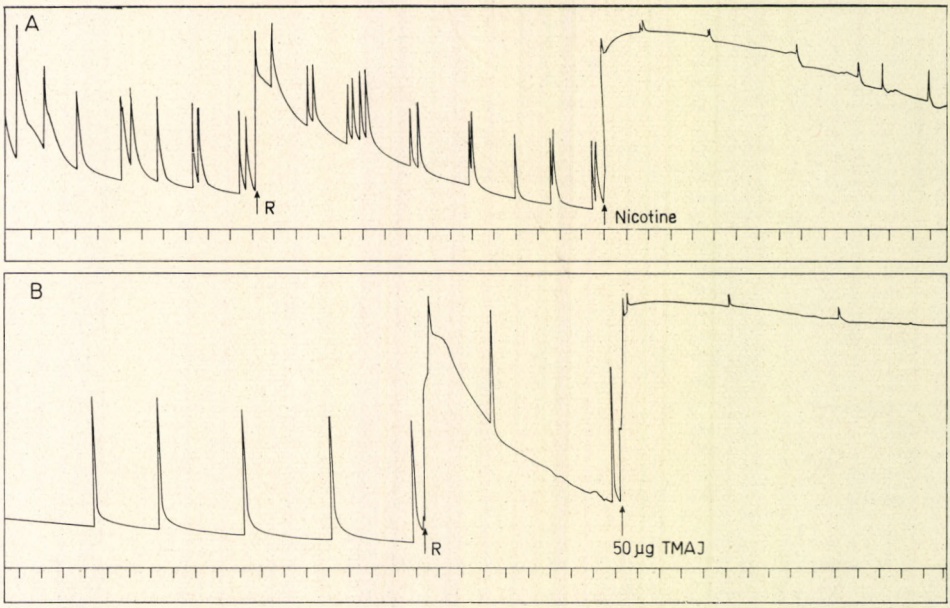

Fig. 2. A — effect of 50 μg nicotine;
B — effect of 50 μg TMA.

100—500 μg mytolon enhanced in most of the cases both the level of the tonus and the frequency of the rhythm *(Fig. 3A)*, but sometimes only the latter was observable *(Fig. 3B)*. Tonic contraction appeared not instantly but as a result of decreasing relaxation after successive phasic contractions.

Large doses of dTC and atropine caused in some of the cases a moderate increase in the frequency of the rhythmic activity. The effect of eserine was not unambiguous. Prostigmine and hexamethonium (100—500 μg) did not alter the tonus or the rhythmic activity.

3. *Effects of adrenergic agonists and antagonists*

Adrenaline in 10—100 μg amount enhanced the level of tonus, and in most of the cases also increased rhythmic activity *(Table II; Fig. 4A)*. Noradrenaline injection caused similar effect *(Fig. 4B)*. The evoked tonic contrac-

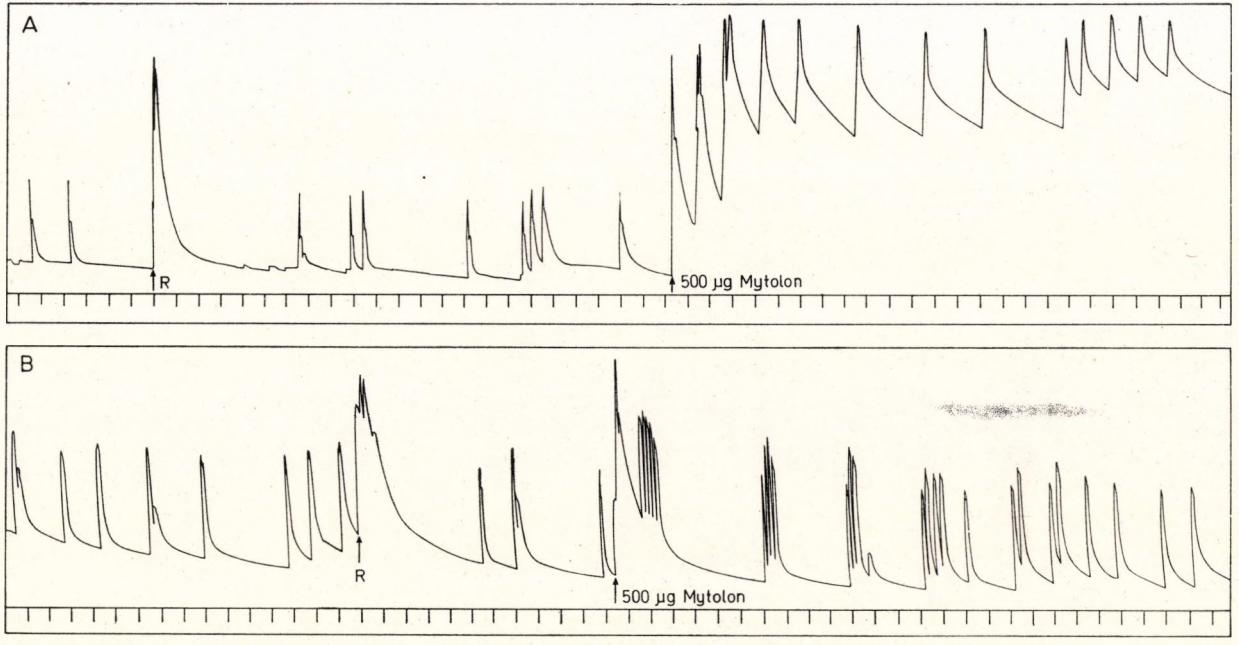

Fig. 3. Effect of 500 µg mytolon.
A — increase of tone and rhythm;
B — increase of rhythm

Table II

	Dose (μg)	Number of experiments	Increase of tone	Decrease of tone	Increase of rhythm	Decrease of rhythm	No effect
Adrenaline	10	7	5		4		1
Adrenaline	100	10	8		5		2
Noradrenaline	10	6	4		3		2
Noradrenaline	100	12	5		5		4
Dopamine	10	12		1	9		3
Dopamine	100	10	3	2	2		5
Dopamine	500	10		7	8		1
Tyramine	100	9		9	3		1
Tyramine	250	6		6	1		
IPNA	100	5	3		4		2
Dibenamine	100	10	1		1		9
Dibenamine	500	10	8		8		2
DCI	100	6	2				4
Ergotamine	100	5			2		3
5HT	10	4			4		
5HT	50	5		5		4	
Tryptamine	100	5		4	3		1
Ergometrine	1	5			5		
Ergometrine	10	5			4		1
Ergometrine	100	5	2		5		
BOL-148	10	4	2				2
BOL-148	100	7	3		5		1

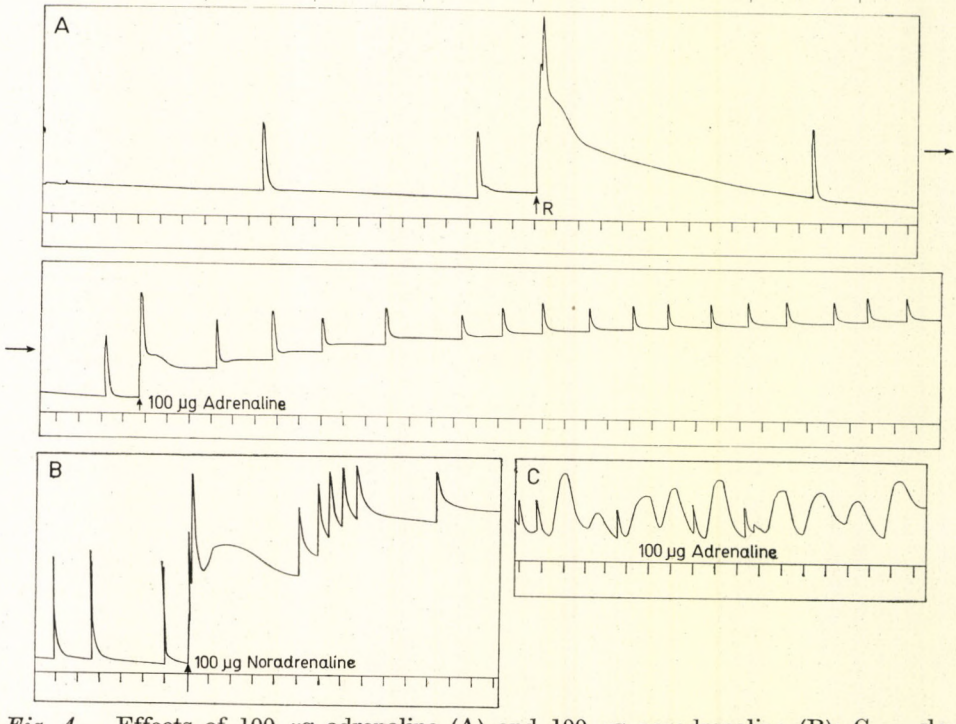

Fig. 4. Effects of 100 μg adrenaline (A) and 100 μg noradrenaline (B); C — slow waving after 100 μg noradrenaline

tion lasted in both cases for a long time, and also the relaxation and rhythmic activity of the foot was observable. Sometimes a slow waving occurred in the level of tonus *(Fig. 4C)*.

The effect of IPNA was similar to that of the noradrenaline.

Dopamine (100—500 μg) increased the rhythm and augmented the amplitudes of the phasic contractions. In *Fig. 5.* demonstrating the effect of the dopamine the phasic contractions have two components, and dopamine influences the second one. 500 μg dopamine decreased the tonus.

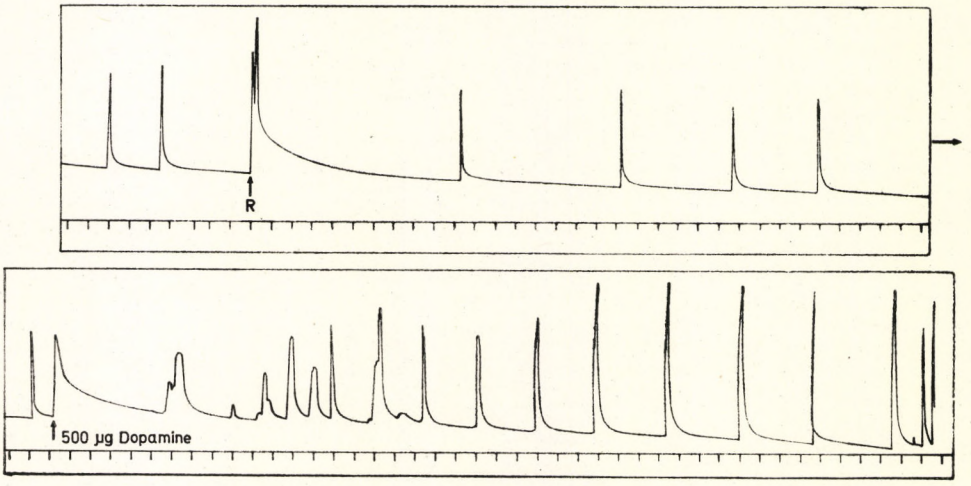

Fig. 5. Effect of 500 μg dopamine. The lower curve is the immediate continuation of the upper one

Tyramine in 100—250 μg amount decreased the tonus, in some cases, however, an increase in the rhythm was observed.

Large amount of dibenamine (500 μg) increased temporarily both rhythmic activity and tonus level.

The effect of DCI (100 μg) was insignificant. Also ergotamine proved to be ineffective.

4. *Effects of 5HT, tryptamine, BOL-148 and ergometrine*

5HT caused in a few minutes a significant relaxation already in 10 μg amount, and also decreased the frequency of the rhythmic activity *(Fig. 6A)*. Tryptamine similarly caused a decrease in the tonus, but in many cases increased the rhythm *(Fig. 6B)*. BOL-148 often increased the rhytmic activity, and seldom did it enhance the tonus *(Fig. 6C)*.

Small doses of ergometrine incraesed the rhythm significantly *(Fig. 7A, B, C)*. The increase of the frequency appears immediately after the injection. Later on parallel with the damping of the increased frequency *(Fig. 8.)* the augmentation of the amplitudes was observable.

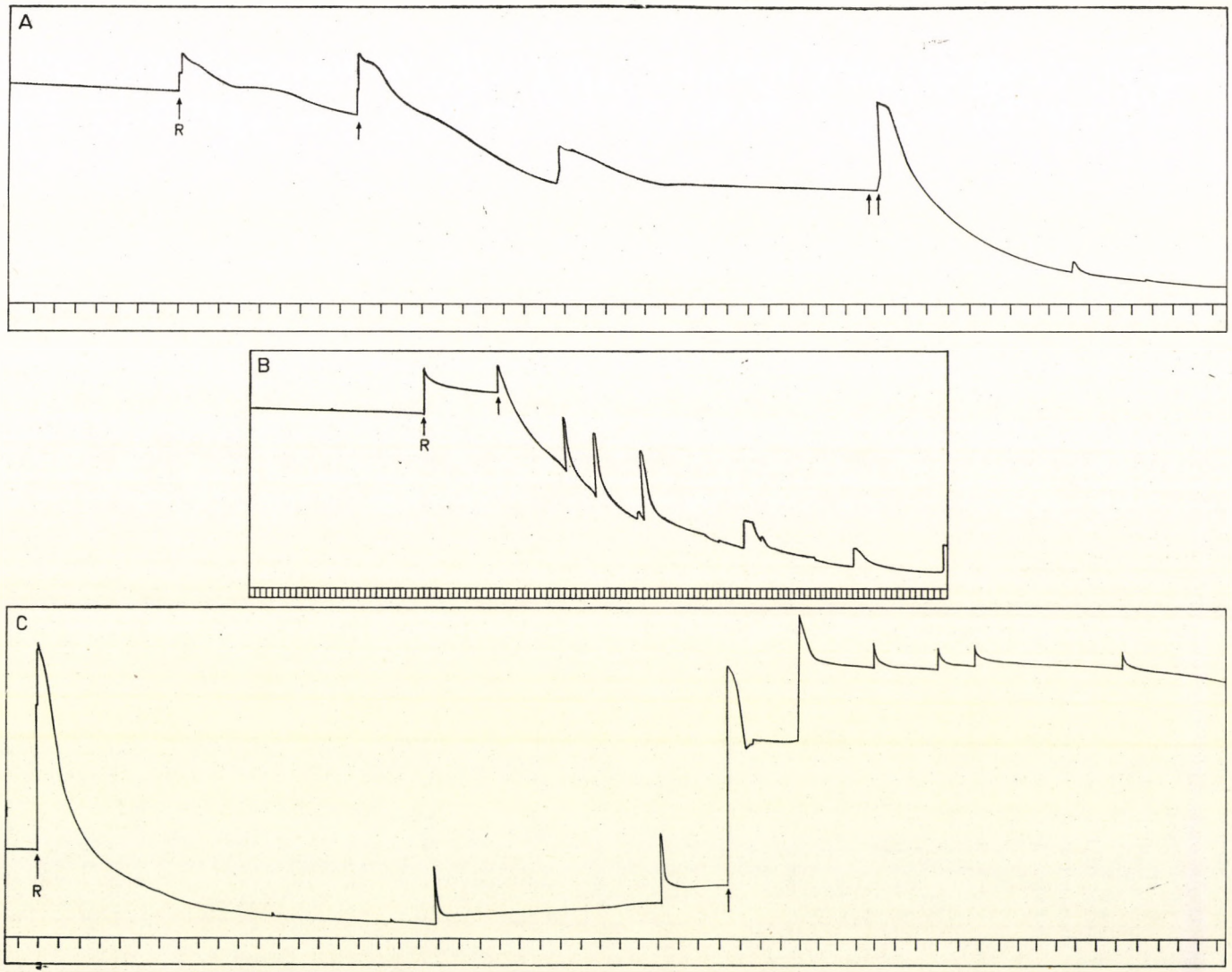

Fig. 6. A — effect of 5HT; ↑ — 10 μg; ↑↑ — 50 μg; B — effect of 100 μg tryptamine; C — effect of 100 μg BOL

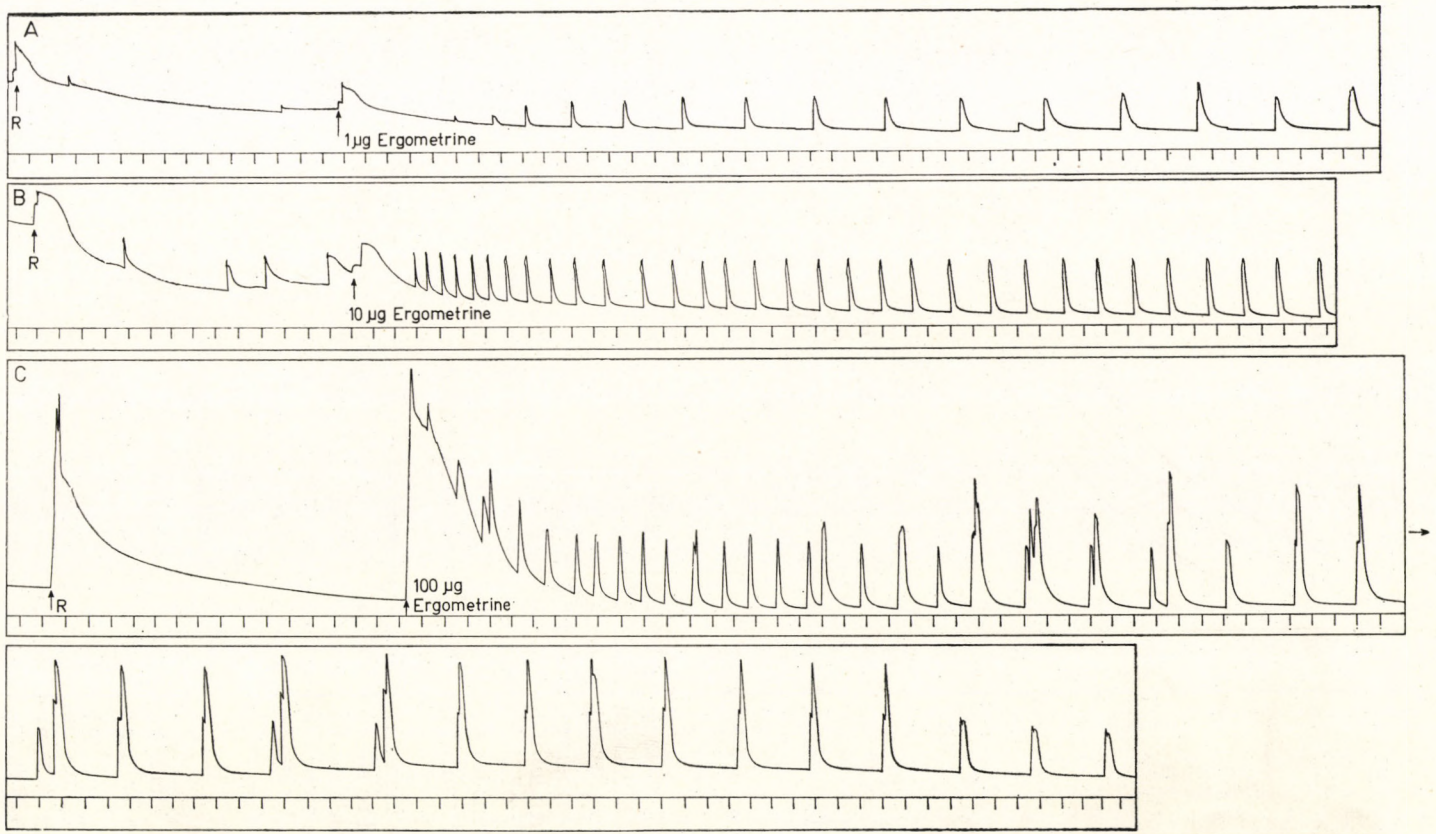

Fig. 7. Effect of ergometrine: A — 1 μg; B — 10 μg; C — 100 μg;

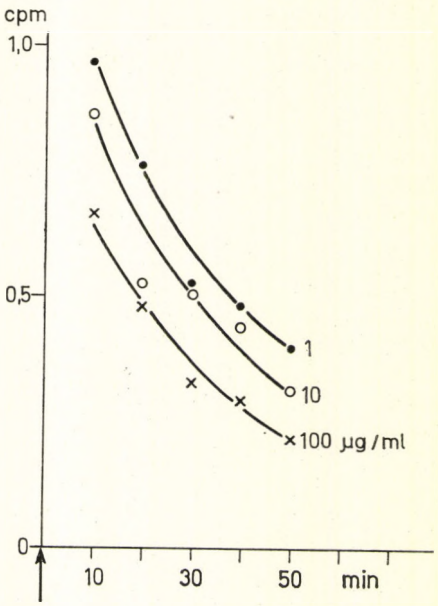

Fig. 8. Change in the frequency of the rhythmic contractions evoked by ergometrine during the first 50 minutes

5. *Effect of drugs on the muscle response evoked by electrical stimulation of the CVc*

With suitable series of impulse applied to the CVc the contraction and subsequent relaxation of the posterior adductor may be effected (SALÁNKI and LÁBOS, 1963). Significant differences were found whether stimulation takes place before or after drug injection. In most of the cases a decrease in the amplitude of the evoked response was observable. In many instances the specificity of this effect is dubious, because the response itself depends to a great extent on the previous stimulations and on the actual level of the tonus. Therefore conclusions were drown only in cases when other phenomena also occurred.

The increased tonus caused by ACh could only be relaxed very poorly as compared to the control, however, with repeated stimulation the tonic contraction diminished by a considerable degree *(Fig. 9)*.

The tonic contraction caused by nicotine and TMA could be relaxed in a less degree *(Fig. 10)*. The rate of relaxation depends on the dose of the drug used.

6. *Combined drug effects*

The increase of tonus caused by 500 µg ACh was not potentiated by 100 µg eserine or prostigmine.

Previous atropine injection (100 µg) prevented the effect of ACh, while 100 µg dTC proved to be ineffective in similar situation.

Both ACh and nicotine responses were smaller than usual, if they were evoked after the injection of 100 µg mytolon.

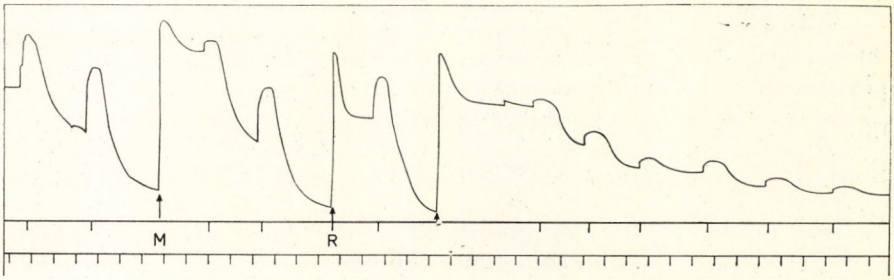

Fig. 9. Effect of stimulation of the CVc before and after ACh treatment. Middle curve — signals of the electrical stimulation. M — mechanical stimulation of the mantle; R — control injection; ↑ — 500 μg ACh

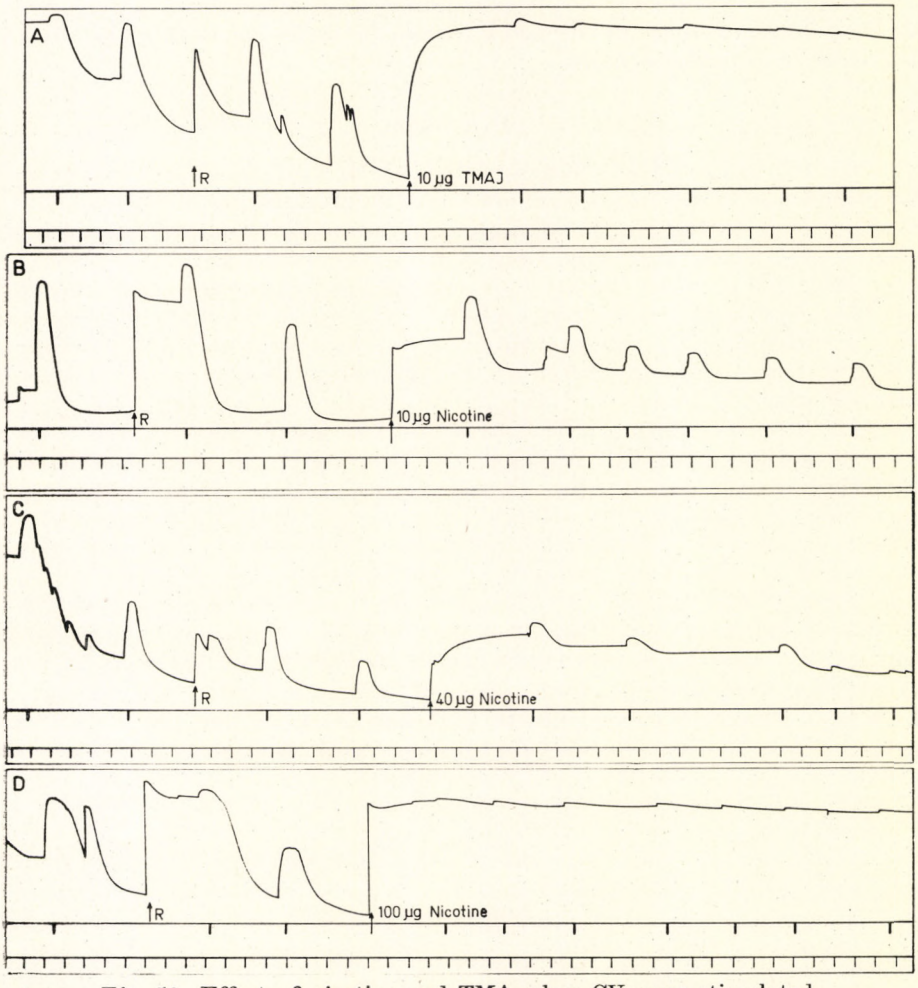

Fig. 10. Effect of nicotine and TMA when CVc was stimulated
A — 10 μg TMA; B — 10 μg nicotine; C — 40 μg nicotine; D — 100 μg nicotine

Combined injection of 5HT and adrenaline caused immediate increase of the tonus, followed by a considerably rapid relaxation. At the same time an increase of the rhythmic activity was observable. In one case *(Fig. 11A)* the increase of the tonus, in others *(Fig. 11B)* the increase of the frequency of the rhythm was dominant. After large doses the relaxation was prevalent *(Fig. 11C)*.

We could not observe any similar effects after applying 5HT and ACh together.

After injecting 5HT the nicotine tonus was either very temporary or it was absent completely, and sometimes the increase of the rhythm occurred.

In case of injecting 10 μg 5HT and 100 μg BOL-148 together, the effect of 5HT was dominant. Increasing the dose of the BOL (500 μg), besides the relaxation a considerable increase in the frequency of the rhythmic activity appeared.

Combined application of adrenaline and dibenamine (200—200 μg) resulted in the decrease of the level of tonus and the increase of the rhythmic activity in most of the cases.

Discussion

On the basis of the results obtained some conclusions can be drawn for the presence of cholinergic, adrenergic and tryptaminergic mechanisms. There are significant differences, as adrenaline, noradrenaline and 5HT were effective in low doses, while ACh acted only in high concentrations.

ACh, TMA, nicotine, mytolon, adrenaline, noradrenaline, dibenamine and BOL-148 caused tonic contraction of the adductor. In contrary to this 5HT, tryptamine, tyramine and in large dose also dopamine evoked considerable decrease of the adductor tone. The rhythmic activity was increased by catecholamines, ergometrine, dibenamine, BOL, mytolon and TEA .

The tonus-inhibiting effect of 5HT is in accordance with the results obtained for the ABRM of *Mytilus* (Twarog, 1967). As after stimulation of the CVc causing the relaxation of the adductors the 5TH content increases in the muscle (Salánki and Hiripi, 1969), the natural relaxing role of 5HT seems very probable. BOL did not antagonize the relaxing effect of 10 μg 5HT even in 500 μg amount, nevertheless increased the rhythmic activity.

Our results on the effect of the ACh are in agreement with other data so far that it increased the tonus in high doses. However, the sensitivity was significantly less than it was found for the ABRM and on Gastropoda preparations (Twarog, 1960; Jaeger, 1962; Burnstock et al. 1967). The effects of TMA and nicotine correspond to that described for the ABRM (Twarog, 1954, 1959). In contrary to this, mytolon and ACh did not cause antagonistic effects, distinguishing the *Anodonta* adductor from the ABRM of *Mytilus* (Twarog, 1960). Jaeger (1962) reported also the ACh-potentiating effect of the mytolon.

Adrenaline is considered to be a less potent relaxant on ABRM as 5HT is (Hoyle, 1964), while according to Jaeger (1963) adrenaline is an effective tonus-increasing and poore rhythm-increasing agent on penis retractor. Geiger (1929) found on *Anodonta* that adrenaline increased the tonus and the rhythmic activity when added to the whole animal, and this effect could be prevented by ergotamine.

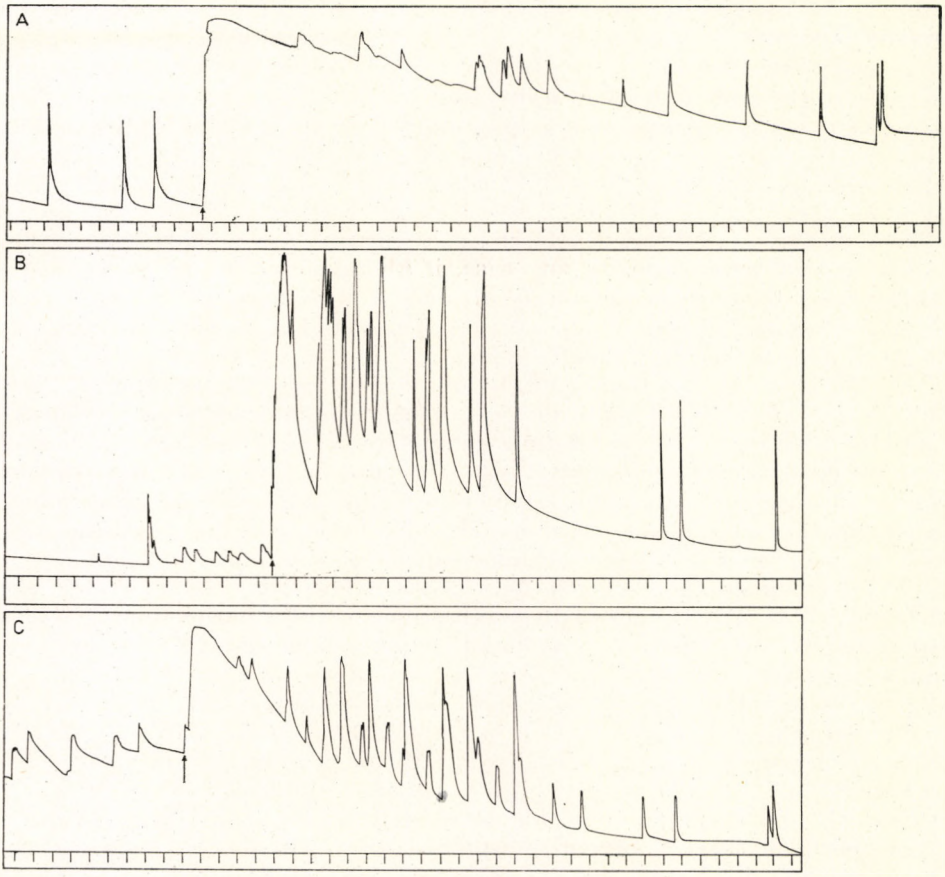

Fig. 11. Effect of 5HT and adrenaline injected together
A and B — 10—10 μg
C — 100—100 μg

In our case both adrenaline and noradrenaline resulted in the increase of the level of tonus and of the rhythmic activity.

When 5HT and adrenaline were injected together there was in the rhythmic activity a high and long lasting, while in the tonic contraction only a moderate and temporary increase, referring to the partial antagonism of these substances. 5HT decreases tonus and under such circumstances the rhythm-increasing effect of the adrenaline becomes prominent. Similar effect was demonstrated on ABRM of *Mytilus* by TWAROG (1954) to the combined application of ACh and 5HT, while on the penis retractor of snails JAEGER (1963) described the decrease of the tonic and the increase of the phasic responses when tryptamine and adrenaline were used together.

On the basis of our experiments we suppose that in the adductors of *Anodonta* an adrenergic-tryptaminergic antagonism exists. Consequently, the pharmacology of the *Anodonta* adductor differs from both the ABRM of *Mytilus* and the radula protractor of *Busycon* and *Buccinum* where ACh-5HT

antagonism was demonstrated (TWAROG, 1954; HILL, 1958; FÄNGE and MATTISSON, 1958).

The physiological role of a cholinergic system remains questionable because of the large dose of ACh necessary to evoke any definite effect, however, it cannot be excluded completely. As the combined effect of ACh and eserine does not differ significantly from that of the ACh, the relatively high cholinesterase activity of the adductor (SALÁNKI et al. 1967) may not be responsible for the ACh insensitivity. The effects of nicotine and TMA may be considered as a result of direct depolarization occurring on non-cholinergic receptor of the muscle membrane. In any case, if a cholinergic system does exist, it differs from the customary, maybe in such a way, that the mediator is not ACh. Among cholinolytics only atropine prevented in some cases the effect of ACh, dTC proved to be ineffective.

The shape of the tonic contractions caused by ACh, nicotine and TMA differs from that caused by adrenaline and noradrenaline. The development of the latters resemble the catch-tone characteristic in physiological conditions introduced by rhythmic contractions. This may indicate, however, that the site of the action of adrenaline and noradrenaline is not the muscle membrane itself but they play a role in the mobilization of a tonus increasing substance or they are triggering a molecular mechanism causing tonic contraction.

Agonistic-antagonistic relations were not unequivocal among adrenergic drugs, e.g. both adrenaline and dibenamine increased the muscle-tone. However, applying 100—100 μg adrenaline and dibenamine together, the decrease of the tonus-level is observed.

Comparing our results with those found in the glochidia of *Anodonta* it is iteresting to note that the lack of the effect of cholinergic drugs on these latter is more expressed, even TMA and nicotine were ineffective on the larvae (LÁBOS et al. 1964). At the same time there is a similarity in the presence of adrenergic and tryptaminergic regulation (LÁBOS et al. 1964; LÁBOS, 1966). In respect of tryptaminergic substances some difference exists between adult mussels and larvae: in the former both 5HT and tryptamine were effective relaxants while in the latter the rhythm was influenced only by tryptamine.

The effect of BOL-148, increasing both rhythm and tone, may be interpreted with the antiserotonin character of the lysergic acid derivatives. This may be valid also for the ergometrine, however, the direct effect of the latter cannot be excluded either. It is interesting to note, that ergometrine proved to be a very potent excitator of rhythm also on the neurones of the *Helix aspersa* (WALKER, 1968).

Summary

The spontaneous activity as well as the evoked responses of the posterior adductor of *Anodonta cygnea* were investigated after intramuscular injection of cholinergic, adrenergic and tryptaminergic substances. We found:

1. Ach in large doses, TMA and nicotine in low concentrations cause tonic contraction. Mytolon and TEAC increase the frequency of the spontaneous phasic contractions. Prostigmine, eserine, curare, hexamethonium and atropine are ineffective or act indefinitely.

2. Adrenaline, noradrenaline, and IPNA cause tonic contraction and increase the rhythmic activity. Dopamine and tyramine in large doses increase the rhythm but inhibit the tone. Dibenamine in large dose acts similarly to adrenaline. Ergotamine and DCI are ineffective.
3. 5HT inhibits both tone and rhythm, tryptamine decreases tone but enhances rhythm. BOL-148 increases both tone and rhythm, while ergometrine results an increase in the rhythm.
4. With stimulation of the cerebro-visceral connective the tonic contraction caused by ACh could be relaxed to some extent while the TMA and nicotine tonus remaind nearly intact.

It is supposed, that in the regulation of the adductor actvity in the fresh water mussel an adrenergic-tryptaminergic antagonism exists.

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KOLINERG, ADRENERG ÉS TRIPTAMINERG ANYAGOK SZEREPE TÓNUSOS MOLLUSCA-IZOM *(ANODONTA CYGNEA* L.) SZABÁLYOZÁSÁBAN

Salánki János és *Lábos Elemér*

Összefoglalás

Anodonta cygnea hátsó záróizmába adott intramuszkuláris injekciókkal vizsgáltuk az izom tónusos és ritmusos működését és a cerebrovisceralis konnektívum (CVc) ingerlésével kiváltott válasz befolyásolhatóságát. Azt találtuk, hogy

1. ACh csak nagy dózisban, a TMA és nikotin kis koncentrációban is tónusfokozó. Ritmusfokozó a mytolon és a TEAC. Hatástalan, ill. nem egyértelmű hatásúak a prosztigmin, ezerin, dTC, hexamethonium és atropin.

2. Az adrenalin, noradrenalin, IPNA tónus és ritmusfokozóak. A dopamin és tyramin nagy dózisban ritmusfokozó és tónusgátló. A dibenamin nagy koncentrációban ritmus és tónusfokozó, ergotamin, DCI hatástalan.

3. Az 5HT tónus- és ritmusgátló, a tryptamin tónusgátló és ritmusfokozó, a BOL tónus- és ritmusfokozó, az ergometrin igen kifejezett ritmusfokozó.

4. A CVc ingerlésével a TMA és nikotin-tónus nem, az ACh-tónus viszonylag jól ernyeszthető.

A záróizomműködés szabályozásában feltehetően adrenerg-szerotoninerg antagonizmus játszik szerepet.

РОЛЬ ХОЛИНЕРГИЧЕСКИХ, АДРЕНЕРГИЧЕСКИХ И ТРИПТАМИНЕРГИЧЕСКИХ ВЕЩЕСТВ В РЕГУЛЯЦИИ ТОНИЧЕСКОЙ МЫШЦЫ БЕЗЗУБКИ

Я. Шаланки и *Э. Лабош*

Внутримышечным введением веществ в заднюю запирательную мышцу беззубки были изучены тоническая и ритмическая реакции мышцы, а также видоизменение ответа, вызванного раздражением церебро-висцерального коннектива (ЦВК). Было установлено, что:

1. ТМА и никотин в низких концентрациях, а ацетилхолин в высоких дозах вызывают усиление тонуса. Митолон и ТЕАС увеличивают тонус. Простигмин, эзерин, d-тубокурарин, гексаметоний и атропин являются неэффективными, вернее их эффект неоднозначный.

2. Адреналин, норадреналин, изо-пропил-норадреналин увеличивают и тонус и ритм. Дофамин в высоких концентрациях увеличивает ритм и угнетает тонус. Дибенамин в высоких концентрациях является усилителем ритма и тонуса. Эрготамин и ДЦИ неэффективны.

3. 5ОТ тормозит ритм и тонус, а БОЛ увеличивает оба процесса. Эргометрин значительно увеличивает ритм.

4. Раздражением ЦВК, тонус вызванный ацетилхолином, почти полностью расслабляется, но эффект ТМА и никотина остаются без изменения.

В регуляции деятельности запирательной мышцы по всей вероятности имеет значение адренергический-серотонинергический антагонизм.

ANNAL. BIOL. TIHANY | 36 | 95—107 | HUNGARIA 1969

DIURNAL RHYTHM OF ACTIVITY IN FRESHWATER MUSSEL (*ANODONTA CYGNEA* L.) UNDER NATURAL CONDITIONS

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Received: February 20th, 1969

It is of common knowledge that the majority of living organisms exhibit a marked daily rhythm not only in their motor activity but also in the activity of certain organs and cells (ASCHOFF, 1965; BÜNNING, 1964; HARKER, 1964, SOLLBERGER, 1965). Periodic rhythmicity was noted in numerous Molluscs, especially in marine species, correlated with ebb and tide and diurnal alternation of illumination (BROWN, 1957; RAO, 1954; SALÁNKI, 1966).

Laboratory experiments showed that a certain diurnal periodicity can be demonstrated in the valve movements (activity of the adductor muscles) of freshwater mussels (SALÁNKI, 1964; SALBENBLATT and EDGAR, 1964) but these investigations are, however, only of a restricted value as regards estimation of the "natural" activity and distribution of activity of these animals. The purpose of the present work was to study whether or not the motor activity (shell movement) in animals kept in natural environment shows a daily periodicity and if it does, what are the factors regulating it.

Method

Experiments were conducted on specimens of freshwater mussel (*Anodonta cygnea* L.) collected from the lakes in the environs of Tata. Prior to the experiments the animals were kept in storage containers placed in Lake Balaton. Recording of the adductor muscles was made by means of a special device which required no fixation of the animal and caused no serious impediment in its movement. The apparatus consists of a resonator coil of an oscillator mounted on one valve and of a sensory resonator coil mounted on the other. As the detected current depends on the distance between the two coils, the valve movements will appear as changes in the current. These changes are then registered by a writing recorder. The technical description of the apparatus was given elsewhere (VÉRÓ and SALÁNKI, 1969). The weight of the coils mounted on the valves is less than 1% of the animal's bodyweight.

The activity of the adductor muscles recorded in the course of the experiments is the main behaviour reaction of the animal (BARNES, 1955) which is closely connected with respiration, nutrition and filtration activity (SALÁNKI and LUKACSOVICS, 1967).

Evaluation of the curves of activity was made on the basis of a division into 4 daily periods (from 0 to 6, from 6 to 12, from 12 to 18 and from 18 to 24 hours) from the viewpoint of periodicity (active and inactive periods) and rhythmic activity (quick rhythmic shell movements in the active phase), as illustrated in *Fig. 1.* On account of the low frequency of shell movement division in shorter periods of time seemed to be unnecessary. Changes in the temperature of the water and in barometric pressure were also noted.

The investigations were performed in 1968, in two periods: during spring (from 7 May to 27 May) and autumn (from 7 September to 9 October). During the experiments the activity of 4 mussels were recorded simultaneously. The animals' environment was identic during the entire period of experiment as they were placed in Lake Balaton at 2 m depth and at 4 m distance from each other, there the animals could freely move.

Results

Considerable differences were noted between the 4 animals *(Fig. 1)* as regards the character and frequency of activity. Great individual variations were noted in the period of activity of the animals examined characterized by rapid adductions of the valves. Detailed analysis of the data indicate that a certain regularity suggesting a daily periodicity of some degree exists in the seemingly irregular and greatly varying activity of the *Anodonta.* In this regard, some differences are noted between the rhythmical behaviour of the animals in spring and in autumn.

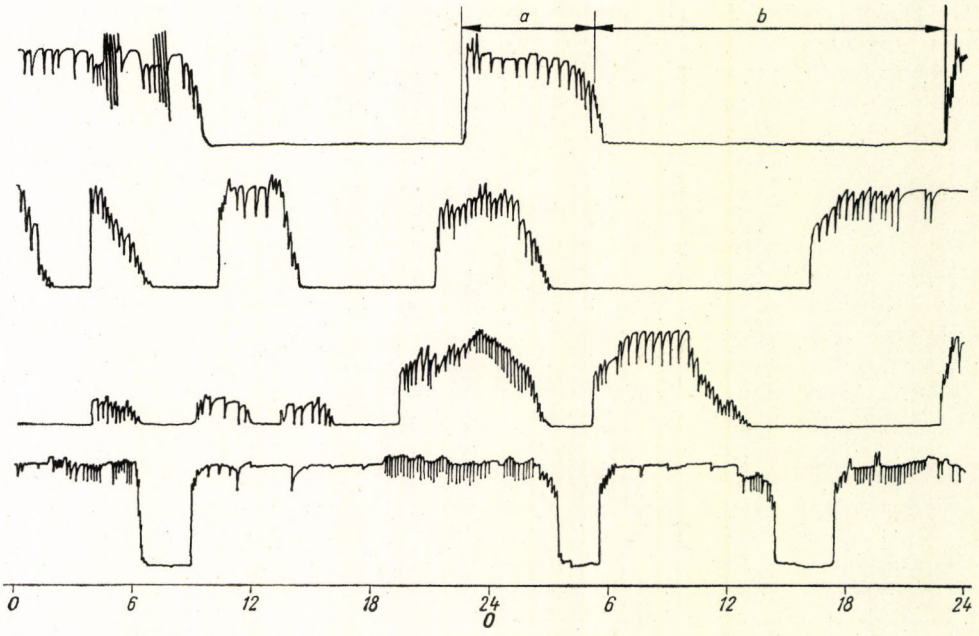

Fig. 1. Recording of activity (2×24 hours) in four animals.
a = active period; b = rest period

1. *Diurnal periodicity of active and quiescent periods*

As the duration of active periods may vary from some hours to several days the diural activity is of mono- or polyphasic in character. In the majority of the cases one or more periods of quiescence were noted in a day *(Fig. 2)*. Study of the daily periods of activity revealed that the animals were somewhat more active in the night hours (from 18 to 24 and from 0 to 06 hours) than in day-time (from 06 to 12 and from 12 to 18 hours). *Fig. 3* shows the data obtained in the spring and autumn experiments considering as 100% the activity measured from 6 to 12 hours. The relatively high deviation in the values is due to the rather great individual differences noted in the animals. The duration of activity corresponded — both in spring and in autumn — to about 50% of the entire experimental period. Comparison of the results obtained in spring and in autumn as regards the duration of active periods in the night and in the day. Mean values of 4 animals showed that in spring the animals were by 40% more active from 18 to 06 hours than in autumn when their activity was by 22.2% longer at night than in the period from 06 to 18 hours.

Individual analyses of active and rest periods of the animals revealed that in spite of great varieties, a certain periodicity of activity can be recognized. This refers first of all, to the time of initiation of the active, respectively rest periods. Out of the 8 animals examined 7 showed more closures in the first half of the day (0—12 h.) as compared to the number of openings. In one animal the distribution of activity and quiescence was exactly 50%. By "closure" we mean cessation of any movement of the animal and firm closure of the valves. This period lasts until the next "opening" of the shell, i.e. the initiation of a new active period. Data regarding the numerical distribution of closures and openings in the two daily periods (0—12 and 12—24 hours) are given in *Table 1* and *Fig. 2*. Apparently, there are individual variations in the number and duration of active and quiescent periods but in none of the cases were noted more openings than closures from 0 to 12 hours. Naturally, the number of openings was higher from 12 to 24 hours.

Table 1

Numerical distribution of closures and openings per animal, in two daily periods, on the basis of data recorded during the entire experimental period

Season	Number	*0—12 hours*		*12—24 hours*	
		Closure	Opening	Closure	Opening
Spring	A-1	10	2	7	15
	A-2	18	15	20	24
	A-3	19	12	12	19
	A-4	3	3	4	4
Autumn	B-1	23	11	14	25
	B-2	10	8	11	12
	B-3	7	6	8	9
	B-4	19	10	6	16
Closure/opening ratio:		*1.63*		*0.66*	

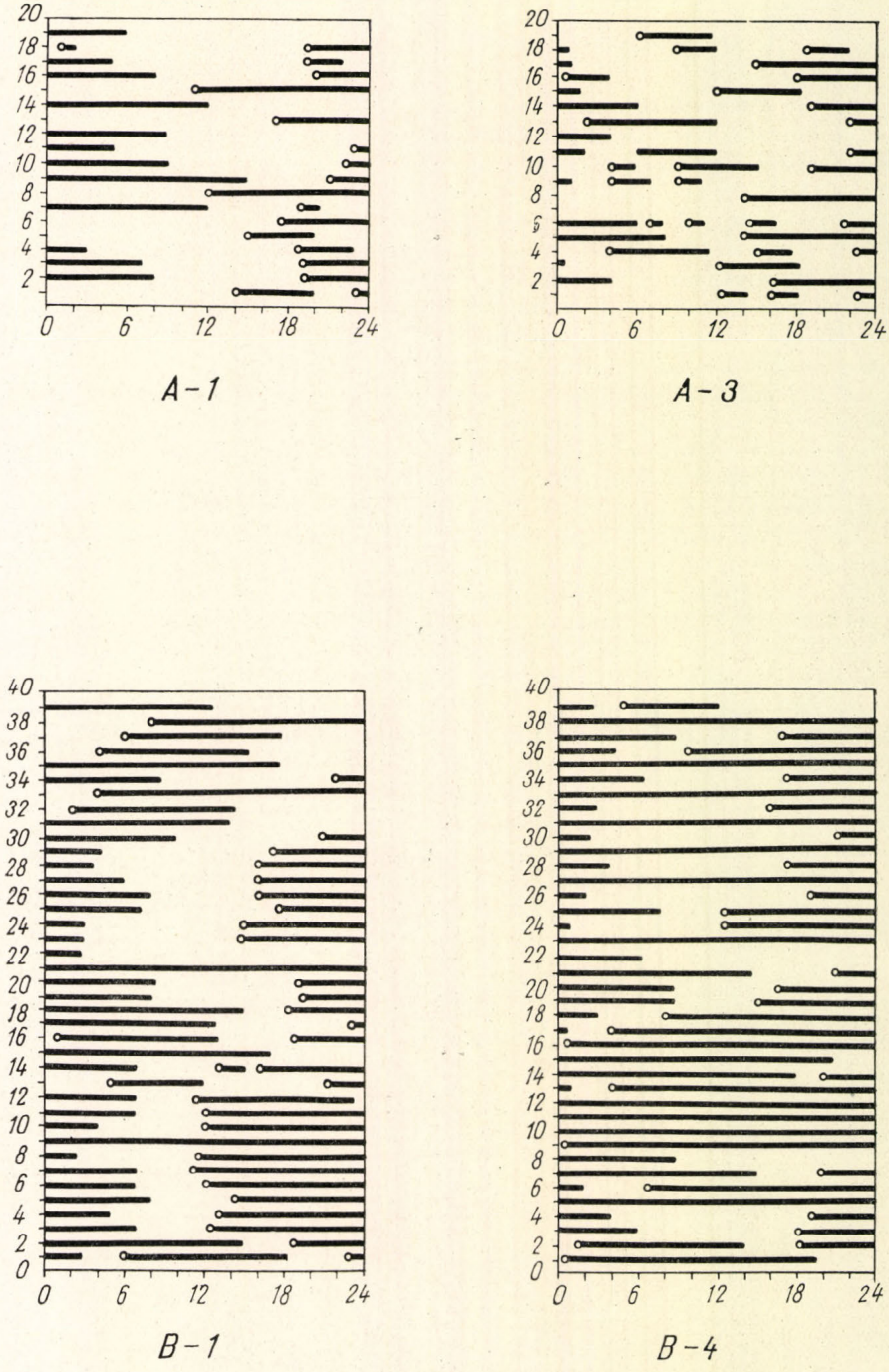

Fig. 2. Graph showing the times of active and rest periods related to 2—2 animals in spring (A-1 and A-3) and in autumn (B-1 and B-4). The heavy line marks the active period.
Ordinate: days of experiment; *abscisse:* hours

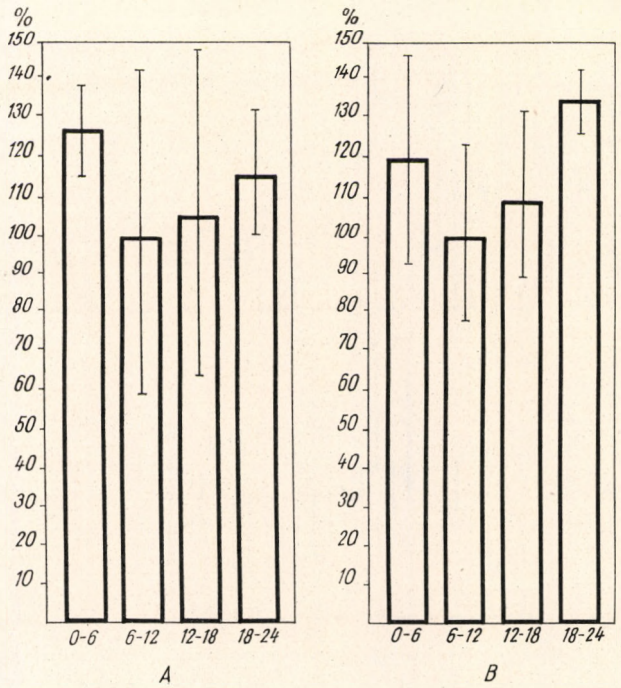

Fig. 3. Daily distribution of total activity of 4 animals in spring (A) and autumn (B) related to the activity recorded in the second daily period

2. *Diurnal distribution of rhythmic activity*

During the period of activity, when the shell is open, from time to time a relatively rapid contraction of the adductor muscles followed by a relaxation occurs. *Fig. 4* shows the daily distribution of this rhythmic activity on the basis of data obtained from parallel recordings on groups of 4 animals. The difference between the total sum of contractions during the noctural and diurnal period was highly significant both in spring and in autumn ($P < 0.001$).

The daily distribution of rhythmical valve movements was studied further from two viewpoints:

a) With each animal we have counted the contractions (c) occurring in each daily period and summed up for the whole experimental period. Then the sum of contractions of all the animals in each daily period was related to one hour of activity:

$$\frac{\sum_{}^{n} c_i}{\sum_{}^{m} t}$$

b) We have calculated for each daily period the mean value of frequency characteristic of the active period $\left(\frac{c}{t}\right)$ and then the mean values for identic daily periods were calculated

$$\frac{\sum_{i=1}^{n} f_i}{n} \quad \text{where } f = \frac{c}{t}$$

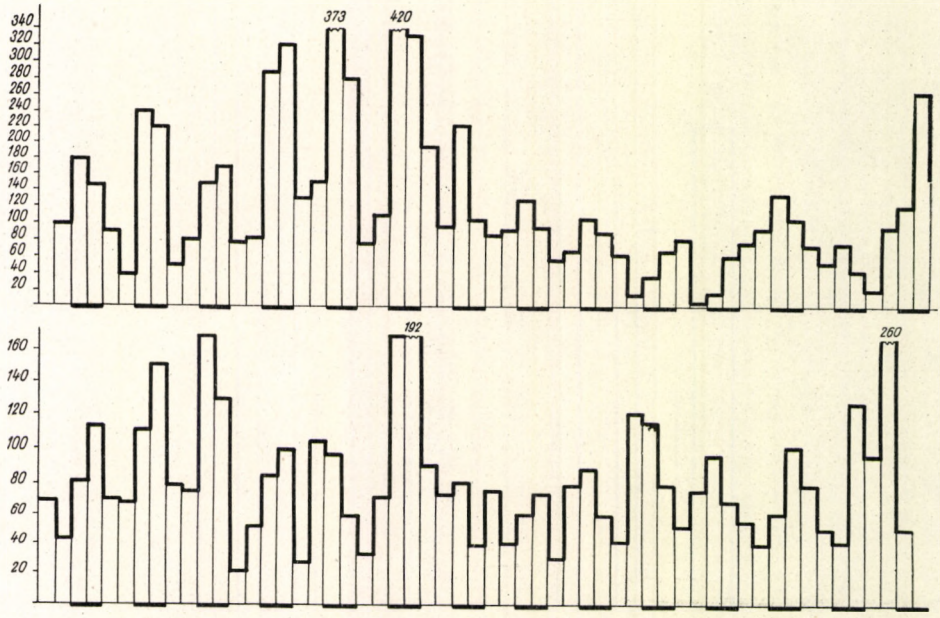

Fig. 4. Daily distribution of rhythmical activity (valvular movements) in the various periods of the day during 14 successive days. Total activity of four animals. Above: spring period; below: autumn period Thick lines on the abscissa — night periods.

In this way we have calculated both the mean value of rhythmical activity per hour in the active periods (mean frequency) and the mean value of frequencies of rhythmical activity per hour observed in a given daily period (frequency-mean) *(Fig. 5* and *6)*. From the data of experiments performed in spring and in autumn it was concluded that the mean frequency of rhythmical activity is higher in the night (from 18 to 06 hours) than in the day-time

Table 2

Diurnal distribution of mean frequency of rhythmical activity related to the length of the active periods

Season	Number	Mean frequency of rhythmical activity (cph)			
		0—6 hours	6—12 hours	12—18 hours	18—24 hours
Spring	A-1	10.661	8.972	7.405	16.200
	A-2	12.173	8.720	9.666	9.672
	A-3	7.983	6.500	7.000	8.450
	A-4	7.556	3.687	3.511	10.179
Autumn	B-1	6.348	2.617	3.647	7.478
	B-2	4.127	5.583	4.067	4.666
	B-3	1.739	0.852	1.138	0.940
	B-4	4.554	5.624	6.304	4.188

(06—18 h) but if the individual values are considered this was a constant finding only in the spring series *(Fig. 5)*. Some partial results obtained in the autumn series were not consistent with these observations *(Table 2)*. —In this regard it should be mentioned that in spring the mean value of rhythmical activity in the daily period from 18 to 06 hours was 50% higher than from 06 to 18 hours, while in autumn this difference was found to be only of 14%.

Study of daily distribution of frequency means likewise revealed higher values in rhythmical activity from 18 to 06 hours than from 06 to 18 hours. This finding too, was constant only in the spring series *(Fig. 6)*. In addition,

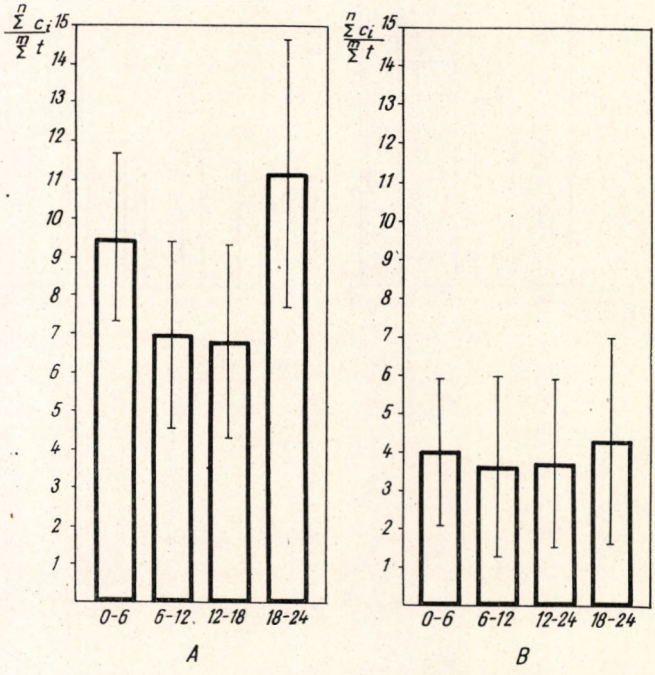

Fig. 5. Daily distribution of mean frequency of rhythmical activity. A = in spring; B = in autumn.

it was noted that in spring the spectrum of frequency-mean had its maximum at a higher value than in autumn. This was equally noted in all the animals examined *(Fig. 7)*.

On the basis of the results it may be concluded that rhythmical activity per hour recorded in the active period shows a daily fluctuation, indicating more activity in the night than in the day-time. This fluctuation is, however, not significant and not even observable in certain periods, e.g. in autumn when there was nearly no difference between the frequencies of rhythmical activity in the dark and light periods. But if the more prolonged nocturnal activity is also considered *(Fig. 4)* the diurnal periodicity of activity is evident.

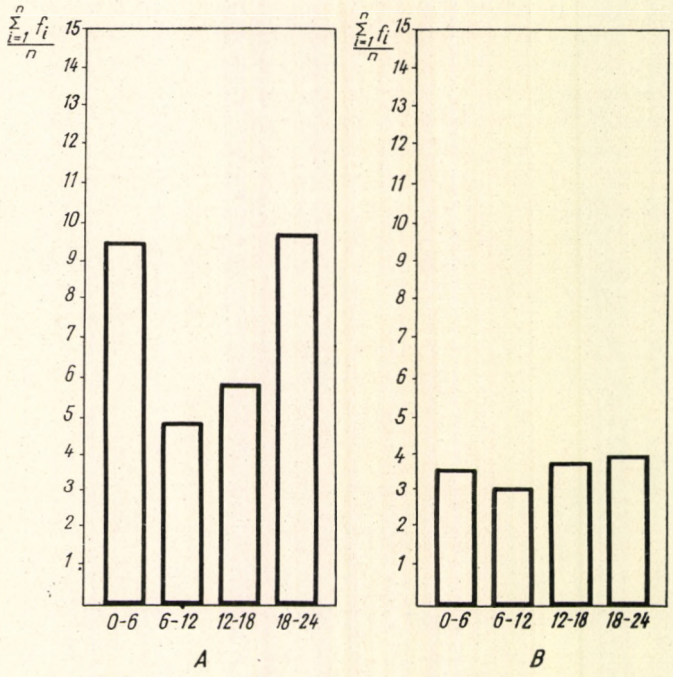

Fig. 6. Daily distribution of frequency-mean of rhythmical activity.
A = spring; B = autumn

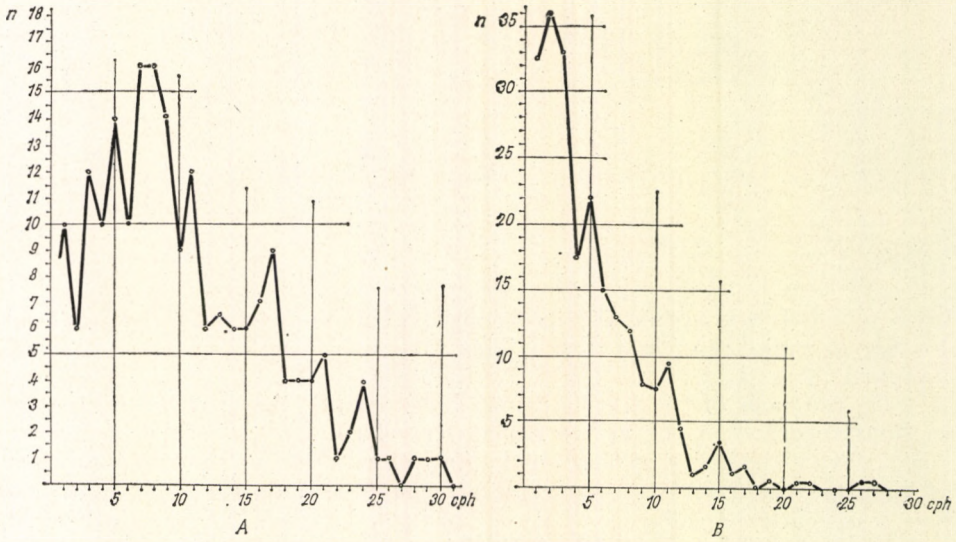

Fig. 7. Frequency spectrum of rhythmical activity.
A = spring; B = autumn

3. *Dependence of rhythmical activity on light, temperature of water and barometric pressure*

Numerous authors have described the daily fluctuations of rhythmical activity depending on illumination, temperature and barometric pressure (BÜNNING, 1964). We have therefore, investigated whether or not the changes in activity can be explained by the fluctuation of physical factors of the environment.

The diurnal fluctuation of light is evident. In addition, the intensity of light can be influenced by sunshine and turbidity of the water. In 1968 244 hours of shunshine were recorded in May, 192 in September and 156 ain October. At any rate, the difference between nocturnal and diurnal illumination may influence the periodicity of activity.

The temperature of the water was measured twice a day: at 8 and at 14 o'clock. The average daily fluctuation of temperature of the water measured at 1 m depth was found to be 2° C in May and 1° C in September. Maximum temperature was usually measured at 14 o'clock. The fluctuation of temperature compared with rhythmical activity showed that parallel with the decrease of temperature in September from 20° C to 11° C the mean frequency of rhythmic activity of *Anodonta* decreased considerably *(Fig. 8)*. The correlation coefficient (r) between the decrease of water temperature and valve activity was 0.62. This difference is significant ($P < 0.001$).

No correlation was demonstrable between barometric pressure and rhythmical activity either in a 24-hour cycle or in a period of several weeks.

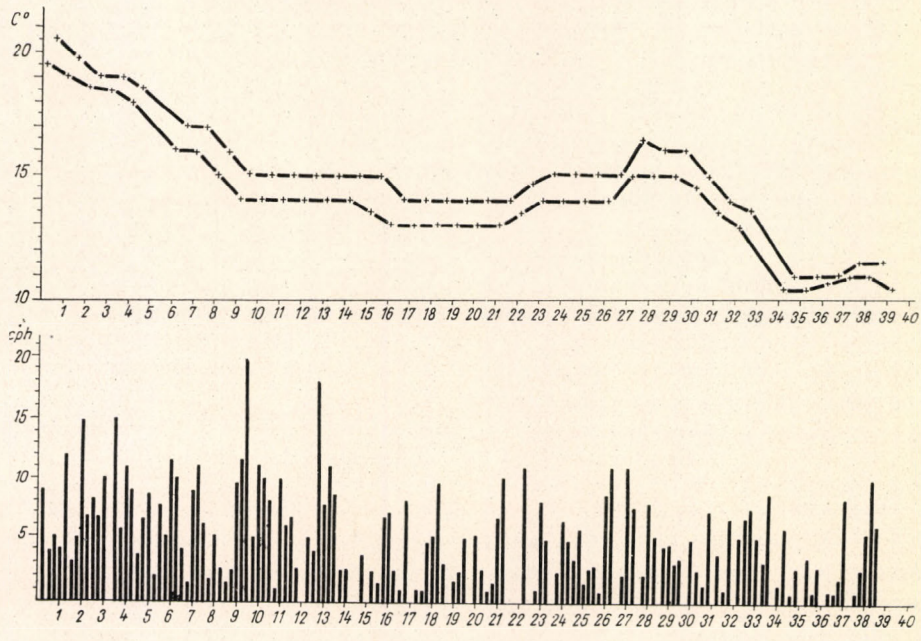

Fig. 8. Temperature of water (above) and frequency of rhythmical activity (B-1 animal) in autumn period. The curves of water temperature have been lined from data of measurements at 8 o'clock and at 14 o'clock.
Abscisse: number of days

Discussion

In the course of previous laboratory experiments we have demonstrated a diurnal fluctuation of low amplitude in the rhythmical and periodical activities of the *Anodonta cygnea* (SALÁNKI, 1964). As the laboratory conditions (t°, illumination and other "laboratory noises") differ considerably from the natural environment of the animal, the diurnal fluctuation observed may be the result of various other circumstances. With the method employed in the present work the recordings were made under natural conditions by which the possibility of external "artificial" effects was reduced to the minimum.

The present investigations also revealed a daily fluctuation in the activity of the adductor muscles of *Anodonta*. Valvular activity was greater in the night than in the day owing to two components acting in one direction. First because the open periods of the shell are longer in the night than in the day and secondly, because the mean frequency of rhythmical activity is higher in the night period. If the actual activity is recorded in which both factors are involved, a marked periodicity in rhythm results, as shown in *Fig. 4*. Similarly, consistent diurnal changes were noted in the times of closures and openings of the valves with all animals examined, though great varieties in frequency of valve movements and in the duration of active periods were noted.

The daily distribution of periods of activity and that of rhythmical activity, as well as that of valvular closures and openings indicate that in spite of an irregular distribution of activity and great varieties in the alternation of active and rest periods, the fresh water mussel in natural environment is more active by night than by day, being thus more related to nocturnal organisms. There are only partial shifts in activity correlated to various daily periods and the extent of fluctuation does not exceed 25% of the total activity.

The most conspicuous daily rhythms influenced by diurnal factors are those characterized by full activity during one part of the day and by complete rest in the other. Though numerous examples of such "phase synchronization" have been described (ASCHOFF, 1964), we usually see the modulating effect of diurnal changes on some kind of activity ("frequency synchronization") as opposed to complete triggering effect (SOLLBERGER, 1965). The manifestation of this partial synchronization is dependent on various factors and so the combination of other effects, the animal's sensitivity, its basic activityt, etc may all have an influence on it. This may explain the individual differences noted in the periodicity of activity and differences between the data of the spring and autumn experimental series.

Illumination, temperature and presumably changes in O_2 concentration of the water seem to be the main factors influencing the diurnal distribution of rhythmical activity in the freshwater mussel.

Daily fluctuation of light is a common natural phenomenon. One has to reckon with the effect of light in spite of the fact that the mussels live at the bottom of the lake, burrowed partly into the mud, whence the caudal end emerges into the water. As has been described on several marine bivalves (HECHT, 1919; KENNEDY, 1960) the syphons localized in the caudal part of these animals are sensitive to light. Light was found to have an inhibitory effect on the rhythmic activity of a marine bivalve (SALÁNKI, 1965) which is in agreement with our findings. According to laboratory investigations (SALBEN-

BLATT and EDGAR, 1964) valvular activity in *Anodonta* is greater in the dark period of the day than in daylight. The data of earlier investigations (SALÁNKI, 1964) showed that more closures occurred in the morning hours than in the second period of the day. As regards distribution of rhythmical contractions, our present results differed from earlier data obtained under laboratory conditions. The difference may be due to factors unrelated with light ("laboratory noise").

The regulating role of diurnal fluctuation of temperature seems to be supported by the finding that in spring when daily t°-fluctuation is greater, the amplitude of activity is likewise greater. This, however, does not explain why at lower temperatures sometimes (at night) increased activity and in other occasions (in autumn) decreased activity can be observed. SALBENBLATT and EDGAR (1964) noted more frequent valvular activity in *Anodonta* with increase of temperature. Our unpublished data confirm these findings. In our opinion it is the illumination and not the temperature that synchronizes the daily rhythm of *Anodonta* but at lower temperature the regulatory effect of illumination asserts itself less markedly. A possible explanation for the indistinct rhythmicity noted in autumn may be provided by the fact that in May there were about 25—30% more hours of sunshine than in September or October. This may produce a more intense daily illumination which may then reflet in rhythmicity regulated by light.

Oxygen supply seems to play an important role in the rhythmical activity of *Anodonta* as this mussel proved to be very sensitive to O_2 (SALÁNKI, 1965) and the oxygen content of the water (photosynthesis) may show a daily fluctuation parallel with illumination. The decrease in O_2 level causes enhanced respiratory activity i.e. an increase in freqency of rhythmicity. Such a mechanism may be involved in the increased activity noted in the dark period.

It is difficult to decide which of all these factors is predominant in the periodicity of activity. According to data in the literature (HARKER, 1964) light, respectively darkness is the most frequent factor regulating phasic activity in rhythms determined by external factors, while the effect of other factors (e. g. temperature) seems to be rather insignificant. Our findings seem to confirm the major role of illumination but a certain influence of other factors cannot be excluded. It may be assumed that the daily periodicity of valvular activity in *Anodonta*, basically of irregular distribution, is the result of the action of several factors.

Summary

From investigations on the periodic and rhythmical activity of *Anodonta cygnea* under natural conditions the following observations were made:

1. The duration of active periods is longer in the night than in daytime.

2. In the active periods the mean rhythmical activity and mean frequency are higher from 18 to 06 than from 06 to 18 hours.

3. In the daily fluctuation of valvular activity illumination seems to play a predominant role but the influence of other factors cannot be excluded.

4. Daily periodicity is not typical of the activity of freshwater mussel. Daily fluctuation of environmental factors synchronize to a certain extent the irregularly distributed activity of the adductor muscles determined by endogenous mechanisms.

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NAPI RITMUS A TERMÉSZETES KÖRÜLMÉNYEK KÖZÖTT TARTOTT TAVI KAGYLÓ *(ANODONTA CYGNEA* L.) AKTIVITÁSÁBAN

Salánki János és *Véró Mihály*

Összefoglalás

Természetes viszonyok között vizsgálva *Anodonta cygnea* periodikus és ritmikus aktivitását azt találtuk, hogy

1. az aktivitás időtartama az éjszakai órákban nagyobb, mint nappal,
2. az aktív periódusban észlelhető ritmikus aktivitás átlaga, valamint a frekvenciaátlag magasabb 18—06 óráig, mint 06—18 óráig;
3. az aktivitás napi ingadozásának kialakításában a fényviszonyok játszhatnak elsősorban szerepet, de nem zárható ki más tényezők jelentősége sem,
4. A napszakosság nem fő jellegzetessége a tavi kagyló aktivitásának, a környezeti tényezők napszakos ingadozása csak bizonyos fokig szinkronizálja az egyébként endogén mechanizmusok által meghatározott, rendezetlen eloszlású záróizomműködést.

СУТОЧНЫЙ РИТМ АКТИВНОСТИ БЕЗЗУБКИ В ЕСТЕСТВЕННЫХ УСЛОВИЯХ

Я. Шаланки и *М. Веро*

Изучая периодическую и ритмическую активность беззубки в естественных условиях, установили, что:

1. Продолжительность активности выше ночью, чем днем.
2. Среднее значение ритмической активности и частоты в активном периоде выше с 18 до 06 часов, чем между 06—18 часами.
3. В сохранении суточного колебания активности прежде всего условия освящения играют роль, но нельзя исключать и участие других факторов.
4. Суточный ритм является не главной характерной чертой активности беззубки: суточное колебание факторов окружающей среды синхронизируют только в некоторой степени безпорядочную деятельность запирательной мышцы, которая вообще находится под контролом эндогенных механизмов.

ANNAL. BIOL. TIHANY | 36 | 109—114 | HUNGARIA 1969

PHOSPHORYLASE ACTIVITY AND ITS INHIBITION WITH GLUCOSE-6-PHOSPHATE OF THE MUSSEL'S ADDUCTOR MUSCLE

GYÖRGY VEREB and MÁRTA CSORNAI

Medical Chemical Institute, Debrecen

Received: 2nd February, 1969

Numerous experiments prove the fact that glycogen phosphorylases of different origin (E. C. 2.4.1.1), though of similar function, significantly differ from one another, even when they originate from different organs of the same animal or when they come from the same organ of several species. First, differences were demonstrated by immunological methods (HENION et al. 1957; JÓKAY et al. 1958), later, even biochemical divergencies were shown in the different organs (BUEDING, 1964; SCHANE, 1965) and in the phosphorylases of the same organ of several species (METZGER, 1968).

In the course of our previous experiments it was found that the phosphorylases of the skeletal muscle in individual species differ greatly from one another in respect of allosteric inhibition (BOT et al. 1969; VEREB, 1969). Because the mussel's adductor muscle, possessing a special function, differs from the mammalian skeletal muscle, it was assumed that its phosphorylase also differs from the well known phosphorylase of the rabbit muscle both in allosteric characteristics and in the regulation of its activity.

During our experiments we investigated the total phosphorylase content of the mussel's adductor muscle both in stimulated and non-stimulated states, furthermore, the effect of stimulation exerted on the proportion of phosphorylase a and b forms, and the inhibition of mussel phosphorylase-a by G-6-P.*

Methods

We have prepared the relaxed adductor muscle of the freshwater mussel (*Anodonta cygnea* L.) in closed or just in opening phase effected by electric shocks on the cerebro-visceral connectivum. The weighted tissue sample was homogenized in glass homogenizator filled with 9 volume of 0.1 M NaF and 0.002 M EDTA buffer (pH 7.0) at 0 °C. The nucleotides also the AMP in high concentration, present in the homogenate were removed by the norite treat-

* *Abbreviations:*

G-6-P	glucose-6-phosphate
G-1-P	glucose-1-phosphate
AMP	adenosine-5-monophosphate
ATP	adenosine-triphosphate
EDTA	ethylendiaminetetraacetate

ment: 100 mg norite was added to 10 ml of homogenate (equivalent to 1 g of muscle) then it was agitated for 5 min at 0 °C, and finally centrifuged.

The activity of the phosphorylase present in the supernatant was determined by CORI method (CORI, 1955). The reaction mixture contained 1% glycogen, 0.016 M G-1-P, 0.05 M NaF, 0.001 M EDTA (AMP — when present — 0.001 M). Total volume 0.8 ml containing a muscle extract equivalent to 40 mg of wet weight muscle. Then incubation followed for 20 min at 30 °C at 6.8 pH value. The reaction was stopped by additional 3.2 ml of 5% TCA. The inorganic P liberated from G-1-P present in the aliquot part of the supernatant, was determined by TAUSSKY-SHORR method (TAUSSKY, 1953). The phosphorylase activity was given in units ;one unit quantity of the enzyme which produces 1 μmol of inorganic P in 1 min.

The Ba-salt of G-6-P was transformed into glucose-6-phosphoric acid by the aid of Varion KS kation-exchanging resin, then its pH was adjusted to 6.8 by adding NaOH. The glycogen prepared from rabbit liver then was freed from nucleotides by means of norite (HELMREICH, 1964).

Results and discussion

1. *Stimulation and phosphorylase activity*

Our first experimental observation was that the mussel adductor contains about one order of magnitude less phosphorylase than the mammalian skeletal muscle.

In order to compare the the relationship between phosphorylase activity and stimulated condition, extracts were prepared from muscles in a contracted state and in a relaxed state elicited by stimulation. The phosphorylase activity was determined both before and after treatment with norite. The values received are presented in *Table 1.*

Table 1

The phosphorylase activity of the contracted and relaxed adductor of the mussel before and after treatment with norite

	Contracted muscle			Relaxed muscle		
	activity	unit/g		activity	unit/g	
	—AMP	+AMP	$\frac{-AMP}{+AMP}$	—AMP	+AMP	$\frac{-AMP}{+AMP}$
Before norite treatment	4.08	4.00	1.0	3.20	3.20	1.0
After norite treatment	2.64	4.08	0.65	1.44	2.96	0.49

It is easy to see that the norite treatment does not cause a decrease in the activity of the total phosphorylase (+AMP), however, it does cause a significant decrease when the activity is measured without AMP. This points to the fact that the muscle extract contains a large amount of AMP which is removed by norite treatment. Thus the phosphorylase-a activity of the extract, as well the proportion of a/b may only be obtained correctly after norite treatment.

The activity of the phosphorylase-a in muscle relaxed is markedly decreased. This change in the phosphorylase-a is well shown in the proportion of values measured with and without AMP. It is clearly seen that while in the contracted muscle 65% of the total phosphorylase activity determined with AMP can be measured without AMP, on the other hand, in the relaxed muscle only 45% can be measured without AMP. Thus it may be assumed that during relaxation, part of the phosphorylase-a, is transformed into b in the relaxed adductor muscle.

It is an interesting phenomenon — differing from that of the mammalian skeletal muscle — that during the long closed period the phosphorylase of the contracted adductor muscle is found permanently in the a form. In the mammals the contraction period is shorter so is the occurrence of the a form of the phosphorylase. From this the following conclusion may be drawn: the phosphorylase-a plays a significant role in the energy supply of the adductor muscle in contraction.

In the mussel's adductor two kinds of muscle bundles — light and dark ones — can be discerned even macroscopically. We have investigated the phosphorylase activity of the lighter and darker muscle bundles in stimulated and unstimulated state. The phosphorylase content of either the lighter or darker muscle bundle did not differ from each other either in relaxed or in contracted state.

2. *The G-6-P inhibition of the phosphorylase-a activity in the adductor muscle*

As regards the high phosphorylase-a activity of the adductor muscle it seemed especially interesting to study the allosteric behaviour of the a form. Because the phosphorylase-a activity in the contracted muscle is high and it stays relatively high even in the relaxed muscle too, the possibility has arisen that the glycogenolysis of the mussel is controlled not only by the interchanging process of the two forms of phosphorylase but also largely by the allosteric regulation of the phosphorylase-a activity. Namely the glycogen in the relaxed muscle could be also mobilized by the phosphorylase-a, thus it would soon be depleted. The fact that this does not ensue is perhaps due to the inhibition of phosphorylase-a activity. For this purpose we have investigated the inhibitory effect of G-6-P on the a form of the phosphorylase-a present in the adductor muscle.

It is well known that the G-6-P exerts a pronounced allosteric inhibition on the b form of the phosphorylase in rabbit muscle. The observations regarding the G-6-P inhibition on the phosphorylase a form of the skeletal muscle in mammals, are rather diverging. Some authors say (Morgan, 1964) that the G-6-P does not exert an inhibitory effect on the a form of phosphorylase, others state (Helmreich et al. 1967) that the effect is uncertain, and it may only be small. Bot and his collaborators (1969), on the other hand, claim that phosphorylase-a of the mammalian skeletal muscle could be inhibited by G-6-P to a marked degree and only such phosphorylase-a, prepared in vitro from crystalline phosphorylase-b does not show G-6-P inhibition (Krebs and Fischer, 1962).

The native, or in vitro specially prepared phosphorylase-a could be inhibited to 30—50% by 10—20 mM G-6-P. This concentration of G-6-P is much greater than a similar inhibitory effect caused by G-6-P in phosphorylase-b.

Thus, considering the foregoing, it was rather unexpected to find that mussel's adductor phosphorylase-a activity is greatly inhibited by G-6-P even at a very low concentration. This is shown in *Fig. 1* where the percental inhibition of phosphorylase-a measured in the norite-treated extract of the mussel's adductor muscle is given *(Fig. 1)*.

It is readily seen that 5 mM G-6-P almost completely inhibits the activity of phosphorylase-a and thus a further 20 mM G-6-P does not cause a marked change in the inhibition. After the examination of this drastic effect of G-6-P we experimented with lower concentrations, too, and simultaneously studied the effect of AMP activator on G-6-P inhibition *(Fig. 2)*.

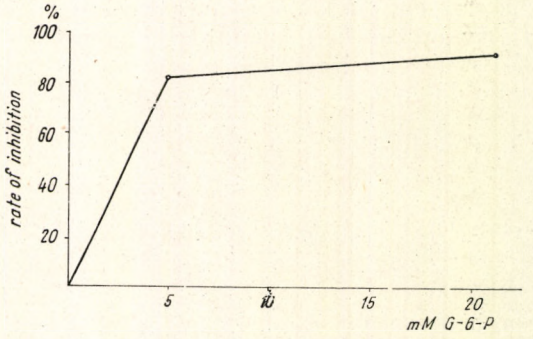

Fig. 1. The inhibitory effect of G-6-P exerted on the phosphorylase-a activity of the adductor muscle of mussel.
The percental inhibition of G-6-P on phosphorylase-a activity measured in the norite-treated extract of the closed mussel's adductor muscle. The measurement of the activity carried out is described in the Methods

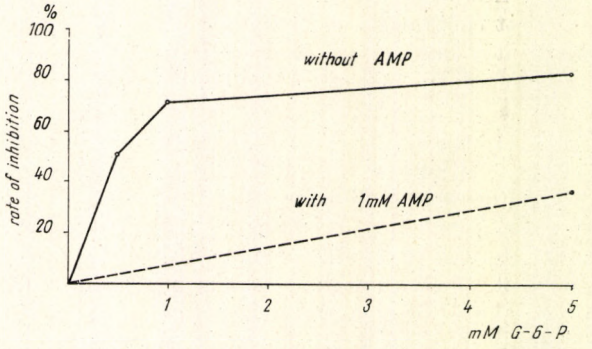

Fig. 2. The inhibitory effect of G-6-P on the activity of phosphorylase in the adductor muscle of mussel in the presence and absence of AMP.
Twice norite-treated adductor muscle extract of closed mussel. The proportion of activity is 0.46 without and with AMP

From *Figure 2.* it is easily perceiveable that a mere 0.5 mM G-6-P also causes strong inhibition. In raising the concentration of G-6-P the inhibition becomes more marked, too. It is obvious also that AMP suspends to a great extent the inhibitory effect of G-6-P on phosphorylase.

0.5 mM G-6-P inhibits mussel phosphorylase to about 50%, while phosphorylase-a of the rabbit skeletal muscle is inhibited, to the same extent only by the application of about 20 mM G-6-P. Thus, the influence of allosteric effectors plays a significant role in the regulation of mussel adductor phosphorylase. G-6-P, by means of its allosteric inhibition can decrease or regulate the rate of glycogenolysis, too. The concentration of AMP in tissue or the change in its concentration also may have an important role in hindering of G-6-P inhibition or in its arresting. Thus, the proportion of these two allosteric effectors may determine the actual activity of phosphorylase-a in the adductor muscle of the mussel.

The importance of this allosteric regulation is enhanced by the fact that 0.5—5.0 mM G-6-P concentration may be supposed to exist in the muscle under physiological, conditions in the different phases of glycogenolysis. Likewise, the concentration of tissue AMP corresponds to the concentration influencing the inhibition.

The extreme sensibility of phosphorylase-a present in the adductor muscle of mussel to the allosteric effectors suggests that in the muscle of mollusks the regulation of glycogenolysis differs from, that of animals capable of fast muscle contraction, e.g. mammals. The lower sensitibility toward allosteric effectors of phosphorylase-a in the higher animals makes it possible that the increasing rate of glycogenolysis may proceed idependently of the concentration of effectors on neural and hormonal influences by the transformation of allosterically sensitive phosphorylase-b into less sensitive phosphorylase-a. In the lower animals it is possible that besides the interchanging of phosphorylase- a and b in the regulation of muscle glycogenolysis an important role is played by the allosteric regulation of phosphorylase-a activity.

Summary

The quantity of phosphorylase-a during the relaxation of the mussel's adductor muscle decreases, and increases during the contraction. No difference can be shown between the activity of the darker and lighter muscle bundles.

The activity of phosphorylase-a in the adductor muscle of mussel could be markedly inhibited by G-6-P. This inhibition is to great extent suspended by AMP. We may conclude that the high allosteric sensitivity of phosphorylase-a plays a rather significant role in the allosteric regulation of glycogenolysis in the adductor muscle of mussels, and most probably, in the muscle of other lower animals too.

Acknowledgement

Authors would wish to thank Professor GYÖRGY BOT, whose altruistic help and useful guidance are greatly appreciated, and Dr. JÁNOS SALÁNKI, director of the Biological Research Institute of the Hungarian Academy of Sciences, Tihany, who has given ample means to carry out our investigations and with his useful advice gave us invaluable help and enables us to conclude our research work.

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KAGYLÓ ZÁRÓIZOM FOSZFORILÁZ AKTIVITÁSA ÉS ENNEK GLUKÓZ-6-FOSZFÁTTAL VALÓ GÁTOLHATÓSÁGA

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Összefoglalás

A foszforiláz-a mennyisége a kagyló záróizom elernyedésekor csökken, kontrakcióban fokozódik. A sötét és világos izomkötegek aktivitásában nem mutatható ki különbség.

A kagyló záróizom foszforiláz-a aktivitása G-6-P-val nagymértékben gátolható. A gátlást AMP felfüggeszti. A nagyfokú alloszterikus érzékenységből a glikogenolízis alloszterikus regulációjának jelentőségére következtethetünk a kagyló záróizomban és feltehetően más alacsonyabbrendűek izmában is.

АКТИВНОСТЬ ФОСФОРИЛАЗЫ ЗАПИРАТЕЛЬНОЙ МЫШЦЫ БЕЗЗУБКИ И ЕЕ ТОРМОЖЕНИЕ ПРИ ПОМОЩИ ГЛЮКОЗО-6-ФОСФАТА

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Общее количество фосфорилазы «а» снижается при расслаблении запирательной мышцы беззубки, а при ее сокращении увеличивается. Не удалось обнаружить разницу в активности фосфорилазы светлой и темной частей мышечных волокон.

Активность фосфорилазы «а» запирательной мышцы беззубки значительно тормозится под действием глюкозо-6-фосфата. Это торможение устраняется при даче АТФ. Принимая во внимание большую степень аллостерической чувствительности, делается вывод о важности аллостерической регуляции гликогенолиза в запирательной мышце беззубки и других мышцах беспозвоночных.

ANNAL. BIOL. TIHANY | 36 | 115—122 | HUNGARIA 1969

HISTOCHEMICAL INVESTIGATION OF CYTOSOMAL LIPIDS IN THE NEURONS OF *ANODONTA CYGNEA* L. UNDER NORMAL AND ANOXYBIOTIC CONDITIONS

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Received: 4th February, 1969

The cytosomes are pigment-containing granules of unknown function, generally occurring in the neurons of Molluscs and other invertebrates (NOLTE et al. 1965). Electron microscopically they proved to be transformed mitochondria (ZS.-NAGY 1968a) showing respiratory enzyme activity, too (ZS.-NAGY 1967a, ZS.-NAGY and KERPEL-FRONIUS 1969). It has earlier been ascertained that these granules contain a lot of lipide and beta-carotene in the neurons of *Anodonta cygnea* (LÁBOS et al. 1966). The cytosomes react upon experimental influences with significant histochemical and ultrastructural changes. For instance the staining of cytosomes with paraldehydefuchsin disappears in anoxia (BARANYI and SALÁNKI 1967) and the matter of high electron density diminishes (ZS.-NAGY 1968a). This latter phenomenon called our attention to the significance of cytosomal lipids. However, there are no data concerning their nature. Only some biochemical reports are available on the phospholipids of Molluscan nervous system (Kreps et al. 1968). This is why the aim of our present work was to analyse histochemically the cytosomal lipid content in normal state and in prolonged anoxia.

Material and methods

The investigations were carried out on the cerebral, visceral and pedal ganglia of 12—20 cm long specimens of *Anodonta cygnea* L. Apart from the normal animals we investigated mussels in prolonged anoxia, too. The anoxia was brought about by covering of the water surface with liquid paraffin layer of 2 cm thickness. The approximately 2 litres of water can be regarded as physiologically anoxybiotic under such conditions on te third day (SALÁNKI 1965). For this reason our observations were made on the 4th—7th days. The majority of animals survived anoxia lasting 6—7 days at 10—15 °C. Anoxia was also brought about by keeping the animals in air at 10—15 °C. In such a case the shells closed and the animal was obviously not able to utilize the oxygen of the air. The majority of animals endured well this type of anoxia, too, for 6—7 days. Our investigations were made in the period of July—January.

The prepared ganglia were cut on cryostat generally in 10 micron thickness, the sections were mounted on slides, generally Ca-formol fixation was used except in cases of controlled chromation. Our material was investigated with the following methods:

1. *General lipid stains*

a) Oil Red O (LILLIE 1944)
b) Aqueous Phosphine 3 R (VOLK and POPPER 1944).

2. *Phospholipid stains*

a) Alcoholic Sudan Black B (SBB) in paraffin sections (MCMANUS 1946. cit: PEARSE 1964).
b) Propylene glycol-SBB (CHIFFELLE and PUTT 1951)
c) Acid haematein (BAKER 1946)
d) Luxol Fast Blue (KLÜVER and BARRERA 1953)
e) Nile blue sulphate (CAIN 1947)
f) Nile blue sulphate (MENSCHIK 1953).

The method mentioned under *c)* was also carried out after pyridine extraction (BAKER 1946) as well as after controlled chromation (ELFTMAN 1954). This latter treatment was also applied to the methods mentioned under *b), d)* and *e).*

3. *Glycolipid stains*

a) Alcoholic alpha-naphtol (DIEZEL 1954)

4. *Acetal lipid stains*

a) Plasmal raction (HAYES 1949)
b) Plasmal reaction (CAIN 1949).

5. *Detection of cholesterol*

a) Investigation of fresh cryostat sections under polarization microscope (LISON 1953)
b) SCHULTZ-method in modification of WEBER et al. (1956).

Results

There are no differences between the three ganglia, thus the following hold good for all three of them. The identification of staining localizations simplified by the yellow pigment content of the cytosomes, which can easily be observed before staining and later can be identified with the stained structures.

A) *Normal animals*

1. *General lipid stains:* The cytosomes generally stain with Oil Red O (LILLIE 1944) in orange-red. In certain nerve cells, especially in the vicinity of the ganglion capsule, definitely red granules can also be seen. The neuropile does not react with this stain at all.

The application of fluorescence method (VOLK and POPPER 1944) is made again easier by the yellow autofluorescence of the cytosomes (ZS.-NAGY 1967b). Its observation in the untreated sections and the investigation of the same cells after staining readily assure the exact comparison of the localizations. The great majority of cytosomes does not react positively with Phosphin 3 R. They not only fail to give the silvery-white fluorescence, characteristic of this method, but even their autofluorescence disappears and only dark brown spots appear on their places. On the places of some cytosomes a weak green fluorescence appears while in others it becomes yellowish red. The periferic part of neurons devoid of cytosomes fluoresces also in yellowis red and the nuclei, nucleoli as well as the whole fibre mass of the neuropile have the same colour.

2. *Phospholipid stains:* The alcoholic SBB in paraffin sections results in an intensive black staining in the cytoplasm of the nerve cells in respect of localization corresponding to that of the cytosomes. The granules are often confluent thus the cytosomes are not always recognizable individually The staining with SBB in proplyene glycol, especially after controlled chromation shows a much better localization *(Fig. 1)*. The intensity of staining differs in the granules, however, the strongly stained forms of cytosomes are predominant. The neuropile also shows a weak sudanophilia of fine distribution.

The cytosomes stain with acid haematein (BAKER 1946) dark blue, bluish grey or black. The staining is particularly strong after controlled chromation. In a few cells only weak staining exists. The neuroppile also stains with the same intensity, localized on the axons *(Fig. 2)*. After pyridine extraction the staining of cytosomes and neuropile disappears completely *(Fig. 3)*.

Luxol Fast Blue stains the cytosomes blue. Using alcohol as solvent the staining is not very intensive, in several places loosenings, unstained spots are observable in the cytosomes. Changing the solvent to chloroform the staining becomes more intensive and the looseni ngs also disappear.

The Nile blue sulphat (CAIN 1947) method gives different results. In the period of July-September the cytosomes stained deep-blue and red spots occurred only sporadically. On the other hand, in the period of October-January the deep blue staining failed to appear and the cytosomes stained generally purplish-red. We failed to get any staining at all with the MENSCHIK (1953) Nile blue method. The sections stained with the dye, however, it completely disappeared during the treatment with aceton at 50 °C.

3. *Glycolipids:* We did not succeed in rendering probable the presence of glycolipids with the method used. We failed to get any red staining in cytosomes and even the extremely pale red colour of the neuropile can not be regarded as being positive.

4. *Acetal lipids:* The majority of cytosomes retained their original yellow colour after plasmal reaction both of HAYES (1949) and CAIN (1949). Some cytosomes however stained with the Schiff raegent. There are cells in which the negative (yellow) and positive (magenta red) cytosomes can be found together but in the majority of them only either the first or the second form occurs. The neuropile also shows an intensive magenta red staining. The staining fails to occur when $HgCl_2$ treatment is cmitted.

5. *Detection of cholesterol:* We did not succeed in observing birefringent structures in cytosomes. No spherits occur in them in unfixed or in fixed state. Likewise negative result was obtained by the Schultz method in every case.

B) *Results in anoxia*

There were no significant differences between the ganglia of animals kept in water closed with paraffin oil or in air.

1. *General lipid stains:* Oil Red O stains the cytosomes less intensively. There is a number of empty cytosomes and the red staining observed in near the ganglion capsule is no longer to be seen. The staining with Phosphin 3 R resulted no significant differences as compared with the situation observed in normal animals.

2. *Phospholipids:* The SBB staining of cytosomes changed. In both methods applied the intensity of staining diminished and the seemingly empty cytosomes increased *(Fig. 4)*. The intensity and area of acid haematein staining also decreased in cytosomes but somewhat increased in the neuropile. Luxol Fast Blue staining was also less intensive and a large number of empty cytosomes occurred. The Nile blue sulphate staining (CAIN 1947) changed in July-September to the effect, that the dark blue colour disappeared and the red staining was predominant. In October-January the red staining became somewhat weaker under the influence of the anoxia and it was localized mainly on the periphery of cytosomes while the inner regions seemed to be empty.

The investigations of glycolipids and acetal lipids gave the same results as in normal animals. Because of our negative results in demonstrating cholesterol in normal animals, we did not repeat these experiments in anoxia.

Discussion

Our results can be considered, in accordance with the generally accepted interpretation of the histochemical methods used (PEARSE 1964), as follows:

The positive results of Oil Red O staining (LILLIE 1944) show that there are neutral lipids in cytosomes. The amount is obviously variable evidenced by the occurrence of differently stained granules. This is in agreement with the electron microscopic observation (Zs.-NAGY 1968b), according to which the cytosomes contain homogeneous areas of medium electron density and of different size, that, as it is generally accepeted, are equivalent to neutral lipids. We can not explain the failure of demonstrating neutral lipids with Phosphin 3 R. Perhaps the carotenoids or other cytosomal components prevent the development of fluorescence. The dye certainly gets into the cytosomes, this is evidenced by the changes observed, but the reaction is not characteristic for the neutral lipids.

Besides neutral lipids the cytosomes contain a significant amount of phospholipids, too. The staining with alcoholic SBB in paraffin sections refers to this even in itself. The results of acid haematein staining and that of the connected controls (pyridine extraction, controlled chromation) unanimously confirm this. This agrees with the biochemical results of KREPS et al. (1968) who found 5 different phospholipids in the ganglia of Unio crassa also belonging to Pelecypoda. The different results with Luxol Fast Blue, using alcohol or chloroform respectively, as solvent, show, that the rate of sphyngomyelins is significant among the phospholipids (KLÜVER and BARRERA 1953). However, it must be considered very carefully, because KREPS et al. (1968) failed to

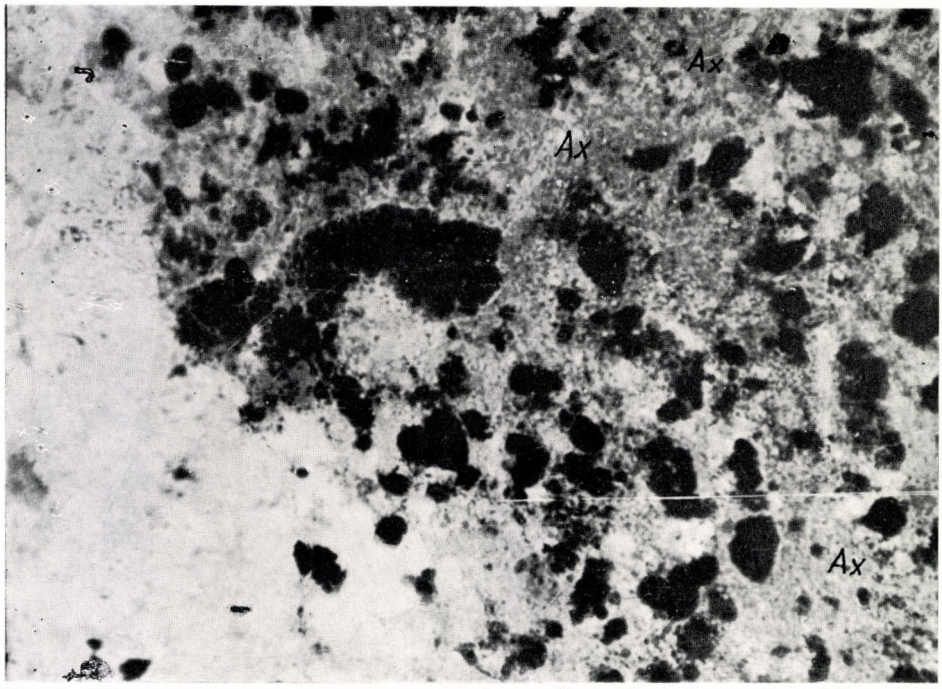

Fig. 1. Visceral ganglion of a normal animal. Controlled chromation followed by SBB staining in propylene glycol. The very intensive black staining is localized on the cytosomes. A weak, fine-granulated staining is to be seen in the axons (Ax), too. ×530.

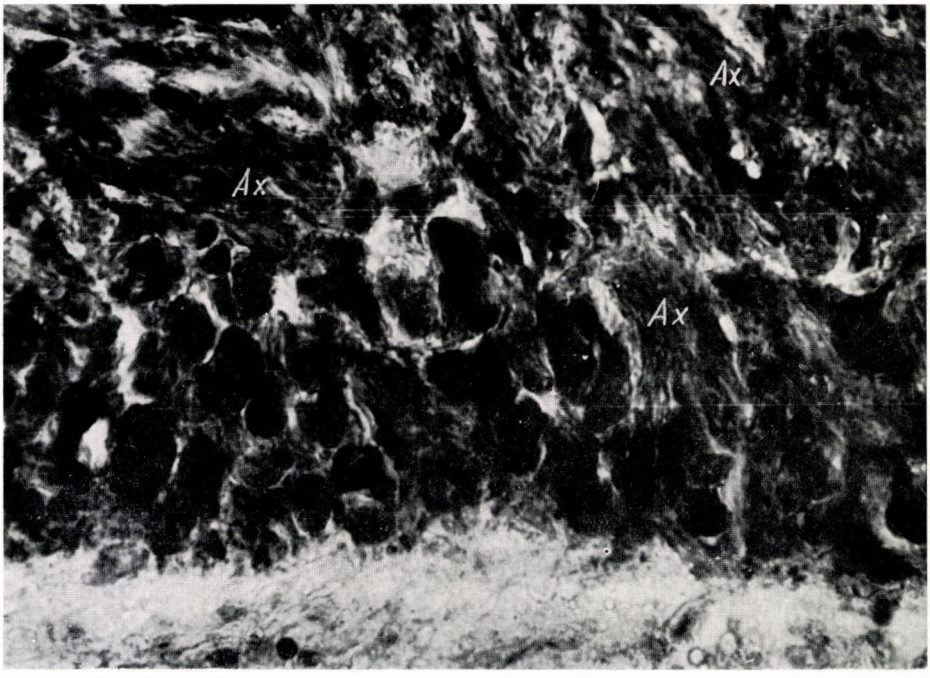

Fig. 2. Pedal ganglion of a normal animal. Controlled chromation followed by acid haematein staining according to Baker. The dark, confluent areas correspond to the mass of cytosomes and there is a staining in the axons (Ax) as well. ×530.

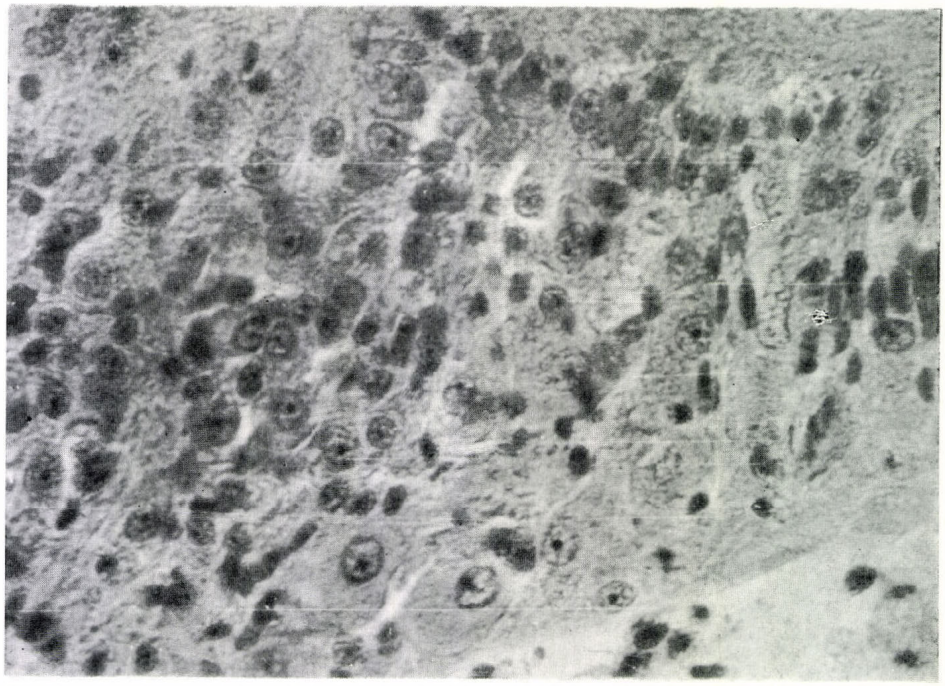

Fig. 3. Another section of pedal ganglion showed on *Fig. 2*, only after pyridine extraction (24 hours, 57 °C). The same staining as on *Fig. 2*. Only the nuclei of nerve and glial cells are stained. ×530.

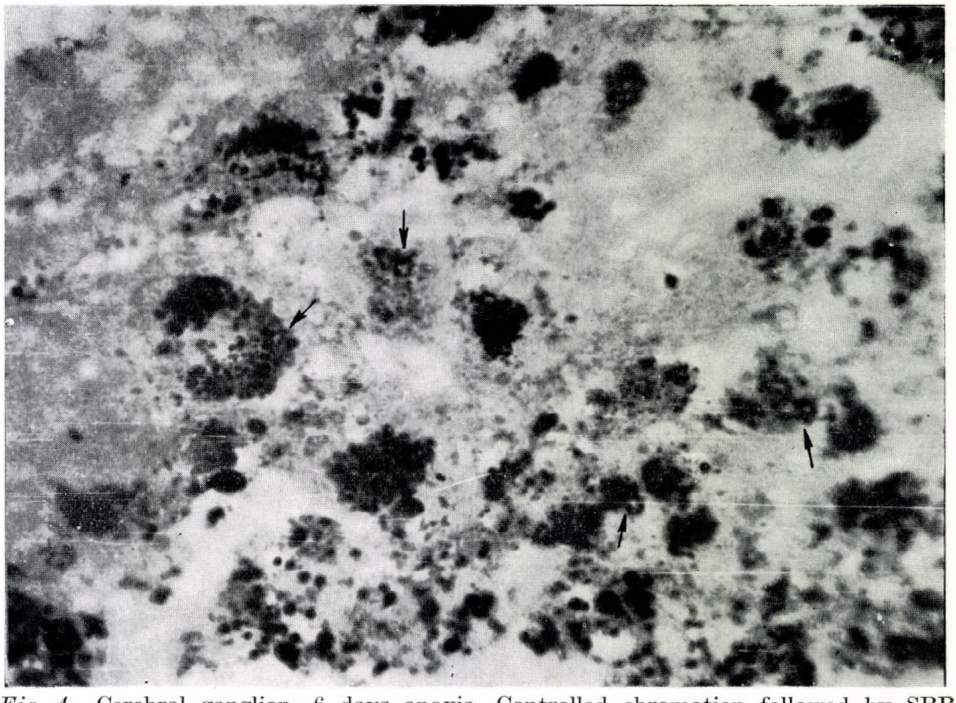

Fig. 4. Cerebral ganglion, 6 days anoxia. Controlled chromation followed by SBB staining in propylene glycol. The granules are more isolated than in normal animals because the staining is weaker. Arrows indicate cytosomes with empty core. ×530.

find biochemically any sphyngomyelins in the central nervous system of Unio crassa. On the basis of Nile blue staining one can conclude, that in the period of July-September the acidic lipids, probably lecithins are predominant in the cytosomes, while in October-January the neutral ones are so. Presumably there is a seasonal change in composition of lipid content. Several earlier data also refer to this effect (Zs.-Nagy 1967a). To the failure of Menschik method no explanation is offered.

From the negative results of Diezel (1954) method one could conclude to the absence of glycolipids either in the cytosomes or in other parts of the central nervous system. This is surprising because glycolipids, more exactly galactolipids amount a significant part of lipid content in the nervous system of higher animals (Pearse 1964). Obviously, certain glycolipids can be present whose carbohydrate component is not galactose, or for other reasons they have such a molecular structure which renders them unsuitable for detection with the method used.

It is obvious from the interpretation of the plasmal reaction (Hayes 1949, Cain 1949, Pearse 1964) and from our results that the Schiff positive forms of cytosomes contain acetal lipids, too. Likeweise the diffuse staining of the neuropile can be considered as a true plasmal reaction, taking into account the SBB and acid haematein positivity observed just here.

The existence of cytosomes showing plasmal positivity in different degrees, as well as their joint occurrence in the same cell, on the one hand, indicate the functional variability of cytosomes, on the other hand, however, can be a state mark of oxydative processes taking place in the cytosomes. Considering the respiratory enzyme activity found in cytosomes (Zs.-Nagy 1967a) as well as the strongly varying level of succinodehydrogenase activity in the single cytosomes (Zs.-Nagy and Kerpel-Fronius 1969), the assumption seems to be justified, that the redox state varies step by step in the cytosomes. This can be the reason of varying demonstrability of acetal lipids (Pearse 1964).

We failed to get any positive evidence for the presence of cholesterol. This obviously does not preclude the possibility of occurrence of small amounts in the nervous system. From this point of view the investigations are of special interest, where 17-beta-hydroxysteroiddehydrogenase was found histochemically in the periganglionic tissues of the ostrea (Mori et al. 1965, 1966). This indicates that the steroid compounds are not totally absent in the tissues of Molluscs, therefore the presence of cholesterol, as one of the starting substances of steroid metabolism, can not probably be left out of account.

The deficiency of oxygen significantly changed the lipid content of cytosomes. From the very low (less than 1 mg/l (Salánki 1965) oxygen level the animals are not able to take up, thus their tissues are physiologically in anoxia. The changes of lipid content can unequivocally be assumed so, that the anoxia caused the decrease both of neutral fat and phospholipid content of cytosomes. This is in agreement with our previous electron microscopic observations, where under the same conditions the decrease of the matter of high electron density was found in the cytosomes (Zs.-Nagy 1968a).

It is not known what is the fate of lipids emptied from the cytosomes. Their oxydative "burning" is not likely to be thought of in the anoxibiotic state. There is no example for lipid consumption without oxygen. Thus one have to assume a cytosomal mechanism offering the suitable conditions for

metabolism of lipids even in the case when no oxygen enters in from outside. As one component of such a mechanism may function even the beta-carotene (LÁBOS et al. 1966), which is well known to be able to transport electrons easily. On the other hand CHALAZONITIS (1961) observed the presence of haem-proteins in the pigmented area of molluscan neurons and this suggests the possibility of the rise of an oxygen reserve. Anyway the surprising fact, that the animals can bear such a long anoxia without damage shows, that there must be a system, making them able to endure this condition in contrast with the neurons of vertebrates, which are widely known to be very sensitive to oxygen deficiency. According to our investigations the lipids can take an important part in such a system.

The changes taking place in anoxia touch the whole function of the neurons. Probably there is a relationship between the increased rhythm of the main integrative function, i.e. of the periodic activity, observed in anoxia (SALÁNKI 1965) and the changed cytological state of the neurons.

Summary

The lipid content of cytosomes found in great quantities in the neurons of fresh-water mussel, is composed as follows: Neutral fats, phospholipids. In summer a lot of acidic lipids can also be found, which in autumn and winter disappears. Certain cytosomes and the neuropile contain acetal lipids, too. There was a failure in demonstrating glycolipids and cholesterol.

In long lasting anoxia (4—7 days) both the neutral fat and phospholipid content decreased. In summer the acidic lipids also disappeared. The acetal lipids did not show any significant changes.

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A CITOSZOMÁLIS LIPIDEK HISZTOKÉMIAI VIZSGÁLATA NORMÁL ÉS ANOXIÁS KÖRÜLMÉNYEK KÖZÖTT *ANODONTA CYGNEA* L. NEURONJAIBAN

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Összefoglalás

A tavi kagyló neuronjaiban tömegesen található citoszomák lipidtartalma következő komponensekből áll: neutrális zsírok, foszfolipidek. Nyári időszakban sok savanyú lipid is található, ami ősszel és télen hiányzik. A citoszómák egy része és a neuropil acetal-lipideket is tartalmaz. Glykolipideket és cholesterint nem sikerült kimutatni.

Tartós oxigénhiányos állapotban (4—7 nap) mind a neutrális zsír, mind a foszfolipid tartalom lecsökkent. Nyári időszakban is eltűntek a savanyú lipidek. Az acetal-lipidek nem mutattak lényeges változást.

ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ЛИПИДОВ ЦИТОСОМЫ В НЕРВНЫХ КЛЕТКАХ БЕЗЗУБОК В НОРМАЛЬНЫХ УСЛОВИЯХ И ПОСЛЕ АНОКСИИ

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Липиды цитосом, обнаруженных в большом количестве в нервных клетках беззубки, содержат следующие компоненты: нейтральные жиры и фосфолипиды. Летом в большом количестве обнаруживаются в них и кислые липиды, которые осенью и зимой отсутствуют. Нейропиль и определенная часть цитосом содержат также и ацеталлипиды. Гликолипиды и холестерин не были обнаружены.

После продолжительной аноксии (4—7 дней) наблюдалось понижение содержания и фосфолипидов и нейтральных жиров. В цитосомах кислые липиды не участвовали и летом.

В количестве ацетал-липидов не было обнаружено существенных изменений.

ANNAL. BIOL. TIHANY | 36 | 123—133 | HUNGARIA 1969

LIGHT AND ELECTRON MICROSCOPICAL INVESTIGATIONS ON THE ADDUCTOR MUSCLE AND NERVOUS ELEMENTS IN THE LARVA OF *ANODONTA CYGNEA* L.

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Received: 14th February, 1969

The physiological investigation of adductor muscle in adult mussel (PAVLOV, 1895, BARNES, 1955, KOSHTOYANTS and SALÁNKI, 1958, SALÁNKI, 1961, 1963) necessiated to study the larval adductor, too. In these investigations light has been thrown on many new physiological facts concerning the response of the adductor (LÁBOS and SALÁNKI, 1963, LÁBOS et al. 1964a, b, c, LÁBOS, 1964, 1966a, b, 1967, 1969, LÁBOS and TURCSÁNYI, 1966). Interpreting these results the fundamental question has arisen whether the adductor muscle is innervated or it functions automatically. There is no unequivocal answer to this question in the old histological works (SCHIERHOLZ 1888, LILLIE, 1895, HERBERS, 1913). Even recently, the investigations with supravital stainings could hardly carry us farther in the question than the data of old authors. Nevertheless, it has turned out well that certain cells having processes can be stained with methylene blue or crystal violet in scattered or characteristic localizations, the neuronal character of wich can not be stated again unequivocally (TÖRÖK and LÁBOS, unpublished). Therefore convincing proofs can only be given by electron microscopic investigations. There are however no such data in the literature.

The aim of our present work was to study the ultrastructure of the glochidia with special interest to the submicroscopic organization of neuromuscular junction. Therefore, our attention was focused first on the adductor muscle and the nervous elements, but several other important characteristics were also touched upon.

Material and method

For experimental purpose the glochidia of *Anodonta cygnea* L. were used between of November and March for in this period glochidia are commonly found in large numbers in the outer gills. They were fixed immediately, together with their mucous groundsubstance. For histological purposes paraffin sections fixed in Susa, stained with routine histological methods (haematoxylin-eosin, azan) and with chrome-haematoxylin-phloxin according to BARGMANN (1949) were used. After a 24 hour fixation even the shells could be cut to a 8—10 micron thickness. For electron microscopy the glochidia were fixed

in 2 per cent OsO_4 solution buffered with s-collidine (BENNETT and LUFT, 1959) for 30 min at 0 °C and subsequently 10 min at room temperature. This was followed by an alcoholic dehydration and through propylene oxyde the material was embedded into Araldite (Durcupan ACM, Fluka). Twenty-thirty glochidia were placed in each block. They all were in closed condition because their adductor contracted immediately on the influence of fixation.

The ultrathin sectioning of the larvae causes great difficulties. The animals at most 200—400 micron size have hard shells of chitin immediately

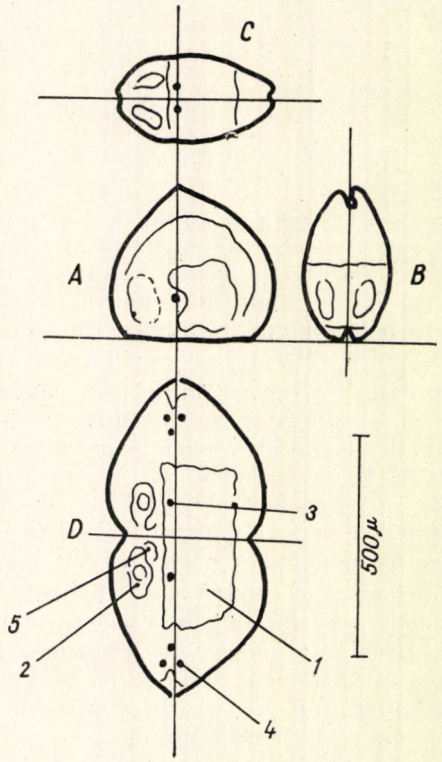

Fig. 1. The proportional sections of glochidia. A — sagittal plane, B — transversal plane, C — horizontal plane, D — opened animal. Meaning of numbers: 1 — adductor muscle, 2 — lateral pit, 3 — solitary sensory cell, 4 — lateral sensory cells, 5 — foot fold.

ruining the glass knives. Thus the following procedure has been adapted: the shells of the suitably oriented larva were trimmed in the block and the surface of cutting was always shaped within the shells of one animal trimming always out the shells. In this way, the sections were made in the mediansagittal, transversal and horizontal planes *(Fig. 1)* on LKB Ultrotom III.

Sections were contrasted with uranyl acetate and lead citrate (REYNOLDS, 1963). Micrographs were taken with TESLA BS 413A electron microscope. To identify the elements found in ultrathin sections easily also half-thick (1—2 micron) sections were cut and investigated in phase contrast.

Results

A) *Light microscopic morphology of the glochidium*

Our histological results coincide essentially with that of the old authors (LILLIE, 1895, HERBERS, 1913). The morphological features most important in rescpect of the interpretation of our electron microscopic results will be related below. The terminology has been wholly taken over from HERBERS (1913).

The larva consists of two halves symmetric to the mediansagittal plane. Its organs are commonly paired except the adductor muscle *(Fig. 2)* adhering on the inner surface of the shells, filling up the middle third of the dorsal half of the body. The adductor consists of smooth muscle cells. Ontogenetically it is not identical with the adductors of the adult animals, namely, it disappears completely during metamorphosis and definitive adductors arise from new anlages (HERBERS, 1913).

The adductor and the inner parts of the shells, unoccupied by the adductor, are covered by the larval mantle *(Fig. 2)*. On the oral and ventral side, the mantle consists of relatively large vacuolated and granulated epithelial cells. It is wholly of ectodermal origin. On its surface a thin stripe of different staining and refraction can be observed. The mantle is not held fast to the shell, there is an interstice between them wherein the myocytes are situated. They keep moving the parts of the mantle and the hooks.

Peculiar organs are situated in the areas behind the adductor *(Fig. 2)*. Here, the lateral pit and the foot fold are found, both of extodermal origin and being continuous with the larval mantle. As compared to them the entodermal sac is in dorsal while the mesoderm is in lateral position. This latter represents the common anlage of the heart, the pericardium and the genital organs. In these areas the cells are small, adhering closely and their nuclei stain strongly. The anlages of all organs of the adult mussel originate from this region. HERBERS (1913) refers to the fact that in overwintered animals the anlages of cerabral and visceral ganglia are to be found in the form of epithelial thickenings localized in the upper wall of the lateral pit.

Special formations of the larval mantle are represented by the so called sensory cells *(Fig. 3)*. There are four pairs of them. One pair is near the adductor, on its ventral side (solitary sensory cells), the other three near the apex of the shell beneath the hooks (lateral sensory cells). These cells overtower the surrounding mantle cells and bear long cilia on their surface. The basal bodies of their cilia are situated side by side and a fibrous bundle extends to the direction of the nucleus from them. This is particularly developed in the solitary sensory cells.

In the vicinity of the lateral pit, cilia can only indistinctly be recognized in histological sections but observing living animals under a microscope it is easy to see that there is an intensive cyliary activity causing an eddy of water here.

Larval nervous centers or diffuse nervous network have been mentioned neither in old literature nor found in our histological material. Chrome-haematoxylin-phloxin (BARGMANN, 1949) stains all the epithelial cells deep blue, therefore no specific elements can be differentiated by this method.

B) *Electron microscopic results*

1. The adductor muscle

The cells of the adductor muscle are of various shape in transversal section. Round, flattened and what is more stelliform as well as irregularly lobulated cross section figures can be found. The greatest diameter of the muscle cells is about 10 micron. The sarcoplasm is very poor in structure, composed by some mitochondria pressed close against the periphery of the cell, a number of larger or smaller vesicles and free ribosomes. Sometimes the sarcoplasm forms radial septa towards the nucleus thus parcelling up the filamentous substance. The cross sections of the nuclei are also irregular in shape. A denser chromatin substance adheres to the inner surface of the nuclear membrane. Relatively big nucleoli and a wide perinuclear space are seen with regular nuclear pores *(Fig. 4)*. The single cells are separated by seemingly empty, extensive intercellular spaces.

The contractile store consists of a thicker (about 200 Å) filament and a thinner (about 40 Å) one. The interfilamentar distance of the thick filaments varies between 800 and 1400 Å. Where the distances are uniform even a regular hexagonal pattern is observable *(Fig. 5)*. This is a frequent but not a common phenomenon. The thin filaments are arranged, however irregularly round the thick ones. In cross sections elements of high density and of about 800—1000 Å thickness occur among the filaments sporadically, wich correspond to the dense bodies of about 5000 Å length observed in longitudinal sections *(Fig. 6)*. Neither is their arrangement regular.

Among muscle cells axon-like structures of about 0,5—1 micron thickness occur frequently. Sometimes they almost completely enclose the muscle cells in cross section pictures *(Fig. 7)*, another occasion only their enlarged knob-like endings attach closely to the sarcolemma or invaginate deep into the muscle cell. They always contain commonly empty-core vesicles of about 400—1200 Å diameter *(Fig. 7)*, rarely, however, dense-core vesicles occur, too. Mitochondria are also present in the enlarged endings. The axolemma is closely connected with the sarcolemma. The two membranes are on certain places nearer to each-other than elsewhere and also a fine stripe appears in the intermembranic space. On the axonal side of these contacts clusters of vesicles *(Fig. 7)* can always be found and, what is more, the coalescence of vesicles into the axolemma can also be observed in some places. These contacts of axons with the muscle cells obviously correspond to the neuromuscular synapses.

The synapses are of various arrangement. There are cases when an axon contacts only one muscle cell, at other times it may even contact two or three muscle cells. We found also a situation when the axon surrounding the muscle cell showed several such junctions with the same muscle cell. It is again a further variation when two or three different axons form synapses on the surface of the same muscle cell.

2. The larval neurones

Flattened cells can be seen in a single layer under the epithelial cells of the larval mantle in close connection with them. These cells bear no resemblance to the mantle cells. In their cytoplasm and processes clumps of vesicles of the

same kind can be seen as in the nerve endings of the adductor muscle. These processes full of vesicles are connected with other ones by synapse-like structures *(Fig. 8)*. The connected membranes thicken and there is a cluster of vesicles on one side. Seldom dense-core vesicles occur, too, the great majority of vesicles, however, appears to be empty.

We could observe a nerve fibre entering the adductor muscle. In the immediate neighbourhood of the adductor there was a thick axon-like process containing mitochondria, a great number of microtubuli, membrane profiles of irregular shape and ribosome-like granules. On its surface 8—10 enlarged knob-like endings were situated. These latter were morphologically identical with the processes of the above related cells. The thick axon enters the adductor at right angles to the direction of muscle cells and its branching gives endings forming the neuromuscular junctions.

Axon-like structures can be found in the whole area of the mantle even among the epithelial cells. Some of them can be recognized as processes of the above related flattened cells, others contain in large numbers dense-core vesicles, again others contain no vesicular components, their inside is structureless and seems to be almost empty. All three kind of processes take part in forming synaptic contacts.

3. The sensory cells

The sensory cells are situated among the epithelial cells of the larval mantle, however, the former greatly differ from the latter. On their surface cilia are found among the microvilli. The cilia contain 9 peripheric and 2 central ciliary tubules *(Fig. 9)*. There is a special structural element near the basis of cilia but still out of cellular surface. The peripheric ciliary tubules fuse in a single tube of high density *(Fig. 9)* having also a closed basal plate. In its plane the cross section picture corresponds only to a dark spot. Under this plate appearing as a transversal dark stripe in longitudinal sections of the cilia, the peripheric ciliary tubules fused into a tube continue again. The outer membrane of cilia closely adhers to the tube of tubules above the dark stripe while under the level of this stripe it draws off again from the tube and join the cellular membrane only farther *(Fig. 10)*. The ciliary tubules penetrating into the inside of the cell, end in the basal body connected with peculiar ciliary rootlets. The rootlets open wide in the shape of a fan and enter deeply the cytoplasm. They are of much higher density than other components of the cytoplasm and there is a dark-bright periodicity in them *(Fig. 10)*. The dark stripe seems to be homogeneous and is of about 200 Å in width. The brighter one is about twice as wide and dark elementary filaments of about 20 Å thickness appear in it, oriented parallel to the longitudinal axis. A number of microtubuli, mitochondria of tubular character as well as multivesicular bodies can be found among the rootlets. All these components are embedded into a peculiar mainly smooth endoplasmic reticulum of a vesicular type. The rootlets get more and more thinner in deeper regions of the cytoplasm and completely disappear at the depth of about 5 microns *(Fig. 10)*.

The nucleus is localized on the cell basis. In its vicinity the cytoplasm contains Golgi apparatuses of luxuriant structure, profiles of granulated endoplasmic reticulum, a lot of free ribosomes, mitochondria and microtubuli. It is remarkable the presence of many mostly empty, sometimes dense-core

vesicles of about 400—1200 Å diameter, they may also constitute large groups *(Fig. 11)*. Such groups of vesicles can be seen attached to the cell membrane in places where axon-like processes surrounding densely the basal part of the cell adjoin to the outer surface of the cell membrane. The cell body of about 25 micron height reaches deeper than the epithelial cells and extends somewhat under them laterally.

4. The cells of the lateral pit and the foot fold

The structure of the epithelial cells of these areas differs in a particular way comparing to that of the larval mantle cells. The cells become narrow and extend to a considerable length into the depth. On their surface they have microvilli. Their nuclei are relatively large, elongate and rich in chromatin. The cytoplasm contains a lot of free ribosomes and mitochondria. There are some weakly developed Golgi apparatuses at places *(Fig. 12)*. In several cells the granular endoplasmic reticulum is also to be found in the form of parallel lamellae or Nebenkern-like structures. It was observed several times that the outer lamella of the nuclear membrane was continuous with the lamellae of endoplasmic reticulum *(Fig. 13)*. In certain cells structures resembling to the precytosomes of the adult mussels (Zs.-Nagy, 1969) were also found *(Fig. 14.)*. Among the epithelial cells there are also cells having no contact with the surface and showing processes as well as a very differentiated cytoplasm. Many dense-core vesicles of about 1000—1500 Å size, well developed Golgi apparatuses, endoplasmic reticulum of vesicular type and mitochondria of tubular character — are the components of these cells *(Fig. 15)*. The dense-core vesicles occur in the processes, too, Sometimes, these cells touch the muscle cells of the adductor directly, but we have never succedded in observing their processes penetrating the adductor.

The processes form a group in several places among the epithelial cells, like a neurpile *(Fig. 16)*. Some processes have no vesicular components, others contain dense-core vesicles, again others are filled up with larger vesicles of about 2000—3000 Å in diameter, resembling morphologically to the elementary neurosecretory granules. The empty vesicles found in the nerve fibres of the adductor do not occur here. In several cases we could see the processes of the epithelial cells join the neuropile directly. There are no synapse-like structures between the fibres. There occurs that one or more processes are closely connected to the surface of certain cells, these contactas, however, show no synaptic specialization.

5. Other submicroscopic features

The abundance of microvilli on the surface of all epithelial cells is remarkable. These are of about 1 micron in length and 0,1 micron thick *(Fig. 17)* and covered by a unit-membrane continuous with the cellular membrane. They generally branch near the cell surface forming a branched candlestick-like structure *(Fig. 18)*. There is a dense stripe of uncertain contour within, which seems to be empty cored in cross-section, being of tubular structure. Between the microvilli there exists a network consisting of very fine filaments and forming small vesicles on the outer surface of villus membrane *(Fig. 17)*.

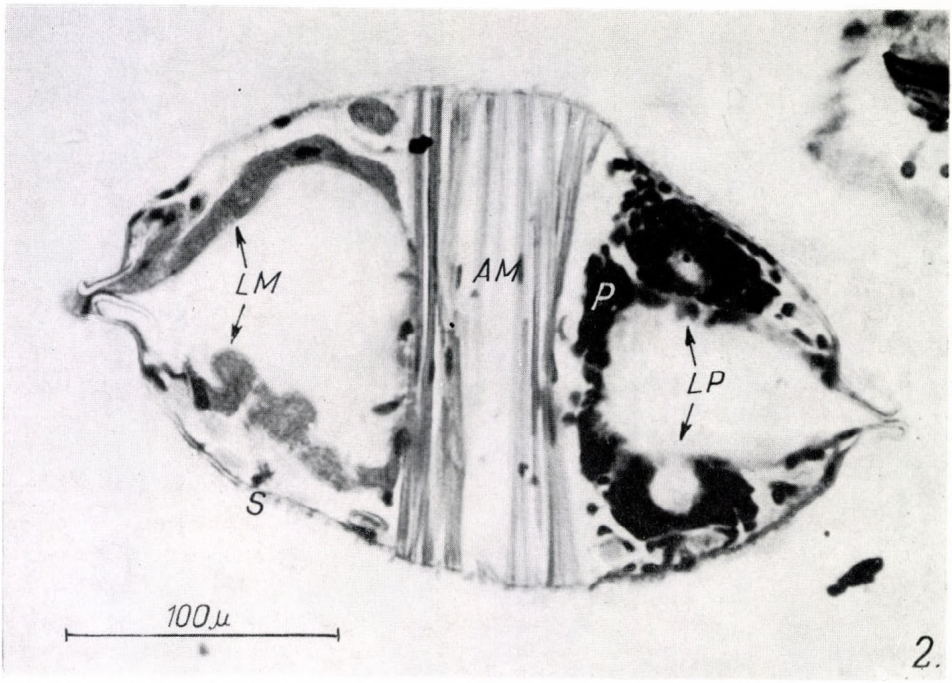

Fig. 2. The histological section of glochidium made in horizontal plane through the lateral pit. AM — adductor muscle, LP — lateral pit, P — foot fold, LM — larval mantle, S — shell, haematoxylin-eosin staining, ×370.

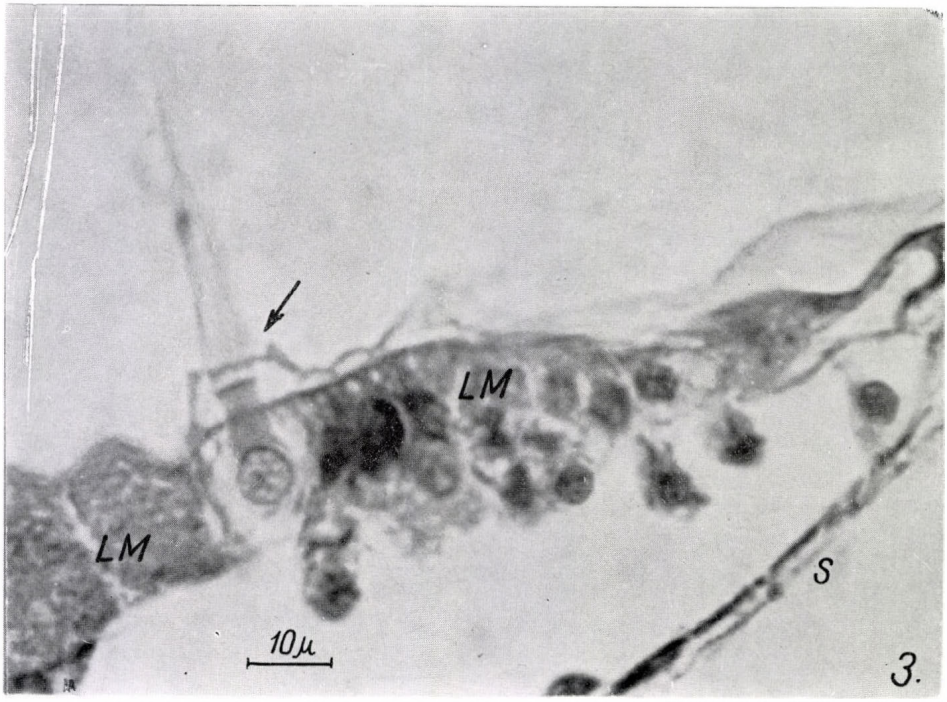

Fig. 3. The histological picture of the solitary sensory cell (arrow). LM — larval mantle, S — shell, haematoxylin-eosin staining, ×1000.

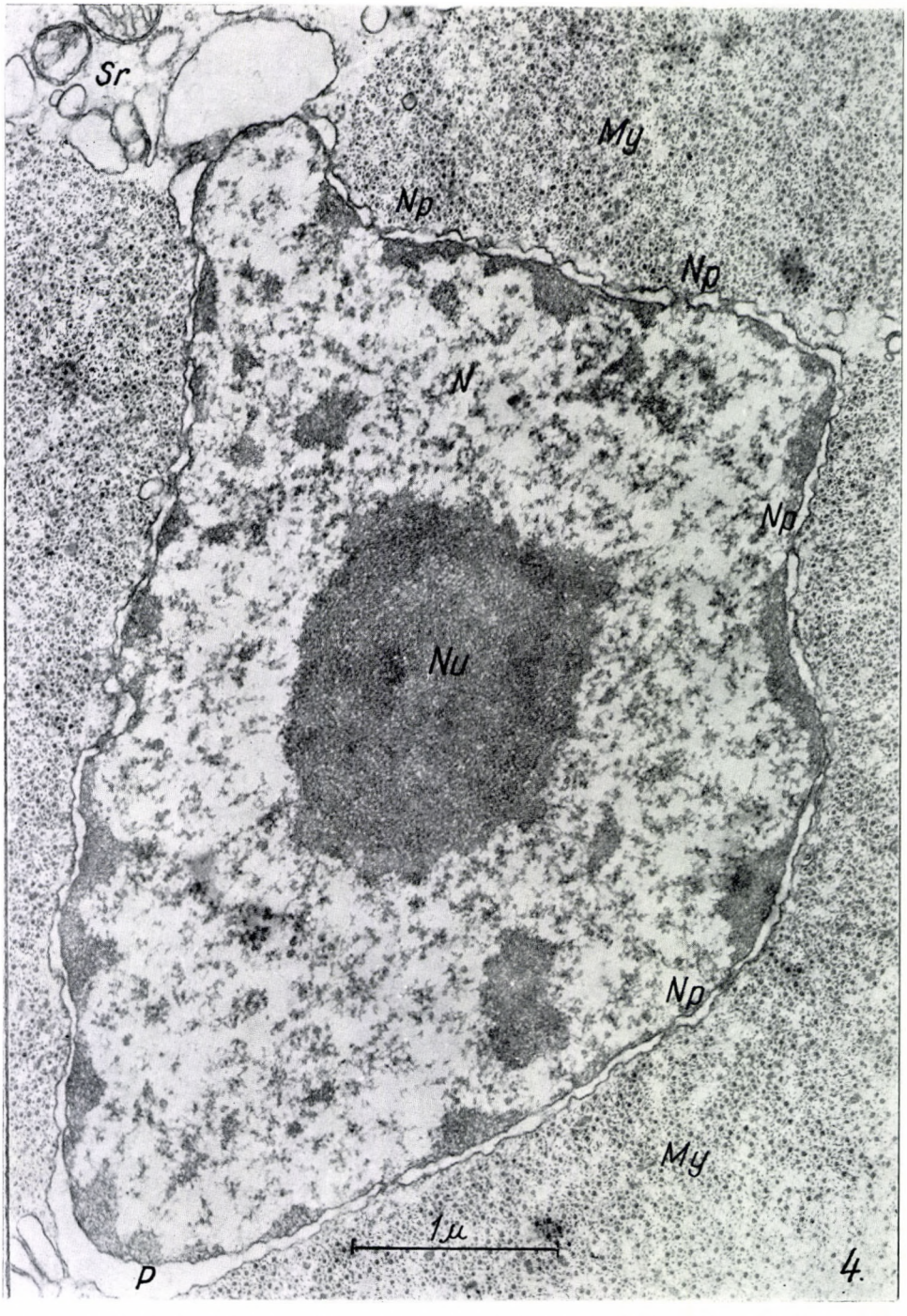

Fig. 4. Nucleus (N) of the adductor muscle cell. Nu — nucleolus, Np — nuclear pores, P — perinuclear space, My — myofilaments, Sr — sarcoplasmic reticulum. ×30 000.

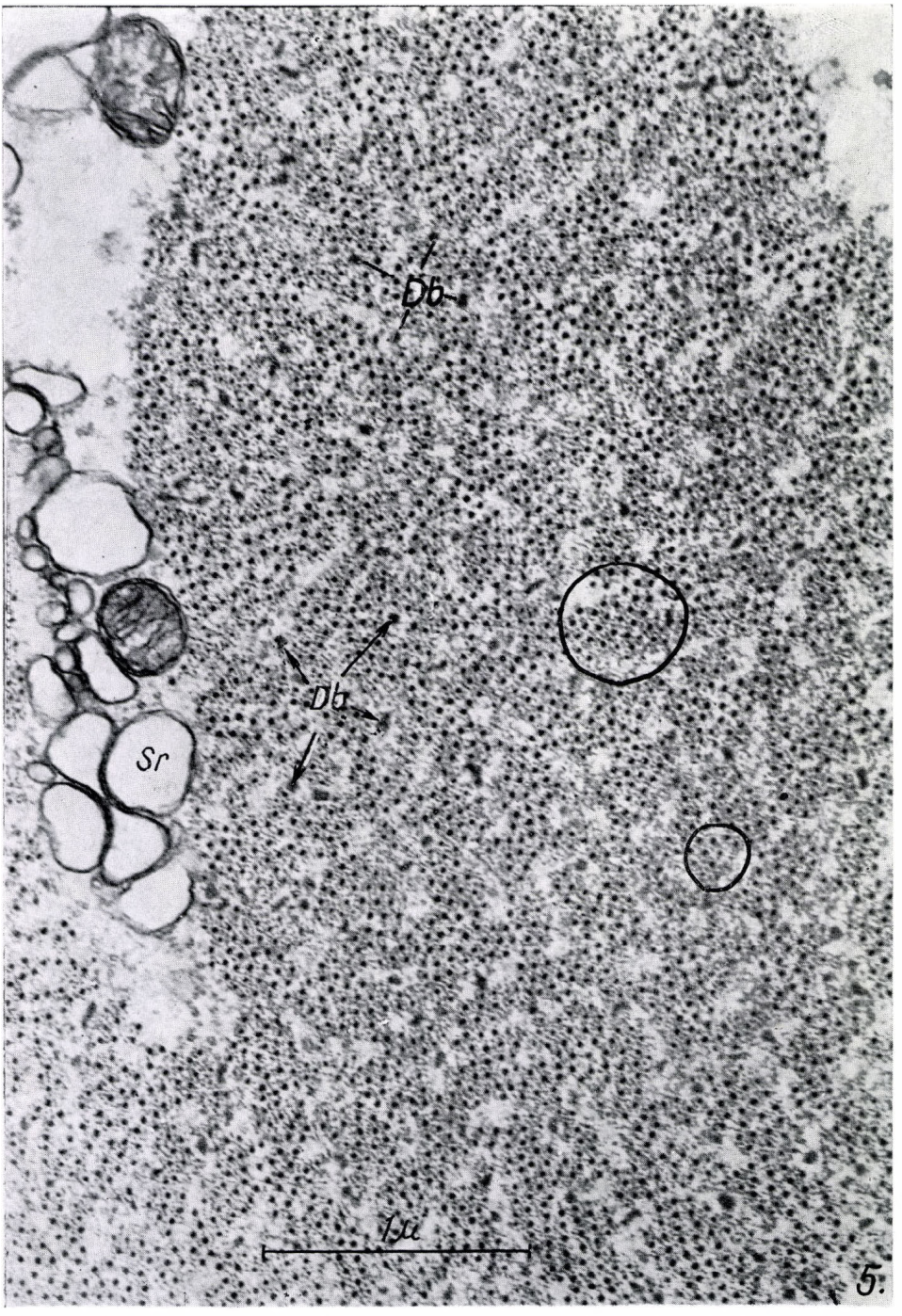

Fig. 5. Cross section of myofilaments in the adductor muscle. The regular pattern is expressed in some places (rings) elsewhere it is absent. Db — dense bodies, Sr — sarcoplasmic reticulum, ×40 000.

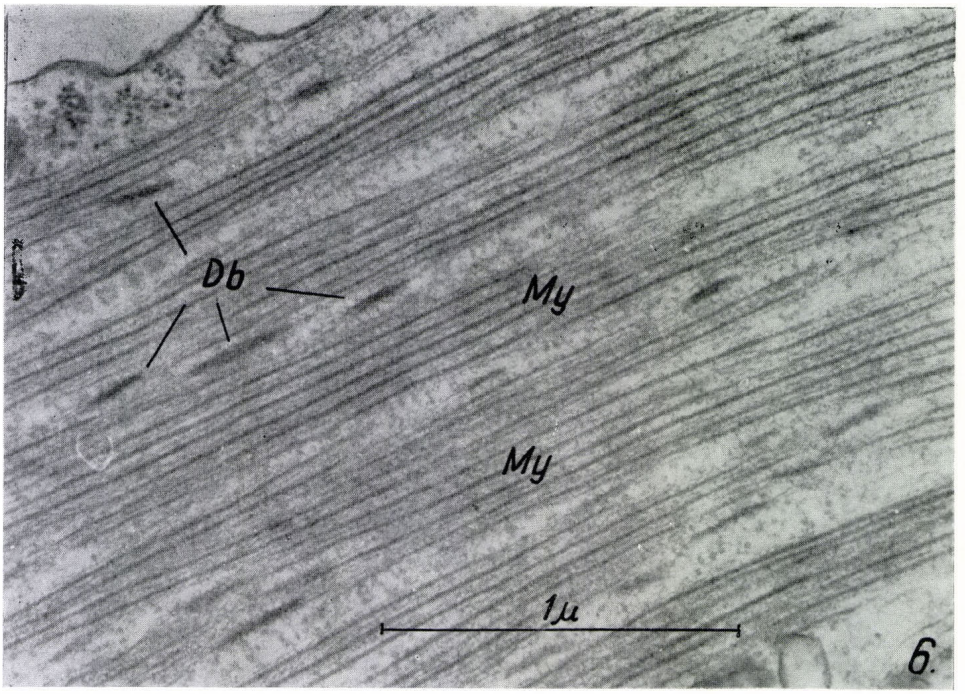

Fig. 6. Longitudinal section of myofilaments in the adductor muscle. Db — dense bodies, ×49 000.

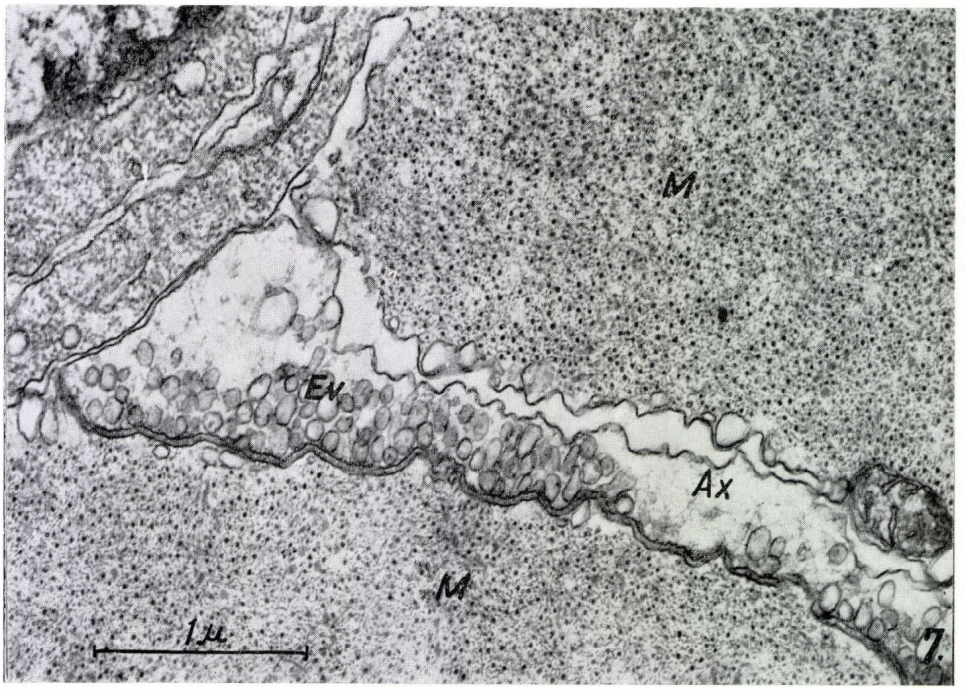

Fig. 7. Picture of a neuromuscular synapse in the adductor muscle. Ax — axon, M — muscle cell, Ev — empty vesicles, ×30 000.

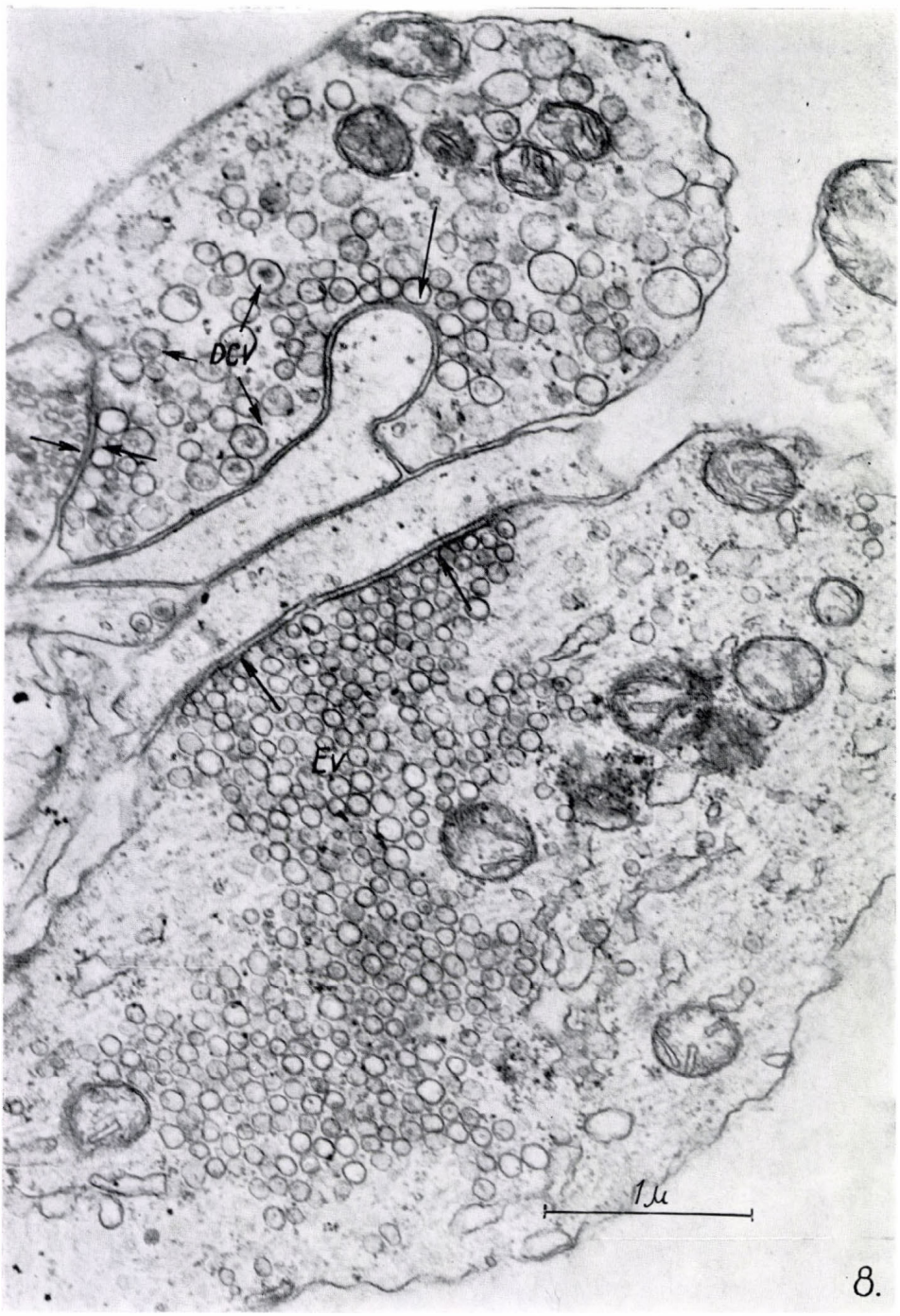

Fig. 8. The synaptic contacts of the larval neurones (arrows). The lower part of the picture shows a soma-like structure, the others are processes. Ev — empty-core vesicles, DCV — dense core vesicles, ×30 000.

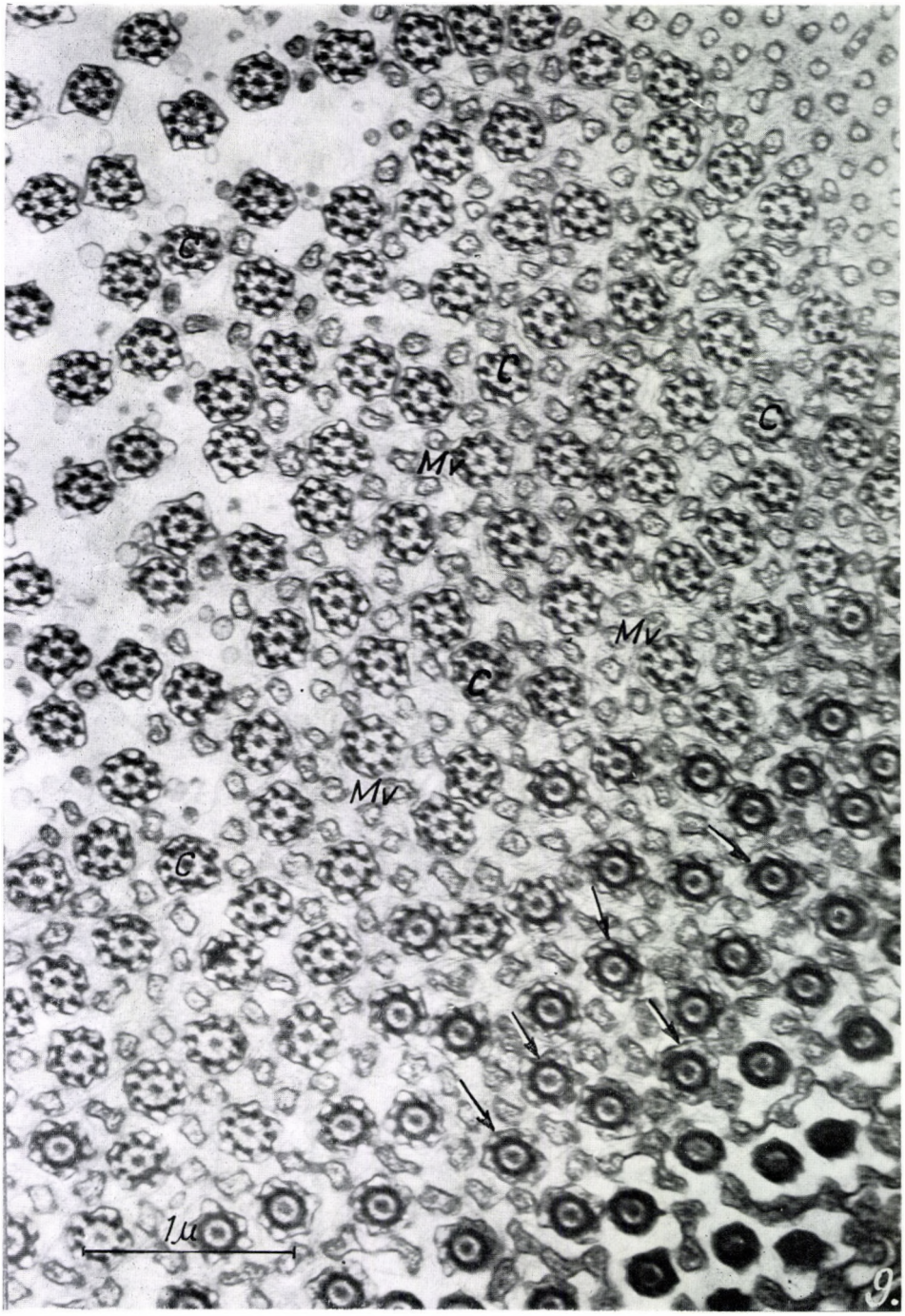

Fig. 9. Transversal, a slightly oblique section of cilia of the sensory cell near the cell surface. Arrows point to cilia in which the ciliary tubules are fused into a tube. Mv — micorvilli, ×30 000.

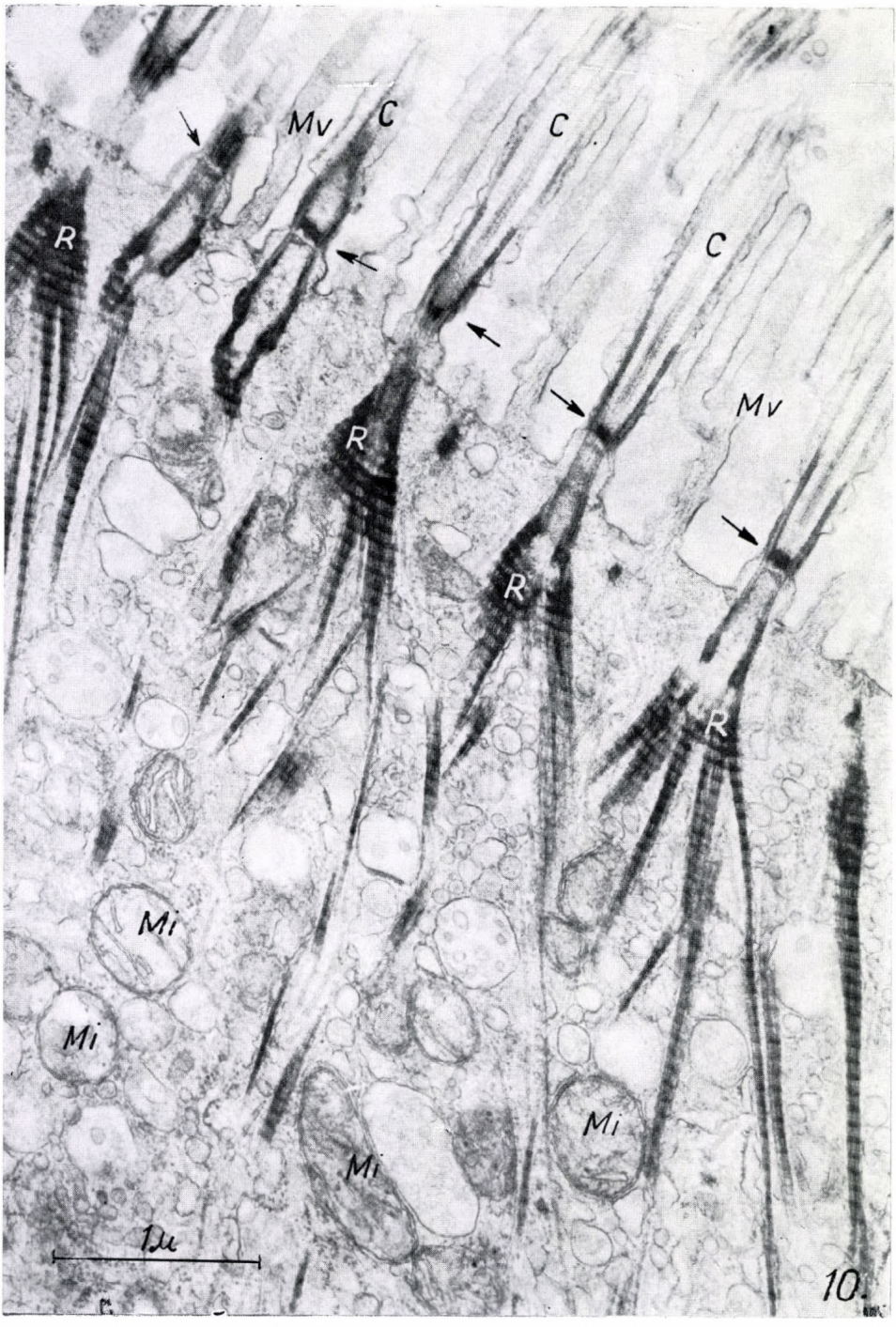

Fig. 10. The superficial part of the solitary sensory cell with the longitudinal section of cilia. Mv — microvilli, R — ciliary rootlets, Mi — mitochondria, Arrows point to the special structures of cilia. ×30 000.

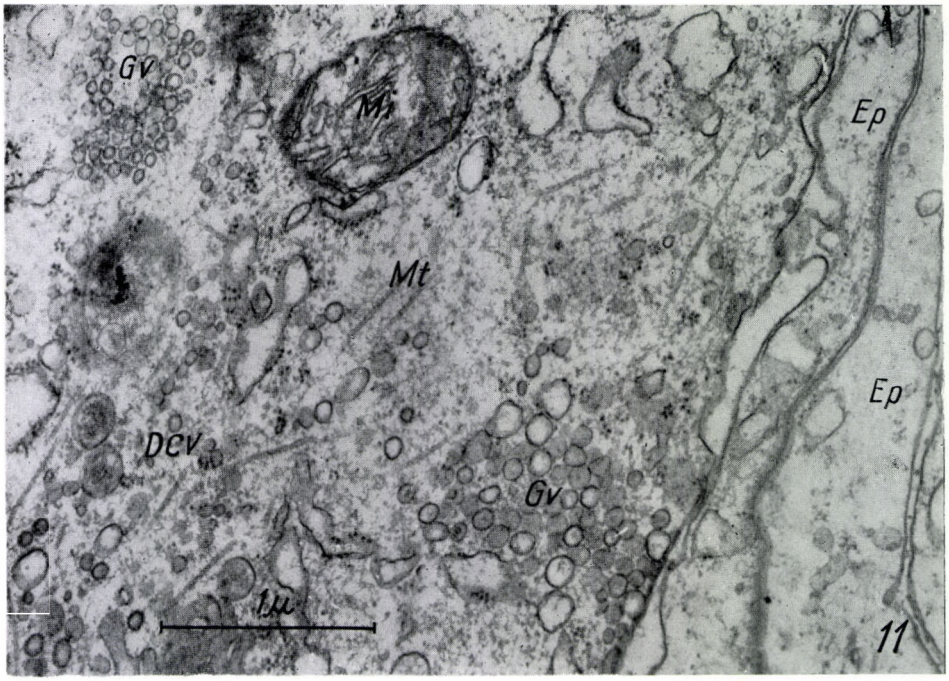

Fig. 11. Detail of basal part of solitary sensory cell. Gv — groups of vesicles, DCV — dense-core vesicles, Mt — microtubuli, Mi — mitochondria, EP — epithelial cell processes, ×30 000.

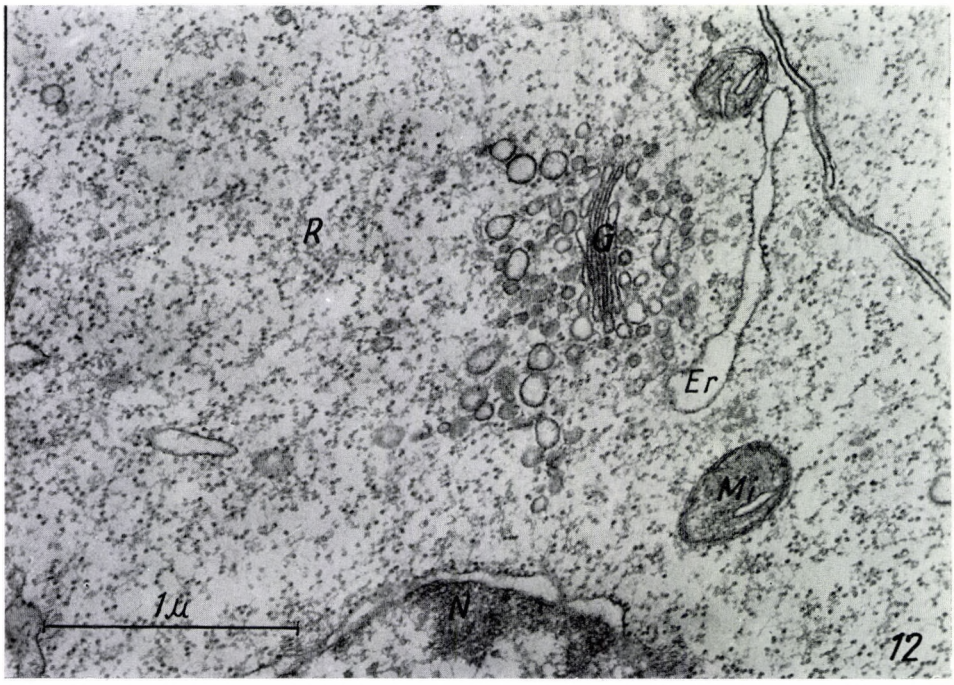

Fig. 12. Detail of the epithelial cells covering the lateral pit. N — nucleus, G — Golgi apparatus, Er — endoplasmic reticulum, Mi — mitochondrium, R — ribosomes, ×35 000.

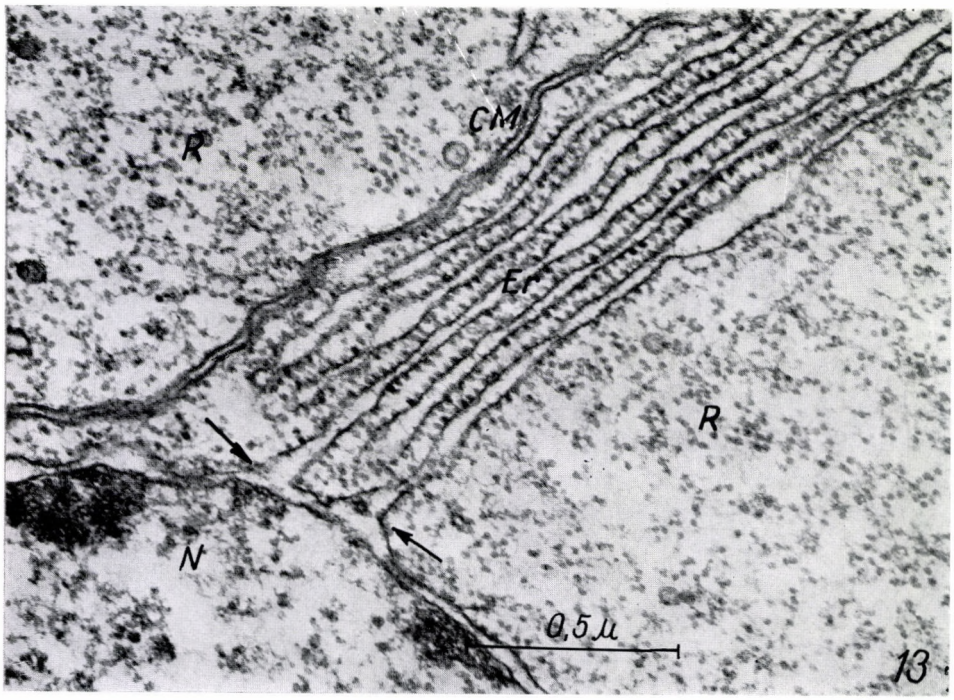

Fig. 13. Parallel oriented lamellae of granulated endoplasmic reticulum (Er) in an epithelial cell of the lateral pit. Arrows point to places where the connection with the nuclear membrane is clearly visible. N — nucleus, CM — cellular membranes, R — ribosomes, ×59 000.

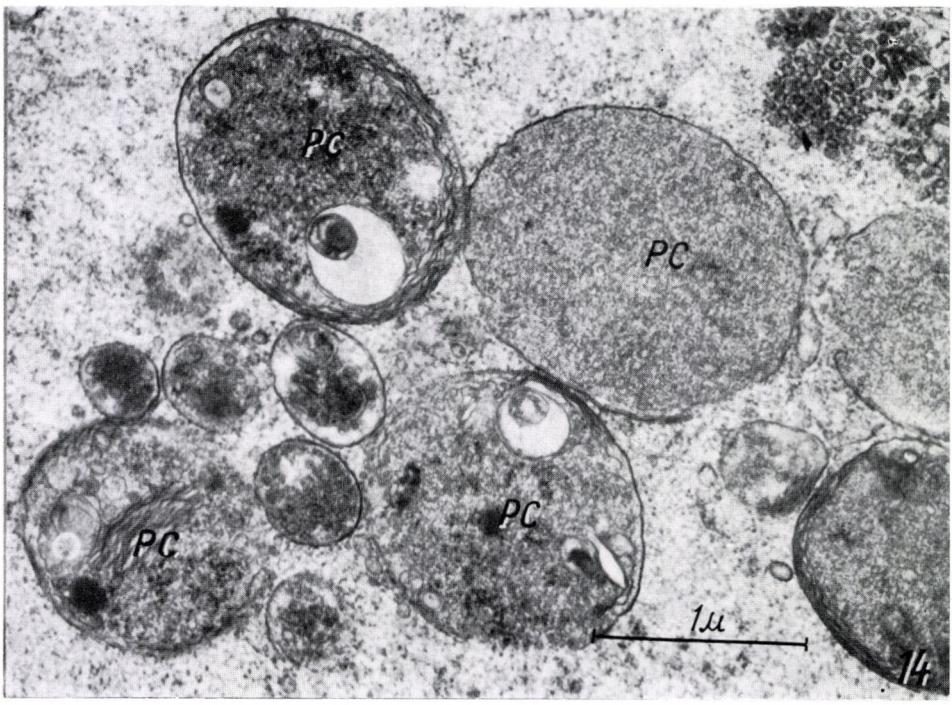

Fig. 14. Precytosomes (PC) in an epithelial cell of the lateral pit. ×30 000.

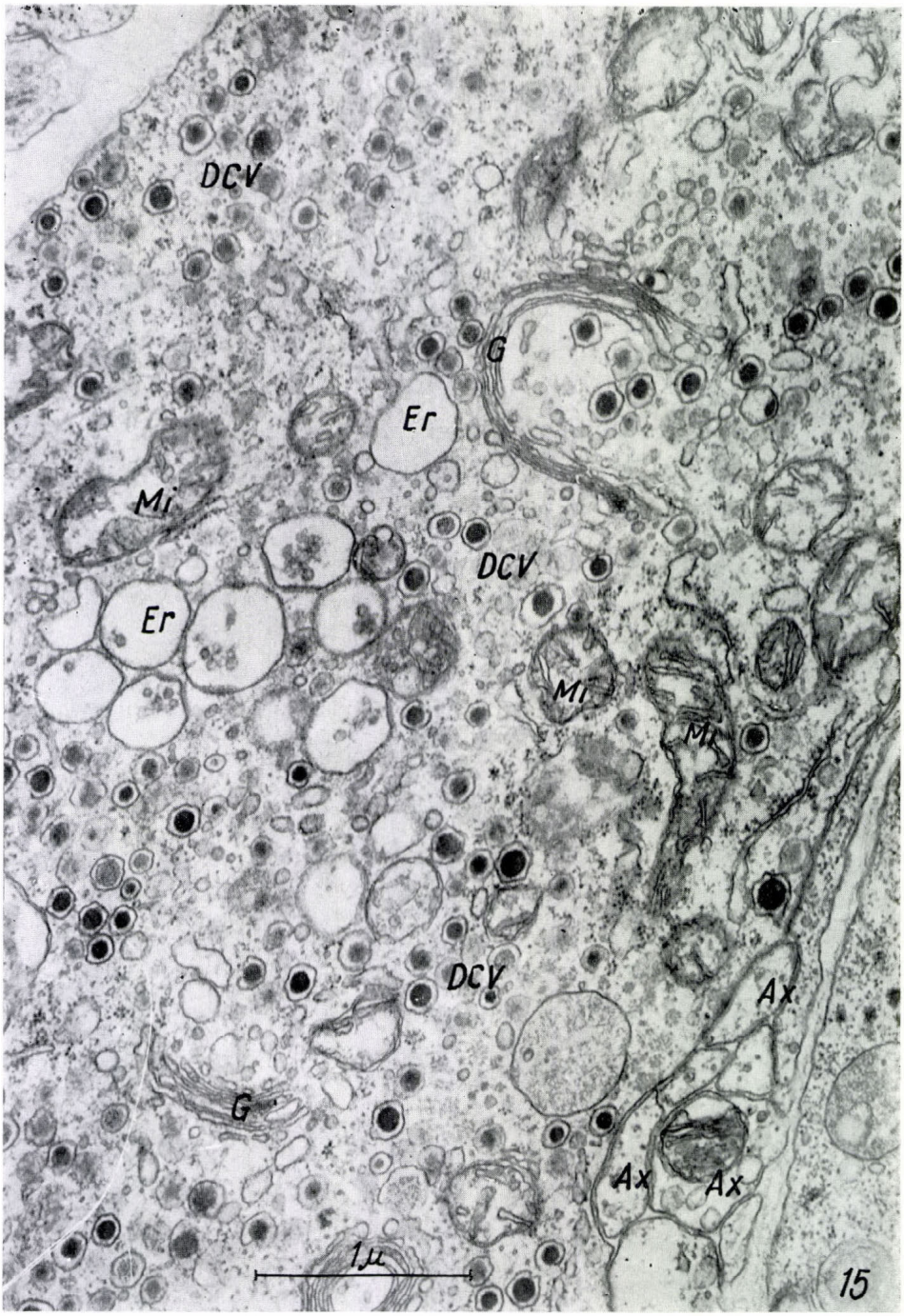

Fig. 15. Detail of a differentiated neuron in the foot fold. G — Golgi apparatus, DCV — dense-core vesicles, Er — endoplasmic reticulum, Mi — mitochondria, Ax — axons coming from other neurons. ×30 000.

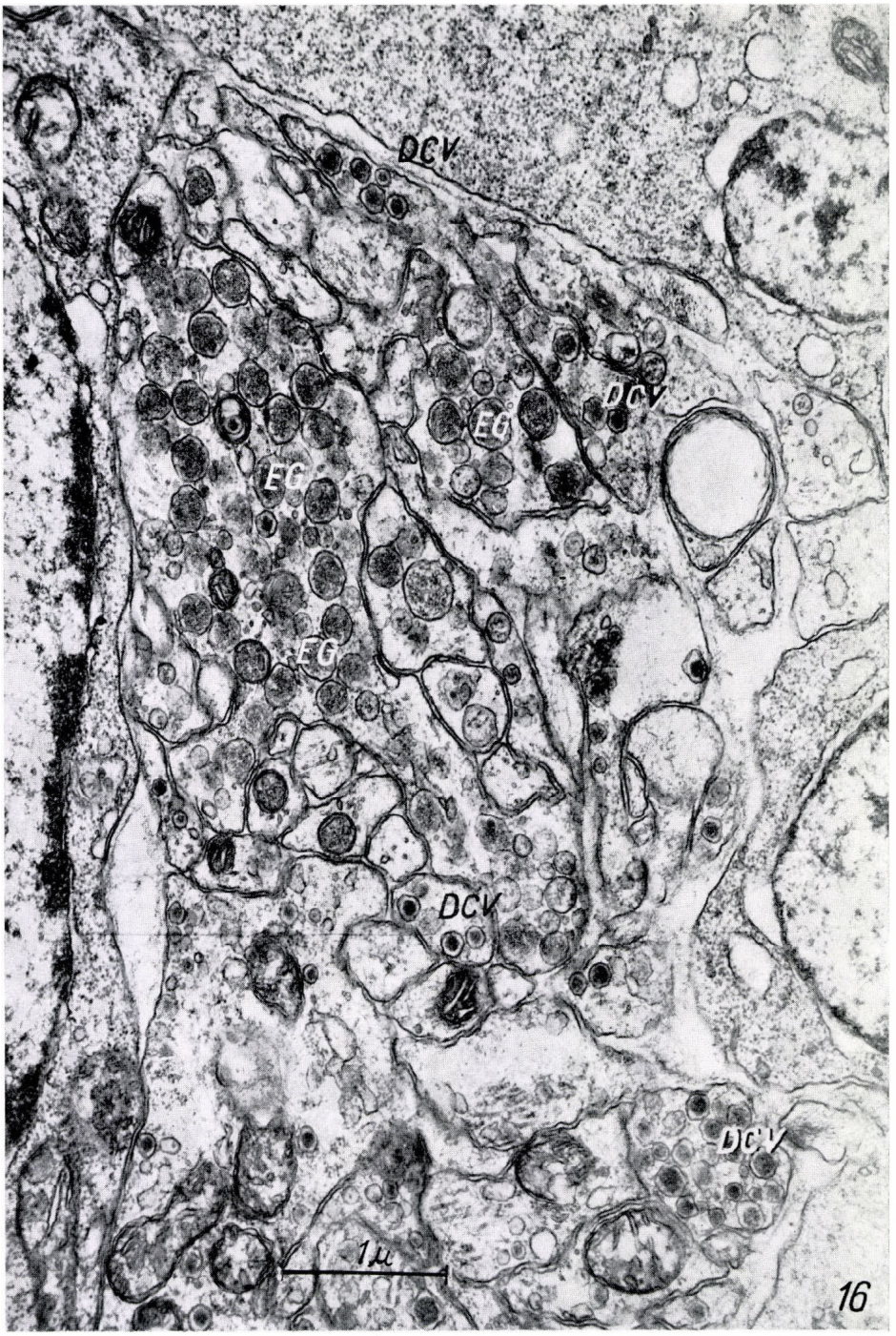

Fig. 16. Neuropile-like clusters of fibres from the foot fold. DCV — dense-core vesicles, EG — elementary neurosecretory granules, ×25 000.

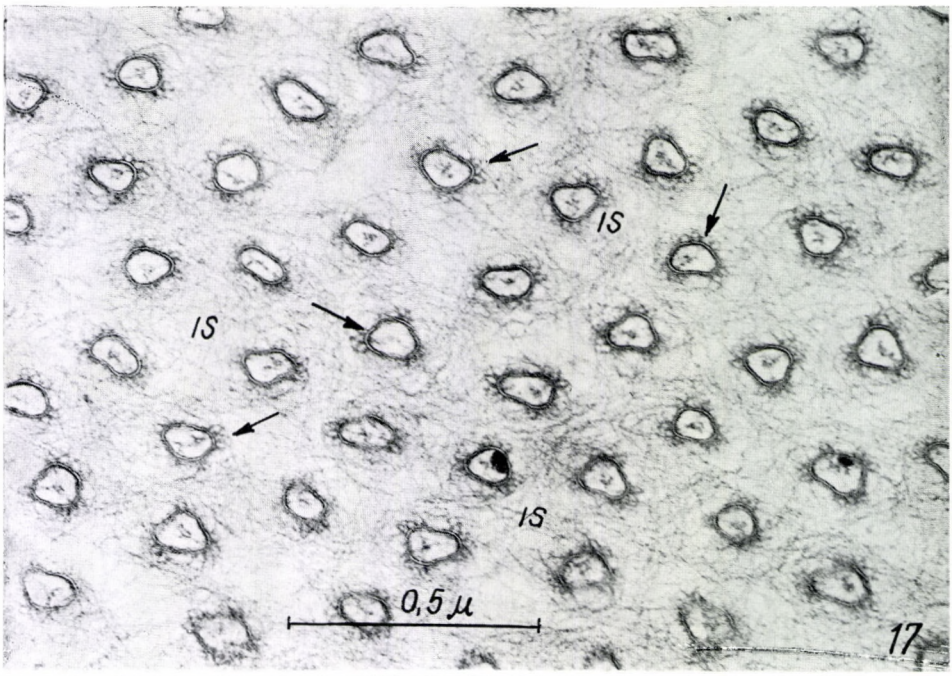

Fig. 17. Cross section of microvilli of epithelial cells from the mantle. IS — intervillous substance forming vesicles near the membrane of microvilli in some places (arrows). ×68 500.

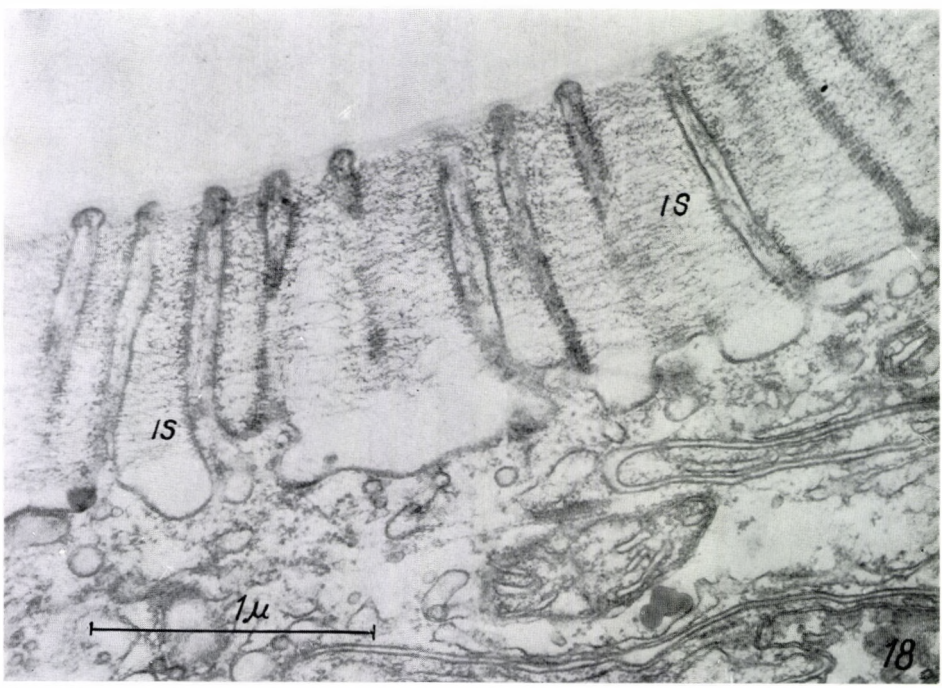

Fig. 18. Longitudinal section of microvilli. IS — intervillous substance. ×39 000

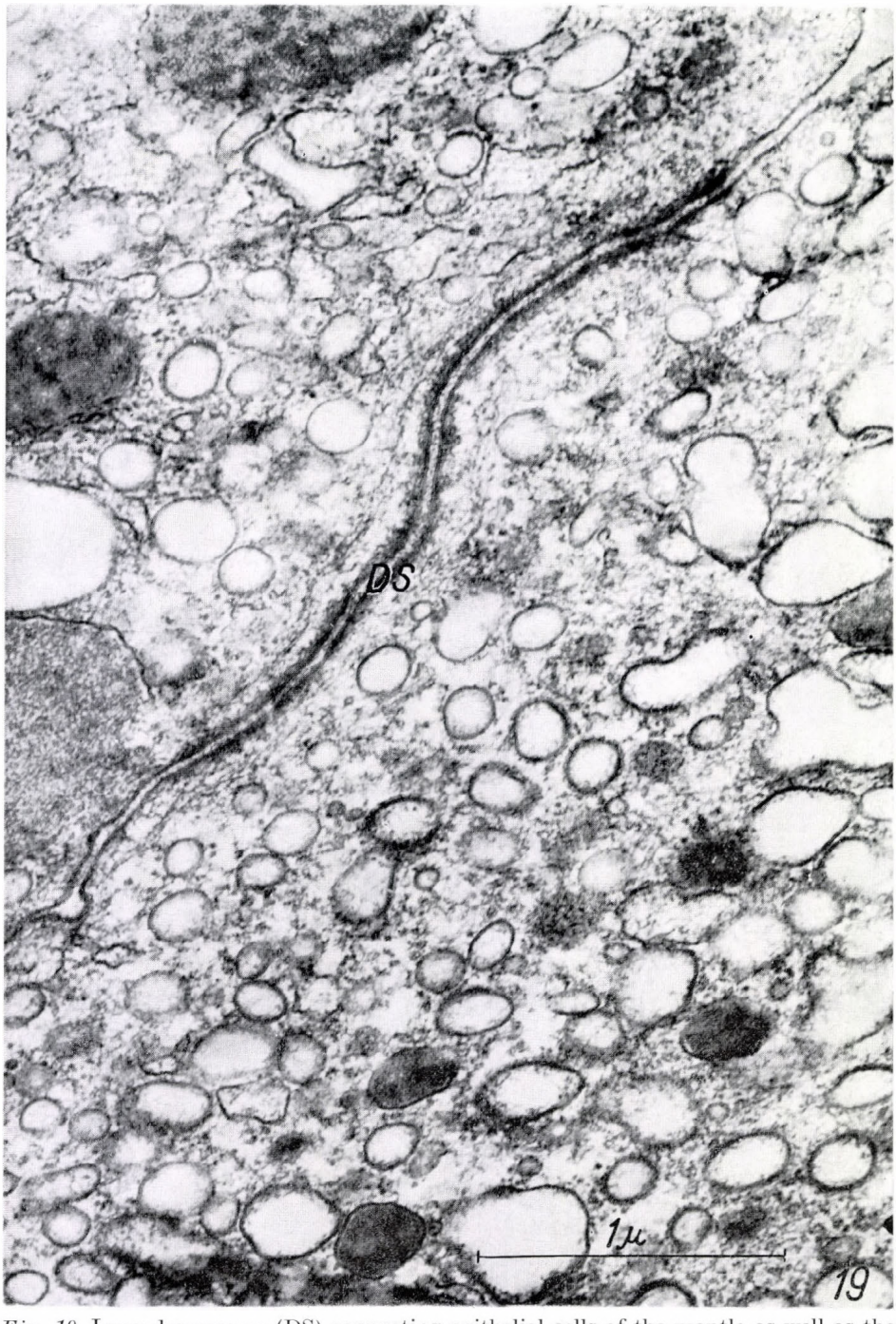

Fig. 19. Long desmosome (DS) connecting epithelial cells of the mantle as well as the typical details of epithelial cells. ×45 000.

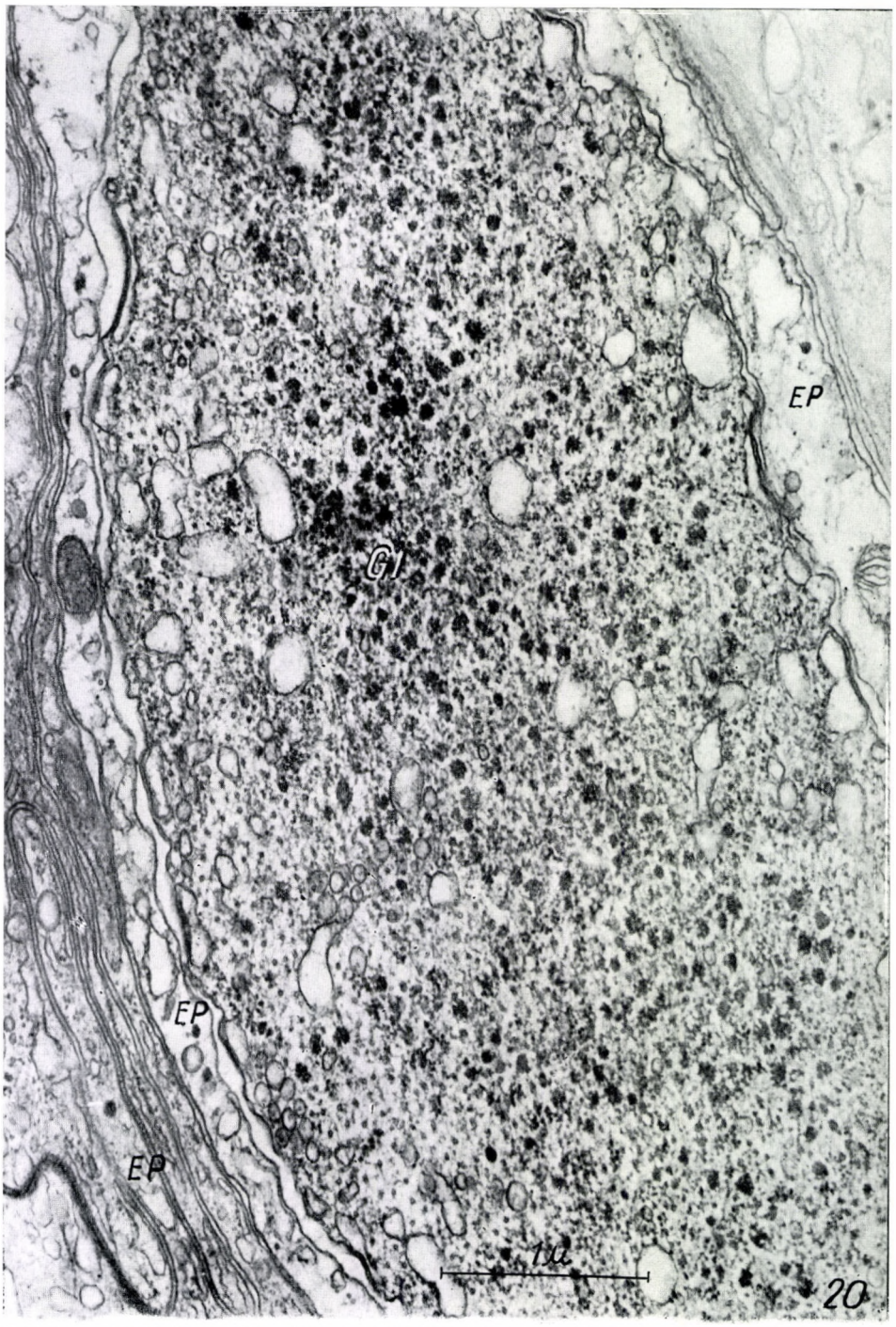

Fig. 20. Epithelial cell having no contact with the surface, containing glycogen granules, surrounded by processes (EP). ×30 000.

The epithelial cells of the mantle contain a great number of vacuoles and granules of different sizes and density. Golgi apparatuses of a very rich in stucture also frequently occur in them. Near the surface the cells are connected by desmosome-like structures while in the deeper regions small intercellular bridges can be seen between the membranes *(Fig. 19)*. Certain epithelial cells last their contact with the surface, became branched and their processes can be traced far in the sections. Sometimes in these cells a number of dense granules is accumulated, resembling to the glycogen granules *(Fig. 20)*.

Discussion

The histological methods used did not help us to know more about the nervous system of the larva than the old authors. Even the chrome-haematoxylin-phloxin staining (BARGMANN, 1949) was not suitable to differentiate between the neural and other elements. Therefore, in the identification of the structures observed we could rely only on the electron microscopic data and the interpretation was based partly on the nervous system of the adult mussels and partly on the general neurobiological knowledge.

On the basis of its morphology the adductor muscle can be classified as a molluscan muscle of the tropomyosin type (KOMNIZ et al. 1957). There is, however, a significant difference in the diameter of myofilaments. While in the adductor muscle of the adult mussel the diameter of thick filaments touches even the 800 Å (own unpublished observation) in the case of glochidia it is not more than 200 Å. The regular arrangement of the filaments is, however, more striking than in the adult animals. At the same time the phasic contraction and relaxation of the larval adductor is significantly faster as compared to that of the adult animals (LÁBOS, 1964b).

The presence of axon-like structures in the adductor unequivocally indicates that the adductor has a motor innervation. The reality of the observed neuromuscular junctions is beyond doubt. It is noteworthy that the synapses are always of the same structure, i. e. the larval adductor has only one kind of innervation at least morphologically.

The presence of motor innervation has been presumed on the basis of response of adductor, under electric stimulation, for there exists a polarized excitability of direction differing from that of the adductor (LÁBOS, 1964a, 1967). The thick axons are probably the equivalent of that excitable structure running perpendicular to the adductor muscle.

During the previous pharmacological investigations of the adductor response there was a failure of distinction between the myogenic and neurogenic character of the response (LÁBOS and SALÁNKI, 1963, LÁBOS et al. 1964, LÁBOS, 1966). Stimulating effects were to be produced with tryptamine, scopolamine, and cocaine as well as with β-adrenergic antagonists (LÁBOS et al. 1964, LÁBOS, 1966, 1969). Acetylcholine, d-tubocurarine and nicotine were ineffective while atropin in large doses (1 mM) had a stimulating effect, (LÁBOS et al. 1964, LÁBOS, 1969). On the basis of all these data a non-cholinergic, tryptaminergic regulation was presumable. The effect of tryptamine, scopolamine and cocaine was markedly potentiated by the catecholamines (adrenaline, noradrenaline, dopamine) but alone they are ineffective. The morphology of

synaptic vesicles, found in larval adductor, renders the cholinergic mediation improbable for they are significantly greater than the well known cholinergic synaptic vesicles. Furthermore, since the nerve endings innervating the adductor contain mostly empty vesicles the failure of direct adrenergic effect becomes clear. Of course on the basis of our investigations we can not decide wether the effect of endogenous or exogenous tryptamine takes place on the site of neuromuscular transmission, on neural elements or directly on the sarcolemma.

The data relating, though indirectly, to the presence of adrenergic regulation (LÁBOS et al. 1964. LÁBOS, 1966, 1969) agree with the mass occurrence of dense-core vesicles in the axons found outside of the adductor muscle, for the adrenergic character of dense-core vesicles is evidenced in adult animals (ZS.-NAGY, 1967).

After all, the neurohumoral regulation of the adductor muscle may be imagined as a diffuse network connected with processes, giving the efferent innervation. Tryptamine exerts its effect on a yet unknown point of this system. The cells and processes containing dense-core vesicles probably control this motor system directly or indirectly or perhaps in a humoral way. Seeing that the cells containing dense-core vesicles are localized in the ganglion anlages while processes of similar nature generally also occur in the larval mantle, it should be assumed the differentiated cells of the ganglion anlages take part in the indirect regulation of the larval adductor muscle. According to our results the ganglion anlages mentioned only as epithelial thickenings by HERBERS (1913), contain also differentiated elements which are functionally not independent from the larval organs. This may be the reason why HERBERS found significantly developed ganglion anlages at the very beginning of the metamorphose, taking over the regulation of functions after desintegration of the larval nervous system.

The sensory cells were known by old authors (LILLIE, 1895), too. Their fine structure is described in our present paper. It is noteworthy that the microvilli characteristic for the common epithelial cells and the cilia appear together on their surface. This refers to the relationship with the epithelial cells of resorptive function as well as to the incomplete differentiation of functions. The tactile excitability of the cilia has been evidenced (AREY, 1921, LUKACSOVICS and LÁBOS, 1965). We are in the dark concerning the role of the peculiar structures of cilia observed. Striated ciliary rootlets have also been found by FAWCETT and PORTER (1954) in the epithelia of gill and intestine of the adult mussels, however, their function is not clear either.

Because of the presence of microvilli the sensory cell may particularly be suitable to intake chemical stimuli in the same way as other resorptive epithelial cells. The quick conduction of the excitation, however, is probably better guaranteed by the special structure and environment of the sensory cell than in the resorptive cells. The processes observed in neighbourhood of the sensory cell most likely represent an afferentation. Thus, the sensory cell must be regarded as a secondary receptor cell.

Our investigations reveal some morphological phenomena interesting also from other points of view. Thus, we succeeded in observing surprisingly celar the direct contact between the outer layer of the nuclear membrane and the membranes of granular endoplasmic reticulum in the undifferentiated cells of the ganglion anlages. It seems likely that these membranes don not come into being de novo, but they develop from the nuclear membrane. We are of

the opinion that our results concerning the structure of microvilli as well as the constitution of the larval mantle are of interest from the point of view of better knowledge of larval functions adapted to parasitic life.

Summary

The larval adductor muscle of *Anodonta cygnea* can be classified as a molluscan muscle of the tropomyosin type. The diameter of its thick myofilaments is about 200 Å, while that of the thin ones is 40 Å. The thick myofilaments show a hexagonal arrangement of about 800—1400 Å lateral length. Some dense bodies also occur. A number of neuromuscular junctions can be observed in the adductor. The nerve endings contain a lot of generally empty-core vesicles of about 400—1200 Å. The main direction of axons is perpendicular to the longitudinal axis of the muscle cells. The axons correspond to the processes of cells situated dispersedly under the larval mantle. The processes of these cells are connected to each-other by synapse-like structures containing the same kind of vesicles as those of the neuromuscular junctions.

In the ganglion anlages, localized in the wall of lateral pits and foot fold, differentiated, branching cells containing dense-core vesicles can also be found. The processes of these cells form primitive neuropile-like clusters of fibres. Processes containing dense-core vesicles can also be found dispersedly outside of the ganglion anlages but never occur in the adductor muscle.

The sensory cells bear specific cilia on their surface, which are connected with striated ciliray rootlets. The sensory cell itself has no processes, however, there is a lot of other processes surrounding it.

The possibility of functional interpretation of the structures observed on the basis of physiological data is discussed and several other ultrastructural characteristics of the glochidia are also pointed out.

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SZÖVETTANI ÉS ELEKTRONMIKROSZKÓPOS VIZSGÁLATOK AZ *ANODONTA CYGNEA* L. LÁRVÁJÁNAK ZÁRÓIZMÁN ÉS IDEGELEMEIN

Zs.-Nagy Imre és *Lábos Elemér*

Összefoglalás

Az *Anodonta cygnea* L. lárvális záróizma a tropomyosin típusú puhatestű izmok közé sorolható. Vastag myofilamentumainak átmérője 200 Å, a vékonyaké 40 Å. A vastag filamentumok többnyire 800—1400 Å oldalhosszúságú, hatszögű elrendeződést mutatnak. Dense-body-k is megtalálhatók. A záróizomban számos neuromuscularis junctió figyelhető meg. Az idegvégződésekben 400—1200 Å átmérőjű, általában üres középű vezikulák vannak. A záróizmot beidegző axonok fő lefutási iránya merőleges az izomsejtek hossztengelyére. Az axonok a köpeny alatt szétszórtan elhelyezkedő sejtek

nyúlványainak felelnek meg. E sejtek nyúlványai egymással is szinapszis-szerű kapcsolatban állnak, amelyekben ugyanolyan vezikulák láthatók, mint a neuromuscularis junctióban.

Az oldalgödör falában és a lábdudorban elhelyezkedő ganglionтelepekben már differenciálódott, dense-core vezikulákat is tartalmazó nyúlványos sejtek is fellelhetők. Ezeknek nyúlványai primitív, neuropil-szerű rosttömörülést is képeznek. Dense-core vezikulákat tartalmazó nyúlványok szétszórtan a ganglionтelepeken kívül is megtalálhatók, de a záróizomban nem fordulnak elő.

Az érzékelősejtek speciális csillókat hordanak a felszínükön, amelyekhez harántcsíkolt gyökérrostok tartoznak. Maga az érzékelő sejt nem nyúlványos, de körülötte számos más nyúlvány található.

Szerzők diszkuttálják a talált struktúrák funkcionális értelmezésének lehetőségeit fiziológiai adatok alapján, továbbá rámutatnak a glochidium ultrastruktúrájának néhány további jellegzetességére is.

ГИСТОЛОГИЧЕСКИЕ И ЭЛЕКТРОННОМИКРОСКОПИЧЕСКИЕ ИССЛЕДОВАНИЯ ЗАПИРАТЕЛЬНЫХ МЫШЦ И НЕРВНЫХ ЭЛЕМЕНТОВ ГЛОХИДИЕВ БЕЗЗУБКИ

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Личиночную запирательную мышцу беззубки можно отнести к тропомиозиновому типу мышц моллюсков. Диаметр толстых миофиламентов 200 Å а тонких 40 Å. Толстые филаменты показывают шестиугольную упорядочность, которая характеризуется 800—1400 Å боковой длиной. Обнаруживаются и dense bodies. В запирательной мышце наблюдаются многочисленные нервно-мышечные контакты. В нервных окончаниях имеются везикулы диаметром 400—1200 Å, средная часть которых обычно пуста. Главное направление нервных волокон, иннервующих запирательную мышцу, перпендикулярно продольной оси мышечных клеток. Волокна соответствуют отросткам клеток, расположенных разброшено под мантией. Эти отростки создают друг с другом контакты, напоминающие синапсы, и содержат такие же везикулы, как и нервно-мышечная связь.

В стене боковой ямки и ганглиозных массах, находящихся в зачатке ноги, обнаруживаются дифференцированные клетки с отростками, содержащие и dense-core везикулы. Отростки этих клеток образуют примитивное нейропиль-подобное накопление волокон. Отростки, содержащие денс-гранулы, расположены рассеяно и вне ганглиозных масс, но в самой запирательной мышце их обнаруживать не удалось.

Поверхность чувствующих клеток снабжена специальными ресничкими, связанными поперечно-полосатыми корешками. Чувствующие клетки не обладают отростками, но в их окружности было найдено большое количество отростков иного происхождения.

Авторы обсуждают возможное функциональное значение обнаруженных структур на основе физиологических данных и дают некоторую дальнейшую характеристику ультраструктуры глохидиев.

ANNAL. BIOL. TIHANY	36	135—149	HUNGARIA 1969

THE SPRING AND SUMMER NUTRITION OF THE 300—500 G PIKE-PERCH (*LUCIOPERCA LUCIOPERCA* L.) IN LAKE BALATON IN 1968. I. DATA BEARING RELATION TO THE NUTRITIONAL CONDITIONS PROCEEDING THE DESTRUCTION OF FISH IN 1965

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Received: 21st December, 1968

The food of pike-perch has been extensively investigated (LUKÁCS, 1932 a, 1932b, ENTZ and LUKACSOVICS, 1957), the hoard of data, however, dwells upon only peculiar characteristics. The detailed study of this subject was first undertaken in 1950. On the basis of investigation extending over the period of three years using a great quantity of material WOYNÁROVICH (1959) elucidated with accuracy the qualitative and quantitative conditions of the nutrition of 300—500 g (fourth grade) pike-perch. The slow and uneven growth rate of the pike-perch became known through the meticulous study carried out by TÖLG (1961). He pointed out that there was a qualitative deterioration of lacustrial stand, drew attention to the disadvantageous conditions of life, furthermore, made mention of the grave conditions caused by scarcity of food and their possible origin.

Before launching our investigations we assumed, partly because of the causes resulting in the destruction of fish in 1965, partly because of the fish fauna complementation (introduction of eel) and of course of the continual eutrofication and the effects of several other factors (anthropogenic effect — SEBESTYÉN, 1967) including the accumulation of pesticide (BARON et al. 1967) in the food-chain, that the nutritional conditions might have changed as it was described by WOYNÁROVICH (1959) some ten years ago.

Accordingly, our work has been informative in nature in order to obtain information on the assumed trend and degree of change having taken place in the nutrition of the fourth grade pike-perch in Lake Balaton.

Material and methods

The material for our investigations has been collected by a special stomach-pump (WOYNÁROVICH, 1958) in 1965 and 1967 (415 and 175 stomach content, respectively), and again in 1968 (920 stomach content). The stomach contents were preserved each in a separate nylon bag containing 4% formalin and the samples were sealed until working up.

Simultaneously with stomach pumping we have taken the weight of each pike-perch in grams, before and after the evacuation of the stomach. The average weight of the fish under investigation was found to be 390 g. We have

identified the fish remains where it was possible, have taken the length of them (longitudo corporis) or when it was not possible simply estimated it together with the appropriate body weight in grams. In cases when identification was impeded by far advanced digestion we entered notes like "unidentifiable fish remains" *(Cyprinida* or *Percida backbone*, etc.).

Knowing the length and weight of fish remains derived from pike-perch stomachs we could draw conclusions on the growth rate and the length distribution of nutritive fish. For the correct body length and weight estimation of fish remains we had as basis a large number of control measurements taken on live fish; for our purposes naturally average values have been used

Results

1. *The quality of food in 1965, 1967 and 1968*

In 1965 the specific distribution of the 332 fish remains the following order of frequency occurred: *Lucioperca lucioperca* > *Acerina cernua* > *Alburnus alburnus*. 67.8% of the fish serving for food were Percids 21.1% Cyprinids and the unidentifiable remains with other families of fishes together amounted to 11.1%. The proportion of feeding pike-perch and those with empty stomachs was 41,2 : 58.8% *(Table 1, Fig 1)*.

Table 1

The monthly and specific distribution of fish remains found in the stomachs of the examined pike-perch in 1965

Period	Aug.	Sept	Oct.	Total
No. of examined specimens	208	99	108	415
Having taken food	86	35	50	171
Empty	122	64	58	244
Lucioperca lucioperca	70	33	27	130
Acerina cernua	63	1	29	93
Alburnus alburnus	27	3	15	45
Abramis brama	7	3	3	13
Pelecus cultratus	5	1	5	11
Lucioperca volgensis	—	2	—	2
Scardinius erythrophthalmus	1	—	—	1
Others	12	7	18	37
Total fish remains	185	50	97	332
Dreissena polymorpha	1	—	—	1

In 1967, 325 fish remains were found in the stomachs of pike-perch. It was interesting to note that compared to the previous year the order of frequency displayed a reversed pattern *Alburnus alburnus* > *Acerina cernua* > *Lucioperca, lucioperca*, furthermore, a great fluctuation could be evidenced concerning the number of consumed species. The percentual proportion of the main groups was as follows: Cyprinids 56,3%, Percids 39.7%, while the rest

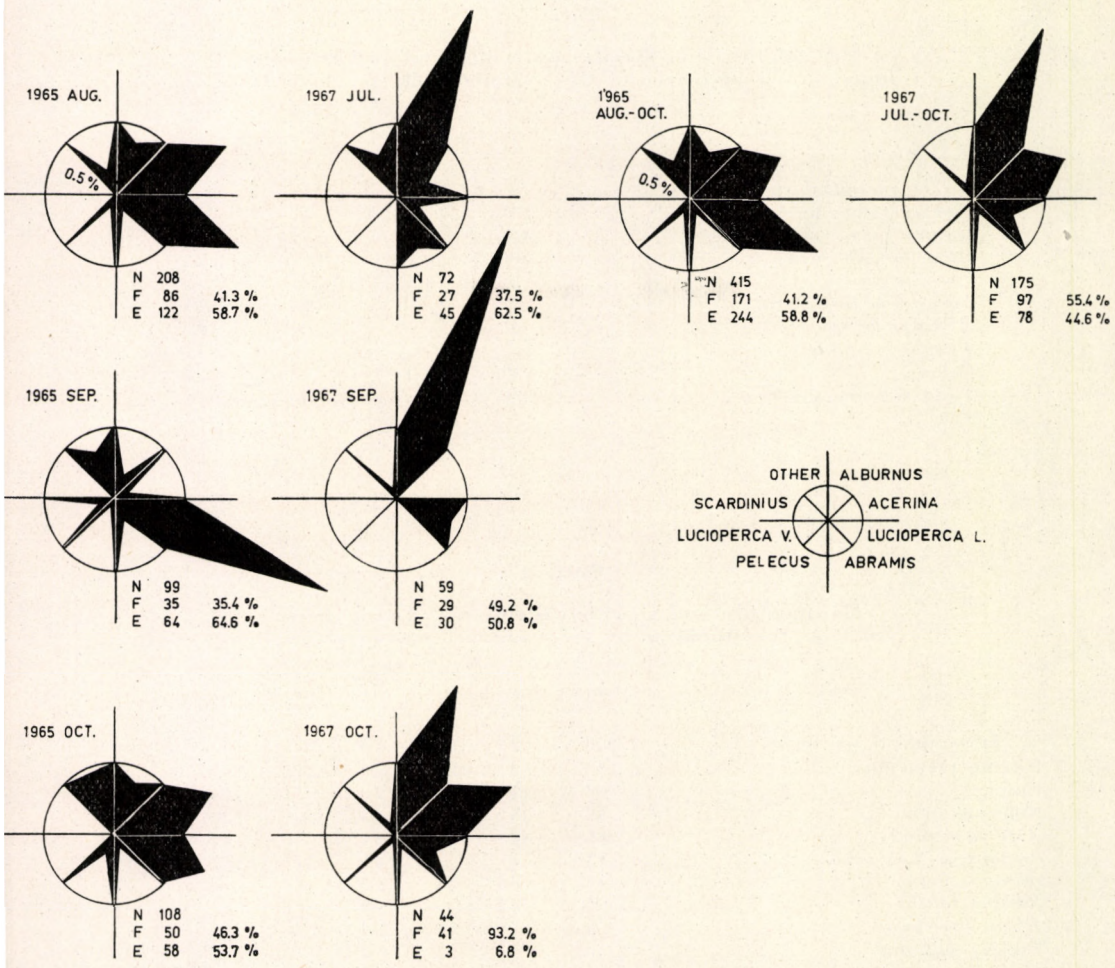

Fig. 1. The food habits of the fourth grade pike-perch 300—500 g in monthly and three monthly contraction. The percentual distribution of fish species serving for food in the years of 1965 and 1967. N = number of examined pike-perch stomachs, F = number of stomachs containing food (feeding pike-perch), E = number of empty stomachs (pike-perch with an empty stomach)

was taken un in the remaining 4%. The proportion of feeding pike-perch and those with empty stomachs was 55.4 : 44.6% *(Table 2. Fig.* 1).

In 1968, out of the 300—500 g pike-perch in all 18 species of fishes were identified after the examination of stomach contents. On rare occasions we encountered a stray specimen of frog skeleton *(Rana* sp.), a newt *(Triturus* v. *vulgaris)* and a leech *(Hirudinea* sp.). Seldom we came across in the food remains of *Dreissena polymorpha* and *Lithoglyphus naticoides*, however, we were able to identify quite frequently the fragments of varecs (mainly of *Potamogeton* spp.). In the stomach contents of 744 feeding pike-perch we determined 1290 fish remains, besides these 226 unidentifiable remains were found. The three most important fishes serving as food occurred in the same order

Table 2

The monthly and specific distribution of fish remains found in the stomachs of the examined pike-perch in 1967

Period	July	Sept	Oct.	Total
No. of examined specimens	72	59	44	175
Having taken food	27	29	41	97
Empty	45	30	3	78
Alburnus alburnus	19	35	113	167
Acerina cernua	3	—	88	91
Lucioperca lucioperca	2	7	29	38
Abramis brama	5	—	8	13
Pelecus cultratus	—	—	3	3
Others	4	1	8	13
Total fish remains	33	43	249	325

Table 3

The monthly and specific distribution of fish remains found in the stomachs of the examined pike-perch in 1968

Period	March	Apr.	May.	June	July	Aug.	Total
No. of examined specimens	206	112	135	65	257	145	920
Having taken food	140	88	121	46	232	117	744
Empty	66	24	14	19	25	28	176
Alburnus alburnus	89	38	70	33	248	120	598
Acerina cernua	65	81	11	11	71	41	280
Lucioperca lucioperca	13	1	—	11	160	12	197
Abramis brama	14	8	24	5	21	7	79
Rutilus rutilus	5	4	28	6	3	2	48
Blicca bjoerkna	4	7	9	2	1	5	28
Pelecus cultratus	16	2	4	1	1	2	26
Lucioperca volgensis	5	—	—	—	3	4	12
Scardinius erythrophthalmus	—	—	—	1	2	1	4
Rhodeus sericeus amarus	1	2	—	1	—	—	4
Perca fluviatilis	2	—	—	1	—	1	4
Leucaspius delineatus	1	—	—	—	—	1	2
Cobitis taenia	1	—	—	—	—	1	2
Gobio fluviatilis	—	1	—	—	—	1	2
Cyprinus carpio	—	1	—	—	—	—	1
Tinca tinca	1	—	—	—	—	—	1
Esox lucius	—	—	—	1	—	—	1
Anguilla anguilla	—	1	—	—	—	—	1
Unidentifiable	57	19	41	18	70	21	226
Identified	217	146	146	73	510	198	1290
Total fish remains	274	165	187	91	580	219	1516
Triturus v. *vulgaris*	1	—	—	—	—	—	1
Rana sp.	1	—	—	—	—	—	1
Hirudinea sp.	—	—	—	—	—	1	1
Dreissena polymorpha	—	4	1	—	—	1	6
Lithoglyphus naticoides	—	1	1	—	1	1	4
Water-weed fragments	1	2	—	—	2	10	15

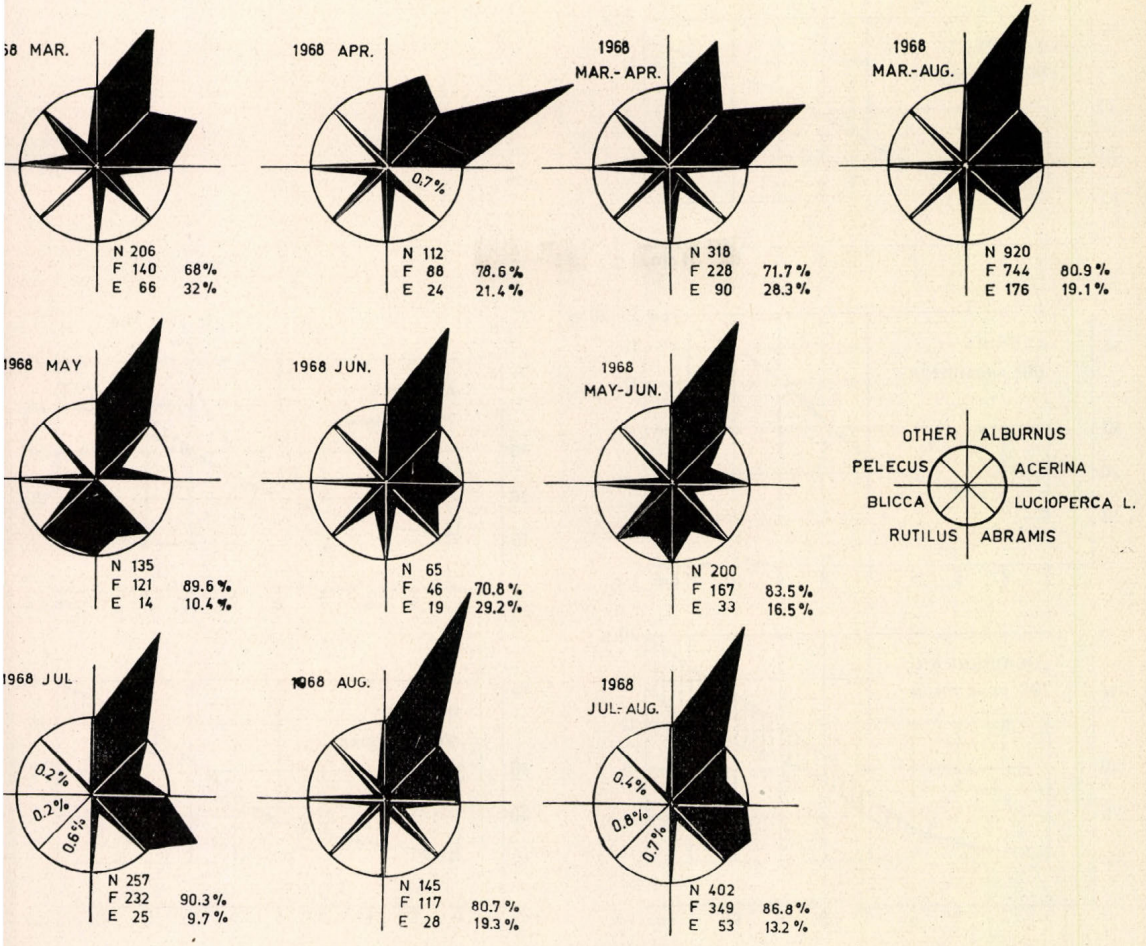

Fig. 2. The food habits of the fourth grade pike-perch 300—500 g in monthly, bimonthly and six monthly contraction. The percentual distribution of fish species serving for food in the year 1968. (N, F, E cf. *Fig. 1)*

of frequency as in 1967. The one heading the list in April was ruffe *(Acerina cernua)*, while in the other months bleak *(Alburnus alburnus)* was the dominant species. Differences could also be observed in the consumption of occasional species for feeding. Among them the most significant is, from frequency point of view, the fry of its own species and bream *(Abramis brama)* together with roach *(Rutilus rutilus)*. A large proportion of the food in July was pike-perch fry and the fry of bream with its one year old specimens, while in May roach was the most dominant occasional prey-fish. The food comprised 61.46% of Cyprinids, 38.2% of Percids and the remaining 0.36% was taken up by several fish families (Cobitidae, Esocidae, Anguillidae). The proportion of feeding pike-perch and those with empty stomachs was 80.9 : 19.1% *(Table 3, Fig. 2)*.

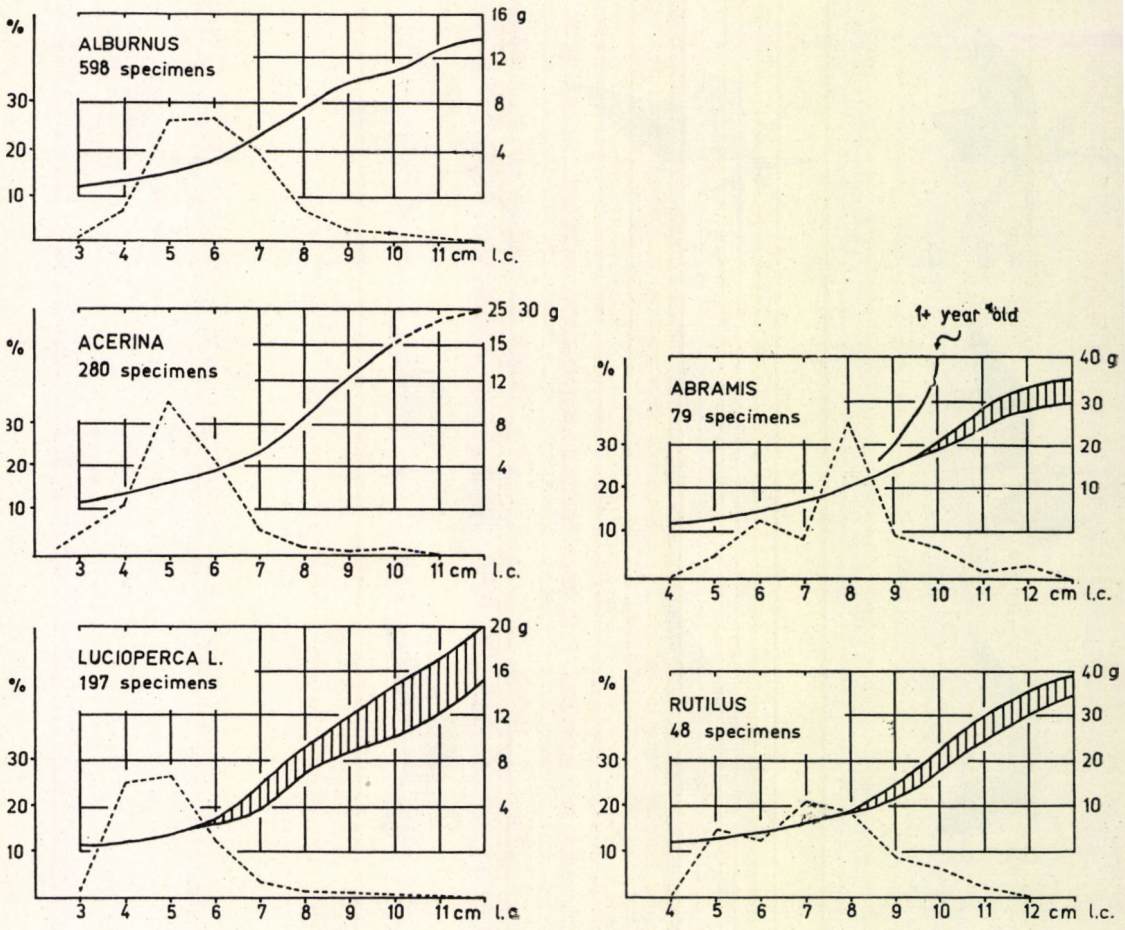

Fig. 3. The percentual distribution in size of the most frequently occurring 5 fish found in the stomachs (broken line), and the weight values corresponding to body sizes (full line) given in grams (1968)

The pike-perch of this order of magnitude mainly consumed specimens of 5—6 cm in length, the somewhat larger ones 7—10 cm came second and these two groups were in fact the basis for their food. They did not consume longer specimens than 12 cm *(Fig. 3)*.

2. *Quantitative conditions in 1968*

The quantitative evaluation of stomach contents was partly based on the number of fish found in the stomach and partly on the measured or estimated weight. According to the number of fish found in the stomachs the percentual distribution data of feeding pike-perch unanimously show that the most frequent type of feeding was to consume only one fish (45.6% in the average of 6 months), less consumed 2 or 3 fish. More than this, 4, 5, 6 . . ., etc. specimens

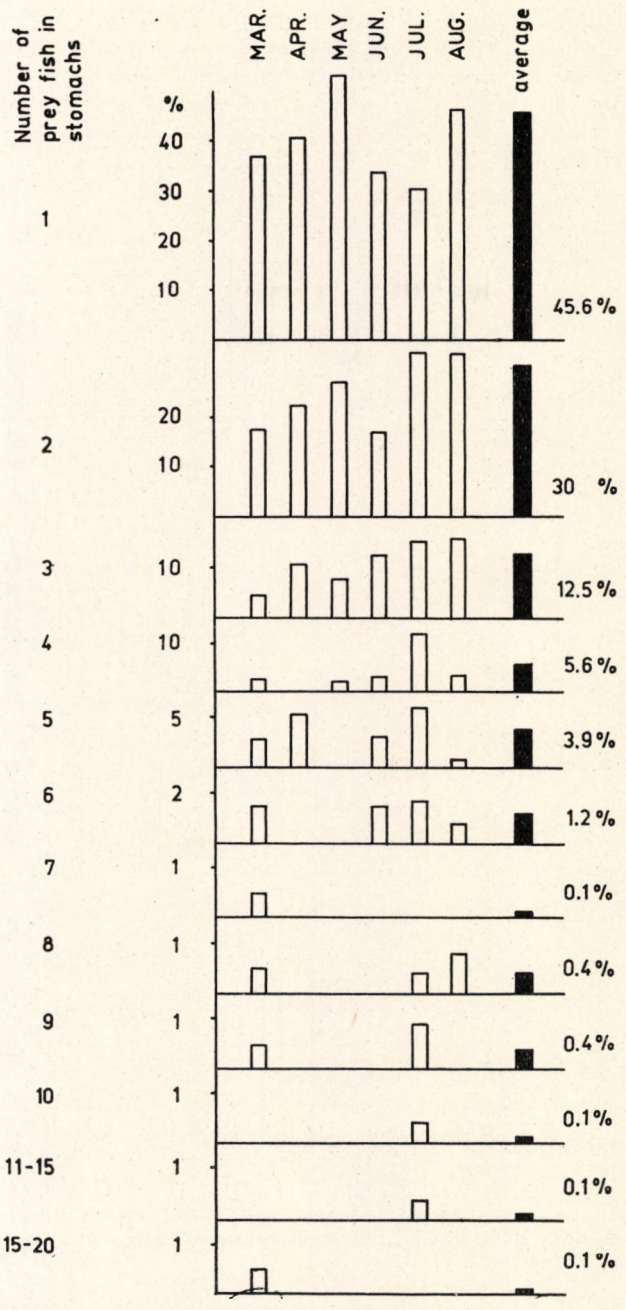

Fig. 4. The percentual distribution of feeding pike-perch according to the number of fish remains found in the stomachs in 1968. The percentual distribution of pike-perch consuming 1, 2, etc. specimens of fish for food, found in the stomachs, is plotted on the ordinate

were only consumed by a small number of pike-perch. At one single occasion we have encountered 19 fish remains in a stomach of a pike-perch in March, this number, however, proved to be the maximum, so far *(Fig. 4)*.

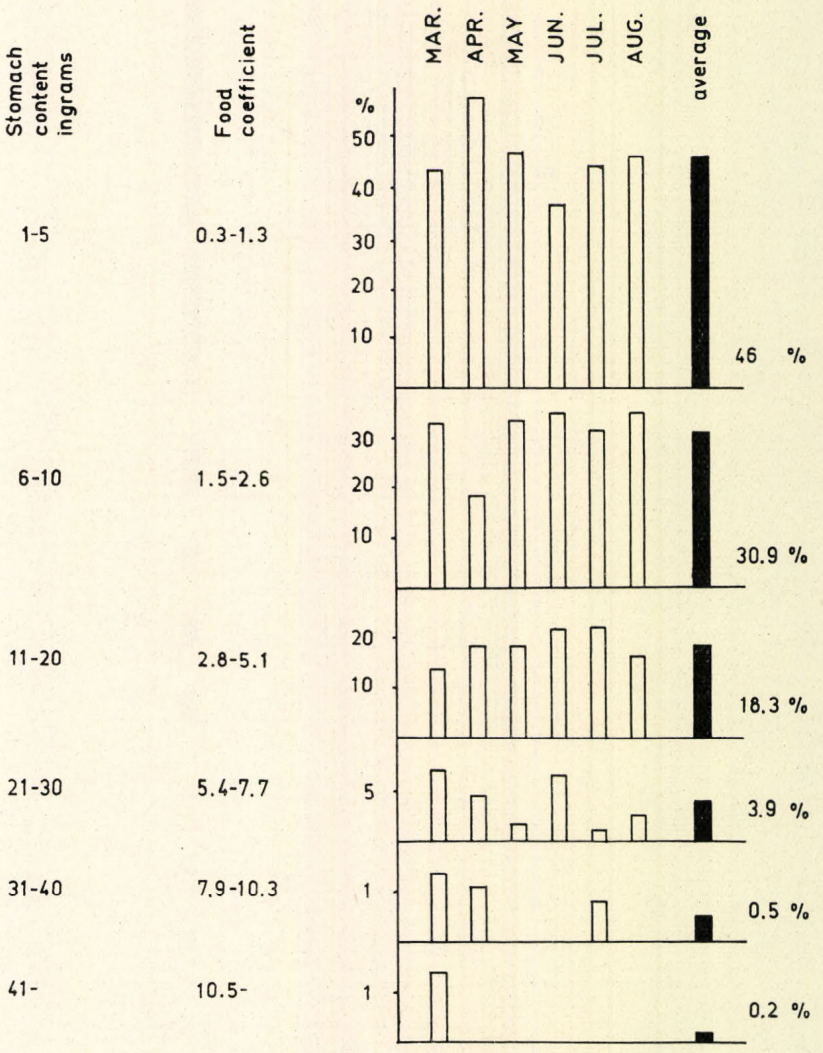

Fig. 5. The percentual distribution of feeding pike-perch in the different stomach-content weight groups (1968). The food coefficient values mean the weight of the food in the average weight percentage of the 390 g pike-perch

The food coefficient values in the case of the fourth grade pike-perch from Lake Balaton are extremely low and they change from one month to the other. Out of the 744 feeding pike-perch this value, calculated in the average of six months at 46%, is 0.1—1.3; at 30.9% it is 1.5—2.6; at 18.3% it is 2.8—5.1. A higher food coefficient has been observed only in a small number of

pike-perch *(Fig. 5)*. The nutritional mean falling to one pike-perch in the spring and summer periods is 6.2 g, while for the same period this number is 7.9 g when a feeding animal is concerned *(Fig. 6)*.

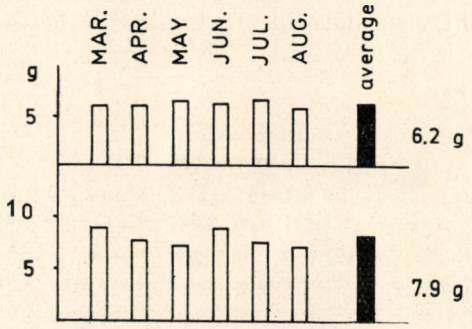

Fig. 6. Upper line: nutritional mean in grams falling on 1 pike-perch (both feeding specimens and those without food).
Lower line: nutritional mean in grams falling on 1 feeding pike-perch

3. *The catch of pike-perch in Lake Balaton in the past 8 years (1960—67)*

From fishery point of view the pike-perch of Lake Balaton is grouped according to the following: fourth grade: 300—500 g; third grade: 500—1000 g; second grade: 1000—1500 g; first grade: above 1500 g. One decade ago the average distribution according to the individual number of the catch was as follows: 75% from the fourth grade, 20% from the third, 2% from the second and 3% from the first grade (WOYNÁROVICH, 1959).

The quantity of annual pike-perch catch between 1960 and 1967 displayed the following variations:

	Grade				Total (q)
	IV	III	II	I	
1960	962	415	73	134	1584
1961	1093	420	73	180	1766
1962	780	362	68	175	1385
1963	990	496	97	133	1716
1964	972	425	108	154	1659
1965*	396	129	33	43	601
1966	283	245	57	65	650
1967	367	238	103	108	816
	5843	2730	612	992	10 177

* *Note:* On the spring of the year marked with an asterisk as the result of pesticide materials poluting the lake, according to the estimation of RIMANÓCZY, about fifty waggons (5000 q) of fish perished, of which about 40% was pike-perch (BARON et al. 1967)

Since 1965 the catch of pike-perch has decreased by more than fifty per cent, and the proportion of catch in the different weight groups has also greatly changed. After 1965, the restriction imposed on the fishing of lower weight categories brought about an increase in the individual number of pike-perch in the groups of the second and first grades taking the total of the catch.

Discussion

A gradual change may be observed in the qualitative composition of pike-perch stomachs examined in 1965, 1967 and 1968. In 1965 the autumn food mainly consisted of *Lucioperca lucioperca* and *Acerina cernua*, while in 1967 firstly bleak *(Alburnus alburnus)*, secondly ruffe *(Acerina cernua)* were dominant for food supply. In the first year the number of specimens with empty stomachs was greater than that of the feeding animals *(Table 1, Fig. 1)*. It is clearly shown in the data of 1968 that the primary food of the fourth grade pike-perch was bleak *(Alburnus alburnus)* and the second in line was ruffe *(Acerina cernua)*. Their proportion in the food was nearly 2 : 1 *(Table 3, Fig. 2)*.

Woynárovich (1959) reports ruffe as the main source of food for pike-perch. In the past few years the *Acerina* population has numerically decreased in Lake Balaton, which fact does not only come from the noted frequency of ruffe in stomach contents but also from the low number of individual specimens in our catch. The population of ruffe, likewise to other quality fish, was drastically decreased by the fish destruction of 1965 (Tölg, personal communication). The constant cause of progressive extinction of stock should be sought for in the gradual change takint place in environmental conditions for years back (Tölg, 1961; Sebestyén, 1967).

Bleak is more important than ruffe as the food of pike-perch, especially for its ideal shape, digestibility and nutritive value: about the double of ruffe's. The habitats of pike-perch and bleak are rather close to one another, from vertical point of view they are readily separable (Woynárovich, 1959; Tölg, 1961). There is about a 2—3 m thick layer of feculent water between bleak inhabiting a water layer near the surface, and pike-perch, rather inhabiting the bottom part of the water (Woynárovich, 1959). The rather low water-level in 1968 might have abolished these border-lines, for it was readily conceivable from the stomach contents that pike-perch consumed from almost every age-group of bleak living in the lake. The most frequent of them were specimens with 5—6 cm long body, living close to the water surface *(Fig. 3)*. In mixed shoals, too, the adult specimens rather frequented places nearer to the habitat of pike-perch, consequently, the preponderance of bleak of 5—6 cm in length in the food of pike-perch just proves an active vertical movement in search for nourishment of the consuming animal. According to the data published by Fortunatova (1949) the majority of pike-perch of the Caspian Sea and in the delta of Volga feed on fish of 10 cm long. It is clearly seen from the above that pike-perch consume for them easily available and the most appropriate sized fish.

Because of the seasonal presence or absence of the other observed species they may only be regarded as incidental nourishment. Further species can be

added to the food-list of the fourth grade pike-perch: *Scardinius erythrophthalmus, Leucaspius delineatus, Tinca tinca* and the eel *(Anguilla anguilla)*. Besides these, it was the first occasion that we noted the consumption of newt *(Triturus* v. *vulgaris)* and frog *(Rana* sp.). In August only in one case did we note the remains of a leech *(Hirudinea* sp.) in a stomach content. FORTUNATOVA (1949) also reported frog consumption of 1.7% in the delta of Volga, and SIROVATSKY (1953) took the same observation at Veselov reservoir with a percentage of 1.1.

According to the literature discussing the most varied water types the nutritional basis of pike-perch comprises fish coming in masses available both regionally and seasonally. The food of pike-perch in seas and in rivers varies with respect to available, thus, during migration from seas to the mouth of the rivers in order to multiply, from regional point of view, their composition can be rather divergent. CHUGUNOVA (1931) reported that the main food of pike-perch inhabiting the Sea of Azov was *Harengula delicatula,* the second place was occupied by the species of *Benthophilus* and *Gobiidae,* and *Percarina maetoica.* In the Ribinsk reservoir and at the delta of Vistula pike-perch mainly consume *Osmerus eperlanus,* when this is absent other littoral species are taken as food *(Rutilus, Abramis, Blicca,* etc.*)* ROMANOVA, 1956; FILUK, 1962; FILUK and ŻMUDZIŃSKI, 1965). The pike-perch inhabiting the Veselov reservoir mainly feed on *Rutilus, Abramis* and *Knipowitschia longicaudata* (SIROVATSKY, 1953), while in Lake Gopło and in Vistula in Poland the greater part usually is Cyprinids (BUDZYŃSKA et al. 1956; HOROSZEWICZ, 1964).

We may conclude in connection with the variety of food that in the food composition of pike-perch living in Lake Balaton considerable gaps occur regionally, that is, at the catching places of pike-perch the bed-profile significantly influences the individual character of the habitat (open water, reed-grass range) accordingly, pike-perch obtain variable food both of quality and quantity. Starting from the northeastern basin passing down towards Balatonszemes, and the environs of Fonyód in the southwest, the examination of stomach contents prove a somewhat better nutritional condition, although, it still fall far from sufficient.

The importance of nutrition of the different fish consumed by pike-perch changes seasonally, thus, the nourishment within the identical water region may be of different constitution (FORTUNATOVA, 1949; SIROVATSKY, 1953; ROMANOVA, 1956). This statement is likewise applicable to the whole area of Lake Balaton (LUKÁCS, 1932a, b; WOYNÁROVICH, 1959).

Our data also emphasize the seasonal role of species found in the food *(Tables 1—3, Figs. 1—2).* Bleak is a very important species for nourishment both in spring and in the summer, but in April ruffe too comes into prominence. The generally narrow nutritional conditions are somewhat eased by the appearance of great shoals of pike-perch fry and of other species' in July. The nutritional importance of roach *(Rutilus rutilus)* has increased compared to previous years. It was rather striking, that species usually inhabiting bottom layer were scare in stomach contents *(Cobitis, Gobio).* The fact, that in what frequency a species may occur as food, is not only the function of feeding intensity of pike-perch but it also depends on the success of spawning of the species in question, of its accessibility and last but not least on the population density of the species. This problem would be well complemented by a close investigation dealing with the nutrition of the introduced eel population.

Consequently, our investigations call for a study of nutritional and populational problems based on a more extensive research work.

The intake of food of fish is a function of environmental and physiological factors (condition, etc.), thus, the number of consumed fish is regulated by the appetite of the predatory fish, the individual density of fish serving as food and by its accessibility (WOYNÁROVICH, 1959). It is very unlikely to assume that those specimens which consume only one fish each suffice their food requirement, of course, it is even wilder to conjecture that they starve on their own accord. The speed of digestion — considering poikilotherm animals — is greatly dependent on the temperature of the environment: in the case of pike-perch in Lake Balaton this speed in the summer period is some 8—9 times faster than in winter (MOLNÁR et al. 1967). The time elapse between two intakes of food can be estimated from the period of time necessary for digestion under the appropriate temperature conditions, consequently, the total amount of food consumed can be calculated duly considering seasonal change for a whole year (POPOVA, 1967; WINDELL, 1967). If for a 100% stomach fullness of pike-perch we take 10% body weight (WOYNÁROVICH, 1959) then in the spring and summer periods of 1968 the nutritional basis for the examined specimens of pike-perch was very low, in actual fact, it was a mere 2% taking body weight.

However, the weight of the actual intake of food in the majority of pike-perch was much below this given value *(Fig. 5)*.

Examining pike-perch WOYNÁROVICH (1959) found food in the stomach in 62.5% of the animals, while the 37.5% of them were without food. He assumes that a thinner pike-perch stock may taken in more food at similar density of nutritive fish but a thin pike-perch population may only gather its food at a scanty stock of nutritive fish with a worse efficacity. According to the data collected during the investigations, it might be assumed that owing to the decrease of stock, the percentual proportion of feeding individuals increased since 1965. On the other hand, the nutritional mean falling to one feeding pike-perch has decreased by 1.2 g compared to the amount consumed ten years ago *(Fig. 6)*. This clearly shows, that although an increase could be observed in the number of feeding individuals the amount of food consumed however, was less in the case of the fourth grade pike-perch in Lake Balaton, indicating an undernourishment.

In connection with the overall low values of the food coefficient for the sake of comparison it is well to mention, that the prey- predatory fish weight proportion of 2—5 years old pike-perch in Gopło Lake, Poland, was 2.4—4.8% BUDZYŃSKA et al. 1956), while this proportion in the delta of Volga was 5.1 (POPOVA, 1967). In other data this value fluctuates between 5—9; the really high food coefficient is around 20 (FORTUNATOVA, 1949).

Under fish breeding conditions or water regions with good supply of food 1 kg increase of weight may only be reached by 5—10 or 15 kg nutritional fish (RIBIÁNSZKY and WOYNÁROVICH, 1962; CHUGUNOVA, 1931). In Lake Balaton, if we consider the nutritional mean (6.2 g) as basis falling to one pike-perch, which is equivalent with about the weight of two 6 cm long bleak then far to many bleak should be consumed of the size and weight just mentioned. Pike-perch of the fourth grade in Lake Balaton at the scanty stock of nutritive fish obtain the quantity necessary for growth at a disproportionately longer period of time — compared to artificial fisheries — which circumstance explains the

slow and uneven growth: they are 1—6 dkg when one year old, 5—15 dkg at a 2 years, 8—50 dkg at 3 years, 15—60 dkg at 4 years, and they only reach one kg at 5 years of age (RIBIÁNSZKY and WOYNÁROVICH, 1962).

The established number of fish calculated on the basis of stomach content investigations gives a fair approximation of the insufficiency of available food. Our data also lead us to the conclusion that the scarcity of food in the past few years, even though the number of pike-perch decreased, is still an existing problem nowadays.

Summary

Fish remains found in the stomachs show the following order of frequency considering only the most important species: *Lucioperca lucioperca* > *Acerina cernua* > *Alburnus alburnus*, this sequence was displayed in 1965, however, in the years 1967 and 1968 the above order was reversed. Significant seasonal and regional fluctuations have been observed in the consumption of occasional species for feeding.

According to the number of fish found in the stomachs the most frequent type was which consumed only one specimen (45.6%), two specimens (30%) while the individuals consuming 3 specimens were scarce (12.5%). More than three specimens, consumed by one pike-perch, gave only 5.6—0.1%.

According to the evaluation of the weight of the consumed food 46% of the feeding pike-perch has taken 1—5 g, 30.9% 6—10 g and only 18.3% has taken 11—20 g of food. The respective values of food coefficient of these weight data are extremely low: only 3.9—0.2% of the pike-perch has reached a food coefficient higher than 5.4.

The proportion of feeding pike-perch and pike-perch with empty stomach from 1965 shifted to the benefit of the feeding type, however, the quantity of available food decreased, compared to the value ten years ago. The number of the feeding animals is not in proportion with the consumed food quantity in spite of the fact that a decrease was observed in the number of the pike-perch population and the feeding conditions of the fourth grade pike-perch in Lake Balaton have not improved considerably.

Acknowledgement

It is the agreeable duty of the authors to thank Dr. J. PONYI (Tihany) for his altruistic help while this paper was under preparation.

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A 300—500 G SÚLYÚ (IV. OSZTÁLYÚ) BALATONI SÜLLŐ [LUCIOPERCA LUCIOPERCA (L.)] TAVASZI-NYÁRI TÁPLÁLKOZÁSA 1968-BAN I. ADATOK AZ 1965. ÉVI HALPUSZTULÁST KÖVETŐ IDŐSZAK TÁPLÁLKOZÁSI VISZONYAIHOZ

Biró Péter és *Elek László*

Összefoglalás

A gyomrokban talált halmaradványok között 1965-ben gyakoriság szerint a *Lucioperca lucioperca, Acerina cernua, Alburnus alburnus* volt a legfontosabb táplálékhalak sorrendje, 1967 és 1968 években ennek fordítottja. Alkalmi táplálékhalfajok fogyasztásában jelentős szezonális és regionális fluktuációt tapasztaltunk.

Gyomrokban talált halak száma szerint leggyakoribbak az 1 db halat evők (45,6%), 2 db-ot (30%) és a 3 db-ot fogyasztók (12,5%) voltak. Nagyobb számú halat a süllőknek 5,6—0,1%-a fogyasztott.

A táplálkozó süllők közül az elfogyasztott táplálék súlyszerinti kiértékelését illetően 46% 1—5 g-ot, 30,9% 6—10 g-ot, 18,3% 11—20 g-ot fogyasztott. E súly-adatoknak megfelelő táplálék-koefficiens értékek igen alacsonyak: 5,4-nél magasabb táplálékhányadost a süllőknek csak 3,9—0,2%-ánál tapasztaltunk.

ПИТАНИЕ БАЛАТОНСКОГО СУДАКА IV.-ОГО РАЗМЕРА (ВЕС 300—500 Г) ВЕСНОЙ И ЛЕТОМ 1968 ГОДА. I. ДАННЫЕ К УСЛОВИЯМ ПИТАНИЯ В ПЕРИОД ПОСЛЕ МАССОВОЙ ГЫБЕЛИ РЫБ В 1965 ГОДУ

П. Биро и *Л. Элек*

Порядок наиболее важных кормовых рыб, установленных на основе остатков рыб в желудке, в 1965 году был: *Lucioperca lucioperca*, *Acerina cernua*, *Alburnus alburnus;* а в 1967 и 1968 годах был найдён противоположен этому ряд. В потреблении случайных кормовых рыб было найдено значительное сезонное и территориальное колебание.

Исследованные судаки по числу рыб, обнаруженных в их желудках, разделялись следующим образом: 45,6% из них одну рыбу, 30% употребляла 2 рыбы. 5,6—0,1 процентов судаков ели больше чем 3 рыб.

Из питающихся судаков по весу принятого корма были получены следующие данные: 46% принимал 1—5 г корма; 30,9% 6—10 г; и 18,3% 11—20 г. Коэффициенты корма соответствующие этим весам очень низкие: это значение превосходило 5,4 только у 3,9—0,2 процентов судаков.

Соотношение питающихся судаков и судаков с пустым желудком изменилось в пользу питающихся с 1965 года, но общее количество питательных веществ было ниже, чем 10 лет тому назад. Количество употребляемого корма не соответствует числу питающихся рыб, и несмотря на то, что общее число судаков понизилось, условия питания балатонского судака IV.-ого размера существенно не улучшались.

ANNAL. BIOL. TIHANY | 36 | 151–162 | HUNGARIA 1969

THE SPRING AND SUMMER NUTRITION OF THE 300—500 G PIKE-PERCH (*LUCIOPERCA LUCIOPERCA* L.) IN LAKE BALATON IN 1968. II. THE CALCULATION OF THE CONSUMPTION, DAILY AND MONTHLY RATIONS

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Received: 10th February, 1969

The study of natural food resource and digestive enzymes of fish began at the end of the last century, then realizing the importance of digestion rate in fish production (RICKER, 1946) the attention was concentrated upon to estimate the quantity of food turnover. Nowadays it became of primary interest to determine on the basis of stomach contents analysis and results achieved in connection with digestion experiments the daily ration and daily consumption of food under natural conditions.

For estimating the daily and monthly ration and the daily (24 hours) food consumption of fish several methods are known (SURBER, 1930; BAJKOV, 1935; DARNELL and MEIEROTTO, 1962; SEABURG and MOYLE, 1964; FORTUNATOVA, 1950 cit. POPOVA, 1967).

MOLNÁR and TÖLG elaborated (1961) an X-ray method to estimated under laboratory conditions the length of time necessary for stomach evacuation on predatory fish belonging to different groups of water temperature. HUNT (1960) measured volumetrically with the method of water displacement the rate of digestion in some species of fish, WINDELL (1966, 1967) determined the dry content of the digestible part of food in individual digestive phases in common sunfish.

Our home literature in this line is rather poor hardly got beyond the study of qualitative-quantitative composition of fish food under natural conditions, and that too is only in connection with the investigation of Lake Balaton.

Among the fishes of Lake Balaton, in respect of stomach contents investigations, pike-perch is the most thoroughly studied species. For the quality and quantity of the seasonal food of 300—500 g pike-perch are fairly well known (WOYNÁROVICH, 1959; BIRÓ and ELEK, 1969), and the periods necessary for the evacuation on stomach at different temperatures are precisely deliniated (MOLNÁR and TÖLG, 1962; MOLNÁR et al. 1967) thus, possibility was offered to estimate the daily and monthly consumption of pike-perch on the basis of food quantity found in the stomachs, further, it became possible to calculate the daily and monthly rations and to infer data concerning the intensity of their feeding.

Methods

In our calculations we made good use of the data already published in a previous paper (BIRÓ and ELEK, 1969). On the basis of measured and estimated total weight of food taken at the time of the last intake of food we divided the stomach contents into 6 groups and calculated the distribution of feeding pike-perch according to the number of individuals within each stomach contents weight-group. We have established by weight-groups the original total body weight of fish serving for food found in the stomachs of pike-perch (WOYNÁROVICH, 1959; BIRÓ and ELEK, 1969), calculated the group means, the standard deviations and variation coefficients. We made good use of *Arrhenius equation*, also employed by MOLNÁR et al. (1967), in calculating the periods necessary for the evacuation of stomachs at different temperatures for the average monthly temperature of the water of Lake Balaton (on the basis of temperatures taken in front of our Institute). The periods necessary for the evacuation of the stomach were given in hours, of which we could calculate the number of monthly intake of food (feeding intensity). The daily and monthly consumption have been calculated, on the basis of BAJKOV (1935) method, grouping according to stomach contents weights, in the case of each feeding pike-perch, by the following formula:

$$\text{daily consumption} = \frac{\text{average amount of food in the stomach}}{} \cdot \frac{24}{\text{rate of digestion}}$$

then the average was taken from the results obtained.

The daily and monthly rations were obtained from the data of consumption. We have estimated the extreme values of consumption and rations on the basis of minimum and maximum weights of the stomach contents.

Results

Feeding intensity

The original body length and weight of the ingested fish for food were estimated to a fair accuracy by the backbone, pharyngeal bones, operculum and the scales. Conclusions were drawn from the stomach evacuation periods in the function of average water temperature to the number of monthly intake of food *(Table 1)*. In March, at a temperature of 5.9 °C in the water the time

Table 1

	t	*y*	*i*
March	5.9	247.2	3
April	15.7	65.0	11
May	20.3	45.7	16
June	19.3	48.9	15
July	22.7	39.2	19
August	21.0	43.6	17

t = average water temperature in °C; y = time of digestion in hours (period of stomach evacuation); i = number of intakes of food per month.

necessary for stomach evacuation, compared to warmer months, was 4—6 times longer, which period, as the temperature rose, became shorter, attaining a 1—3 day period; the intake of food recurred in every 1—2 days. In March, the length of time necessary for digestion exceeded 10 days, rendering about 3 intakes of food; in April, this number lessened to 3 days which means that 11 intakes of food may take place. The most intensive period for intake of food occurred in July when the total amount of ingested food required only about a day and a half to pass from the stomach to the pylorus fulcrum and the intestine, calculating the time length in 6-month average: this value was the shortest, in actual fact in meant about 19 intakes of food per month.

Consumption

The measured and estimated total weight of stomach contents of feeding pike-perch showed varying values between March and August, the highest values were reached in March and July, while the lowest occurred in June. The nutritional mean calculated for one pike-perch (Σ x/n) may only be informative in the light of real consumption *(Table 2)*. During the period of investigation most pike-perch stomachs contained not more than 1—5 g food, 6—10 g or more was only found sporadically. The stomach contents within the appropriate weight group, the divergence from average and variational coefficients were almost the same. The food weight per individual distribution is

Table 2

	N	*n*	Σx	$\Sigma x/n$
March	206	140	1265	9.35
April	112	88	682	7.75
May	135	121	889	7.35
June	65	46	410	8.91
July	257	232	1746	7.52
August	145	117	826	7.06

N = total number of examined pike-perch; n = number of feeding pike-perch; Σx = total weight of stomach contents of n-number of pike-perch in grams Σx/n= average of food falling to one feeding pike-perch in grams.

falling short of normal considering feeding pike-perch *(Figs 1—6)*. In March, the calculated daily consumption was rather low in spite of the fact that the total food weight found in the stomach was comparatively high (1265 g). In the following months the amount of daily consumption increased until July. The consumption was most intensive in July: in average 4.6 g/ individual per day, while the August value fell back to the level of May. According to our calculations the food consumption for March was 2.7g/ individual per month, this value greatly increased in April, and gradually reached the value 87.5 g/individual per month by July, then in August this value decreased. The total consumption calculated for one pike-perch in the spring and summer months

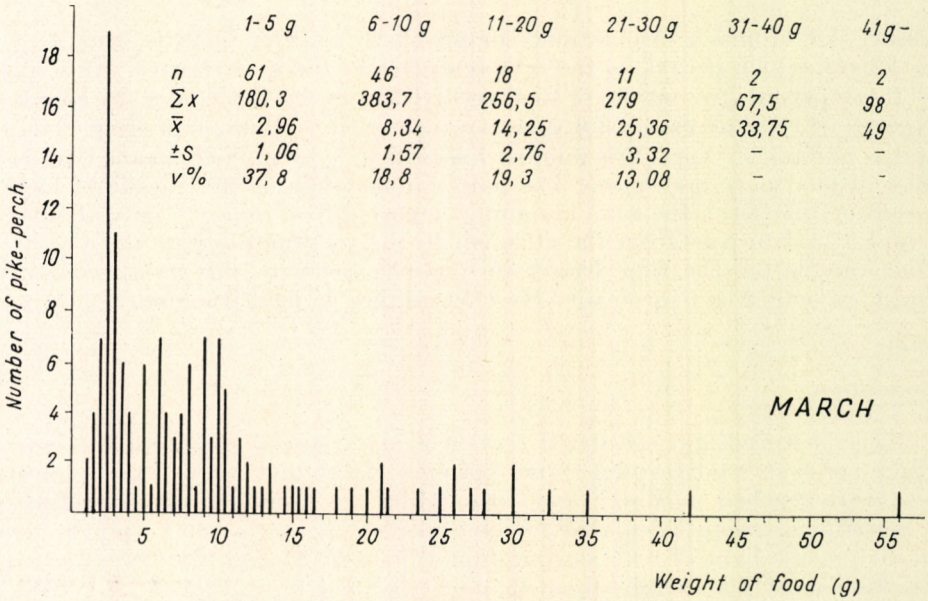

Fig. 1. The distribution of feeding pike-perch according to the weight of stomach contents per number of individuals in different months.
Sequence of numbers within the weight groups of stomach contents:
n = number of feeding pike-perch;
Σx = total weight of stomach contents of n-number of pike-perch in grams;
x̄ = arithmetical mean of food weight groups;
±s = standard deviation;
v% = variational coefficient

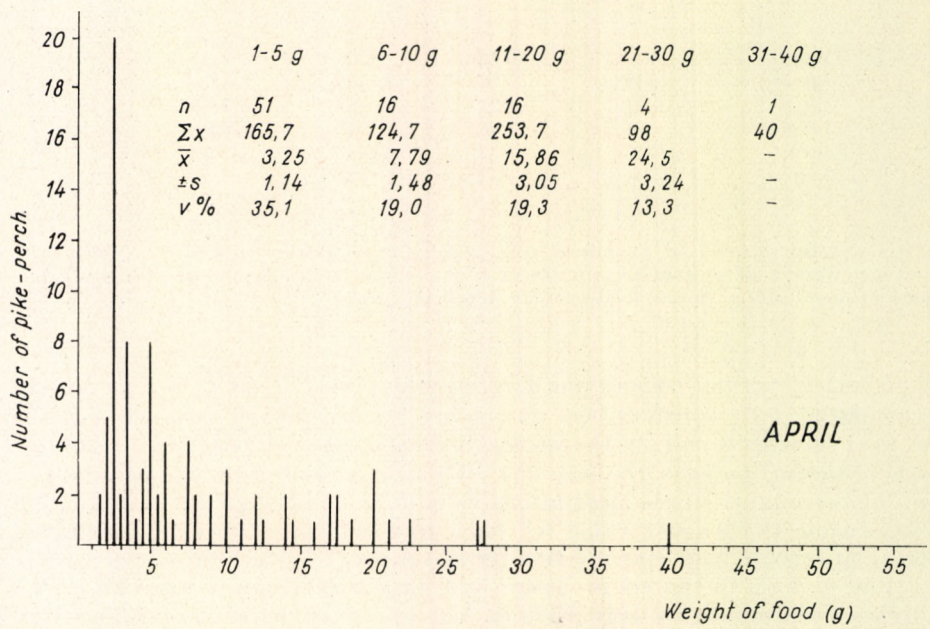

Fig. 2

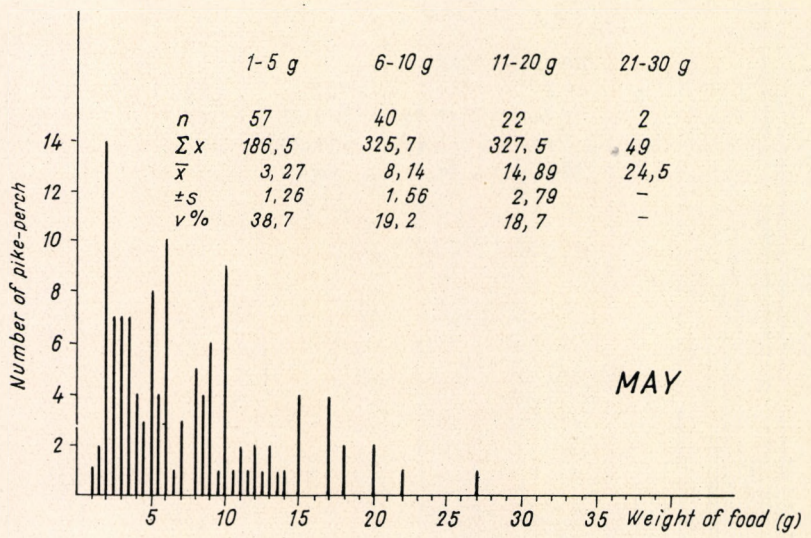

Fig. 3

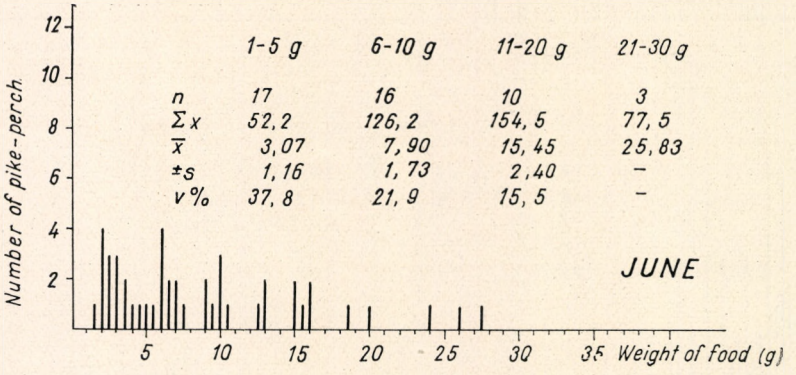

Fig. 4

was 315 g *(Table 3)*. The estimated minimum and maximum daily and monthly consumption data diverge to a great extent from the average when the extreme values of the weights of stomach contents are considered *(Table 4)*.

Daily ration

According to our data on fourth grade pike-perch in Lake Balaton the daily ration (i.e. the weight of the daily consumed food per the percentual average body weight of 390 g pike-perch) in the spring and summer months of 1968 was 0.87%; monthly ration 13.45%. On six monthly totalizing we found that the amount of ingested fish reached only 80.73% of the average

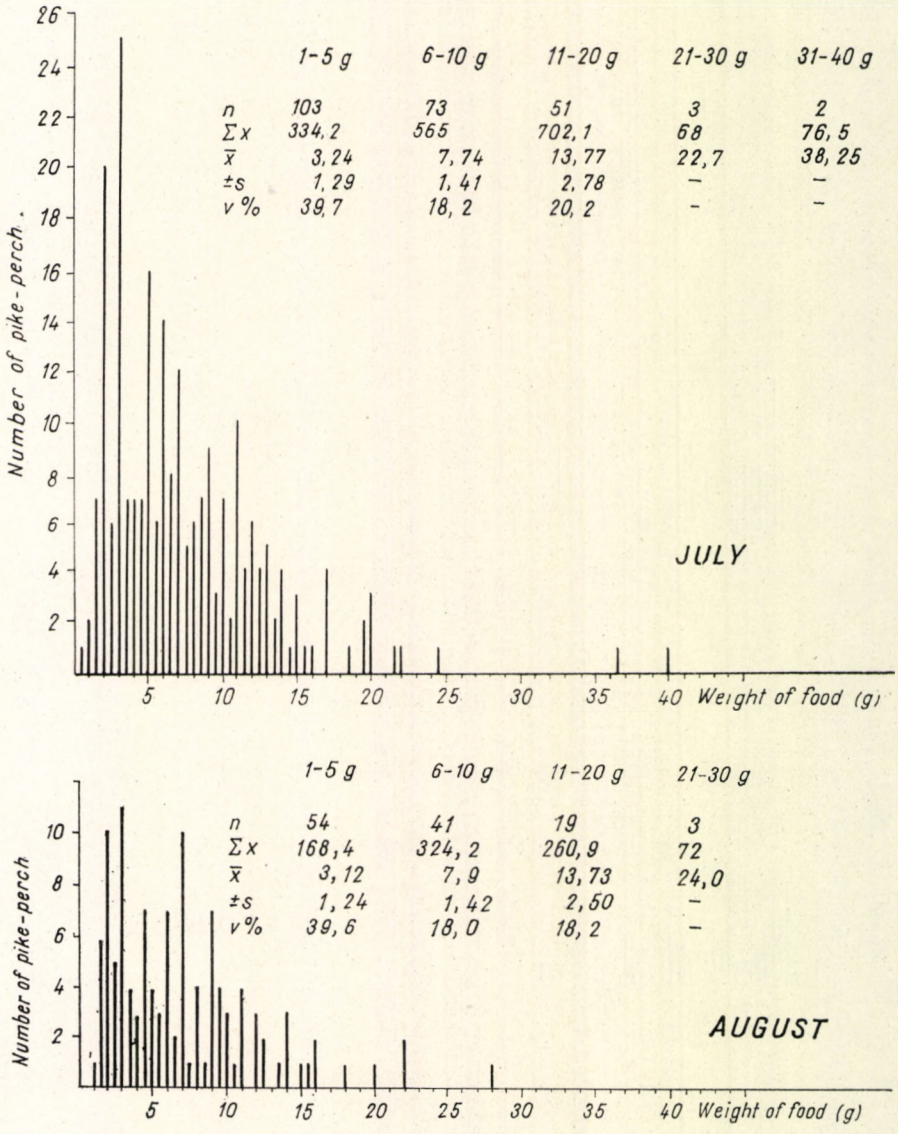

Fig. 5

Figs 2—6. Explanation see under *Fig. 1*

body weight of pike-perch *(Table 3)*. The relative indices of consumption in average extreme values gave 0.24—3.76% per day and 1.75—57.05% per month *(Table 5)*.

Table 3

	D_c	M_c	D_r	M_r
March	0.9	2.7	0.23	0.69
April	2.9	31.5	0.74	8.07
May	3.9	61.7	1.00	15.82
June	4.4	65.6	1.12	16.82
July	4.6	87.5	1.17	22.41
August	3.9	66.0	1.00	16.92
Total	20.6	315.0	5.26	80.73
Average	3.4	52.5	0.87	13.45

D_c = daily consumption in grams; Mc = monthly consumption in grams; Dr = daily ration; M_r = monthly ration

Table 4

	D_c		M_c	
	min.	max.	min.	max.
March	0.09	5.44	0.29	16.33
April	0.55	14.77	6.09	162.45
May	0.53	14.18	8.40	226.84
June	0.73	13.47	11.02	202.04
July	0.31	24.50	5.82	465.27
August	0.55	15.41	9.36	262.04
Total	2.76	87.77	40.98	1334.97
Average	0.46	14.63	6.83	222.50

D_c = daily consumption in grams; M_c = monthly consumption in grams (minimum and maximum values)

Table 5

	D_r		M_r	
	min.	max.	min.	max.
March	0.03	1.40	0.07	4.20
April	0.14	3.80	1.56	41.65
May	0.13	3.64	2.15	58.16
June	0.19	3.45	2.83	51.80
July	0.79	6.30	1.50	119.30
August	0.14	3.95	2.40	67.20
Total	1.42	22.54	10.51	342.31
Average	0.24	3.76	1.75	57.05

D_r = daily ration; M_r = monthly ration (minimum and maximum values in the average body weight percentage of 390 g pike-perch)

Discussion

Observations in connection with the intake of food as well as the investigations concerning this draw attention to the decisive importance of temperature influencing the length of time necessary for the digestion of food. MOLNÁR et al. (1967) stressed that generally pike-perch only consume further amount of food if the stomach contents are already liquefied and passed down into the pylorus fulcrum and intestine. There are observations to the effect that the digestive period is a function of the quality of food (WINDELL, 1966, 1967; POPOVA, 1967) and that a smaller amount of food is digested in shorter period of time than a bigger amount (BARRINGTON, 1957; HUNT, 1960). DARNELL and MEIEROTTO (1962) in investigating black bullheads *(Ictalurus melas)* came to the conclusion that the digestion of the standard food is an indicator to the digestion of other food-items.

According to our observations the majority of pike-perch in Lake Balaton ($\gtrapprox$ 76%) only consumed 1—2 fish consisting mainly of bleak *(Alburnus alburnus)* ($\gtrapprox$ 46%) (BIRÓ and ELEK, 1969). Thus taking bleak as the standard food, its relative stomach evacuation periods serve as essential footing to estimate the average ration and consumption (MOLNÁR and TÖLG, 1962; MOLNÁR et al. 1967).

Under experimental conditions the rate of digestion is the function of different stress-effects depending on the sensibility of the fish (WINDELL, 1966). Under natural conditions besides temperature the rate of consumption and the time necessary for digestion are the function of the physiological conditions (condition, age, body length, etc.). It has been established that the turnover of food in young fish in comparatively higher than in the older specimens, and that their rate of digestion is faster. The differences existing between the qualiative and quantitative composition of stomach contents bear revelance to the body length, growth, density of population and the inter- and intraspecific competition of the fish (RICKER, 1946; SEABURG and MOYLE, 1964).

According to investigations under experimental conditions carried out on pike-perch it was found that the rate of digestion was 8—9 times higher in summer than in winter (MOLNÁR et al. 1967), therefore it might be expected that the rate of consumption increase by a similar scale.

The feeding intensity and consumption determined on the basis of stomach evacuation periods are to a great extent influenced by the environmental factors existing in Lake Balaton. The differences between the calculated and measured values may be explained in the physiology of pike-perch, in the accessibility of natural food resources, in its distribution as well as in the selection.

Pike-perch is a seratim feeding animal, in its rhythm of life longer on shorter starving periods may be observed stemming perhaps from physiological reasons, e.g. in April before spawning (WOYNÁROVICH, 1959). Intensive intake of food occurs in the summer months perhaps because the vast shoals of fry of the nutritive fish appear and because of the higher water temperature (BIRÓ and ELEK, 1969). RADAKOV (1961, 1965) says that it is more difficult for the predatory fish to gain its prey from shoals than taking from sporadically occurring individuals, however, in certain instances the opposite is true, e.g. in winter when the motility of fish is somewhat impeded. The accessibility of

food, besides temperature, fundamentally influences the number of pike-perch with a full stomach and the ones with an empty stomach. Certain species (individuals) appear in greater numbers at sites where the nutritional conditions are more favourable (RICKER, 1946).

The ingested prey's original weight indicates that the greatest amount of food reaches the stomach in early spring and at the end of summer *(Table 2)*. The "factor of voracity" in the pike-perch of Lake Balaton (WOYNÁROVICH, 1959) is low mainly because of environmental and food accessibility circumstances. In six monthly average only a more 0.2—0.5% of the examined fish reached food intake of 10% of body weight, or of a near value (BIRÓ and ELEK, 1969: food coefficient). The distribution of pike-perch according to the mass of stomach contents shows that only a small number of the fourth grade pike-perch reaches satiety *(Figs 1—6)*. Two-year old specimens of *Abramis* and *Blicca* would mean sufficient amount of nourishment, of which one specimen would suffice to reach the degree of satiety, on the other hand, the significantly smaller bleak *(Alburnus alburnus)* and ruffe *(Acerina cernua)* on which pike-perch feed means of course under-nourishment.

It is a well known fact that pike-perch is a cautious pedatory animal fastidious in respect of shape and size and usually consumes the same type of fish throughout its life (WOYNÁROVICH, 1959; SEABURG and MOYLE, 1964).

The amount of the consumed food of the 300—500 g pike-perch rarely exceeds two per cent of the body weight *(Table 3)*. The divergences from average (individual variations) may be attributed to the nutritional possibilities for the food stock of the Lake seems to be sharply deliniated to certain areas as based on the investigations carried out on stomach contents (BIRÓ and ELEK, 1969). According to the investigations of DAWES (1930) it may be assumed that a smaller quantity of food be better utilized, i.e. the utilization of food in the case of pike-perch of Lake Balaton must be of a good efficiency. From this it follows that at different water temperatures full stomachs do not realize the same values in respect of fish production. The relative index of daily consumption indicates a low level of food turnover in the case of pike-perch. According to WINDELL (1966, 1967) the "actual daily rations" falling between the extreme values of maximum and minimum of the daily rations are in close connection with growth and production *(Tables 3—5)*. The daily and monthly rations are decisive factors on the rate of uneven growth influenced by under-nourishment (TÖLG, 1961).

In comparing our data to foreign results it is revealed that pike-perch of Lake Balaton from quantitative point of view are poorly fed. The yearly consumption of pike-perch inhabiting Volga delta is 2.02—2.39 kg taking one kilogram body weight (POPOVA, 1967). KARPEVICH, A. F. (cit. in: RICKER, 1946) in investigating pike-perch of Sea of AZOV *(Stizostedion lucioperca)* found that the monthly consumption in the winter-summer period varied between 0 and 1.5 times of body weight. In summer the daily ration reached 5% of body weight (average 0.58, taking into consideration the total quantity it was near 7.0 per year). The annual consumption of the whole population was 309.000 tons, i.e. 7.8 tons per km^2, according to his estimation.

SEABURG and MOYLE (1964) in investigating the summer nutrition of fish inhabiting two lakes of warm water situated in west Minnesota, observed that the centrarchid panfishes, among them walleye *(Stizostedion vitreum)*, had a daily ration of 1—2% of the body weight.

To give a sufficiently correct answer on the question that how inter- and intraspecific competition influence the rations in the function of fish-population density is only possible after an examination of food consumed by different species of fish (primarily of the introduced eel) and the estimation of fish stock.

Summary

Author established on the basis of six monthly experimental periods that the 300—500 g pike-perch inhabiting Lake Balaton feed most intensively in July: stomach evacuation occurs every day and a half meaning that the animal is capable to consume food 19 occasions per month.

The total weight of stomach contents of feeding pike-perch was 410 g in June, the lowest value, while in March and July it was 1265 and 1745 g, respectively, having been the highest value. The nutritional average falling to one pike-perch is only informative in nature because great differences occurred when compared with the calculated daily consumption.

The daily and monthly consumption of one pike-perch is small, in an average it only yields 3.4 g and 52.5 g. The quantity of food consumed over a period of six months was 315 g, which is 80.73% of the body weight.

On the basis of the data obtained it became clear that the food turnover of the fourth grade pike-perch inhabiting Lake Balaton is rather low.

Acknowledgements

Author wishes to express his sincere gratitude to Dr. JENŐ PONYI for his help and useful advice while preparing this paper and also to Dr. ELEMÉR LÁBOS for the invaluable guidance rendered during the evaluation of the statistical data.

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A 300—500 G SÚLYÚ (IV. OSZTÁLYÚ) BALATONI SÜLLŐ *(LUCIOPERCA LUCIOPERCA* L.) TAVASZI-NYÁRI TÁPLÁLKOZÁSA 1968-BAN II. A NAPI ÉS HAVONKÉNTI TÁPLÁLÉKFOGYASZTÁS, TÁPLÁLÉK ARÁNY SZÁMÍTÁSA

Biró Péter

Összefoglalás

Szerző hat hónapra terjedő vizsgálatok során megállapította, hogy a 300—500 g súlyú balatoni süllők júliusban táplálkoztak legintenzívebben: másfél naponkénti gyomorkiürülések mellett havi 19 esetben fogyaszthattak újból táplálékot.

A táplálkozó süllők gyomortartalmának összsúlya júniusban volt a legkisebb (410 g), márciusban és júliusban a legtöbb (1265 g, illetve 1746 g). Az egy süllőre jutó táplálékátlag csak tájékozódásra alkalmas adat, mert a számított naponkénti fogyasztással összevetve nagy különbségek adódtak.

Egy süllő napi és havi táplálékfogyasztása kis mennyiségű, átlagosan 3,4 g, illetve 52.5 g volt. Hat hónap alatt elfogyasztott táplálék 315 g-nak adódott, ami a testsúly 80,73%-át jelenti.

A kapott adatok alapján is nyilvánvaló a IV. osztályú balatoni süllők táplálékforgalmazásának alacsony szintje.

A táplálkozó és éhező süllők aránya 1965-től a táplálkozók javára tolódott el, azonban a táplálék mennyiségileg kevesebb volt, mint tíz évvel ezelőtt. A táplálkozók számával az elfogyasztott táplálékmennyiségek nem állnak megfelelő arány bn, sőt a balatoni IV. osztályú süllők táplálkozási lehetőségei a süllőállomány számbeli csökkenése ellenére sem javultak számottevően.

ПИТАНИЕ БАЛАТОНСКОГО СУДАКА IV.-ОГО РАЗМЕРА (ВЕС 300—500 Г) ВЕСНОЙ И ЛЕТОМ 1968 ГОДА. II. РАСЧЕТЫ СООТНОШЕНИЯ КОРМА И ПОТРЕБЛЕНИИ ПИЩИ В ДЕНЬ И МЕСЯЦ

П. Биро

В ходе исследований, продолжавшихся шесть месяцев, было установлено, что питание балатонского судака весом 300—500 г самое интенсивное в июле: предполагая, что через полтора дня их желудок опустошается они могли принимать пищу в 19 раз по месяц.

Вес содержимого желудка питающихся судаков самым низким оказался в июне (410 г), а самым высоким, — в марте и июле (1265 г и 1746 г). Среднее количество корма, расчитанного на одного судака, носит только ориентировочный характер, т. к. существует большая разница между расчитанными и потребленными количествами пищи.

Дневное и месячное потребления судака были низкие: в среднем 3,4 г и 52,5 г. Пища, потребленная в течении 6 месяцев, равняется 315 г, что соответствует 80,73% веса тела рыб.

На основе полученных данных ясно, что оборот пищы балатонских судаков IV.-ого размера находится на низком уровне.

ANNAL. BIOL. TIHANY | 35 | 163—171 | HUNGARIA 1969

EFFECT OF AGENTS MODIFYING THE LEVEL OF CYCLIC 3', 5'-ADENOSINE MONOPHOSPHATE IN ADIPOSE TISSUE ON MOBILISATION OF FATS IN FISH AND FROGS

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Received: January 20th, 1969.

It is accepted that catecholamines and some other lipolytic hormones stimulate the decomposition of triglycerides in adipose tissue of mammals by increasing the intracellular level of cyclic3', 5'-adenosine monophosphate(cAMP, SUTHERLAND et al. 1965, WEISS et al. 1966, BUTCHER et al. 1966). The effect of catecholamines is to first increase the cAMP content of adipose cells and this is followed by an increase in the lipolytic activity (BUTCHER et al. 1965). The cAMP enhances the activity of the adequately prepared adipose tissue homogenates too (RIZACK, 1964). It appears likely that the lipolytic activity of mammalian adipose tissue is partly a function of the level of cAMP in adipose cells.

Investigating the effect of catecholamines, adrenocorticotroph hormone and glucagon on the lipolytic activity of fish (FARKAS, 1967), green frog (FARKAS, 1966) and grass snake (FARKAS, 1968) adipose tissue under in vivo or in vitro conditions it was found that these hormones in contrast to those of mammals do not increase but actually decrease the free fatty acid production. From observations of the effect of the same hormones on frog adipose tissue phosphorylase (FARKAS, 1966), as well as from the observations that dichloroisoproterenol is able to antagonize the above effect of catecholamines (FARKAS, 1968) it may be inferred that there is also a biochemical system consisting of adenyl cyclase cAMP in the adipose tissue of lower vertebrates. It is certainly clear that the catecholamines at least decrease the free fatty acid production through this mechanism. It is possible that catecholamines enhance the formation of α-glycerophosphate by stimulating the decomposition of glycogen and that this in turn leads to an increase in the reesterification processes. On the other hand it is questionable whether the cAMP plays such a role in the maintenance of the lipolytic activity in the adipose tissue of lower vertebrates as it was supposed to do in mammals.

In the present study the effect of two agents is investigated on the mobilisation of fats in fish and frogs which were shown to modify the lipolytic activity of catecholamines i.e. to influence the cAMP level in mammalian adipose tissue. Theophylline and nicotinic acid were selected for this purpose, the former affects the mobilisation of fats in the same way as the catecholamines, the latter in the opposite direction (TRINER and NAHAS, 1966, CARLSON, 1963, FARKAS et al. 1964, BOMBELLI et al. 1965, BJORNTROP, 1965).

Material and methods

The experiments were carried out on the bream *(Abramis brama* L.), adult male marsh frogs *(Rana ridibunda* L.*)* and on grass snakes *(Natrix natrix* L.) weighing 200—250, 60—70 and 80—100 grams respectively. The fish were collected from Lake Balaton and kept in suitable aquaria, the frogs were purchased and the grass snakes collected. The animals were brought into the institute one week before the experiment. No food was given to the animals in captivity.

The drugs, dissolved in physiological saline, were injected intraperitoneally into the fish, and into the ventral lymph sac of the frogs. The control animals received physiological saline only.

Blood was withdrawn by cutting the caudal vein from the fish and by decapitation from the frogs and collected into prechilled heparinized centrifuge tubes containing 0.1 ml of 2% heparin solution.

The in vitro experiments were carried out on adipose tissues taken from freshly killed animals. The adipose tissues were cut up in 5—10 mg pieces and incubated at room temperature and at pH 7.4 in albumin free frog-Ringer solution. The free fatty acid content of the adipose tissues were determined at the start and at the end of the experiments, the difference giving the amount of fatty acids produced during the experiment.

Results

After injection of theophylline into fish and frogs —in contrast to the mammals (Hynie et al. 1966, Triner and Nahas, 1966) — no increase was obtained in the plasma free fatty acid levels *(fig. 1.)*. Both fish and frogs responded to the drug with a decrease in the plasma free fatty acid level but as the *fig. 1.* shows the fish reacted more sensitively than did the frogs. 40 mg/kg theophylline evoked a maximal plasma free fatty acid response in the fish, but in the frog a dosage of over 100 mg/kg was needed to produce the same effect. The blood sugar level was increased in both animals. This effect of theophylline is the same in fish and frogs as in mammals.

Under in vitro conditions in the presence of theophylline the free fatty acid production is diminished *(fig. 2.)*. Incubating the adipose tissue of the fish pike perch *(Lucioperca lucioperca* L.) in the presence of both catecholamine and theophylline the production of fatty acids was further diminished, but the effect was only additive.

The in vivo administration of nicotinic acid into untreated rats results in a decrease of plasma free fatty acids (Farkas et al. 1964, Bombelli et al. 1965). When nicotinic acid was injected into fish no decrease was obtained in the plasma free fatty acid level within 6 hours after the injection even when the dose was increased ten fold *(fig. 3.)*. In the experiments presented in the *fig. 3.* the animals were killed 3 hours after the administration of nicotinic acid. After nicotinic acid treatment the plasma free fatty acid level has increased in the frog. In both species blood glucose was decreased by nicotinic acid.

As shown above and presented earlier, both theophylline and epinephrine decreases the level of free fatty acids in the blood of lower vertebrates. *Fig. 4.*

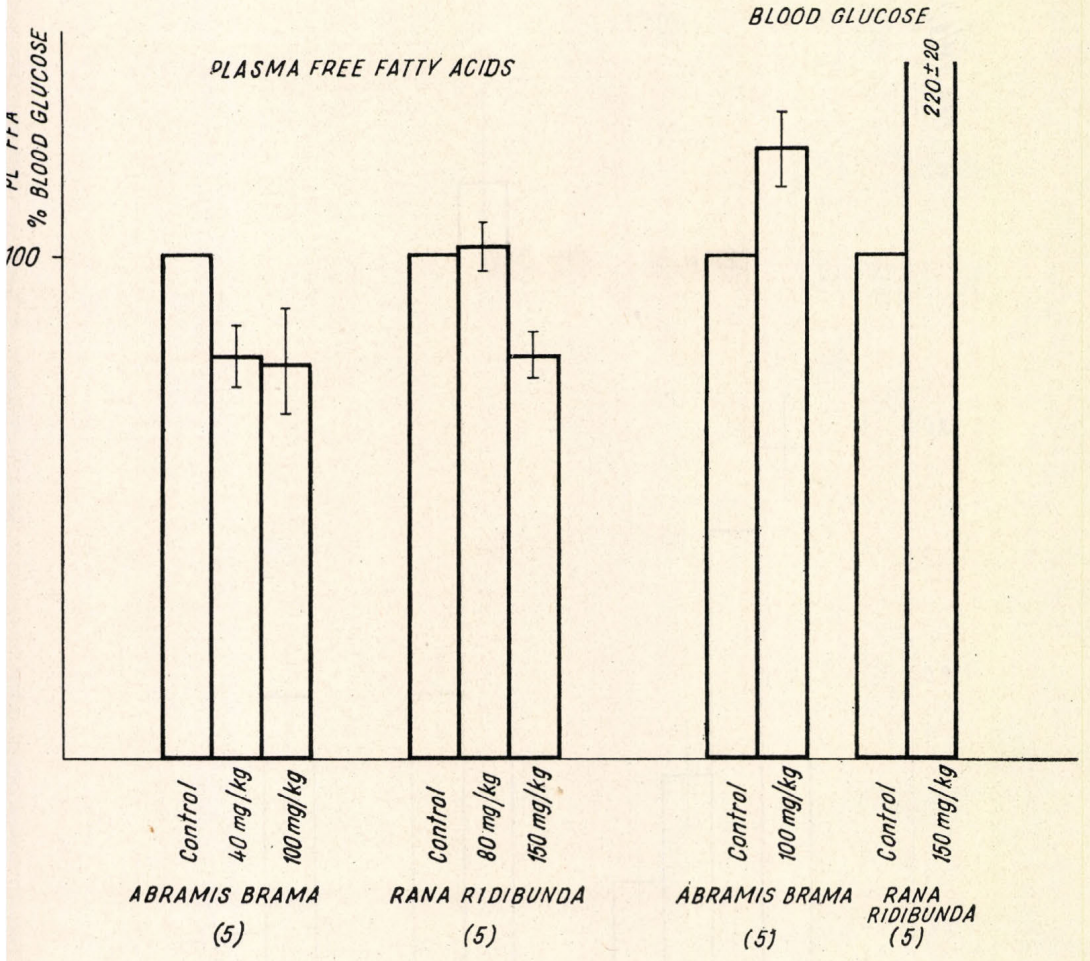

Fig. 1. Effect of theophylline on the mobilisation of fats and glycogen in fish and frog. The animals were killed two hours after injecting the drog. The results are expressed in the per cent of the control.

demonstrates that nicotinic acid is able to antagonize this effect of both agents. Thus the effect of nicotinic acid is essentially the same in the lower and the higher vertebrates except that in mammals it antagonises the lipolytic activity of catecholamines. Injecting both nicotinic acid and epinephrine in the fish produced an increase in the plasma free fatty acid level. A similar result was obtained when dichloroisoproterenol was used to antagonise the catecholamines in fish (FARKAS, 1958).

Discussion

The intracellular level of cAMP in the adipose tissue of mammals is regulated by the relation of two processes: the formation and decomposition of the nucleotide. Adenyl cyclase is responsible for its synthesis and a specific

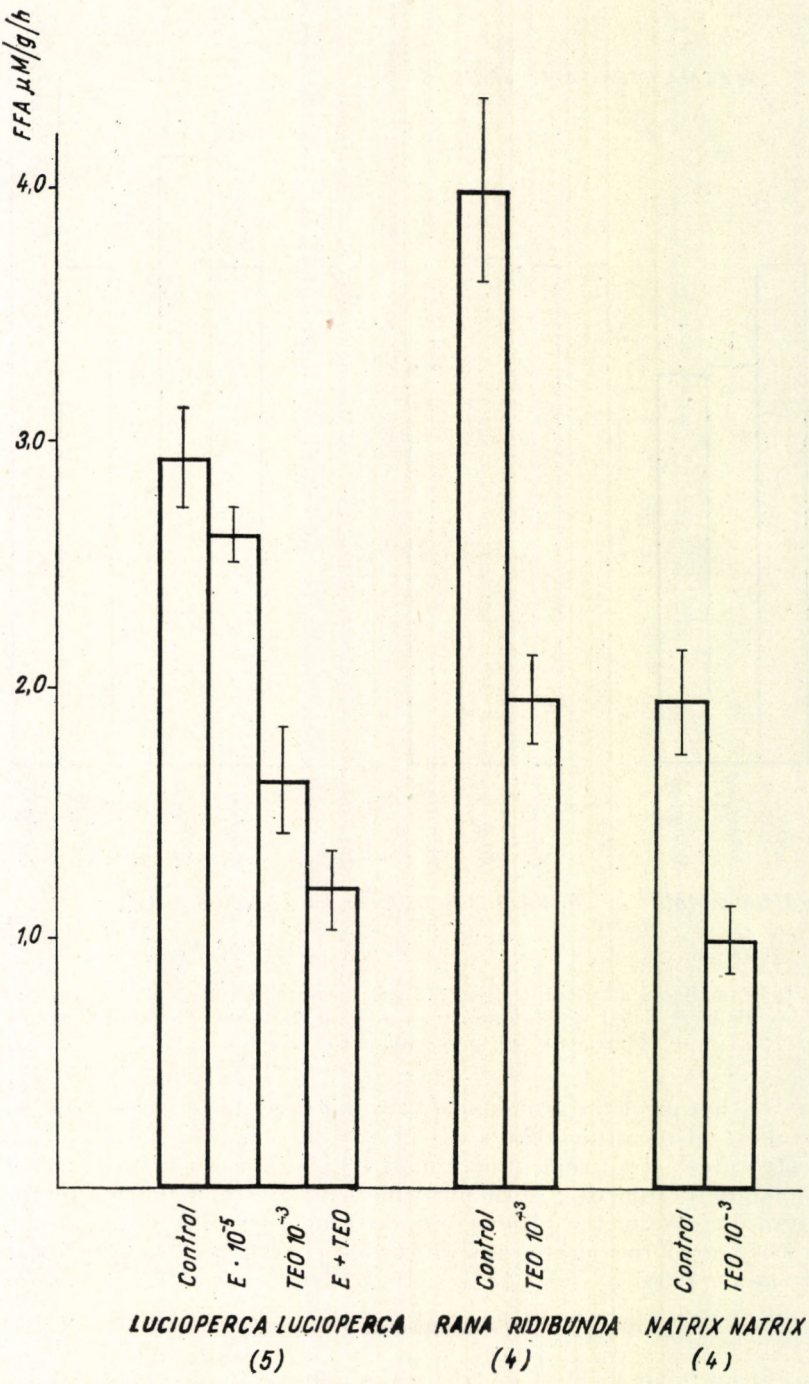

Fig. 2. Effect of theophylline on the free fatty acid production in vitro. The adipose tissues were incubated 60 minutes in albumin free frog Ringer solution at room temperature and at pH 7.4.

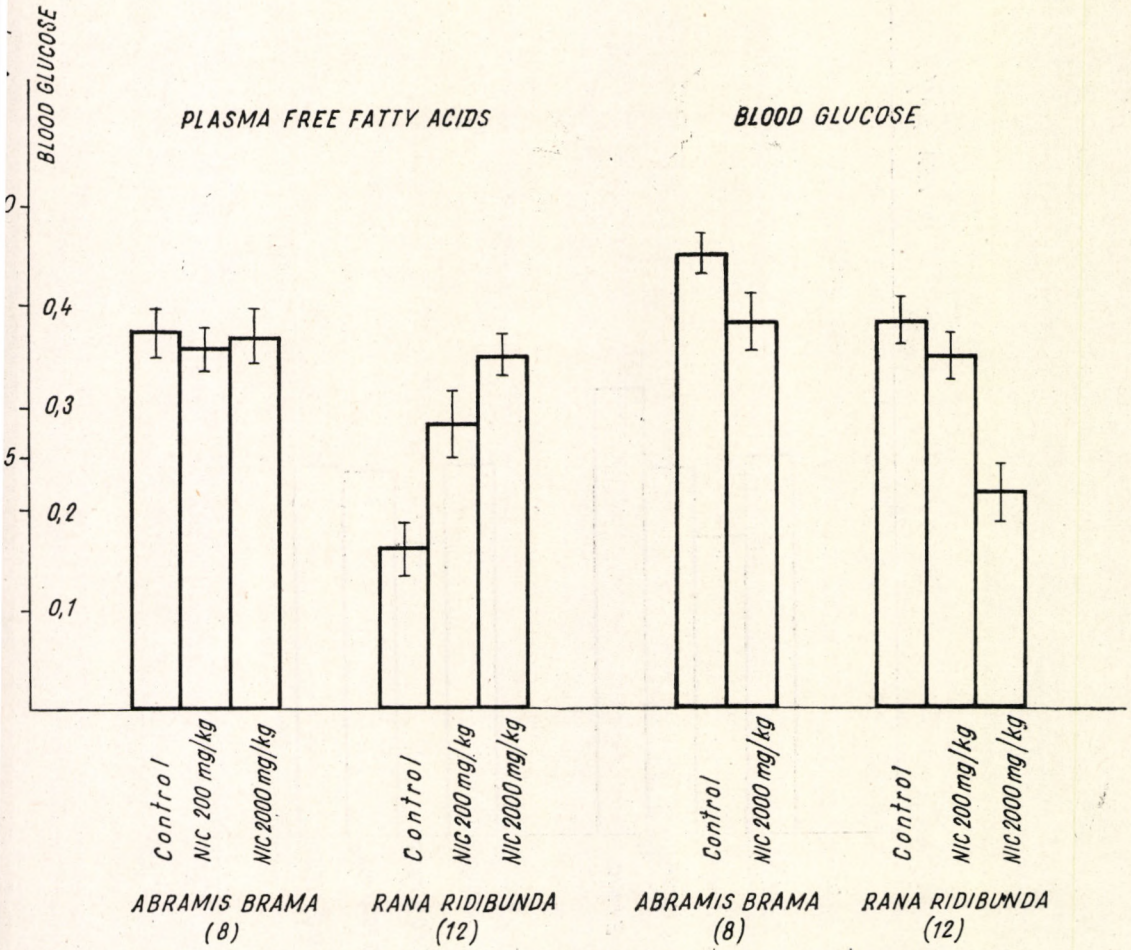

Fig. 3. Effect of nicotinic acid on plasma free fatty acid level and blood glucose in fish and frog.
The animals were killed 3 hours after administration of nicotinic acid.

phosphodiesterase for its decomposition. The former, in the presence of Mg^{++} ions, removes two phosphate moieties from ATP and joins the remainder to the 3rd carbonic atom of the ribose the latter splits the ring formed by the cyclase reaction and forms 5AMP.

The drugs employed in the present study affect the intracellular level of cAMP in different ways. Methylxantines (theophylline, cafeine) are able to block the phosphodiesterase and in this way to increase the cAMP level in the adipose tissue. Theophylline increases the level of free fatty acids in the blood of rats and potentiates the lipolytic effect of catecholamines both under in vivo and in vitro conditions (Hynie et al. 1966, Triner and Nahas, 1966). Nicotinic acid by stimulating the above enzyme (Krishna et al. 1966) may decrease the production of free fatty acids and antagonize the lipolytic effect

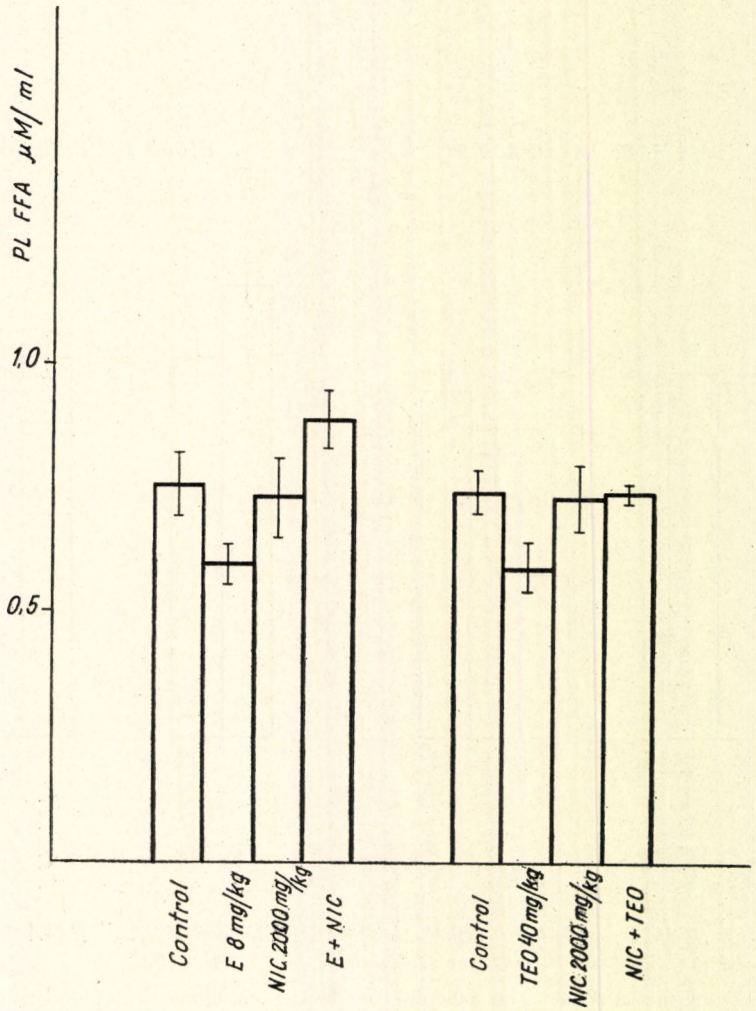

Fig. 4. The reversal of epinephrine and theophylline induced decrease of plasma free fatty acid level in fish by nicotinic acid.

of catecholamines (CARLSON, 1963, VERTUA et al., 1964, BJORTROP, 1965) and dibuthyryl cAMP (MELVIN et al. 1968) under in vitro and in vivo conditions.

If cAMP played any role in the maintenance of the lipolytic activity in fish and frog adipose tissue theophylline should increase and nicotinic acid decrease the plasma free fatty acid level. If, however, the catecholamines decreased the free fatty acid production in fish and frog adipose tissue by increasing the intracellular level of cAMP, it could be expected that:

1. theophylline would affect the free fatty acid production in the same and nicotinic acid in the opposite direction as the catecholamines and

2. nicotinic acid would antagonise the effect of catecholamines and theophylline on the mobilisation of fatty acids.

The experiments have shown, that theophylline decreases the production of free fatty acids in adipose tissue in vitro and the level of plasma free fatty acids in vivo. The adipose tissues incubated in the presence of both catecholamines and theophylline produced less free fatty acids than if they had been incubated only in the presence of epinephrine or theophylline.

In vivo administration of nicotinic acid did not lead to the expected decrease in the plasma free fatty acid levels. It left unchanged the plasma free fatty acids in the fish and inreased their level in the frogs. The blood glucose level was decreased in both cases.

The hypoglycemic activity of nicotinic acid has been described in the literature and evidence has been presented that-like insulin-it stimulates the uptake of glucose into the adipose tissue (LEE et al. 1961, BJORNTROP, 1965). Its effect on the plasma free fatty acid level was opposite to that which the hypoglycemic activity would have suggested, however. Increased uptake of glucose results in an increase in reesterification of the liberated fatty acids and this in turn leads to a decrease in the plasma free fatty acids. No final explanation can be offered at present as to why nicotinic acid does not lead to a decrease in the plasma free fatty acid level. It is possible that this may be related to its effect on the direction of glucose decomposition. Nicotinic acid-like insulin-directs the glucose into the pentose phosphate shunt (LYNN et al. 1960, LEE et al. 1961) which results in the formation of free fatty acids and not α-glycerophosphate.

The observation that nicotinic acid antagonised the effect of epinephrine and theophylline on the free fatty acid level furnishes further evidence that catecholamines, and probably the other lipolytic hormones too, decrease the plasma free fatty acid level in lower vertebrates by increasing the intracellular level of cAMP in the adipose tissue.

It may be inferred from the above work that cAMP formed by the lipolytic hormones does not play any role in the maintenance of the lipolytic activity in the adipose tissue of lower vertebrates. How this nucleotide stimulates the triglyceride lipase in the adipose tissue of mammals is not clear. It is possible that the enzyme, like the phosphorylase, has an active and an inactive form (HYNIE et al. 1966) and that it affects the conversion of the inactive lipase to an active form. On the other hand, it is not clear whether this is the only lipase in the adipose tissue of the mammals. The presence of a lipase independent of catecholamines supports the results of RUBINSTEIN et al. (1964) showing that besides the lipoprotein lipase and hormone sensitive lipase there also exists a lipase in the adipose tissue of the rat which does not require catecholamines for its activity. The effect of nicotinic acid on the in vitro free fatty acid production of rat adipose tissue suggests the presence of such a catecholamine-independent lipolytic activity as well. Under in vitro conditions, nicotinic acid is not able to antagonise the free fatty acid production of the untreated tissues, only that of the catecholamine treated tissues (CARLSON, 1963). From the observation that under in vitro conditions maximally only a 70% decrease was obtained in the plasma free fatty acids of rats treated with nicotinic acid (BOMBELLI et al. 1965) it may be inferred that about 30% of the fatty acids is the product of such a catecholamine-independent lipolytic activity. Because the catecholamines did not increase the rate of decomposi-

tion of triglycerides in the adipose tissue of fish, frog and grass snake, it is thought that this catecholamine independent lipolytic activity is the only one ensuring the decomposition of triglycerides in these animals.

Summary

Theophylline under in vivo and in vitro conditions decreased the plasma free fatty acid level and adipose tissue free fatty acid production in fish *(Abramis brama* L., *Lucioperca lucioperca* L.), frog *(Rana ridibunda* L.*)* and grass snake *(Natrix natrix* L.*)* After in vivo administration of nicotinic acid, the plasma free fatty acids remained unchanged in the fish, while in the frog, their level was raised. Nicotinic acid antagonised the effect of epinephrine and theophylline on the free fatty acid level in the fish. Because theophylline is known to increase and nicotinic acid to decrease the intracellular level of cyclic 3', 5'-adenosine monophosphate in the adipose tissue it is supposed that this nucleotide does not play role in the control of the lipolytic activity in the adipose tissue of lower vertebrates.

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A ZSÍRSZÖVET cAMP SZINTJÉT BEFOLYÁSOLÓ ÁGENSEK HATÁSA ZSÍRMOZGÓSÍTÁSRA HALAKBAN ÉS KÉTÉLTŰEKBEN

Farkas Tibor

Összefoglalás

Teophyllin hatására in vivo vagy in vitro körülmények között csökken a plazma szabad zsírsavak szintje, ill. a zsírszövet szabad zsírsav termelése halban *(Abramis brama)* és kétéltűben *(Rana ridibunda)*. Nikotinsav in vivo adagolása után a halban változatlan maradt, kétéltűben pedig emelkedett a plazma szabad zsírsavak szintje. Nikotinsav antagonizálta adrenalin és teophyllin hatását a plazma szabad zsírsavszintre halban. Minthogy teophyllin növeli, nikotinsav pedig csökkenti a zsírszövet cAMP szintjét emlősökben, jelen és korábbi eredményeink alapján feltételezzük, hogy ez a nucleotida nem játszik szerepet a lipolytikus aktivitás fenntartásában alacsonyabbrendű gerincesekben.

ВОЗДЕЙСТВИЕ ВЕЩЕСТВ, ИЗМЕНЯЮЩИХ УРОВЕНЬ ЦИКЛИЧЕСКОЙ АМФ ЖИРОВОЙ ТКАНИ, НА МОБИЛИЗАЦИЮ ЖИРА РЫБ И АМФИБИЙ

Т. Фаркаш

Под влиянием теофиллина in vivo и in vitro снижается уровень свободных жирных кислот плазмы и синтез свободной жирной кислоты в жировой ткани рыб *(Abramis brama)* и амфибий *(Rana ridibunda)*. После введения никотиновой кислоты in vivo уровень свободных жирных кислот плазмы у рыб останется без изменения, а у амфибий — увеличивается. Никотиновая кислота оказала антагонистическое адреналину и теофиллину действие в изменении уровня свободных кислот плазмы рыб. Так как теофиллин увеличивает, а никотиновая кислота снижает уровень циклической АМФ жировой ткани млекопитающих, на основе этих и предыдущих данных автор предполагает, что этот циклический нуклеотид не играет роли в сохранении липолитической активности низщих позвоночных.

ANNAL. BIOL. TIHANY	36	173–177	HUNGARIA 1969

GAS CHROMATOGRAPHIC STUDIES ON THE SEASONAL CHANGES IN THE FATTY ACID COMPOSITION OF THE COPEPOD (CRUSTACEA) PLANKTON

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Received: February 10th, 1969

In earlier investigations we found that the fat of crustacean plankton has much higher iodine values in winter, than in summer (FARKAS and HERODEK, 1964). That time the fatty acid composition was analysed still by paper chromatography. From such a complicated mixture after developing on paper fatty acids differing in chain length and in degree of unsaturation get to the same spots. However by hydrogenating the samples before chromatography, it was possible to check the seasonal changes at least in the distribution according to the chain length. This way a definite increase in C_{20}, C_{22} fatty acid content of copepod crustaceans by decreasing water temperature was demonstrated. We supposed, that the bulk of these long-chain fatty acids consisted of poly-unsaturated-ones. Later by gas chromatography these long, highly unsaturated fatty acids were really demonstrable from several crustacean species (HERODEK and FARKAS, 1967).

In present work seasonal changes of the fatty acid composition are followed up by gas-liquid chromatography, in order to determine exactly which fatty acids are involved in the temperature adaptation of crustaceans.

Material and methods

The crustaceans were sampled with a No. 6 plankton net from Belső-tó pond in the peninsula of Tihany. The animals were transferred alive in water to the laboratory. The samples were examined under microscope, and used only if besides Cyclopids *(Cyclops vicinus, Acanthocyclops* sp.) other species were not present in more than one per cent. Fresh weight of the collected material was several grams. The animals were blotted and grounded by anhydrous Na_2SO_4. The samples were extracted three times with 30 ml petroleum ether/g sample under reflux in N_2 atmosphere. The lipid content of the pooled solvents was determined gravimetrically, after evaporating an aliquot. Accordingly another aliquot containing 40 mg lipid was taken, evaporated under N_2, and the lipid redissolved in hexane. Transmethylation was carried out with absolute methanol containing 5 per cent cc. HCl. Five ml of this hydrochloric acid-methanol mixture was added to the 40 mg lipid dissolved in 1 ml hexane, and the mixture was sealed into test tubes under N_2. The tubes were kept at 80°

Table 1.

Seasonal changes in the

Date of sampling	Water temperature t °C	14 : 0	14 : 1	15 : 0	15 : 1	16 : 0	16 : 1	16 : 2	16 : 3	18 : 0	18 : 1
1967. X. 11.	17	2.5	1.8	1.1	—	14.0	15.5	—	—	6.1	9.3
X. 25.	12	2.0	0.7	0.8	—	11.7	12.0	—	—	5.7	7.9
XII. 6.	4	1.9	1.0	1.7	—	8.4	10.8	—	—	3.8	7.9
1968. II. 27.	3	1.4	1.0	0.9	—	7.8	4.2	3.4	—	2.2	4.5
III. 13.	2	1.6	0.6	0.5	1.3	5.9	1.7	—	—	1.1	4.5
IV. 3.	14	2.5	1.7	0.4	0.9	10.8	7.8	3.8	2.2	6.6	8.4
VII. 6.	24	1.8	1.4	1.7	0.8	11.0	8.3	2.0	1.5	6.0	13.7

C for 4 hours. After cooling down the upper phase containing the methyl ester was separated.

The analysis was carried out by a Chrom III. IKZ (Laboratorni Pristroje CSSR) gas chromatograph. The equipment operated with flame ionization detector. Column length was 3 m, inner diameter 6 mm. It was filled with 20% ethylene glycolsuccinate on 80—100 mesh Chromosorb W. Column temperature was 184° C. Carrier gas was N_2, its flow rate 100 ml/min. Standard fatty acids (NIH) and mixtures of known fatty acid composition were used for peak identification. Peak areas were determined by triangulation. Correction factors for individual fatty acids were determined by means of known fatty acid mixtures of similar composition as the samples. With these factors the fatty acid composition was calculated in weight per cent.

Results

Altogether 25 different fatty acids were detected in the crustaceans *(Table 1)*. Some of them were not present in all samples in measurable quantities. There were 11 fatty acids surpassing at least in some months the 5 per cent of the total quantity of fatty acids. Each of the fatty acid 16 : 0, 16 : 1, 18 : 1, 18 : 4, 20 : 5 and 20 : 6 represented in some months more than 10 per cent. Only by 22 : 6 was the 20 per cent exceeded in winter. The longer the chain, the more prominent are the unsaturated acids. Among fatty acids with 14 carbon atoms the saturated myristic acid predominates. Among those with 16 carbon atoms 16 : 0 and 16 : 1 show about the same values. All C_{18} fatty acids were detected in quantities above 5 per cent, but the most unsaturated acid was already found in the highest amount. Among the C_{20} acids 20 : 4 and even more 20 : 5 dominate. Finally among the longest, 22 carbon atom long fatty acids only the most unsaturated acid was found above 5 per cent, but this 22 : 6 acid is the most abundant of all acids in the winter.

Investigations started with samples collected from still warm water, and changes in the fatty acid composition were followed during the cooling down of the water and its warming up in the next year. Accordingly data show in the middle of the *Table* the fatty acid composition in the coldest period and on the whole they change symmetrically upwards and downwards. It was

fatty acid composition

18 : 2	18 : 3 ω 3	18 : 4	20 : 1	20 : 2	20 : 3 ω 6	20 : 3 ω 3	20 : 4 ω 6	20 : 4 ω 3	20 : 5 ω 3	22 : 4 ω 6	22 : 4 ω 3	22 : 5 ω 6	22 : 5 ω 3	22 : 6 ω 3
4.6	5.1	16.1	—	2.4	—	—	6.1	—	10.6	—	—	—	—	4.8
1.4	3.5	9.7	—	2.3	—	—	4.9	0.8	12.8	0.7	2.2	1.8	1.7	17.4
1.9	5.7	11.7	—	4.1	—	0.7	3.7	1.0	13.2	0.4	1.7	1.1	0.6	18.7
6.0	2.6	6.2	—	9.9	1.2	0.3	2.7	2.4	13.9	1.4	1.7	—	1.1	25.2
4.5	5.1	9.0	4.2	4.6	05.	1.7	2.0	2.2	12.3	4.8	3.0	2.9	1.7	24.4
4.6	4.6	4.2	—	0.9	—	—	4.4	0.6	10.1	0.3	2.9	2.5	—	19.8
5.3	1.1	11.4	—	3.6	—	0.7	5.4	1.1	10.4	1.0	1.7	—	1.1	8.9

found in cases of C_{14}, C_{16} and C_{18} acids equally a decrease of saturated and monoenoic acids by the cooling down of the water. No changes paralleling that of the temperature were found in the di-, tri-, and tetraenoic acids. The 20 : 5 showed a definite increase from the summer value 10.5 to the winter 13.9. The most essential change was revealed in the 22 : 6 content, which by the cooling down of the water increased from 4.8 to 25.2 per cent.

Discussion

The significance of changes in the fatty acid composition for the survival of plankton crustaceans was indicated already earlier (FARKAS and HERODEK, 1964). The more unsaturated is the fat, the lower is its melting point. Melting point determinations of the fat of plankton samples collected at different times showed it to be always with 1—2° C lower, than the lake temperature. This means, that these animals are capable to regulate the composition of their fat in such a way, that it remains in a just liquid state the whole year through. In this process — as revealed by the present gas-liquid chromatographic results — to a lesser extent 20 : 5 and principally 22 : 6 acids are involved. The total of these two acids is high enough in winter, that even every glycerolipid molecule could get to one of them. The incorporation of fatty acids with 5 or 6 double bonds can alter basically the physical properties of the lipid molecules and the state of their mixture. The inter and intra-molecular distribution of these fatty acids seems to be worthy of further examination.

The elucidation of the mechanism regulating 22 : 6 level in the fat according to the changing requirements in these some millimeter long animals seems a difficult task. It is possible, that the synthesis of fatty acids or their incorporation into the depot fat is influenced by the temperature or by the physical state of fat. In green algae 22 : 6 was not yet detected (KLENK, 1963) and until it is not demonstrated from the food it can be supposed, that crustaceans produce it themselves. In 22 : 6 acid, isolated from fishes, the positions of double bonds are identical with those of decosahexaenoic acid of mammals. Fish oil originates by far greater part in plankton crustaceans. It is therefore most likely that 22 : 6 acid of plankton crustaceans is of divinyl-methane

structure too. If so, its synthesis may proceed in the same way that was demonstrated in mammals (KLENK, 1960; MEAD, 1960) i.e.:

$$18 : 3 \rightarrow 18 : 4 \rightarrow 20 : 4 \rightarrow 20 : 5 \rightarrow 22 : 5 \rightarrow 22 : 6.$$

Crustaceans can take up plenty of linolenoic acid from algae. Whether these animals can synthetize this acid or not is not yet known. The two acids involved in the temperature adaptation are closely related, as 22 : 6 is formed from 20 : 5.

Fatty acid composition of plankton crustaceans can correspond to the temperature so well only because the temperature changes are rather slow owing to the great heat capacity of the water. Poikilothermic land animals are subject to much higher temperature changes during short periods, sometimes exceeding 10 °C a day, and of course, they are not expected to change the composition of their fat so rapidly that its melting point should be always with 1—2 °C lower than the external temperature.

Summary

For the winter months the amount of saturated and monoenoic acid content of the fat decreased.

In di-, three- and tetraenoic acids no changes paralleling that of the temperature were observed. The 22 : 5 content somewhat increased for winter.

The greatest change was found in 22 : 6, the most unsaturated fatty acid. In summer it amounts to 5 per cent, while in winter to 25 per cent of the total fatty acids. It seems that the variations in the quantity of 22 : 6 ensures the constant optimal physical state of fat in crustaceans in spite of seasonal changes of the environmental temperature.

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A COPEPODA (CRUSTACEA) PLANKTON ZSÍRJÁBAN MUTATKOZÓ ÉVSZAKOS VÁLTOZÁSOK GÁZKROMATOGRÁFIÁS VIZSGÁLATA

Herodek Sándor

Összefoglalás

A téli hónapokra lecsökkent a zsírban a telített és egyszer telítetlen zsírsavak mennyisége.

A kétszer, háromszor és négyszer telítetlen zsírsavak esetében nem észleltünk a hőmérséklet alakulásával összefüggő változást. A 22 : 5 mennyisége télire kissé megemelkedett. A legnagyobb változást a 22 : 6, a legtelítetlenebb zsírsav mutatta. Ez nyáron az összes zsírsavak 5, télen 25%-át tette ki. Úgy látszik, hogy a 22 : 6 mennyiségének változása biztosítja, hogy a hőmérséklet évi változása ellenére a rákokban a zsír mindig optimális halmazállapotú maradjon.

ИССЛЕДОВАНИЕ СЕЗОННЫХ ИЗМЕНЕНИЙ ЖИРОВ РАКООБРАЗНОГО ПЛАНКТОНА ПРИ ПОМОЩИ ГАЗОВОЙ ХРОМАТОГРАФИИ

Ш. Херодек

Зимой в составе жиров происходит снижение количества насыщенных и однократно ненасыщенных жирных кислот.

В составе двух—, трех—, и четырехкратно ненасыщенных жирных кислот не были найдены изменения, зависящие от температуры: соотношение 22:5 зимой несколько повысилось. Самое значительное изменение было обнаружено в случае наиболее ненасыщенной жирной кислоты 22:6. Эта жирная кислота составляла летом 5 процентов от всего количества жирной кислоты — а зимой 25%. Вероятно, изменение соотношения 22:6 обеспечивает в раках, несмотря на годичные колебания температуры, постоянное оптимальное состояние жира.

ANNAL. BIOL. TIHANY	36	179–184	HUNGARIA 1969

THE METABOLISM OF THE INTRACARDIALLY INJECTED 1—^{14}C PALMITIC ACID IN THE CARP *(CYPRINUS CARPIO L.)*

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Received: February 10th, 1969

In mammals basic importance is ascribed to the blood free fatty acids for their very short turn over time in the transport of lipids and in the energy providing system of the organisms. Papers on this subject are accordingly numerous (ANNISON, 1964).

In our knowledge no experiments of this type have been carried out on fish up till now. The aim of the present study was to investigate whether after injecting labeled palmitic acid into the circulation of fish similar phenomena occur as in mammals, and that how far fatty acid transport among tissues of fish resembles to that in mammals.

Material and methods

Carps of 0.8—1.2 kg weight were used. They were transferred from the fish-pond at Irmapuszta on 29 October 1968. Before experiment fish were kept in aquarium for a day.

1-^{14}C palmitic acid (REANAL, Budapest) was used as labeled fatty acid. Its specific activity was 1 mCi/mmol. The blood plasma of one fish was separated. The labeled fatty acid was saponified by a small excess of 0.1 N NaOH. The fish blood plasma was poured to this optically clear, warm, radioactive soap solution, the mixture was vigorously shaken and filtered. The plasma obtai nedthis way contained 2 μCi/ml labeled palmitic acid bound to albumine. Of this plasma 0,5 ml i. e. 1 μCi fatty acid was intracardially injected into each fish

The injection was carried out slowly (in cca. 10 sec). The fish were then placed in a 100 liter aquarium for 5, 20 and 60 min respectively. After this interval the animals were removed and exsanguinated by cutting off their tail. The blood was immediately collected in chloroform: methanol 2 : 1. The blood obtained varied between 9 and 17 g. The liver was first rinsed in 0.49% NaCl, then weighed. An about 3 g part of the liver was cut off, weighed and homogenized in chloroform: methanol 2 : 1. In the case of the fish killed 60 minutes after the injection some flesh and some intestinal adipose tissue were also homogenized. Lipids were extracted from all tissues according to FOLCH et al. (1957). Solvents were evaporated under N_2 in Claisen apparatus, the lipids were dissolved in chloroform and stored under N_2 at —20 °C until further use.

The lipid classes were separated by thin layer chromatography as described earlier (HERODEK, 1968).

Radioactivity measurements were carried out with a Packard Tri-Carb scintillation spectrometer. The lipid samples were dissolved in 10 ml scintillation solution consisting of toluene containing 0.4 per cent 2,5-diphenyloxazole and 0.01 per cent 1,4-di-/[2-/5-phenyloxazolyl/-]benzene.

Results are expressed in per cent of the injected radioactivity, i.e. in 10^{-2} μCi. Data represent averages at 5 min of 3, at 20 min of 2 and at 60 min of 4 animals.

Results and discussion

Radioactivity of blood free fatty acids at different points of times after injecting labeled palmitic acid into fish is demonstrated in *Fig. 1.* For comparison data obtained in experiments with rats by GÖRANSSON and OLIVECRONA (1964) are also shown in this *Figure.* The disappearance of the labeled fatty acids from fish blood was so much slower that the time scale for fish

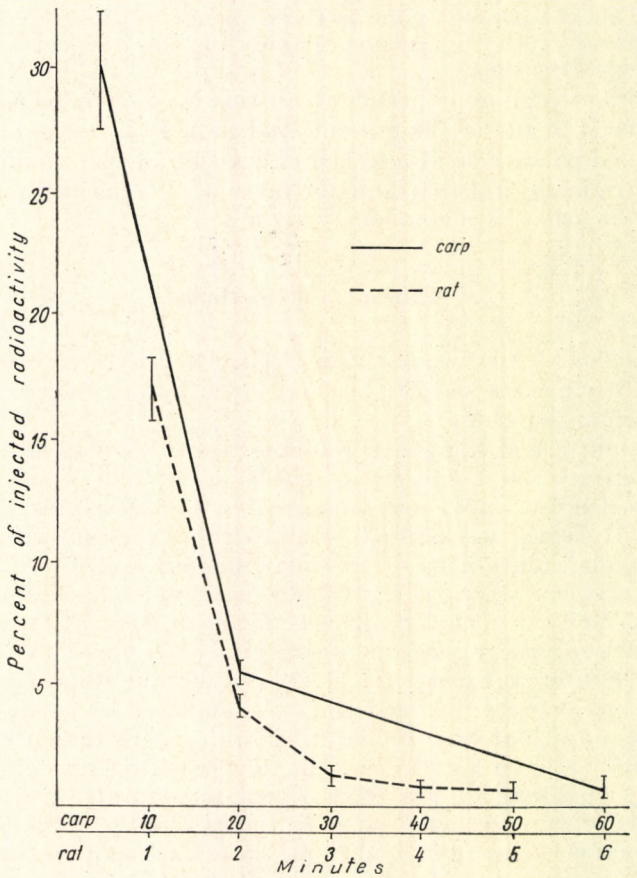

Figure 1.
Radioactivity in the blood free fatty acids after injection of 1-^{14}C-palmitic acid

had to be reduced to the tenth of that of rats to obtain curves of matching slopes. While in rats labeled free fatty acids practically disappeared from the circulation within 5 min, in carps about 30 per cent of the injected radioactivity is still present in the blood free fatty acids after the same period. In carps the radioactivity of free fatty acids decreased in 20 min to the same extent as in rats within 2 min and even after one hour a well measurable activity — about one per cent of the injected dose — was present in their blood free fatty acids. The turn over time of free fatty acids seems to be therefore in carps about ten times longer than in rats. For calculations the weight of blood was taken as 3 per cent of the weight of the whole body (KORZHUYEW and NIKOLSKAYA, 1951). The concentration of free fatty acids in the blood of carps is 0.6—1.2 μmol/ml (FARKAS, 1967) which is similar to that of mammals. From the data above it seems, that related to body weight in carps the turn over rate of blood free fatty acids is about twenty times lower than in rats. Ratio of their oxygen consumption is similar, it is in rat 2000 ml 0_2/kg/h and in carp 104 ml 0_2/kg/h *(Handbook of Biological Data,* 1956). In mammals free fatty acids of the blood originate in the adipose tissue. The adipose tissue amounts in mammals to 10—20 per cent of the body weight. The adipose tissue of fishes releases in vitro the same quantity of free fatty acids as the mammals' adipose tissue of the same weight (FARKAS, 1967). However fishes differ from mammals in having no subcutaneous adipose tissue, and that their intestinal adipose tissue amounts only to 1—2 per cent of their body weight. From this amount of adipose tissue the fatty acid inflow into the circulation is with one order of magnitude smaller than in mammals agreeing to the fact that the free fatty acid outflow of fish blood is also one order of magnitude lower due to the longer turn over time.

In mammals about one third of labeled fatty acids injected into the circulation is taken up by the liver. In liver of carps only a much lower radioactivity was detected *(Table 1).* As demonstrated above from t e blood of carp

Table 1.

Radioactivity in liver lipid fractions in per cent of the injected radioactivity

Lipid fraction	Time after injection		
	5 min.	20 min.	60 min.
Cholesterol esters	0.079 ± 0.015	0.221 ± 0.076	0.079 ± 0.013
Triglycerides	0.551 ± 0.202	3.023 ± 0.944	1.040 ± 0.204
Free fatty acids	0.654 ± 0.220	0.324 ± 0.188	0.669 ± 0.185
Diglycerides	0.246 ± 0.058	0.450 ± 0.346	0.317 ± 0.037
Phospholipids and Monoglycerides	0.733 ± 0.434	1.999 ± 0.478	1.260 ± 0.505

Mean values ± S. E. of the mean.

the free fatty acids disappear very slowly, thus even after a whole circulation time the bulk of labeled acids remains still in the blood. The liver's poor fatty acid uptake therefore can be really interpreted as a sign of a smaller or different role of the liver in the fat transport in carp than in rat. Also in carp liver

most of the labeled fatty acids are present in triglycerides and phospholipids. A considerable radioactivity is in the diglycerides observable too, as it was found already earlier in tissue slices of carp liver incubated in vitro with labeled palmitic acid (HERODEK, 1966). It seems that the formation of diglycerides of very long turn over time (HERODEK, 1967) represents a quite general phenomenon of triglyceride synthesis.

In the liver relatively much of labeled palmitic acid is present in free form. The question may arise whether these fatty acids are not those of the blood, remaining in the liver in spite of the thorough washing? In case of the group of one hour it can be unequivocally seen that these free fatty acids really belong to the liver, for at this time the total blood shows no more than 1 per cent of the injected radioactivity, and this value in the liver is 0.6.

Hardly any activity is present at first in the lipid esters of the blood after injecting labeled fatty acids into normal rats, but after 15 min their activity abruptly rises exceeding in a few minutes 1 per cent of the injected activity. In hepatectomized animals no such rise occurs, proving that these lipids are synthetized in the liver (BORGSTRÖM and OLIVECRONA, 1961). In carp blood only very low radioactivities were found in the lipid esters *(Table 2)* and in

Table 2.

Radioactivity in blood lipid fractions
in per cent of the injected radioactivity

Lipid fraction	Time after injection		
	5 min.	20 min.	60 min.
Cholesterol esters	1.357 ± 0.161	0.368 ± 0.036	0.232 ± 0.042
Triglycerides	0.353 ± 0.095	0.044 ± 0.012	0.033 ± 0.006
Free fatty acids	29.923 ± 3.640	5.231 ± 0.232	1.028 ± 0.140
Diglycerides	0.175 ± 0.034	0.054 ± 0.001	0.052 ± 0.019
Phospholipids and Monoglycerides	0.219 ± 0.056	0.126 ± 0.021	0.058 ± 0.020

Mean values ±S.E. of the mean.

contrary to the mammals, even these values decreased with time. Conceivably while in mammals great part of free fatty acids gets first into the liver, where it is incorporated into the different lipids of lipoproteins and transported in this form to the consuming tissues, in fishes the tissues take up the free fatty acids directly from the blood.

One hour after injection 1 g flesh contained 0.045 ± 0.004 per cent, and 1 g adipose tissue 0.021 ± 0.010 per cent of the radioactivity. According to this even the specific activity of flesh is higher than that of the adipose tissue, and as the total mass of the flesh is much larger than that of the adipose tissue, there must be an even higher difference in the total radioactivity uptake. The flesh of carps, living in fish ponds, may contain 10 per cent fat, demonstrating that in fishes the musculature is more important as fat depot than the adipose tissue.

Summary

Labeled palmitic acid, bound to plasma was injected into the circulation of carps, and 5, 20 and 60 minutes thereafter radioactivity of different lipid classes of the blood and liver was determined.

The blood free fatty acids contained in the 5th minute thirty per cent, in the 20th five per cent and in the 60th minute one percent of the injected radioactivity.

The liver took up much less fatty acids than usual in experiments with mammals.

The esterified fatty acids of the blood showed all the time low radioactivities.

In fish examined the turn over of blood free fatty acid was with one order of magnitude slower than in mammals. The free fatty acids seem to get directly to the consuming tissues without previous incorporation into the lipoproteins in the liver.

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A PONTYBA INTRACARDIALISAN INJEKTÁLT 1-^{14}C PALMITINSAV ANYAGCSERÉJE

Herodek Sándor

Összefoglalás

Pontyok vérébe plazmához kötött jelzett palmitinsavat juttattunk, majd meghatároztuk vérük és májuk különböző lipid frakcióinak rádióaktivitását 5, 20, ill. 60 perccel az injekció után.

A vér szabad zsírsavai a beadott dózisnak az ötödik percben 30, a huszadikban 5, a hatvanadikban 1 százalékát tartalmazták.

A máj sokkal kevesebb jelzett zsírsavat vett fel, mint amennyit az emlősök mája szokott.

A vérben az észterezett zsírsavak mindvégig alacsony aktivitást mutattak.

A vizsgált halakban a vér szabad zsírsavainak forgalma egy nagyságrenddel kisebb, mint az emlősökben. Úgy látszik, a szabad zsírsavak zömmel közvetlenül jutnak el a felhasználó szövetekhez, és nem épülnek be a májban a lipoproteidekbe.

ОБМЕН ВЕЩЕСТВ I-^{14}C ПАЛЬМИТИНОВОЙ КИСЛОТЫ ВВЕДЕННОЙ ВНУТРИСЕРДЕЧНО КАРПУ

Ш. Херодек

В крови карпа было введено связана плазмой меченная пальмитиновая кислота; затем через 5, 20 и 60 минут была определена радиоактивность различных липидных фракций крови и печени.

Свободные жирные кислоты содержат 30 процентов введённой радиоактивности через 5 минут, 5 процентов — через 20 и один процент — через 60 минут.

Печень карпа накапливает значительно меньше меченых жирных кислот, чем печень млекопитающих.

Эстеры свободных жирных кислот крови характеризовались низкой активностью в ходе всего процесса.

В изученных видах рыб обмен свободных жирных кислот ниже на один порядок, чем в млекопитающих. Вероятно, что большинство свободных жирных кислот транспортируются непосредственно к тканям, и так они не входят в состав липопротеидов печени.

ANNAL. BIOL. TIHANY | 36 | 185–195 | HUNGARIA 1969

THE QUANTITY, VERTICAL AND HORIZONTAL DISTRIBUTION OF THE TOTAL BACTERIOPLANKTON OF LAKE BALATON IN 1966/67

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Received: 20th January, 1969

The important role of the diversified mineralizing activity of the microorgansms in the material and energy turnover of water bodies is well known (KUZNETSOV, 1952; BROCK, 1966). However, the significance of bacterial biosynthesis concomitant with decomposition was emphasized only recently (SOROKIN, 1967).

Energy liberated during mineralization represents in fact the basis of the synthesis of full value proteins (in relation to nutrition) accumulated in the bacterial cell. And as the sole source of food, the bacterial cell is utilized by a wide range of aquatic animals (GORBUNOV, 1946; RODINA, 1948, 1950; SOROKIN, 1966). Since bacterial biosynthesis is directly founded on organic substances eliminated from the chain of utilization of aquatic animals, the entire process will, in an ecological sense, hardly differ from primary production (SOROKIN, 1965).

This direct trophic role of the bacteria corroborates the justification of approaches which seek to connect the trophity of waters and their bacterial biomass. Owing to earlier methodological difficulties, however, little is known on the qualitative and quantitative conditions of the bacterioplankton.

As RAZUMOV's (1932) direct method, now is general use, renders a possibility for the exact quantitative survey of the bacterioplankton, the present work attempted a survey by this method of the seasonal, quantitative conditions of the bacterioplankton in the open water of Lake Balaton.

Data submitted by ZIH (1929) and HARANGHY (1941) give information on the heterotrophic bacterial flora of Lake Balaton. However, the results obtained by an indirect method, though valuable from several points of view, fail to present a complete picture of the quantitative conditions of the total microflora.

Material and methods

The material of the present study consists of samples taken for an algological investigation during a period of fifteen months (August, 1966—November, 1967). Samples fixed previously by acetic acid and lugol's solution were conserved in formalin. The samples derive from 3 points each of the 5 standard sections of Lake Balaton (TAMÁS, 1967). The period of time of the monthly

collectings at the 15 sample localities did not exceed 48 hours, thus it was possible to work on approximately synchronous samples — an essential feature in view of the rapid changes occurring in bacterioplankton.

The total number of bacteria present in the samples was established by RAZUMOV'S method. 10—40 ml of the well shaken sample was filtered through a N 2 Soviet membrane filter, stained by carbolic eritrozine and counted under an apochromate lens (100 × 25).

Results

Seasonal vertical distribution

In section M, the number of bacteria varied between 1 — 9 · 10^5/ml in the samples originating from different water depths. The vertical distribution is extremely diverse *(Fig. 1)*. The maximum number of individuals appeared in the surface samples in September, and in the middle ones during October and November; on the other hand, a reverse vertical distribution showed at locality Mo in September and October. At 2 m depth, maximum values appeared in November only at size M1 and in September at Mo. During spring, the distribution of the baterioplankton was uniform in all three localities.

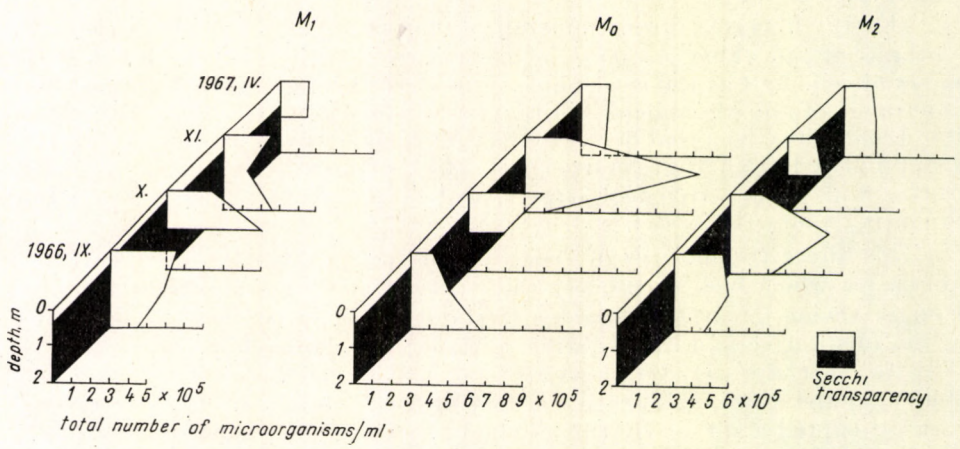

Fig. 1. The seasonal vertical distribution of the bacterioplankton at three points of section "M"

In section K, *(Fig. 2)*, the number of bacteria fluctuated between 1 — 8 · 10^5/ml at different water levels during autumn. In November, the entire water column is characterized by a relatively uniform 2 · 10^5 bacteria/ml. In April 1967. an identical vertical distribution was found at all three points of the section. Maximum values showed at the surface and at a depth about 2 m.

In section G, samples were taken also in August, 1966 *(Fig. 3)*. The summer month was characterized by a conspicuously uneven vertical distribution. The number of bacteria varied between 2 — 5 · 10^5/ml; maxima appeared at the surface and, excepting site G1 at a depth of about 3 m. In November, the water body was homogeneous also here, similarly to the situation in section

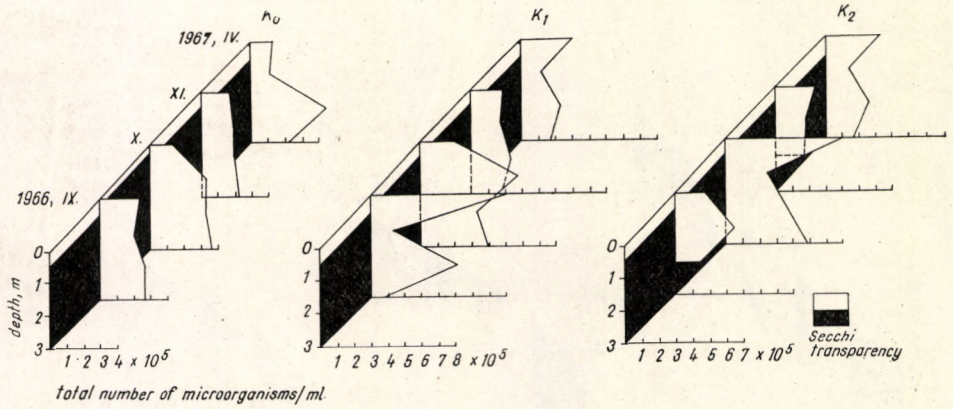

Fig. 2. The seasonal vertical distribution of the bacterioplankton at three points of section "K"

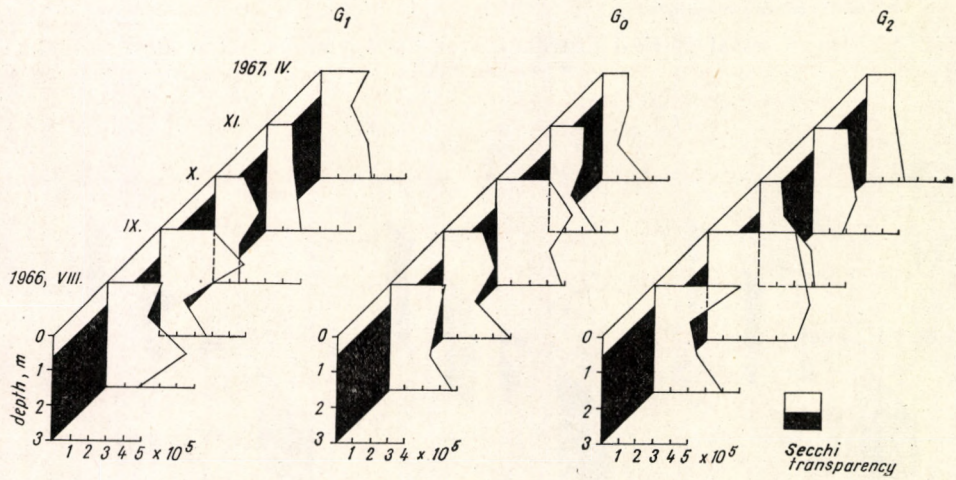

Fig. 3. The seasonal vertical distribution of the bacterioplankton at three points of section "G"

K, with an average number of bacteria $2 \cdot 10^5$/ml. The September—October distribution is characterized by the maximum evolving at about 1 m, divergently from that of the summer month. The vernal vertical distribution, characterizing section K, appeared here also partly.

In section A, considerable differences in the number of individuals occurred in the samples of both summer and autumnal samples. The amount of bacteria varied between $1 - 8 \cdot 10^5$/ml, and the maximum values were found most frequently on the surface. No picture, characteristic of sections K and G, emerged in the spring month, a nearly uniform vertical distribution showing with an average $1{,}5 \cdot 10^5$/ml number of bacteria *(Fig. 4)*.

As compared to the preceding sections, the distribution of bacteria was more uniform in section E. The uniform vertical distribution was usually distorted by the higher number of bacteria formed at greater water depths *(Fig. 5)*.

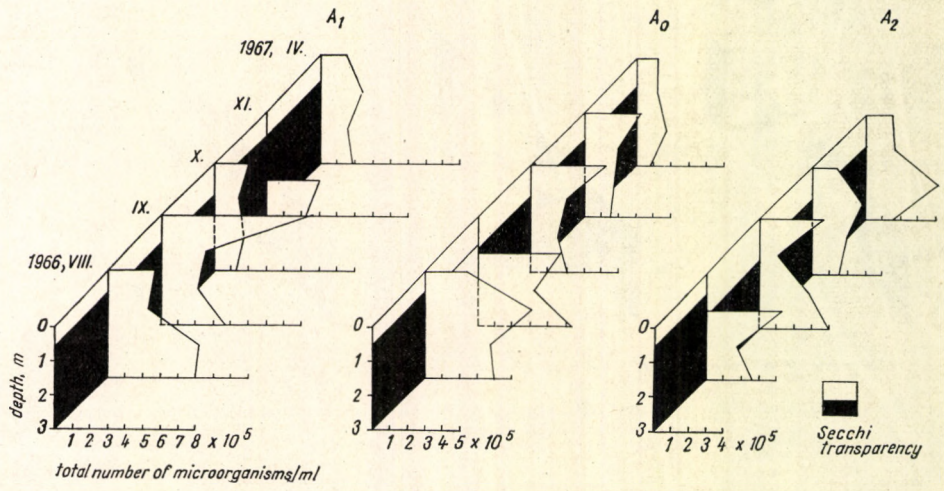

Fig. 4. The seasonal vertical distribution of the bacterioplankton at three points of section "A"

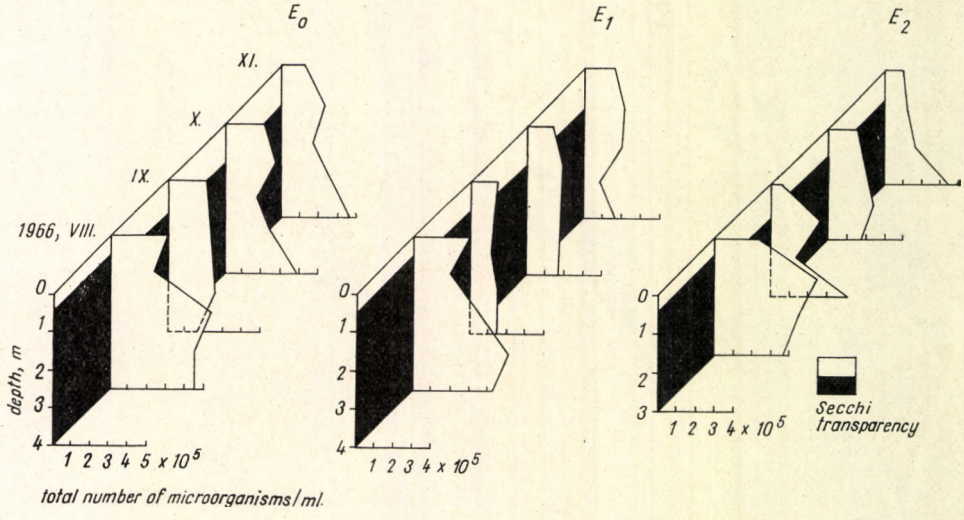

Fig. 5. The seasonal vertical distribution of the bacterioplankton at three points of section "E"

Seasonal horizontal distribution

Two prominent values appeared in section M during the investigated period *(Fig. 6)*: a smaller value (slightly more than $4 \cdot 10^5$ bacteria per ml) in October, 1966, and a higher one (nearly 1 million bacteria per ml) in August, 1967. Aside of these, the differences between the sites within the section were smaller. In the period studied, the average number of bacteria was $2{,}3 \cdot 10^5$/ml, and the number of individuals increased significantly at all three localities only in July, 1967.

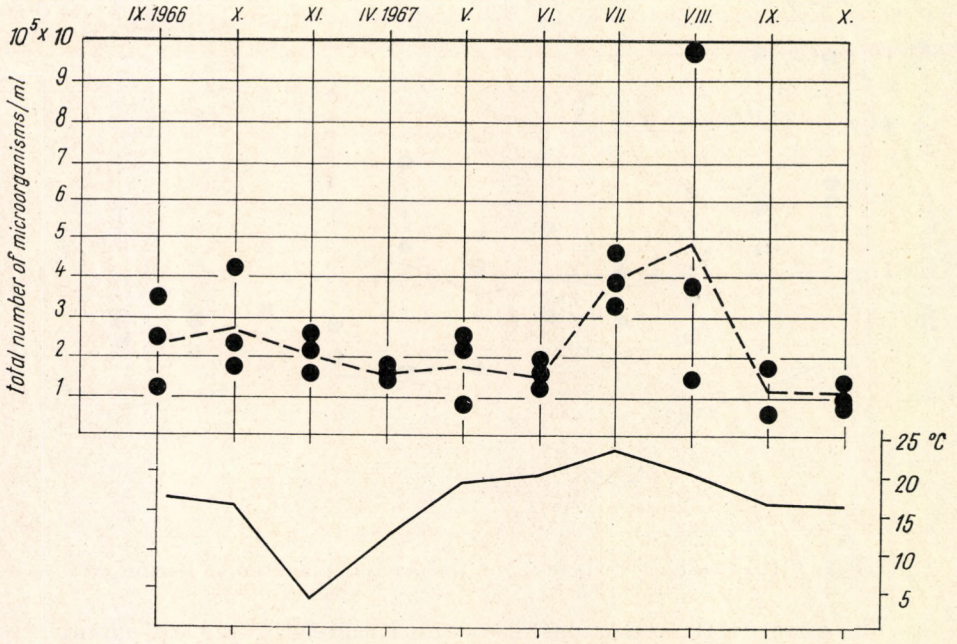

Fig. 6. The seasonal dynamism of the bacterioplankton in section "M"

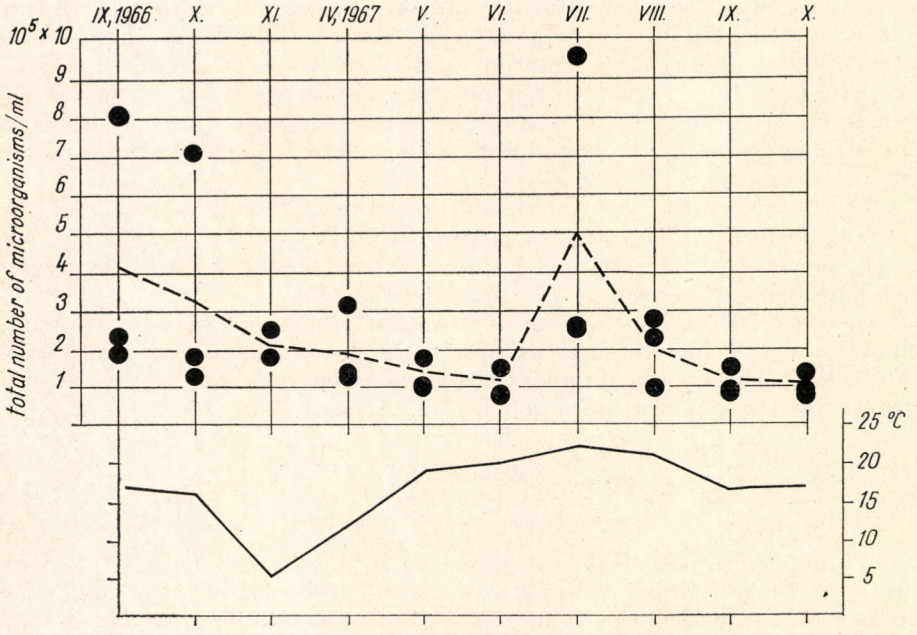

Fig. 7. The seasonal dynamism of the bacterioplankton in section "K"

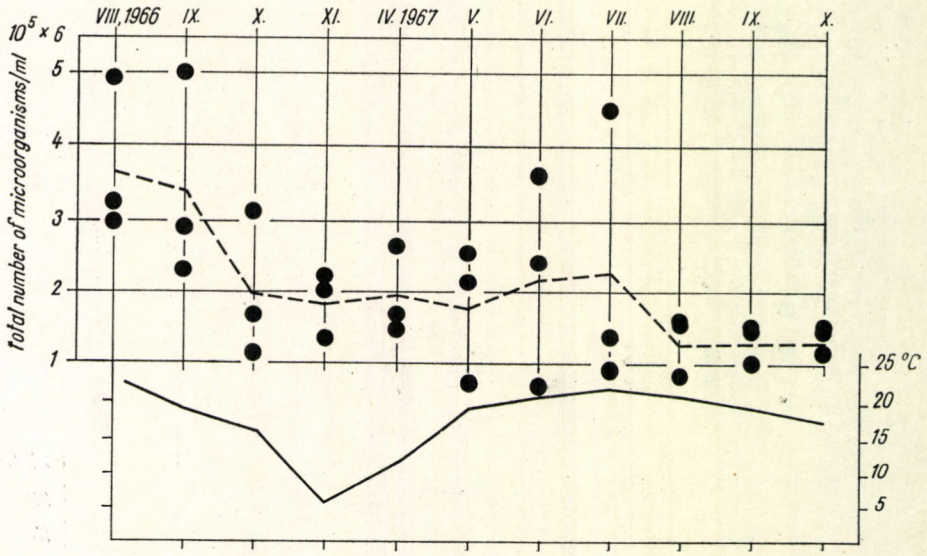

Fig. 8. The seasonal dynamism of the bacterioplankton in section "G"

The quantitative conditions of section K agreed to a great extent with those found in section M. However, prominent values were more frequent at the sites within the section and their order of magnitude also approached 1 million *(Fig. 7)*. Aside of these, the amount of bacteria was nearly identical in the investigated period.

According to the 1966 data, the number of bacteria was high at all three points of section G during August—September *(Fig. 8)*. A characteristic feature of the amount of the bacterioplankton was the considerable differences measured at the three sites within the section, appearing in half of the surveys. The amount of bacteria became more uniform during the colder months. The order of magnitude of the prominent values reached $5 \cdot 10^5$ bacteria per ml, while the average was $2 \cdot 10^5$/ml.

The number of bacteria increased in section A during September, 1966, and May, 1967. The fluctuation of values within the section was smaller, with the sole prominent value having been $8 \cdot 10^5$ bacteria per ml in September, 1966. The average value was $2{,}1 \cdot 10^5$/ml *(Fig. 9)*.

In section E, maxima evolved in August, 1966, and May, 1967. Values within the section hardly vary, excepting the prominent figure $4{,}4 \cdot 10^5$/ml in April, 1967. The average number of bacteria, as compared to that of the other sections, was the smallest in section E ($1{,}6 \cdot 10^5$/ml) *(Fig. 10)*.

Discussion

Seasonal vertical distribution

Available data are meagre with respect to the vertical distribution of the microflora of lakes. In his comprehensive work, KUZNETSOV (1952) points out that the vertical distribution is highly variable depending on the character of the lake and the prevailing season. In lakes with a temperature and chemical

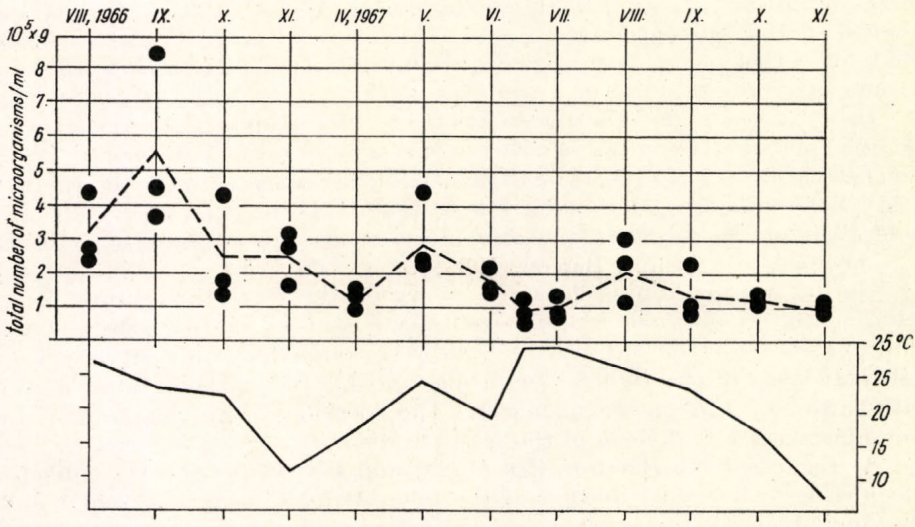

Fig. 9. The seasonal dynamism of the bacterioplankton in section "A"

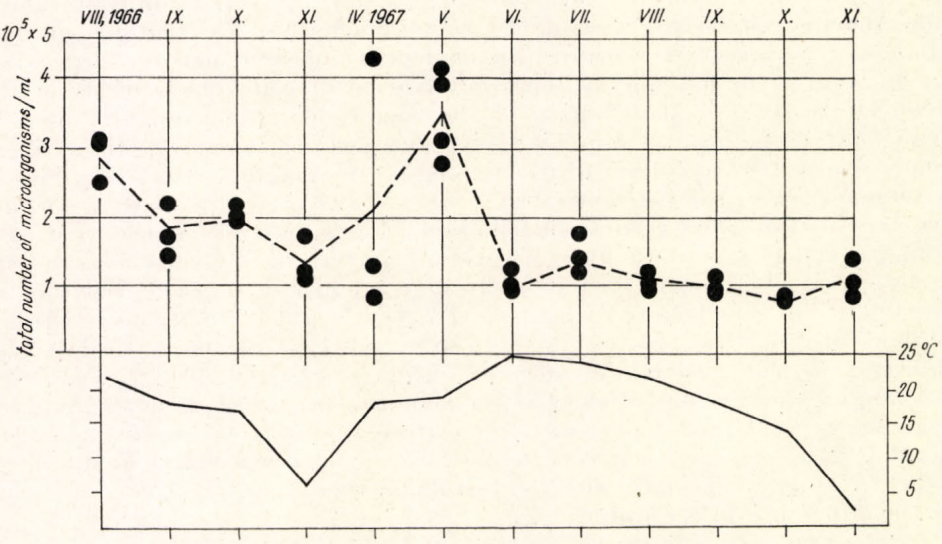

Fig. 10. The seasonal dynamism of the bacterioplankton in section "E"

stratification , the bacterioplankton displays a definite and stabile vertical distribution. The maximum of the total microflora evolves generally in the metalimnion (YEGOROVA, 1951; OTZEVSKI, 1966; OVERBECK, 1966, 1968). According to KUZNETSOV's data deriving from the Beloie Lake, the maximum of the bacterioplankton was also in the metalimnion in August, 1932, but it

became definitely delimited to the epilimnion in August, 1933. The data also reveal that the bottom water contains invariably more bacteria. During periods of complete circulation, the distribution of bacteria, apart from the bottom maximum, is uniform in the water body.

In stratified lakes, the distribution of heterotrophic bacteria shows a different picture. The most significant maximum is in the surface layers (POTTER and BAKER, 1961), and this is usually correlated with the distribution of the phytoplankton (OVERBECK, 1966, 1968). The high number of heterotrophic bacteria in the bottom water layers is also an apparently universal phenomenon (POTTER and BAKER, 1961; OTZEVSKI, 1966).

Hardly any pertaining data are known on the vertical dynamics of the bacterioplankton of shallow and constantly disturbed lakes (RAZUMOV and ZAHAROVA, 1948; OTZEVSKI, 1966). OTZEVSKI conducted some investigations on several lakes of the Balkan Peninsula, and his results point to a definite stratification of the bacterioplankton. The location of the maximum and minimum layers was diverse in the lakes studied.

As regards Lake Balaton, ZIH (1929) and HARANGHY (1941) published data on the vertical distribution of the heterotrophic bacteria. Their results show unequivocally the uneven vertical distribution of the bacteria. Owing probably to the disordered position of the maximum—minimum layers, HARANGHY renounces stratification, basing his inferences on surveys made in the "Well", the deepest point of the lake.

The results of our 65 vertical surveys made in Lake Balaton irrefutably show the uneven distribution of the bacterioplankton. Fluctuations in the number of bacteria in the diverse water depths appeared mostly during the summer and autumnal months, with only November being characterizable by a comparatively even distribution of the bacterioplankton. Similarly homogenous distribution can be found in the various sections also during the springtime surveys. If the position of the maximum—minimum layers is examined in details, the vertical distribution will be found strikingly diverse even within the same section. The vertical distribution is thus most dynamic is nature, changing and forming in a state of constant movement. By ZIH's (1929) and HARANGHY'S (1941) data, the heterotrophic microflora of Lake Balaton is characterizable by a similar dynamism. OTZEVSKI'S (1966) studies made on the shallow eutrophic lakes of the Balkan also revealed the uneven vertical distribution of the bacterioplankton. According to RAZUMOV and ZAHAROVA (1948), the vertical distribution of the bacterioplankton in a shallow but extensive water reservoir shows a highly intricate picture, especially during the summer months. It follows that the vertical distribution of the bacteria in standing bodies of water with a large surface area and a small depth is widely effected by the dynamism of the water.

The extensive and shallow waters of Lake Balaton is deeply agitated even by moderately strong wind. Depending on the force of the wind, particles of diverse size and rate of sedimentation enter the water-body. Variation is further increased by the different quality per sections of the sediment, indeed, sediment may considerably vary even within the same section (ENTZ et al. 1963). All these results in the diversity of available food of the various sections of water, allowing the formation of bacterial populations highly different both qualitatively and quantitatively. However, details of these processes can be exposed only by short-term studies.

Seasonal horizontal distribution

On the basis of the average amount of the bacterioplankton, not great differences can be found between the sections investigated. The number of bacteria was the highest in sections M and K ($2{,}3 \cdot 10^5$) and the smallest in section E ($1{,}6 \cdot 10^5$). The highest values reached 1 million per ml in sections M, K and A, and only about $5 \cdot 10^5$/ml in sections G and E.

Literature has some summarized data on the amount of the bacterioplankton of diversely trophic lakes (KUZNETSOV, 1952; ROZUMOV, 1962; RODINA and KUZMITSKAYA, 1963; THSERBAKOV, 1967). Accordingly, on the basis of the amount of the bacterioplankton, Lake Balaton approached the oligotrophic waters, even though the maximum values reached the order of magnitude of the bacterioplankton of mesotrophic lakes. ZIH'S (1929) and HARANGHY'S (1941) statement therefore, namely that Lake Balaton is poor in heterotrophic bacteria, still holds true with respect to the total of the microflora.

If the excessive values are disregarded, the differences are small in the seasonal course. Maxima evolved in sections G, A and E at the end of the summer and the beginning of the autumn in 1966; a strong maximum appeared in section E, evolving at a smaller rate also in section, A, in May, 1967; and the 1967 maximum showed in sections M and K in June.

Increasingly more data become available concerning the seasonal fluctuation of the bacterioplankton (KUZNETSOV, 1960, 1961, 1965; OVERBECK and BABENZIEN, 1964; POTAYENKO, 1968); however, investigations extending over a number of years are still needed to exactly determine the annual course of the bacterioplankton of Lake Balaton. It should be born in mind that the regular seasonal dynamics of the microflora present in the deeper lakes is probably wanting or appearing in a very intricate form owing to the constant but irregular disturbance of the sediment rich in nutritive substances. To know more about it, investigations extending over several years, including also short-period surveys, are needed.

Summary

1. Applying RAZUMOV'S direct method, the total number of bacteria at 15 standard sites in the open water of Lake Balaton has been determined; the studies were based on the vertical and horizontal samples taken monthly in 1966 and 1967.

2. Compared with the earlier data referring to the heterotrophic bacteria, the number of total bacteria proved to be 10,000 times higher.

3. In 1966 and 1967, the average number of bacteria was $2{,}2 \cdot 10^5$/ml in the open water of Lake Balaton, and the most prominent values reached one million.

4. The seasonal course of the total bacterioplankton was different in the diverse section in both years. In the case of the shallow, cnnstantly disturbed waters of the lake changes involving shorter periods play a presumably greater role in the quantitative evolvement of the bacterioplankton.

5. In the open water of the lake, the vertical distribution of the bacterioplankton is characterizable by unevenness. Owing to the dynamism of the water the distribution is extremely mobile and short-lived.

Acknowledgement

The author is indebted to Dr. J. PONYI for his valuable critical remarks during the preparation and reading of the manuscript.

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A BALATON TELJES BAKTERIOPLANKTON MENNYISÉGE, VERTIKÁLIS ÉS HORIZONTÁLIS ELOSZLÁSA 1966/67-BEN

Oláh János

Összefoglalás

1. Szerző Razumov direkt módszerével meghatározta a Balaton nyíltvizének 15 standard pontján az össz baktérium-számot az 1966. és 1967. évek havonkénti vertikális és horizontális mintáiból.

2. A korábbi, heterotróf baktériumokra vonatkozó adatokkal összevetve az össz baktérium szám 10 000-szer magasabbnak bizonyult.

3. Az 1966. és 1967. években a Balaton nyílt vizében átlagosan 2,2 . 10^5/ml baktérium volt és a kiugró értékek elérték az 1 milliót.

4. A különböző szelvényeken a szezonális lefutás mindkét évben eltérő volt. A sekély, állandóan felkavarodó Balaton esetében a bakterioplankton mennyiségének alakításában a rövid periódusú változásoknak feltehetően nagyobb a szerepük.

5. A Balaton nyíltvizében a bakterioplankton vertikális eloszlását az egyenlőtlenség jellemzi. Az eloszlás a víz dinamizmusa miatt rendkívül mozgékony, rövid életű.

ОБЩЕЕ ЧИСЛО БАКТЕРИОПЛАНКТОНА ОЗЕРА БАЛАТОН ПО ВЕРТИКАЛЬНОМУ И ГОРИЗОНТАЛЬНОМУ РАСПРЕДЕЛЕНИЮ В 1966—67 ГОДАХ

Я. Олах

1. Автор с помощью прямого метода Разумова определил общее число бактерий на 15 стандартных станциях озера Балатон из вертикальных и горизонтальных проб, собранных в 1966/67 годах в каждый месяц.

2. По сравнению с прежними данными, относящимся к сопрофитам, общее число бактерий в озере Балатон оказалось в 10 тысяч раз больше упомянутых данных.

3. В открытой воде озера Балатон в 1966/67 годах в среднем было найдено 2,2.10^5/мл бактерий, а самые высокие значения равнялись 1 миллионам.

4. На различных поперечных сечениях сезонное колебание было различно в обоих годах.

В озере Балатон, характеризующемся частыми перемещениям и низким уровнем воды, в количественных изменениях бактериопланктона важную роль играют прежде всего кратковременные изменения.

5. Вертикальное распределение бактериопланктона в открытой воде озера неравномерное, чрезвычайно вариабельное и эфемерное ввиду подвижности воды.

ANNAL. BIOL. TIHANY	36	197–212	HUNGARIA 1969

A QUANTITATIVE STUDY OF THE SAPROPHYTIC AND TOTAL BACTERIOPLANKTON IN THE OPEN WATER AND THE LITTORAL ZONE OF LAKE BALATON IN 1968

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Received: 6th February, 1969

Little is known on the annual changes enacted in the bacterioplankton population of lakes. The results of investigations conducted for several years refer to considerable annual changes in the total bacterioplankton (RAZUMOV, 1962; THSHERBAKOV, 1967; IVATIN, 1968) and in the proportion of the total and saprophytic bacterioplankton (KUZNETSOV, 1960).

The surveys of the years 1966—67 were made on conserved samples deriving from the open waters of Lake Balaton (OLÁH, 1969), hence the number of saprophytes could not be established. However, the results of direct countings may serve for the basis of investigations aimed at the annual changes enacted in the amount of the total bacterioplankton.

With this aim in mind, we have continued determinations of the total bacterioplankton, and by the counting of saprophytes we have attempted to gain information about the order of magnitude of the proportion between the total and saprophytic bacterioplankton suitably reflecting the pollution and supply of readily assimilable organic substances (KUSNETZOV, 1960).

The bacteriological examination of the open water presupposes, however, the demarkation of the open water and the littoral zone. Since the amount of bacteria satisfactorily reflect the trophic state of a lake (RAZUMOV, 1962), the study of the trophic conditions of diverse parts of the lake is also concurrently given.

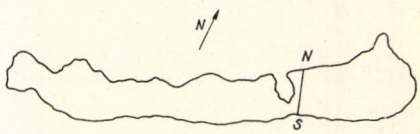
Investigating the amount of bacterioplankton present in the littoral zone with a macrophytic vegetation and the open waters in the lakes of the Ladoga Lake district, RODINA (1961) emphasizes the higher biological productivity of the coastal zone. The morphology of the shallow Balaton with its extensive body of water also resulted in a considerable length of shoreline which, with respect to the magnitude of the coast — water body index (SEBESTYÉN et al. 1951), may increasingly influence the food supply of the lake.

The annual production of the considerable macrophyta stand of the littoral zone is estimated at 8000 tons (ENTZ, 1954). Through the detritic drift (SEBESTYÉN, 1949, 1964), a part of this amount becomes linked up with the matter turnover of also the open waters and during the microbial mineralization appears in the form of detritus at the diverse stages of the foodchain. All this corroborates the importance of the littoral zone of Lake Balaton in the material turnover of the entire lake.

With due attention to these considerations, we began the seasonal quantitative survey of the saprophytic — and the total bacterioplankton participating in these processes, in the five standard sections and one investigated detailed section of Lake Balaton.

Localities and methods

The samples, deriving from 16 sites of the Balatonfüred — Zamárdi transverse section *(Fig. 1)*, were taken monthly or in fortnightly periods, from April till October, 1966. Open water surveys were made at three localities each



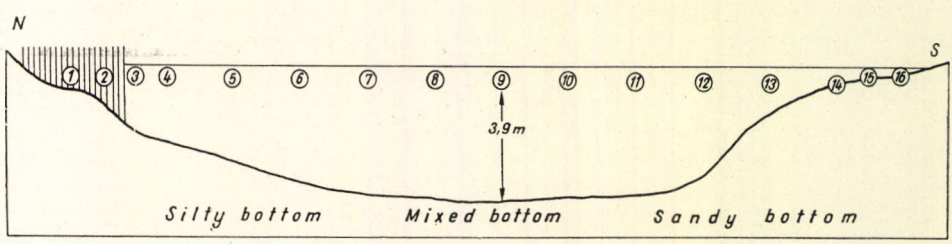


Fig. 1. Sites of sampling, depth and sedimentation conditions in the investigated detailed transect.
Site 1: the Scirpeto-Phragmitetum fontinalosum zone of the reeds;
Site 2: the Scirpeto-Phragmitetum phragmitetosum zone of the reeds;
Site 3: the edge of the reeds;
Site 4: 50 m from the edge of the reeds;
Site 5: 500 m from the edge of the reeds;
Sites 6—13: sampling points removed 6—700 m from one another in the open water;
Site 14: 400 m from the shoreline;
Site 15: 5 m from the shoreline;
Site 16: 2 m from the shoreline.

of the five standard sections of Lake Balaton; the data of the sites of collecting are given in TAMÁS's paper (1967).

Water samples were taken by FRANCEV's sampler (KUSNETZOV, 1952) from a depth of 50 cm, and transferred into a sterile, 250 ml glass with a glass stopper. Applying RAZUMOV's direct method (1932), the amount of the total bacterioplankton was calculated from the samples. The saprophytic microorganisms were counted on nutrient agar and, concurrently with the actinomyces, on JENSEN's casein-glucose agar, in repetitions of five.

With recourse to the two kinds of nutrient media a more exact survey of the quantitative distribution f the saprophytes was made possible. On the nutrient agar, the number of colonies was counted on the seventh day, on

casein-glucose on the tenth day, and the number of actinomyces on the twentieth day. Incubation took place at 25 C° and the number of colonies was established at a magnification of × 10. Pouring was made directly after the collecting, on board ship.

Results

The investigated detailed section

In April the total bacterioplankton was uniformly 1 — 1,5 million/ml a long the entire section, excepting the Scirpeto-Phragmitetum fontinalosum zone of the reeds (Tóth, 1960) where it reached 3 millions *(Fig. 2)*. The amount

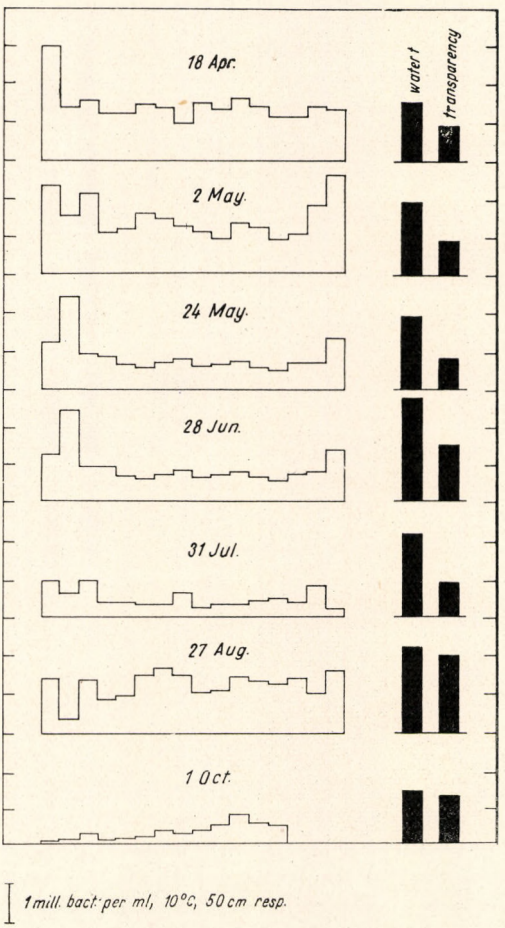


Fig. 2. The seasonal dynamism of the amount of the total bacterioplankton at 16 points of the transect

of saprophytes showed the same distribution on both culture media (*Figs* 3, 4), but higher values were obtained on casein glucose agar. As related to the entire survey, the number of colonies grown on the nutrient agar was only about half of that appearing on the casein-glucose agar.

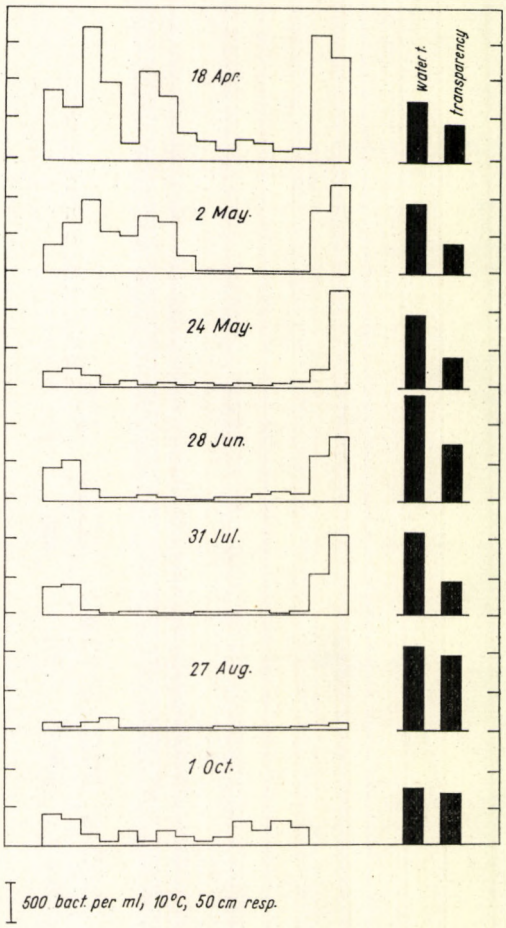


Fig. 3. The seasonal dynamism of the amount of saprophytes counted on nutrient agar at 16 points of the transect

In the open water, the number of saprophytes was 2—300/ml; with respect to the littoral zone, the maximum was 3000/ml on casein-glucose agar. The high number of saprophytic bacteria of the northern shore showed up even 1500 m away from the edge of the reeds. On the southern shore, free of reeds, the high number of saprophytes was delimited to a relatively narrow zone compared to the northern shore.

At the beginning of May, the amount of the total bacterioplankton still exceeded 1 million/ml in the open water, and was above 2 million/ml in both

coastal zones, but the number of saprophytes decreased below 100/ml in the open water. However, the maximum values remained high in the littorals, and reached 4200/ml on casein-glucose agar. The extension of the zones was identical with the situation found in April.

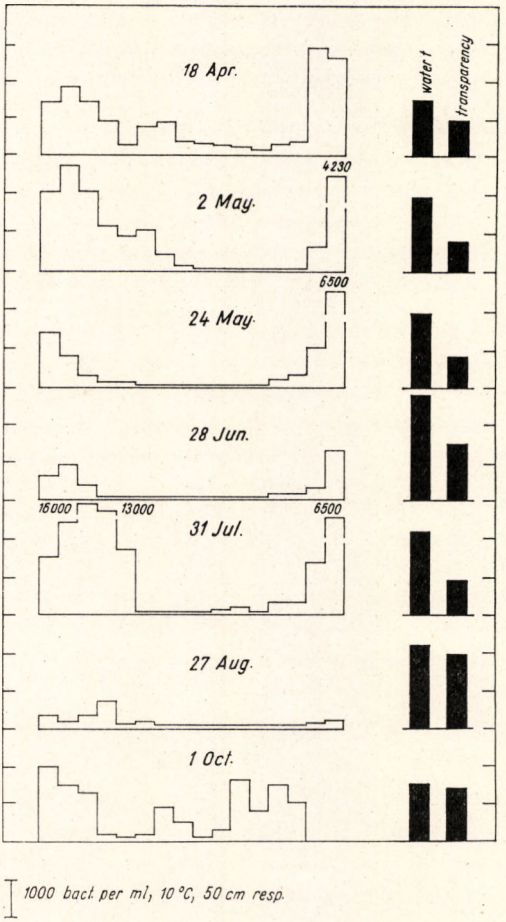


Fig. 4. The seasonal dynamism of the amount of saprophytes counted on casein-glucose agar at 16 points of the transect

An essential change occured by the end of May. The total bacterioplankton varied about $5 \cdot 10^5$/ml in the open water, it remained above 1 million/ml in the littoral zones, and reached 2.5 million/ml in the Scirpeto-Phragmitetum phragmitetosum zone of the reeds. In the open water, the number of saprophytes was below 100/ml; it continued to increase along the southern shore free of reeds, but a considerable decrease showed on the northern shore with a maximum of 1400/ml on casein-glucose agar. The littoral zone of high saprophytic content, as related to that of the open water, became narrower and delimited to the area of the reeds.

By the end of June, the amount of the total bacterioplankton and the number of saprophytes decreased even further. The number of saprophytes sunk below 50/ml in the open water and diminished from 6500 to 1300 in the littoral of the southern shore. In the shallow water of the sandy bottom along the southern shore, the slight increase found at the end of May still persisted.

By the end of July, the amount of the total bacterioplankton decreased to $3 - 4 \cdot 10^5$/ml in the open water, and remained below 1 million in the littoral zones. The number of saprophytes on nutrient agar exhibited the picture shown in June, but a most conspicuous rise could be observed in those developing on casein-glucose agar. The ratio of the littoral zones and the open water was identical with that experienced in the spring, whereas the maximum number of saprophytes reached 16,000/ml.

At the end of August, the amount of the total bacterioplankton increased over 1 million/ml both in the open water and the coastal zones. The number of saprophytes was low on both culture media, and the littoral zones were quantitatively hardly differing from the open water.

At the beginning of October, it could be seen from the data of the 14 points along the transect that the amount of the total bacterioplankton decreased to its lowest level with respect to the investigated period, and that it was essentially higher in the open water than in the northern littoral zone. As related to August, the number of saprophytes increased and by their quantity the coastal zone and the open water hardly differed.

The relatively high springtime values of the actinomyces present in the plankton gradually decreased and appeared in insignificant numbers during the summer months.

Table 1

The amount of actinomyces present in the plankton of the detailed section

Sites of sampling	Date of sampling						
	18 April	2 May	24 May	28 June	31 July	27 Aug.	1 Oct.
1.	75	40	0	8	7	2	3
2.	70	10	3	1	3	1	0
3.	50	30	10	3	0	0	0
4.	50	20	0	0	1	1	0
5.	3	20	0	0	0	2	0
6.	10	3	0	1	1	0	2
7.	60	20	1	0	1	0	1
8.	3	10	0	0	3	0	0
9.	10	3	2	3	0	0	1
10.	15	0	0	2	0	0	1
11.	0	3	3	0	1	3	0
12.	10	0	1	0	0	0	2
13.	20	3	1	4	3	0	4
14.	10	10	2	1	2	1	0
15.	130	3	20	0	0	0	0
16.	90	120	40	12	9	4	8

The open water

In May, the amount of the total bacterioplankton was above $5 \cdot 10^5$/ml *(Fig. 5)*, reaching or exceeding in some cases one million per ml. It was only at two points of section K that lower values were found ($3 - 3{,}5 \cdot 10^5$/ml).

The number of saprophyta grown on nutrient agar *(Fig. 6)* varied between 10 and 80 per ml the lowest occurring in section A. On casein-glucose agar *(Fig. 7)*, values below 100/ml were found for sections G, A and E, but above 100/ml for sections M and K.

In June, the amount of the total bacterioplankton sank below $5 \cdot 10^5$/ml; a figure about 1 million was found only in one site. Saprophytes deriving from

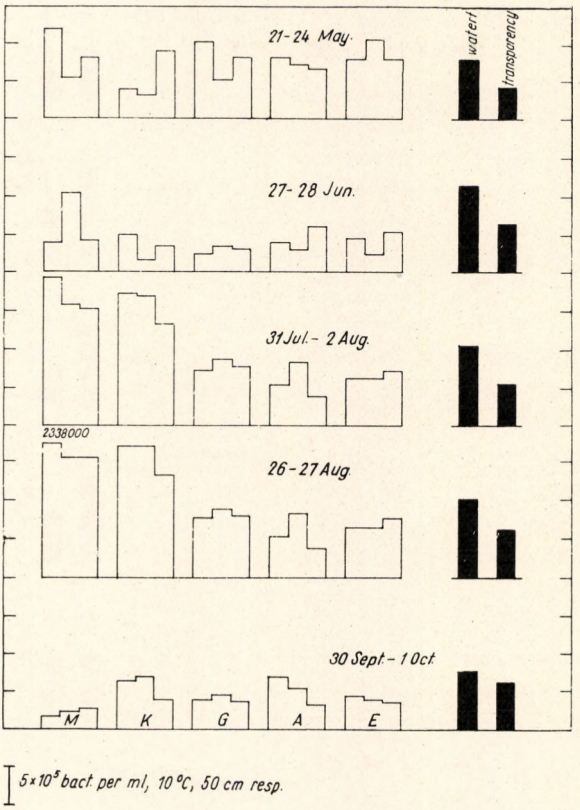


Fig. 5. The seasonal dynamism of the amount of total bacterioplankton at three sites of the 5 standard sections (M, K, G, A) of Lake Balaton

sections G, A and E decreased in numbers on both culture media, but for sections M and K the value obtained in May increased nearly by a thousand fold. On nutrient agar, 40—16,000/ml, on casein-glucose agar, 44—19,000/ml saprophytic bacteria were counted.

In July, the amount of the total bacterioplankton rose above $5 \cdot 10^5$/ml in sections G, A and E and to 1.5 millions per ml in sections M and K. The number of saprophytes increased further on casein-glucose agar for sections M and K, reaching 120,000 per ml, while on nutrient agar the value remained high, similarly to the situation in June. The exceedingly high values extended also to section G, but no essential changes occurred in sections A and E.

In August, the amount of the total bacterioplankton of sections G, A and E remained unchanged as related to that of July; in sections M and K the high value characterizing July again persisted, indeed, it reached 2.3 millions per ml at one point of section M. The high saprophyte numbers, characterizing June and July, ended in sections M, K and G, so that low values were received, excepting a slight rise in section E, for the entire area of the lake.

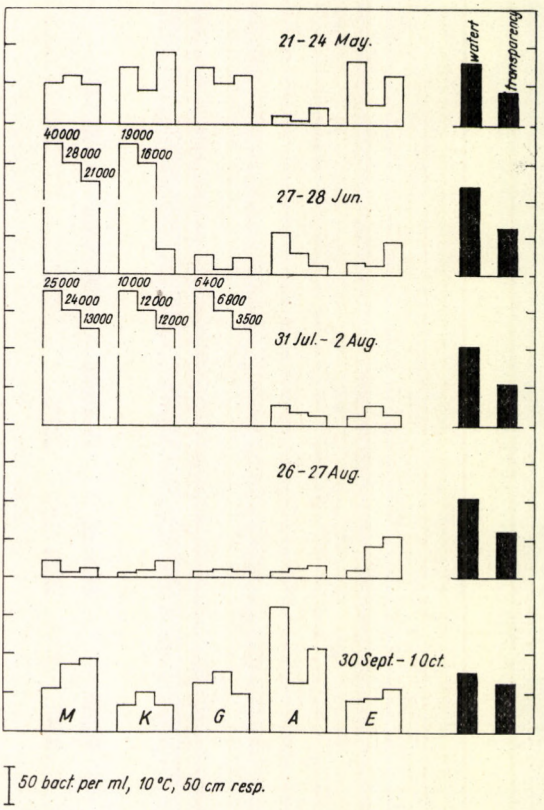


Fig. 6. The seasonal dynamism of the amount of saprophytes counted on nutrient agar at three sites of the 5 standard sections (M, K, G, A, E) of Lake Balaton

Table 2

The amount of actinomyces present in the plankton of the open water

Date of sampling	Sites of sampling														
	M			*K*			*G*			*A*			*E*		
	1	0	2	0	1	2	1	0	2	1	0	2	1	0	2
	number of individuals per ml														
21—24 May	16	10	13	3	18	13	3	0	3	2	2	1	0	0	3
27—28 June	8	7	16	4	0	3	1	4	1	0	0	3	1	0	0
31 July—August	2	0	8	0	1		0	0	1	1	0	1	3	0	1
30 Sept.—						4									
1 October	0	2	11	0	3	2	1	0	1	1	0	2	1	2	0

By the end of September and the beginning of October, the amount of the total bacterioplankton decreased, with the lowest value showing in section M ($2 \cdot 10^5$/ml). On the other hand, the number of saprophytes increased as related to the preceding month, reaching 400—800/ml on casein-glucose agar.

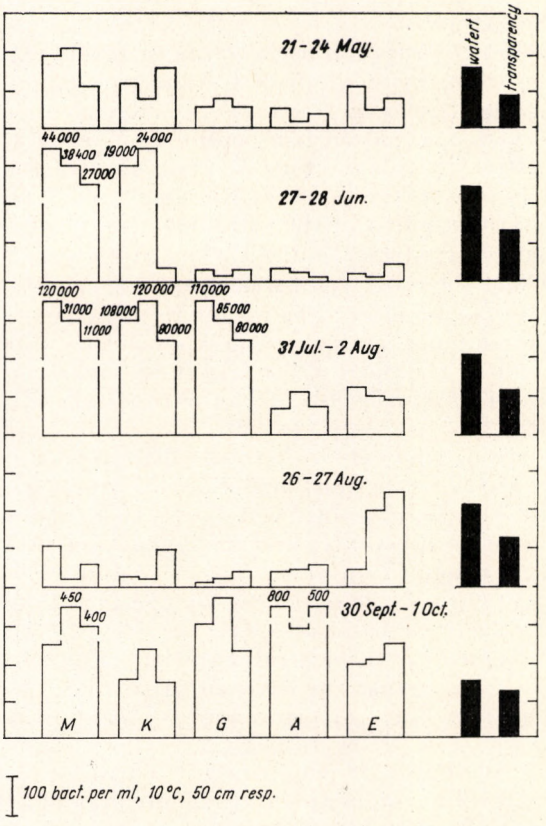


Fig. 7. The seasonal dynamism of the amount of saprophytes counted on Jensen's casein-glucose agar at three sites of the 5 standard sections (M, K, G, A, E) of Lake Balaton

Discussion

The investigated detailed section

The comparative bacteriological conditions of the open water and the littoral zone of Lake Balaton were studied by HARANGHY (1941); his data, referring to saprophytes developing on gelatine and HEYDEN-albumose agar (HESSE and NIEDER, 1898), reveal a higher number of bacteria present in the coastal zone.

Within the section studied here, a definite distribution with respect to the amount of both the saprophytes and the total bacterioplankton can be found. Similarly to the situation 30 years ago (HARANGHY, 1941), the content of saprophytic bacteria was low in the open water, being 50/ml during the sum-

mer months. On the other hand, values about, or occasionally considerably exceeding, an average 1000/ml was obtained in the littoral zones. Whereas merely double or triple amounts of differences existed in the total bacterioplankton prevailing between the open water and the coastal zones, the number of saprophytes was found to be, especially on casein-glucose agar, 40 to 120 times higher in the littorals.

It is known that the saprophytic microflora reacts rather sensitively in respect of quantity to changes in concentration of organic substances easily assimilable by bacteria. It was on this basis that KRISS (1968) studied, by the amount of saprophytes counted on fish-pepton agar, the distribution of "unstable" organic matter in pelagic waters. Indubitably, the analysis of the number of saprophytes makes possible the study of processes no further traceable merely by the number of the total bacteria (OLÁH, to be published).

The subdividing or configuration according to bacteria and their numbers of the littoral zone and the open water was most conspicuous during the spring months. By the end of summer and the beginning of autumn, it decreased to a certain extent. The summer minima, well known from literature, occurred regularly at all points of the open water (HENRICI, 1938; POTAYENKO, 1968), but smaller to greater maxima evolved in the reeds also during the summer months.

The stronger springtime extension of the littoral zone richer in bacterioplankton along the reed covered northern shore and the springtime maximum appearing generally also in the open water can be explained by the higher concentration of nutritive substances and the higher temperature of the water.

During autumn, a significant amount of organic substances gets from the reeds into the water; part of them arrives, by the detritic drift in a disintegrated state, also to the open water areas (SEBESTYÉN, 1964) so a sedimentary zone rich in organic matter is formed (MOON, 1933; ENTZ et al 1963).

Our data clearly show that the extent of the wide coastal zone, of high bacterial content evolved during springtime, coincides with this mud zone richer in organic substances *(Figs. 1, 3, 4)*.

Owing to the low winter temperatures, the utilization of the nutritive substances deriving from the mineralization of the organic substances having arrived into the water during autumn is slower, thus an accumulation of food substances occurs, as related to the summer months. With the rising springtime temperature, this causes a more intense bacterial activity. However, by the rise of temperature, the food-supply is quickly utilized and the quantity of bacteria significantly decreases, that is, the coastal zone becomes delimited to the area of the reeds, by the end of May. EDMONDSON (1968) states that the high winter and early spring concentration of nutritive substances is characteristic of most waters. The springtime maximum of the phytoplankton can be explained by similar phenomena (LUND, 1965). According to KONSTANTINOV (1967), the essential element of the biological spring in these waters are the higher concentration of biogeneous materials and the stimulating effects of the increasing temperature.

The mineralization of the macrophytic vegetation of the littoral zone, especially as regards shallow waters, is an important ecological process — come about by the rise in trophic level of the water area — also with respect to the entire lake (GORBUNOV, 1953; KRASENINIKOVA, 1958). The higher biological production evolved by these means (RODINA, 1961) appeared to any extent,

according to our data, only during the spring months (within the investigated period) in the specific conditions of Lake Balaton.

In the constantly disturbed water of Lake Balaton, strongly heated during summer and saturated with oxygen even on the surface of the muddy bottom, bacteria rapidly utilize the easily assimilable nutritive substances. The high lime content also quickens mineralization (PROWSE, 1966), favouring, at the same time, the formation of insoluble precipitation complexes more stable against bacterial activity.

If KRISS's contention (1968), namely that the proliferation of bacteria in a closed glass container filled with lake-water is in direct proportion with the amount of "stable" organic matter present in the water, is accepted then the preponderantly greater portion of organic substances present in Lake Balaton exists in this form. According to ZIH (1929), the amount of the saprophytic bacterioplankton contained in the waters of Lake Balaton increases to 400—500 times of its original quantity in a few days in a closed glass container.

In our opinion, this is the explanation of the intensive self-purifying ability of the water of Lake Balaton. It is due to this process that during the summer months the open water conditions with a saprophyte content characterizing oligotrophic lakes prevail already at a distance of no more than a few meters from the reedless southern coast, even in waters excessively disturbed by bathing people. The shrinking of the northern littoral zone into the stripe of reeds during the summer months can be explained by the same causes.

The open water

The results of monthly investigations conducted in 1966/67 failed to give an unequivocal picture of the seasonal dynamics of the total bacterioplankton (OLÁH, 1969). The entire process can in details be exposed only by short-period surveys; however, some rules seem to be inferable also from the monthly surveys of the three years in question.

Changes occurring in the amount of the bacterioplankton present in the diverse sections of Lake Balaton cannot be dovetailed into each other. Within the investigated period, the maximum appeared in a very definite form in sections A and E in May, whereas the maxima of July and August showed in sections M and K. The June and November minima formed in all sections.

In 1968, the amount of the total bacterioplankton was higher than in the years 1966/67. Whereas the values received for these latter varied about $2{,}3 \cdot 10^5$/ml (OLÁH, 1969), they have been over $5 \cdot 10^5$/ml — excepting June — in 1968. The increase was especially large in Keszthely-bay and its neighbourhood, with their maxima above 1.5 millions and 2.3 millions, respectively, in July and August, — values which belong to the order of magnitude of the mesotrophic lakes (KUZNETSOV, 1952; THSHERBAKOV, 1967).

Agreeing with ZIH's (1929) and HARANGHY's (1941) data, our also show a low saprophyta content of Lake Balaton. On the other hand, the exceedingly high numbers appearing in sections M and K in June, and in sections M, K and G in July, refer to the more intricate conditions of Keszthely-bay and its neigbourhood, at the same time ensuring the intensive self-purifying ability of the water.

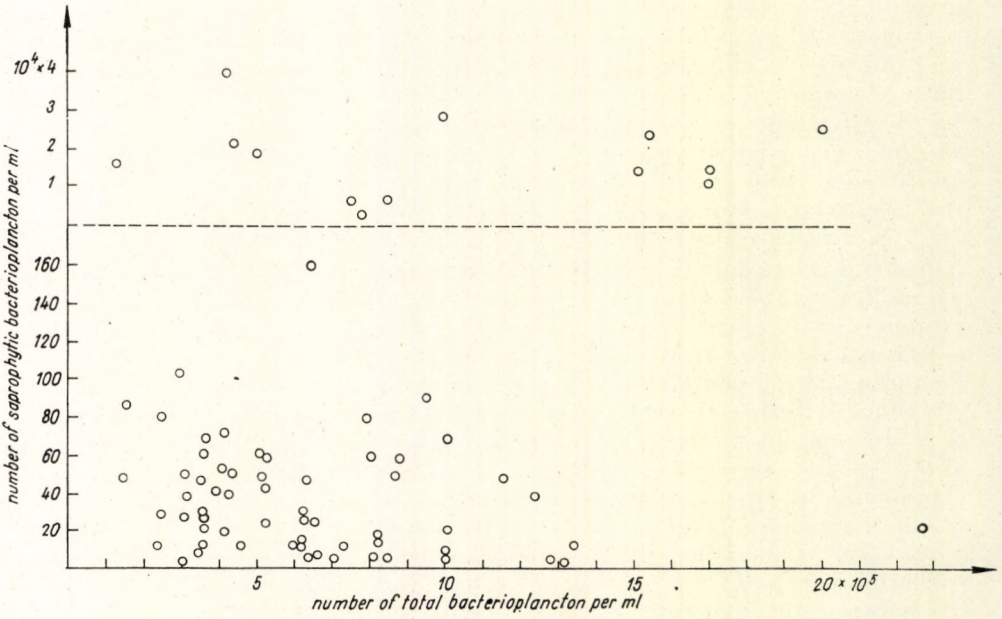


Fig. 8. The ratio of the total bacterioplankton and the number of saprophytes counted on nutrient agar

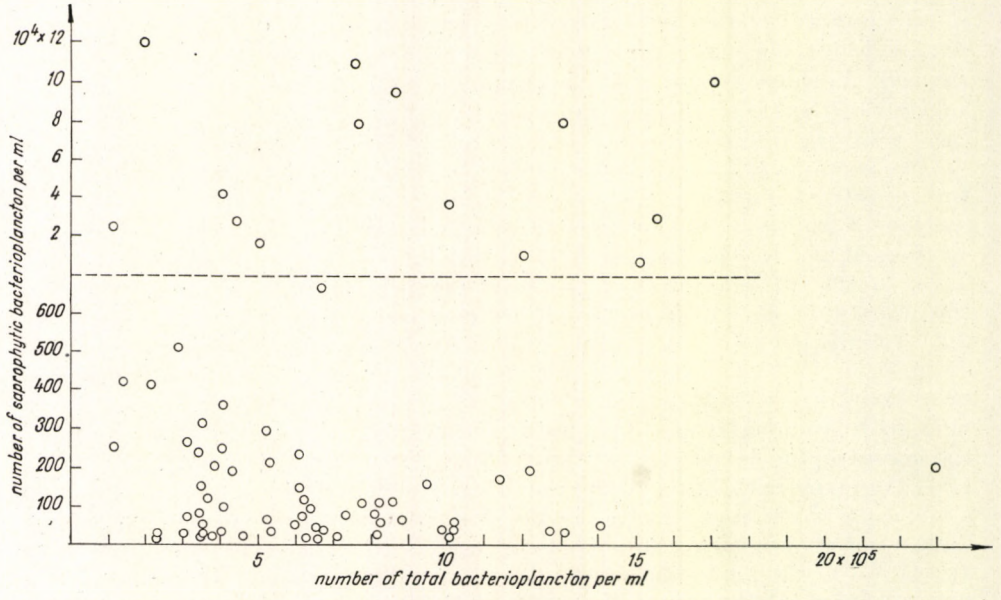


Fig. 9. The ratio of the total bacterioplankton and the number of saprophytes counted on casein-glucose agar

Similarly to some other saprophyta groups, the actinomyces *(Table 2)* are excellent indicators of the quantity of decomposing organic substances. The oxygen consumption of Lake Balaton water is 2 — 4 mg/l (ENTZ, 1949—50, 1953) so that the quantity of the actinomyces in the plankton is not significant, according to our measurements. They have been present in relatively higher numbers in the plankton of sections M and K during the spring.

The ratio between the total bacterioplankton and the number of saprophytes was used by KUZNETSOV (1960) as an index of the maturity of water reservoirs, since the saprophytes react most sensitively to changes in the concentration of easily assimilable organic substances (KRISS, 1968). Owing to the low concentration of nutritive substances in Lake Balaton, the ratio is about ten thousand, indicating a favourable water purity; however, the ratio decreased to 40—50 in Keszthely-bay and its neighbourhood in June, July, 1968, and then reached 100,000 in August.

It seems therefore that the generally low bacterial content of Lake Balaton implies a not continuously available accumulation of nutritive supply, especially in Keszthely-bay and the neighbouring waters, giving rise to an extremely high number of the saprophytes or the mass proliferation of algae.

Concomitantly with the rise in quantity of the total bacterioplankton, the number of saprophytes generally also increase (RODINA and KUZMITSKAYA, 1968). The data of our investigations conducted in Lake Balaton do not follow this relationship but refer to rather an inverse ratio *(Figs. 8, 9)*. Probably this phenomenon is also explainable by the special physico-chemical properties of Lake Balaton.

Summary

1. By the application of plate-pouring and the direct method, the seasonal quantitative conditions of the saprophytes and the total bacterioplankton have been investigated in five standard and one investigated detailed sections of Lake Balaton.

2. On the basis of the amount of both saprophytes and the total bacterioplankton, a definite differentiation can be found between the litoral zone and the open water. The quantity of the bacterioplankton was 2—3, that of the saprophytes 40—120 times greater in the former zone. The differentiation was the most conspicuous during the spring.

3. The littoral zone of high bacterial content was narrow along the reedless southern shore during the entire period of investigation. On the other hand, the littoral zone of high saprophyta content along the northern shore margined with reeds is wide during springtime (1500 m), but shrink to the area of the reeds during summer. The considerable springtime expanse of the northern coastal zone coincides with the wide sedimentary zone, rich in organic substances, extending before the shoreline.

4. The quantitative dynamics of the saprophytes and the total bacterioplankton reveals the intensive self-purifying ability of Lake Balaton; its most important factors are the high summer temperatures of the water, the full oxygen saturation, the constant movement of the water, and the high lime content.

5. In the open water, the amount of the total bacterioplankton was above $5 \cdot 10^5$/ml, and the maximum value reached was 2.3 millions. These data are essentilly greater than those obtained in 1966/67.

6. The saprophyta content of the open waters of Lake Balaton is generally low, 10—100/ml on nutrient agar, and 20—500/ml on casein-glucose agar. Both mediums yielded very high values (10,000—40,000/ml) for Keszthely-bay and its neighnourhood during June and July.

7. The amount of actinomyces present in the plankton is not significant.

8. The ratio of the total bacterioplankton and the saprophytes is favourable with respect to the purity of the water (10,000); however, this ratio decreases to 40—50 in Keszthely-bay and its neighbouring waters during June and July.

9. The increase of the amount of the total bacterioplankton was not followed by a similar increase in the number of saprophytes.

Acknowledgements

The author is indebted to Dr. J. PONYI for his valuable critical remarks concerning the composition of the manuscript.

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A SZAPROFITA ÉS TELJES BAKTERIOPLANKTON MENNYISÉGI VISZONYAI A BALATON NYÍLTVIZÉBEN ÉS PARTI ZÓNÁJÁBAN 1968-BAN

Oláh János

Összefoglalás

1. A Balaton öt standard szelvényén és egy részletes keresztszelvényén lemezöntéssel és direkt módszerrel vizsgáltuk a szaprofita és teljes bakterioplankton szezonális mennyiségi viszonyait.

2. A szaprofita és a teljes bakterioplankton mennyisége alapján egyaránt határozott tagolódást találtunk a parti zóna és a nyíltvíz között. Az előbbi zónában a teljes bakterioplankton 2—3-szor, a szaprofiták 40—120-szor nagyobb mennyiségben fordultak elő. A tagolódás a tavaszi hónapokban volt a legszembetűnőbb.

3. A nádasmentes déli parton a magas baktériumtartalmú parti zóna az egész vizsgált periódusban keskeny volt. A nádassal szegélyezett északi oldalon a magas szaprofita-tartalmú parti zóna a tavaszi hónapokban széles (1500 m), nyáron a nádas területére zsugorodik. Az északi parti zóna erőteljes tavaszi kiterjedése egybeesik a part előtt húzódó széles, szervesanyagokban gazdag üledék-zónával.

4. A szaprofita és a teljes bakterioplankton mennyiségi dinamikája bizonyítja a Balaton intenzív öntisztuló képességét, amelyben a magas nyári vízhőmérséklet, a teljes oxigén telítettség, a víz állandó mozgása és a magas mésztartalom játszanak döntő szerepet.

5. A nyíltvízben a teljes bakterioplankton mennyisége milliliterenként 5 . 10^5 fölött volt és a csúcsérték elérte a 2,3 milliót. Ezek az adatok az 1966/67. éviekhez viszonyítva lényegesen magasabb értékek.

6. A Balaton nyílt vizének szaprofita tartalma általában alacsony, nutrient agaron 10—100/ml, kazein-glükóz agaron 20—500/ml. A Keszthelyi-öbölben és környékén június-július folyamán rendkívül magas értékeket mértünk mindkét táptalajon (10—40 ezer/ml).

7. A sugárgombák mennyisége a planktonban nem jelentős.

8. A teljes és szaprofita bakterioplankton aránya a víz tisztasága szempontjából kedvező (10 ezer), a Keszthelyi-öbölben és környékén azonban az arány június-július hónapokban 40—50-re csökken.

9. A teljes bakterioplankton mennyiségének növekedését a szaprofiták számának növekedése nem követte.

КОЛИЧЕСТВЕННЫЕ ОТНОШЕНИЯ СОПРОФИТОВ И ОБЩЕГО БАКТЕРИОПЛАНКТОНА В ОТКРЫТОЙ ВОДЕ И ЛИТОРАЛЕ ОЗЕРА БАЛАТОН В 1968 ГОДУ

Я. Олах

1. Сезонные количественные отношения сопрофитов и общего бактериопланктона были изучены на 5 стандардных поперечных сечениях озера Балатон с помощью посева и прямого метода.

2. По количеству сопрофитов и общего бактериопланктона побережная и открытая вода сильно различаются друг от друга. В побережной зоне общее количество бактериопланктона в 2—3 раза, а количество сопрофитов в 40—120 раза выше, чем в открытой воде. Эта разница было наиболее выражена в весенние месяцы.

3. На южном литорале лишенном тросника зона, характеризующаяся высоким числом бактерий, была низкая в изученном периоде. На северном литорале покрытом тросником зона с высоким числом бактерий была широкая в весенние месяцы (1500 ск), а потом ограничивалась зоной зарослей тросника.

Весной широкая зона высоким содержанием бактерий северного литорала совпадает зоной ила богатой органическими веществами.

4. Количественное изменение сапрофитов и общего бактериопланктона свидетельствуют об интенсивности процесса самоочищения воды озера Балатон. В этом процессе важную роль играют высокая летняя температура, насыщение с кислородом, постоянное движение воды и высокое содержание $CaCO_3$.

5. В открытой воде количество общего бактериопланктона было в среднем около 5.10^5 мл/мл, а самое высокое значение было 2,3 мил.

6. В открытой воде содержание сапрофитов довольно низко, на МПА 10—100/мл, а на агаре с казеином и глюкозой 20—500/мл. В Кестхейском заливе и его окрестности численность сопрофитов в июне и июле была неожиданно высокой на обеих средах (10—40 тыс/мл).

7. Количество актиномицетов в планктоне незначительно.

8. Соотношение числа сопрофитов и общего бактериопланктона в отношении чистоты воды благополучно (10 тыс.) в заливе Кестхей и его окрестностях: это соотношение снижается до 40—90 в июне и июле.

9. Не был найден параллелизм в возрастании количества общего бактериопланктона и числа сапрофитов.

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QUANTITATIVE INVESTIGATIONS ON MUD-LIVING CRUSTACEANS IN THE OPEN WATERS OF LAKE BALATON

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Received: 4th February, 1969

Two earlier publications (ENTZ et al. 1963; PONYI, 1966) give a preliminary survey on the qualitative and partly quantitative conditions of "micro-crustacea" found in Keszthely Bay and in other sections of Lake Balaton. In want of adequate collecting apparatus, the employed EKMAN—BIRGE type dredger, in the case of small crustaceans, was only suitable for informatory quantitative investigations, for this machine is hardly capable to secure the very soft upper layer of the bottom. Notwithstanding, the data coming to light drew attention to "micro-crustacea" living in abundance in the Lake, therefore, a more accurate method to be worked out to ascertain their number was rather desirable. The results achieved in the field of methodological investigation of the past few years (PONYI et al. 1967) rendered possible the more accurate quantitative measuring of crustaceans living in the mud of open water in Lake Balaton. This study is to give information on the results having achieved so far.

Conditions of collecting, sample taking and working-up.

Mud samples were taken at the South-western part of the Lake (M, K, G) at three points, at the North-eastern part at two (A, E) and at three-three points of the transversal sections being at a right angle to the longitudinal axis of the Lake (cf. TAMÁS, 1967, pp. 233—234). The samples were secured by a modified version of the CRAIB type apparatus (more information in: PONYI et al. 1967). The time of collecting: 14th—15th June, 1966, 26th—27th August, 21st—22nd September, 18th—19th October, 15th—16th November, 11th—12th April, 1967 and 16th—18th May. Data bearing relation to collecting (temperature of water and atmosphere, depth of water, water transparency) and other comments may be found in TAMÁS'S paper (1967, p. 235 and 1968. p. 229).

At each of the 15 collecting sites of the 5 sections 3 samples were taken (total surface was 40 cm^2), which were put into a common container. The samples contained about 300 ml mud and 100 ml water coming from just above the surface of the mud. In the laboratory the sample was carefully stirred, then half of it was divided into equal portions of 20 ml each and were poured into conic No. 25 plancton nets. Into the net thin stream of sieved tap water was allowed to pour in order to eliminate fine mud and colloid-size particles. The prepared material was then transferred into a quadratic-latticed dish and a binocular microscope was used for selection (PONYI et al. 1967).

A survey and some comments on the species found during the investigation

The selected crustaceans of some 4—5 thousand belonged to 29 species, the distribution according to larger taxonomic units is as follows: Cladocera 15, Ostracoda 4, Copepoda 10 species. From ecological and frequency points of view two main groups may be distinguished valid for Lake Balaton:

(a) *mud (partly reed-grass and reed-grass coats) inhabitants:*

Cladocera:

Macrothrix laticornis (JURINE), *Iliocryptus sordidus* LIEVIN, *Alona rectangula* G. O. SARS, *Alona quadrangularis* (O. F. MÜLLER), *Alona affinis* LEYDIG, *Alonella rostrata* (KOCH), *Leydigia leydigii* (LEYDIG), *Leydigia acanthocercoides* (FISCHER), *Pleuroxus uncinatus* var. *balatonicus* DADAY, *Monospilus dispar* G. O. SARS.

Ostracoda:

Candona balatonica DADAY, *Candona* sp. (not *balatonica)*, *Ilyocypris gibba* (RAMDOHR), *Darwinula stevensoni* (BRADY et ROBERTSON).

Copepoda:

Paracyclops fimbriatus (FISCHER), *Acanthocyclops viridis* (JURINE), *Microcyclops varicanas* (G. O. SARS), *Ectinosoma abrau* (KRITSCHAGIN), *Attheyella* (s. str.) *crassa* (G. O. SARS), *Nannopus palustris* BRADY.

(b) *Plankton members (and well swimming littoral inhabitants):*

Cladocera:

Latona setifera (O. F. MÜLLER), *Diaphanosoma brachyurum* (LIEVIN), *Daphnia hyalina* var. *galeata* G. O. SARS, *Daphnia cucullata* G. O. SARS, *Bosmina longirostris* f. *pellucida* STINGELIN.

Copepoda:

Eudiaptomus gracilis (G. O. SARS), *Cyclops vicinus* ULJANIN, *Acanthocyclops vernalis* (FISCHER), *Mesocyclops* (s. str.) *leuckarti* (CLAUS).

Two species, *Attheyella* (s. str.) *crassa* (G. O. SARS) and *Microcyclops varicans* (G. O. SARS), are new to the fauna of Lake Balaton. Both species — excepting one specimen — come from the A and E sections of the Lake. *A. crassa* occurs in different waters (both in stagnant and in running), including even subsoil waters. *M. varicans*, according to the literature, is the inhabitant of the macrovegetation of the littoral zone.

The quantitative distribution in the different regions of the lake

(a) *Mud inhabitants*

From quantitative point of view the majority of species ranged into the group of open water mud inhabitants, in the course of the investigation, seemed to play only a minor role. Although among them were real mud inhabitants

(Iliocryptus sordidus, Pleuroxus uncinatus var. *balatonicus, Leydigia leydigii, L. acanthocercoides,* etc.) we shall disregard them in the following pages of this paper. Likewise shall we dispense with the description of such crustaceans which are rather the inhabitants of the macrovegetation and in a restricted sense of the littoral zone, occurring in the mud of the open water only sporadically. Respective data on them may be found in various papers (PONYI, 1957, 1960, 1962; SEBESTYÉN, 1947, 1948, 1965).

In the view of quantity; the first place is occupied by *Ectinosoma abrau.* Though at different points of the identical sections the number of individual specimens significantly diverged, its ubiquity in the whole of Lake Balaton seems to be certain. Section K is the poorest in any month of sample taking, showing an increase of number towards Tihany (G—E) and Keszthely (M). The greatest population was observed in early spring months *(Fig. 1)*; the numbers given indicate a distribution in 20 cm² per exemplars:

M	*K*	*G*	*A*	*E*
13 ± 2	7 ± 2	34 ± 9	16 ± 7	19 ± 7

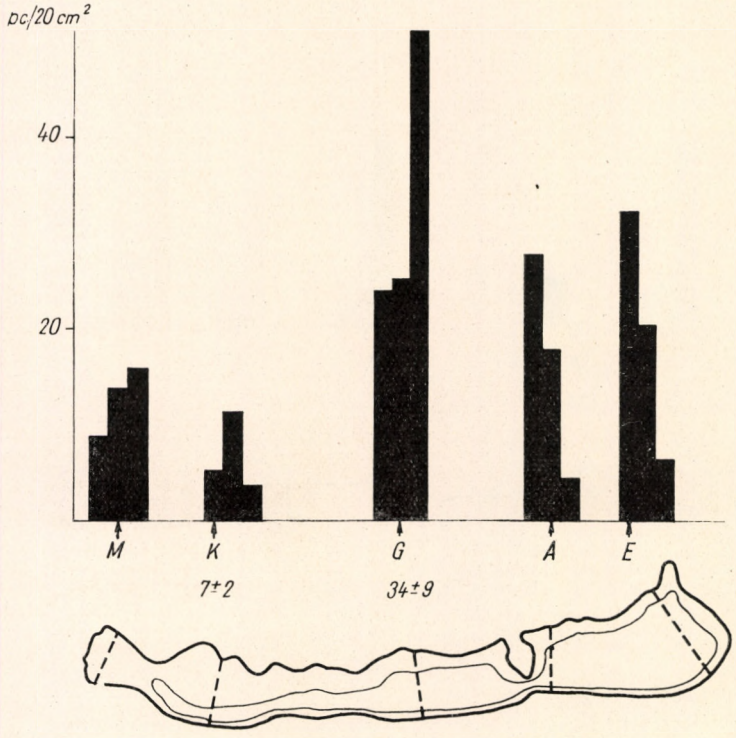

Fig. 1. The quantitative conditions of *Ectinosoma abrau* in June 1966 in the different sections of Lake Balaton.
† = samples from the axis of depth.
The numbers below the abscissa mean the average of the three samples and the deviation from the average

where it is readily observable that K and G significantly differ from each other calculated as means on the basis of three sample taking points. The quantity decreased in July, the biggest values were found in sections M and E. The smallest number of individuals was detected in August *(Fig. 2)*, however, no

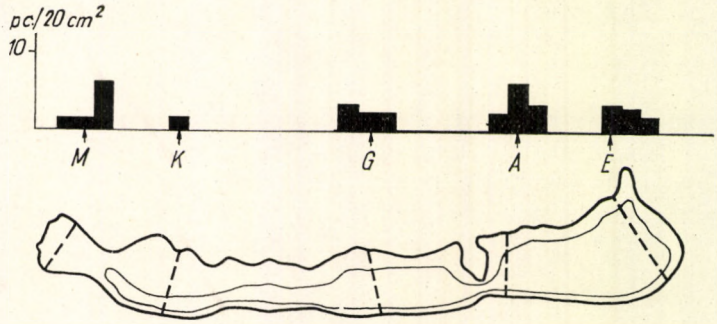

Fig. 2. The quantitative conditions of *Ectinosoma abrau* in August 1966 in the different sections of Lake Balaton.
↑ = samples from the axis of depth

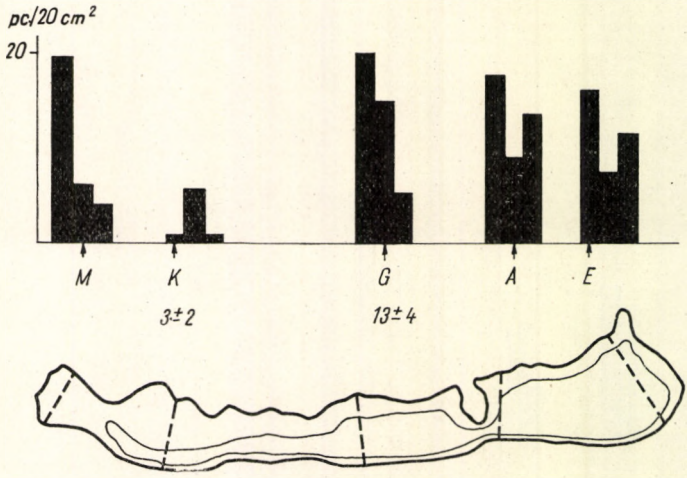

Fig. 3. The quantitative conditions of *Ectinosoma abrau* in November 1966 in the different sections of Lake Balaton.
↑ = samples from the axis of depth

quantitative differentiation could be ascertained. Starting from September, in the course of October and November *(Fig. 3)* section K strikingly differs from all the other sections. The same phenomenon also occurs in April and May.

The highest number of specimens with eggs (on an average in respect of all sections) was found in April, their number gradually decreased until July,

only sporadically appearing in August and September, while in October and November examples with eggs were not found. In respect of sectional distribution A and E proved to be the richest.

The monthly mean calculated from the transversal sections is as follows (exemplars per dm^2):

1966						1967	
June	July	Aug.	Sept.	Oct.	Nov.	Apr.	May
89	38	10	21	47	51	47	18

The data indicate that the greater population of *Ectinosoma* falls on the early summer period.

As respect of quantity the second place is occupied by *Paracyclops fimbriatus*. Its distribution is very uneven within the individual sections *(Fig. 4)*. However, the significant values always come from samples of the axis of depth. The fluctuation of monthly values calculated from all sections shows that the highest number of individuals in the early summer period (47 specimens per dm^2) suddenly decrease by August (5 specimens per dm^2), then from September a gradual increase sets in (13—23 specimens per dm^2).

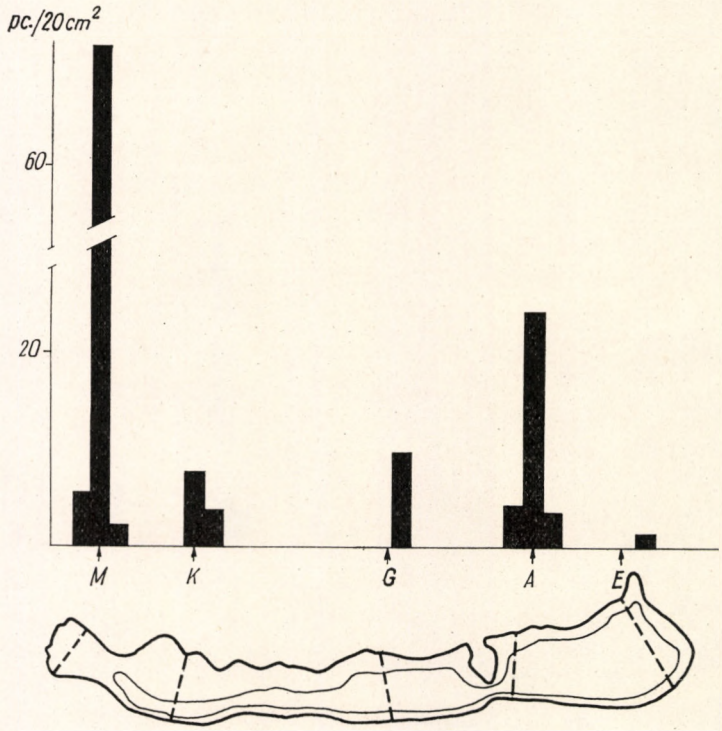

Fig. 4. The quantitative conditions of *Paracyclops fimbriatus* in June 1966 in the different sections of Lake Balaton.
↑ = samples from the axis of depth

Darwinula stevensoni occurs in small number of individuals, though its distribution is rather even in every section and even in sectional points. The 13—14 specimens per dm^2 in the months of June and July by September decrease to 5 specimens per dm^2, then showing rise in number in October nearly reaching the summer level.

A *Candona* sp., whose identification could not yet be accomplished for in the last few years only juvenile exemplares have been collected, may also be mentioned here as quantitatively significant. Great numbers (7—14 specimens per dm^2) were found in summer months and in September. It should be noted here, that this Ostracoda is not synonymous with *Candona balatonica*. This latter was found only in October and November within the period of investigation, mainly in sections M, K and E, and even here only very sporadically.

The quantitative distribution according to collecting points of *Alona quadrangularis* and *Alona affinis* is rather uneven. It came to light that these two species bear less significance beside the aforementioned species in respect of quantity, taking into account the whole of the investigational period, calculated separately on the basis of the five sections both in the warm and cold water period (June—September and October—May). Both species were found in the greatest number in June, near the middle of the Lake (G section) in less towards the shores in greater numbers. By August their number drastically decreased showing a rise only in autumn. *A. quadrangularis* both in the warm and cold water period produced an average of 11 specimens per dm^2 and 4 specimens per dm^2, respectively. *A. affinis*, on the other hand, occurred in an average of 9 specimens per dm^2 and 2 specimens per dm^2, respectively. While in the littoral zone and in its vegetation generally *A. affinis* appears more frequent as compared to *A. quadrangularis* (Sebestyén, 1947, 1948, 1965; Ponyi, 1962;

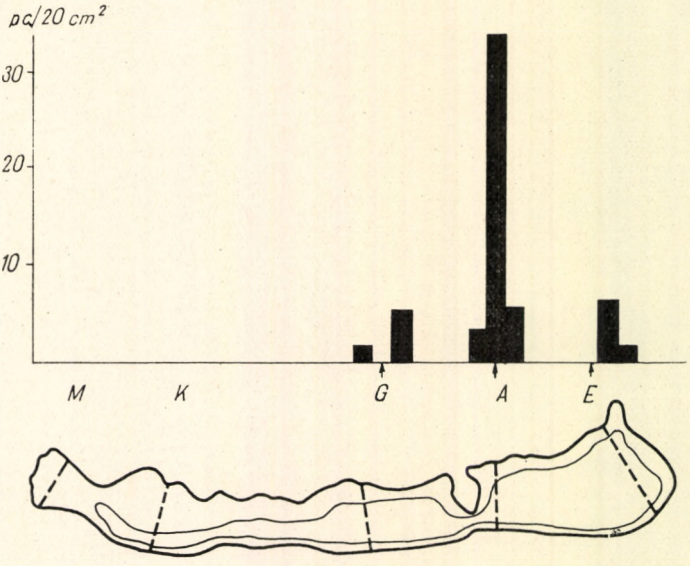

Fig. 5. The quantitative conditions of *Monospilus dispar* in October 1966 in the different sections of Lake Balaton.
† = samples from the axis of depth

data of other habitats: BERG, 1929; FLÖSSNER, 1962, 1964, etc.), in the mud of open water, taking into consideration all five sections — it seems — that this proportion is inverted. It is to be noted, that during the period of investigation *A. affinis* occurred in sections A and E with greater frequency than in sections K and M.

The mosaic-like distribution of *Monospilus dispar* in Lake Balaton has been known some time (SEBESTYÉN, 1965) which has been nowproved with greater certainity by these quantitative investigations *(Fig. 5)*. The results further show that in the period of investigation the North-eastern basin (A, E) both in the warm (June—September) and cold period (Oct.—Nov., Apr.—May)

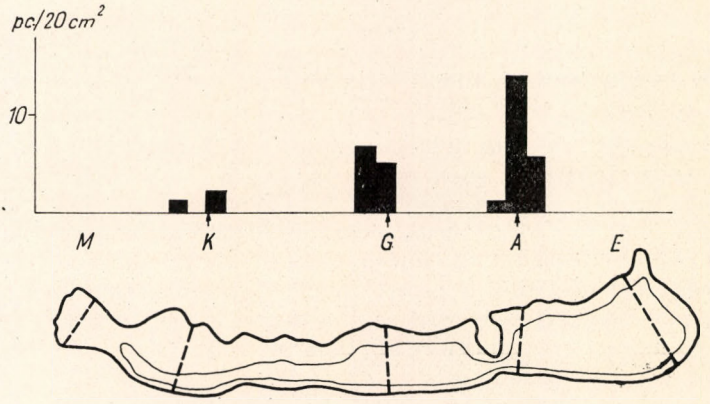

Fig. 6. The quantitative conditions of *Nannopus palustris* in June 1966 in the different sections of Lake Balaton.
↑ = samples from the axis of depth.

possesses a higher number of individuals than does the South-western. While the former produces for both periods 12—16 specimens per dm², the other only 0.5—1 specimen per dm². Whether this quantitative distribution is stable for the two basins or has only been characteristics for the year when the experiment was carried out is uncertain, and only future research of the question may decide unambiguously.

The distribution of *Nannopus palustris* in the Lake has been very similar to that of *Monospilus (Fig. 6)*. Quantitatively it was only notable in June and July (2—7 specimens per dm²).

(b) *Notes on plankton members found in the mud samples*

In the mud, on its surface and on the border of water and mud considerable quantity of plankton crustaceans were found. Out of the 4—5 thousand animals which were selected out some 35% were plankton organisms (juveniles of *Mesocyclops* (s. str.) *leuckarti*, *Eudiaptomus gracilis* and *Cyclops vicinus)*. During the 8 months in each of the five sections the plankton members appeared with a frequency of above 50%:

M	*K*	*G*	*A*	*E*
3	6	2	1	1

These data indicate, especially in the South-western basin of the Lake, that the juvenile forms of planktonic copepods play an important role in the mud surface. The frequency distribution of *Mesocyclops* and *Eudipatomus* genera seems to be proportional to the relative quantity of planktons. For the benthic distribution of *Cyclops vicinus* see an other paper (PONYI, 1968).

The comparison of quantitative data between 1965 and 1966

Although the previous quantitative investigations (PONYI, 1966) were only informative in nature, their comparison with the present results offers to be profitable for two reasons *(Table 1)*. The number of individuals per dm^2 of the collected mud inhabiting crustaceans from the same period and sections of the two years increased 3—4 times by employing the method just described. This number most probably is nearer to the real values than the earlier ones, although we should not disregard the damaging effect of chlorinated carbon hydrogen remmants (cf. PONYI et al. 1968, p. 185).

Table 1.

The quantitative distribution of mud inhabiting "micro-crustaceans" in the open water mud of Lake Balaton (specimens per dm^2)

Sections	*M*		*K*		*G*		*A*		*E*		
Dates	specimen per dm^2	%	specimen per dm^2	%	specimen per dm^2	%	specimen per dm^2	%	specimen per dm^2	%	%
1965. VI—X.	46	30.1	14	9.1	25	16.3	34	22.2	34	22.2	
1966. VI—X.	148	24.1	43	7.0	118	19.2	148	24.1	158	25.1	

In spite of the difference between the two methods of collecting the relative "micro-crustacean" values in per cent in respect of the same sections show great similarity. On the basis of this it may be expressed with certainty that the distribution of mud inhabiting crustaceans of the open water is heterogeneous. The richest regions are the North-eastern basin and Keszthely Bay (section M), while the middle region of the Lake is the poorest, meaning that the mud of open water in the Lake, taking into account other qualitative characteristics, may be devided at least into three great units.

Summary

1. Author established, in respect of the mud of open water, that from quantitative point of view the following species are most important: *Ectinosoma abrau*, *Pracyclops fimbriatus*, *Darwinula stevensoni* and a *Candona* sp. The species of *Alona* and *Monospilus* mainly play a more significant role in the, North-eastern basin.

2. The fry forms of Copepoda plankton members appear in greater quantities in the mud, especially in section K, therefore their activity should not be neglected.

3. On the basis of comparative examinations carried out in 1965 and 1966 it became evident that the mud of open water of great extent may be devided into three large units.

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MENNYISÉGI VIZSGÁLATOK A BALATON NYÍLTVÍZI ISZAPJÁBAN ÉLŐ RÁKOKON

Ponyi Jenő

Összefoglalás

1. A tó nyíltvízi iszapjára vonatkozóan a szerző megállapította, hogy mennyiségi szempontból az *Ectinosoma abrau*, *Paracyclops fimbriatus*, *Darwinula stevensoni* és egy *Candona* faj a legjelentősebb. Az *Alona* és *Monopsilus* fajok elsősorban az ÉK-i medencében látszanak jelentősnek.

2. A tó iszapjában, különösen a „K"-val jelzett területen, a juvenilis Copepoda-planktontagok jelentős mennyiségben fordulnak elő, így azok tevékenysége nem elhanyagolható.

3. Az 1965. és 1966. évi vizsgálatok összevetése alapján bizonyossá vált, hogy a nagyterületű nyíltvízi iszap 3 nagyobb részre tagolódik.

КОЛИЧЕСТВЕННЫЕ ИССЛЕДОВАНИЯ РАКОВ, ЖИВУЩИХ В ИЛЕ ОТКРЫТОЙ ВОДЫ ОЗЕРА БАЛАТОН

Й. Поньи

1. Было установлено, что в иле открытой воды озера в наибольшем количестве обнаруживаются *Ectinosoma abrau*, *Paracyclops fimbriatus*, *Darwinula stevensoni* и один вид *Candona*. Виды *Alona* и *Monopsilus* найдены в значительном количестве в северно-востокном бассейне.

2. В иле озера особенно в области «К» обнаруживается значительное количество недорозвитых Copepoda— планктонов, так что их деятельность надо принимать во внимание.

3. Сравнивая исследования 1965 и 1966 годов доказывают, что ил открытой воды разделяется на 3 больших составных части.

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ON SOME PROPERTIES OF THE EXOPEPTIDASE OF *GAMMARUS (RIVULOGAMMARUS) ROESELI* GERVAIS (AMPHIPODA) AND *ASELLUS AQATICUS* (L.) (ISOPODA)

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Received: 7th February, 1969

Earlier investigations (KLEINE and PONYI, 1967; KLEINE, 1967; DEVILLEZ, 1965) revealed that the stomach juice and the hepatopancreas of some Decapod species contain a carboxypeptidase with highly similar effects to the pancreatic carboxypeptidase of the mammals, though exhibiting but meagre substrate specificity.

Within the class Crustacea, there are no data on the exopeptidase conditions of groups phylogenetically removed from the Decapods. The present paper proposes to discuss some properties of the carboxypeptidase of one representative each of the orders Amphipoda and Isopoda.

Material and method

1. *Material under investigation and the preparation of the ferment extract*

The animals used in our investigations *(Gammarus, Asellus)* had been collected from one of the tributary streams (Aszófői patak) of Lake Balaton, and kept, for at least 2 weeks prior to their preparation, in a well aerated aquarium supplied with through-flow water system.

The ferment extract was taken from the intestinal tract of the animals; the dissecting technique has already been published in an earlier paper (PONYI and P.-ZÁNKAI, 1967). The intestinal tract of 50 *Asellus* and 25 *Gammarus* specimens was used per each experimental series. The prepared organs were collected in 2 ml distilled water and immediately placed in ice or in refrigerators, respectively, and kept there, except for the period of centrifuging, until their employment. The material was homogenised by Potter's glass homogenisator. Centrifuging took 10 minutes, at 5000 r.p.m.

The stomach and the stomach juice of *Gammarus* and *Asellus*, as well as the midgut, are infinitesimally smaller in comparison to the relatively large hepatopancreatic (HP) tubes and to the liquid content in them. Thus the extract obtained from the animals originates mainly from the HP tubes and from their contents.

2. *Substrate incubation conditions methods of identification*

Two peptide substrates have been used in our investigations: carbobenzoxy-L-glutamyl-L-tyrosine (CGT) (pepsine and carboxypeptidase-A-homospecific substrate) (Sas et Son Ltd.), and carbobenzoxyglycyl-L-phenylalanine (CGP) (carboxypeptidase-A-specific substrate) (Fluka).

The substrate concentration in 1 ml of reaction mixture is shown in *Table 1.* The composition of the incubational mixture is as follows:

	Enzyme	Asellus substrate ml	Buffer	Enzyme	Gammarus substrate ml	Buffer
CGT	0.08	0.12	0.80	0.03	0.10	0.87
CGP	0.08	0.10	0.82	0.02	0.05	0.93

The buffers employed were: McIlvaine (0.1 M; between pH 3.1—6.5), tris (HCl) 0.1 M; between pH 7.2—9.1). The homogenizates were incubated for 30 min at 37.5 $\pm$ 02. °C. The value of the control was 0 for both species.

During the quantitative paper chromatography (Hanson, 1966), the ninhydrin-copper complex was measured with Beckman spectrophotometer at 495 $\mu\mu$. The protein content of the enzyme preparation was determined by

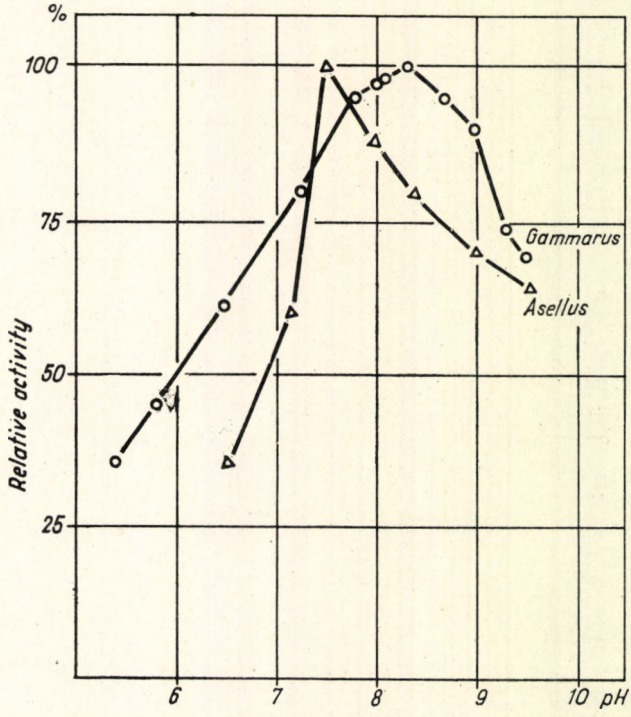

Fig. 1. The pH activity curve, with respect to CGP, of the ferment extract of *Gammarus (Rivulogammarus) roeseli* Gervais and *Asellus aquaticus* (L.)

the LOWRY method modified by GLÄSSER and KLEINE (1962). Crystallized bovine serum-albumen (Albumine bovine (fraction V) B grade (Calbiochem)) was used for standard. The albumen content of the *Gammarus* and *Asellus* enzyme perparations varied between 2.5—6.4 mg/ml and 3.6—6.2 mg/ml, respectively.

During the preliminary investigations, we used a freshly prepared sephadex-G-100 column, set at 7.5 pH by a 0.05 phosphate buffer. The size of the tube was 1.5 × 24 cm. The fractions were collected by a home-made automatic microfraction collector built in a refrigerator. 1.25 ml fractions were collected.

Results and discussion

For the demonstration and characterization of the carboxypeptidase-A effect CGP was used. The pH optimum of the clevage in *Asellus* (between 7.5—7.7) agrees with that of *Astacus* and *Cambarus*, but rather deviating (between 8.0—8.2) in *Gammarus (Fig. 1)*. CGT was applied to show the eventual pepsin or cathepsin-A effect. The pH optimum is identical (7.2 pH; *Fig. 2.)* for both species and agrees with the conditions found in the two Decapod species studied earlier; the presence of a carboxypeptidase is also obvious here (KLEINE and PONYI, 1967).

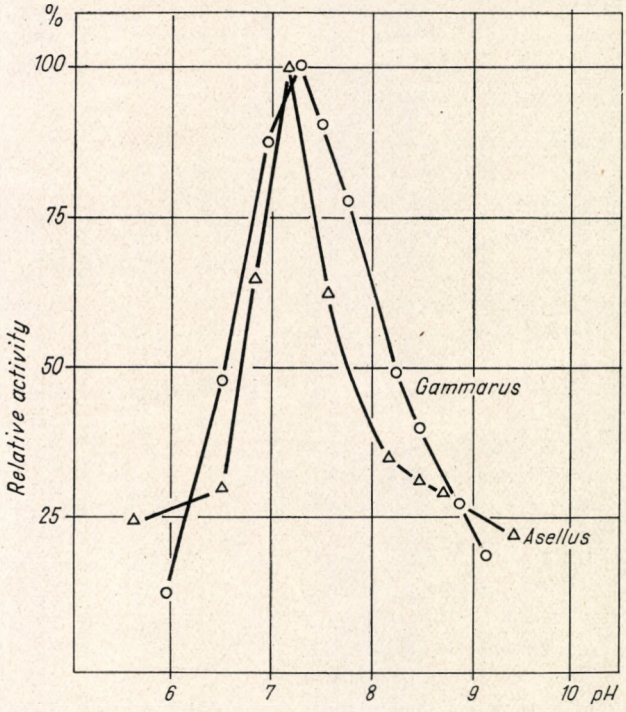

Fig. 2. The pH activity curve, with respect to CGT, of the ferment extract of *Gammarus (Rivulogammarus) roeseli* GERVAIS and *Asellus aquaticus* (L.)

The quotient of CGP/CGT activity of *Asellus* and *Gammarus* points to the same small substrate specificity as was observed also in the case of *Astacus* and *Cambarus:*

Astacus	1,01
Gammarus	1.23
Asellus	1.28
Cambarus	1.42

The CGP/CGT quotient is 8.3 in the case of the mammalian pancreas (HOFMAN and BERGMANN, 1940).

The specific activity of the *Gammarus* and *Asellus* carboxypeptidase (v_s) significantly differs, according to the investigations hitherto conducted, from that of the Decapods. Whereas this value concerning the HP of *Astacus* is 2.00 (CGP) and 1.64 (CGT), respectively, and for the stomach juice 5.21 (CGP) and 5.15 (CGT), respectively, the figures are about ten times higher for the species investigated. *(Table 1).*

Table 1

The exopeptidase activity of the hepatopancreas of *Gammarus (Rivulogammarus) roeseli* GERVAIS and *Asellus aquaticus* (L.). Activity data are expressed by the amino acids μMol/mg albumen (60') = v_s), formed at 37.5 °C. $\bar{x}$ = arithmetic mean, n = number of investigations, s = standard error of mean

Species	Concentration of substrate	pH	$\bar{x}$	*n*	*s*
Gammarus	CGP $5 \cdot 10^{-3}$M	8.1—8.3	54.926	11	1.677
	CGT $5 \cdot 10^{-3}$M	7.0—7.1	44.481	4	3.601
Asellus	CGP 10^{-2}M	7.5—7.6	43.184	7	4.922
	CGT $6 \cdot 10^{-3}$M	7.1—7.2	33.602	7	1.171

Concerning the v_s values, significant differences can be found also between *Gammarus* and *Asellus* (for CGT : $P < 0.01$; for CGP: $0.02 > P > 0.01$). As related to Decapods some deviations in the stability of the enzyme can also be observed. In the case of *Gammarus*, an activity decrease of 20—25 per cent is perceiveable after 2 weeks for both CGT and CGP (at + 2 °C), and 35—40 per cent after 3 weeks in the case of *Asellus*. Even a storing of 5 weeks fails to evoke any considerable decrease in activity for the ferment of Decapods (cf. p. 45, ibid.).

In the gel filtration of the *Gammarus* extract we received consequently 3 fractions (peak) — against that of the Decapods (KLEINE, 1967) — of which the middle one was active for CGP and CGT, respectively *(Fig. 3).*

On the basis of the available data it may be inferred that one has to count with smaller or greater differences (e.g. deviating properties of the isoenzymes) in the proteolytic ferments present in the different groups of Crustacea (Decapoda, Amphipoda, Isopoda etc.) and even in the various species (C. MANWELL et al. 1967).

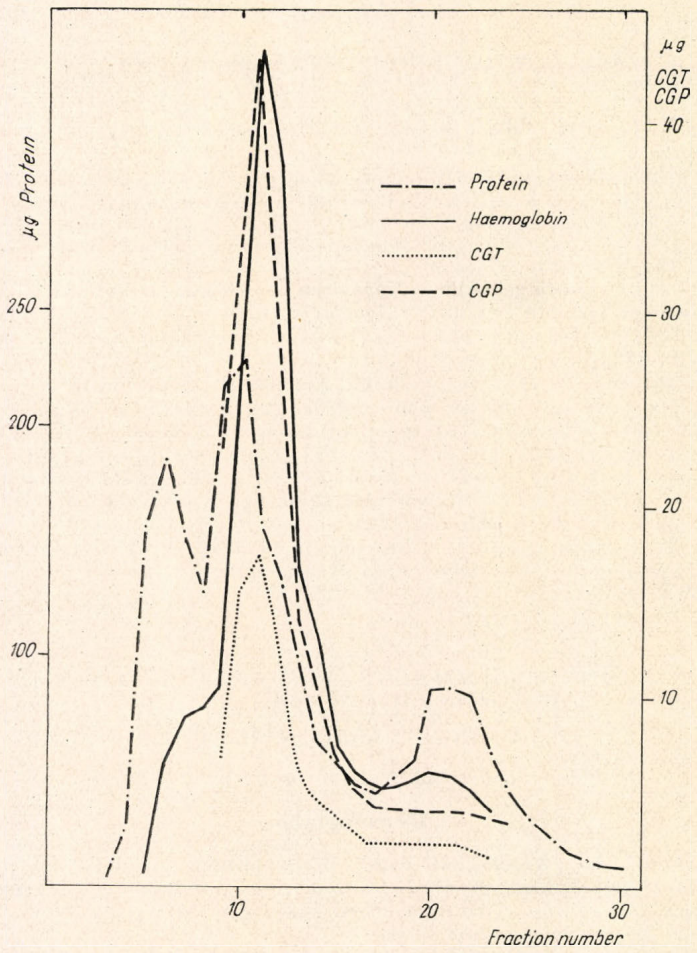

Fig. 3. The gel filtration on sephadex G-100 of the ferment extract of *Gammarus (Rivulogammarus) roeseli* GERVAIS. (For details see text)

Summary

1. The specific activity (v_s) of the hepatopancreas of *Gammarus (Rivulogammarus) roeseli* GERVAIS and *Asellus aquaticus* (L.) on carbobenzoxyglycyl-L-phenylalanine (CGP) and carbobenzoxy-L-glutamyl-L-tyrosine (CGT) is more than tenfold as that known for the other Decapod species.

2. The activity quotient of CGP/CGT (1.23 and 1.28, respectively) of the two investigated species refers, similarly to the case of *Astacus* and *Cambarus* (1.01 and 1.42, respectively), to a slight substrate specificity.

3. Of the 3 albumen fractions (peak) received during the sephadex gel filtration of the enzyme preparation of *Gammarus* only one was active with respect to CGP and CGT, respectively.

Acknowledgement

It is our agreeable duty to express our gratitude to Dr. R. KLEINE (Physiologisch-chemisches Institut, Halle /Saale(DDR) for his cordial help in our work.

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GAMMARUS (RIVULOGAMMARUS) ROESELI GERVAIS (AMPHIPODA) ÉS *ASELLUS AQUANTICUS* (L.) (ISOPODA) EXOPEPTIDÁZÁNAK NÉHÁNY TULAJDONSÁGÁRÓL

Ponyi Jenő, Biró Kálmán és *P.-Zánkai Nóra*

Összefoglalás

1. A *Gammarus (Rivulogammarus) roeseli* Gervais és *Asellus aquaticus* (L.) hepatopankreász carbobenzoxyglycyl-L-phenylalanin (CGP) és carbobenzoxy-L-glutamyl-L-tyrosin (CGT)-re vonatkoztatott specifikus aktivitás (v_s) több mint a tízszerese az eddig ismert Decapoda fajokhoz képest.

2. A két vizsgált faj CGP/CGT aktivitás quotiense (1,23 ill. 1,28) — az *Astacus* és *Cambarus*-hoz hasonlóan (1,01; 1,42) — csekély szubsztrát-specifikusságra utal.

3. A *Gammarus* enzimpreparátumának sephadex gélfiltrációja során kapott 3 fehérje frakció (peak) közül CGP ill. CGT-re vonatkozóan csak egy volt aktív.

НЕКОТОРЫЕ ХАРАКТЕРНИЕ СВОЙСТВА ЭКЗОПЕПТИДАЗ *GAMMARUS (RIVULOGAMMARUS) ROESELI* GERVAIS *(AMPHIPODA)* И *ASELLUS AQUATICUS* (L.) (ISOPODA)

Й. Поньи, К. Биро и *Н. П.-Занкаи*

1. Специфическая активность (v_s) гепатопанкреаса *Gammarus (Rivulogammarus) roeseli Gervais* и *Asellus aquaticus* L., расчитанная на карбобензоксиглицил-Л-фенилаланин (КГФ) и карбобензокси-Л-глутамин-Л-тирозин (КГТ) больше чем в 10 раз выше описанных до сих пор для Decapoda величин.

2. Соотношение КГФ/КГТ активности (1,23 и 1,28) этих видов также как у *Astacus* и *Cambarus* (1,01 и 1,42) указывает на незначительную специфичность субстрата.

3. В ходе очистки фермента *Gammarus* с помощью гельфильтрации было получено 3 белковых фракции, из которых в отношении КГТ только одна фракция обладала активностью.

ANNAL. BIOL. TIHANY | 36 | 229–256 | HUNGARIA 1969

KLADOCERA TANULMÁNYOK A BALATONON
IV. SZUBFOSSZILIS MARADVÁNYOK BALATONI ÜLEDÉKEKBEN I.

SEBESTYÉN OLGA

Magyar Tudományos Akadémia Biológiai Kutatóintézete, Tihany

Érkezett: 1969. február 11-én

A tavi kladocera-fauna ismeretének új jelentősége paleolimnológiai vizsgálatok során tűnt ki (FREY, 1955, 1958, 1959, 1960 b, 1964). A mikrofauna tagjai közül e csoport maradványait jelentős mennyiségben s általában jó megtartásban őrzik az üledékek, úgyannyira, hogy ezek számbavétele és értelmezése alapvető, mondhatni nélkülözhetetlen a hajdani tavi élet rekonstruálásában. (FREY, 1964: 35, DECOSTA, 1964: 66, GOULDEN, 1964: 2, MUELLER, 1964: 2).

Kitűnt az is, hogy az általánosan használt határozókulcsokban tekintetbe vett — ép példányokra vonatkozó — faji bélyegek legtöbbje nem elegendő vagy nem használható szubfosszilis anyagon. Az utóbbit ui. többnyire a kitin külsőváz különböző testtáji részei és azok töredékei teszik. D. G. FREY ismert fel oly bélyegeket, melyek lehetővé teszik a maradványok faji hovatartozásának pontos megállapítását. Ezeknek filogenetikai értéke is van. E bélyegek jelenléte biztosítja az identifikálás helyességét récens anyagon is (FREY, 1958, 1959, 1962 a, 1964: 48—49, l. még DECOSTA, GOULDEN, MEGARD, MUELLER felsorolt munkáit);

Ma már nyilvánvaló, hogy a paleolimnológia ekológiai vonatkozásait (pl. a klíma finomabb szakaszai, állatföldrajz, stb.) közvetlenül, sőt — közvetve — kulturális kapcsolatait is a kladoceramaradványok illetőleg ezek együttesei és mindezekben beálló változások értelmezése hathatósan viszi előre, alapot nyújtva a tó s a tavi élet múltjának, alakulásának felvázolásában, a messzi múltban történt emberi tevékenység felidézését is beleértve.

Tavunk kladocera faunájának megismerésében még van tennivaló. DADAY JENŐ számos tanulmánya az alap, melyre építhetünk. Kevés azoknak a fajoknak a száma, melyet DADAY nem jegyzett fel. A mai helyzet pontos ismerete paleolimnológiai tanulmányokban igen szükséges. Ezért párhuzamosan a fosszilis anyag feldolgozásával, a Tihany—Balatonfüred profil több pontját az év különböző szakában ismételten felkerestem mintagyűjtés céljából. Ez a profil különböző élőhelyeket (s. l.) (habitat) foglal magában: növényzetnélküli térségeket, melyekre különös súlyt helyeztem, nádasok szomszédságát, a tihanyi Kisöbölnek a parttól ill. a nyíltvíztől különböző távolságban fekvő, más és más környezetet nyújtó részleteit (SEBESTYÉN, 1965: 190—191). Néhány mintát Keszthely parti vizeiből vettem.

1. táblázat

A tanulmányban említett külföldi tavak és a
Data of paleolimnological bearings of lakes mentioned in the

	1 Tó	2 Földrajzi helyzet	3 Méretek	4 Típus
Európa	Wauwiler tó neolitikus	Svájc Luzern közelében?		Mohás terület
	Längsee Carinthia	Alt. 548 m	0,76 km² max. m. 21 m k. m. 11 m	Meromiktikus
	Wallensen É-Németország	Lignit terület	h. 23 m m. 3,4 m 1948	Növényzetben gazdag mocsár vízfoltokkal 1958
	Schleinsee Németország	Langenargen közelében	149 ha max. m. 11 m k. m. 6,5 m	Eutrof?
	Herning mellett	Jutland, Dánia		Sphagnumláp
	Esthwaite Water Anglia	Angol tóvidék	h. 2 km max. m. 15,5 m	Eutrofikáció folyamatban
Ázsia	Lake Nojiri Japán	alt. 654 m N. lat. 36°49′ E. long, 138°13′		
	Lake Zeribar Irán	alt. ± 1300 m N lat. 35°35′ E long. 46°08′	4 km × 1,5 km m. 4—5 m t ± 8 km²	Sekély hegyi tó
É-Amerika	Dead Man Lake New Mexico USA	Chuska Mt alt. 2800 m	380 × 300 m 11 ha m. ± 1 m, változó	Sekély hegyi tó
	L. Whimpy Wyoming, USA	Wind River Mt alt. 2438 m	30 × 40 m m. 1—2 m, változó	Kis hegyi tó
	Madison lakes Wisconsin	5 tó láncolata USA	0,8—39,40 ha max. m. 4,3—25,6 m k. m. 1,6—12,1 m	Különböző harmónikus tavak
	45 tó a Mississippi völgyében	N lat. 47,0—29,92 USA	Különböző	Természetes + oxbow + mesterséges tavak
	L. Winona L. Wyland L. Lawrence Indiana	USA	t, k. m. 203,7 ha 9,07 m 24,7 ha 3,82 m 3,43 ha 7,00 m	Eutrof

Table 1.
Balaton paleolimnológiai szempontból fontosabb adatai
text. For explanation of headlines see English text p. 5.

5 Furat	6 Vizsgált réteg geológiai kora pollenzóna	7 Megjegyzés	8 Szerző
Üledékekből kimetszett darabok	Kultúr-réteg, tavikréta Schötz 1 Egolswill II.	1850. megszünt mint tó	Zemp. 1941
5 m 9 m	VI VII	Biogén meromixis kezdete az Atlanticusban	Frey 1955
Firbas I. sz. furata	Jégkorszak vége Idősebb tundrakor, Alleröd Fiatalabb tundrakor	Preborealban megszünt mint tó	Frey 1958
11 m	Furat felső 6 m VII—X	Tó kezdete[8]	Frey
		Idősebb tundrakor Ib	1961
11 m tavi üledékből 2,20 m vizsgálva	Eemi eljegesedésközti szakasz	Lúgos vízű tó később neutrálissá v. kissé savanyúvá vált	Frey 1962*b*
2 furat	II—VIIb	Természetes úton emberi emberi behatásra eutrofikáció	Goulden 1964
2,4 furat megfelel 12000 évnek	Jégkorszak vége Postglacialis (R I., II., III.)	Vulkáni hamueső ± 4600 B. P. ± 3600 B. P.	Tsukada 1967
Leghosszabb 25,4 m	Pollen- és Chydoridazónák: 23 000—12 000 A 12 000— 5 000 B 5 000— B. P. C	Hideg száraz steppe Pistacia-Amygdalus savanna, kevés változás	Megard 1967
	Tavi üledék 11 m: 7—10 Jégkorszaki + 1—3 dm Holocén szerves iszap	Minimális emberi beavatkozás, nyári sekély vízben helyenként Charamező	Megard 1964
325 cm	Jégkorszak vége + Postglaciális	Csap. terület emberi behatás nélkül	DeCosta 1968
15 cm felületi üledék (Ekman)	Megfelel 50—70 évnek B. P.		Frey 1960*a*, *b*
3 cm felületi üledék (Ekman)	Megfelel ± 15 évnek B. P.		DeCosta 1964
2—3 cm felületi üledék (Ekman)	Megfelel 15 évnek B. P.		Mueller 1964

	1 Tó	2 Földrajzi helyzet	3 Méretek	4 Típus
Közép Amerika	La Aguada de Santa Ana Vieja	Guatemala N lat. 16°34′ W long. 89°50′	⌀ 200 m m. ± 1 m	Kis trópusi tó törpefüves szavannában
	Laguna de Petenxil	Guatemala N lat. 16°55′ W long. 89°50′ alt. 200 m	1 × 1,5 km max. m. ± 5 m	Tóláncolat tagja, trópusi erdőben, egészében trofogén
	Balaton	N lat. 46°42′—47°3′ E long. 17°14′—18°10′ alt. 104,075 m	h 70 km t 594 km² max. m. 11 m k. m. 3,25 m vol. 1,8 km²	Jól pufferolt lúgos vízű, széljárta sekély tó, egészében trofogén

Hangsúlyozni szeretném, hogy a Balatonon, csekély mélysége ellenére, terjedelmes nyíltvízi területek vannak, melyeknek — limnológiai szempontból — vegetációnélküli eprofundális területek felelnek meg. Ilyen területeken élő együttesekben kevesebb faj van képviselve, mint a parti övben. A nyíltvízi benthikus Cladocera-együttest felfoghatjuk úgy, mint egy litorális jellegű fauna elszegényedett maradványát, illetőleg mint a parti övből nyíltvízi térségekre behatolt faunatöredéket. A feldolgozott legrégibb (j é g k o r s z a k v é g i)* szubfosszilis maradványok inkább az előbbi lehetőségre utalnak. Hogy a Balatonon van ilyen elkülönülés a tó sajátosságai következtében, kitűnt már a negyvenes évek derekán végzett vizsgálatok során, amikor részben csupán nyíltvízi területek benthikus kladocera-faunájának megismerésére szorítkoztam (SEBESTYÉN, 1947). Meg lehetett állapítani, hogy az ott talált fajok némelyikének partközeli populációja bizonyos vonatkozásban eltérő alaktani jelleget mutat, a nyíltvízi populációval szemben. A litorális kladocera-együttes felépítéséhez oly tipikus parti fajok is járulnak, melyek nyíltvízi habitatokból hiányzanak (SEBESTYÉN, 1948).

A Balaton parti öve igen változatos termőhelyekben a víztükör terjedelmes voltának, a partkifejlődés magas értékének, a déli és északi part eltérő limnológiai és topográfiai jellegének megfelelően. A Balaton Bizottság felvételei során CHOLNOKY JENŐ a természetes partok hosszát 225 km-nek mérte. Ma, részben a tükör összehúzódásával, részben a különböző emberi beavatkozás kihatásaként, a part hosszát 180 km-nek veszik a hidrológusok. Változik a partszakaszok limnológiai jellege is.

A jelenlegi faunaismeret kiegészítését egyelőre a különböző lelőhelyekről vett minták feldolgozásától lehet várni. FREY új módszere különösen alkalmas valamely tó kladocera-faunájának megismerésére. Ez a módszer a tavi üledékek felületi rétegéből feltárt morfológiai maradványok — a külső váz részei — rendszertani feldolgozásán alapszik (FREY, 1960 a, MUELLER, 1964, DECOSTA, 1964: 66, GOULDEN, 1966: 398, MEGARD, 1964: 535). Az így nyert

* Balatoni vonatkozásban a geológiai korokat jelző szakkifejezések ritkítva vannak, külföldi vonatkozásban — kevés kivétellel — az eredeti megjelölést használtam, ritkítás nélkül.

5 Furat	6 Vizsgált réteg geológiai kora pollenzóna	7 Megjegyzés	8 Szerző
25 cm		Utóbbi 4000 évben klímában lényeges változás nem volt Cowgill and Hutchinson	Goulden 1966*b*
225 cm 250,5 cm	C^{24}2170 ± 85 3990 ± 160 B. P.		Goulden 1966*a*
B 28 410 cm, 9 minta	Jégkorszak vége I*a*, I*b* Pleistocén és Holocén határa III—IV Ó-Holocén—Új-Holocén V—X	Öregedő tó, erős anthropogén hatás	Sebestyén ez a tanulmány

faunalistákból az előfordulás gyakorisága is kitűnt. Az adatok tótörténeti értelmezése a fajok ekológiájáról való tájékozottságot tételezi fel. A természetes vizekben való előfordulás adatainak ekológiai értelmezéséhez nagyban hozzájárulnak az újabb tótörténeti kutatások eredményei. Természetesen még több szükséges és várható is e tekintetben tenyésztési kísérletek tapasztalataitól.

A tótörténeti vizsgálatok tárgya általában kisméretű vagy eltűnt tavak üledékei *(1. táblázat)*. Találunk utalást arra, hogy kisméretű és ekológiai szempontból egyszerű tavak kladocera-faunája nem olyan változatos, mint nagy kiterjedésű tavakéi (Megard, 1964: 529, DeCosta, 1968: 409—411). Ilyen vonatkozású megállapítás tavunkon még korai volna.

Ez a tanulmány csak szerény kezdetét jelentheti a nagy feladatnak, hogy mikrofossziliák tótörténeti értelmezése alapján bepillanthassunk a tavi élet múltjába. Több furat szubfosszilis anyagának egybevetése vethet fényt ebben a kérdésben, s ha majd a tótükörről leszakadt hajdani tavi területek üledékeinek biogén maradványairól is fogalmat alkothatunk. Balatoni üledékek nem-pollen mikrofossziliáinak eddigi vizsgálata csak kezdeti tájékoztatónak tekinthető.

E tanulmányban ismertetendő balatoni kladocera maradványok legtöbbje a Balatonboglár—Révfülöp szelvény közepéről vett (B 28) furatból, néhány az Akali I furatból ered (Sebestyén, 1965: 189, 1968: 203, 1969). Kladocerákra és *Pediastrum*okra elemeztem e furatból két p l e i s t o c é n (160, 140), hat h o l o c é n (100, 80, 60, 40, 20, 1) mintát és egyet e két geológiai kor határának megfelelő rétegekből. A korazonosítást Zólyomi Bálinttól vettem át (p. c. és in litt.), aki azt palynologiai alapon határozta meg. Zólyomi professzor volt szíves furatmintáit is rendelkezésemre bocsátani (v.ö. Sebestyén, 1969: 203, 1969: tábla). A B 28 furat helye körülbelül 25 km-nyi távolságban van a Tihany—Balatonfüred profiltól, ahol a hatvanas években üledékmintákat vettem.

A balatoni maradványok között eddigelé egyetlen teljes vagyis ép külső vázat nem találtam (kezelt minták, Sebestyén, 1968: 203), s a különböző testtáji részek legtöbbje töredék. Különösen a héjpár töredezik össze,

úgyannyira, hogy jórésze (15—31%) nem alkalmas faji identifikálásra. A legszebb és legkönnyebben felismerhető maradványokat j é g k o r s z a k i mintákban találtam. Általában az erősen kitinizált részek maradnak meg, Chydoridákon pl. a fejpajzs, különösen annak rostrális fele, az utópotroh karma, a ♂ első láb kampója, az utópotroh, főként annak dorzális szegélye s a mandibulák. Ez megfelel az irodalmi megállapításoknak is.

Az ún. alsóbbrendű állatok közül tavunk üledékei legnagyobbrészt a Chydoridae család maradványait őrizték meg. Nagyon kevés a pelagikus kladocera maradvány. A kvantitatív lemezeken kereken 3500 maradványt vettem számba.

Bosmina 91 db = 2,6%
Sida 16 db = 0,45%
Daphnia szűrőláb négy, ennek fele qualitativ anyagból.
a többi Chydoridae.

Utóbbi csoport maradványainak gyakorisága, fogyó sorrendben: **S** = héj, **H** = fejpajzs, **P** = utópotroh, **C** = utóbbi végkarma, **Md** = mandibula, ♂ = a ♂ első lábának kampója, **E** = egyéb. Utóbbi csoportba esnek pl. antenna ízek stb. Két mintában volt több **P** mint **H** (ó h o l o c é n 80, nagy különbség, ú j h o l o c é n 60, igen kevés különbség). Öt mintában több volt a **Md** mint a **C**, kettőben (40, 140) kevesebb, kettőben (20, 60 ú j h o l o c é n) nem volt karom. A 160. mintában *(pleisztocén)* az antennula és az antenna ízek száma *(Eurycercus)* került az **Md** elé.

A maradványok faji meghatározásában Frey alapvető tanulmányai (rajzok, fényképek, maradványok leírása) nyújtanak nagy segítséget (Frey, 1958, 1959, 1960 a, 1962 a). Egyes fajokra vonatkozó megjegyzéseket, rajzokat csaknem valamennyi, kladocerákkal foglalkozó tótörténeti munkában találunk E rajzokat összegyűjtve s kiegészítve balatoni anyagról készült eredeti vázlataimmal, könnyen kezelhető ún. típuslapokat állítottam össze valamennyi számbajöhető Chydorida-fajról. Nagy segítséget nyújtott az a készítménysorozat, melyet N. N. Smirnow (Borok) volt szíves felajánlani 1965-ben: 27 Chydorida-fajra vonatkozó 56 kanadabalzsamos készítmény. Ezúttal is legyen szabad hálás köszönetemet kifejeznem szívességéért.

Ez alkalommal *Sida crystallina*val, *Alonella* és *Chydorus* nemek 3-3-fajával, *Oxyurella*, *Graptoleberis* és *Monospilus* nem egy-egy képviselőjével foglalkozom. A fajok között vannak olyanok, melyeknek récens balatoni előfordulásáról keveset vagy semmit sem tudunk; két olyanra is utalnom kell, melyekkel 1965. dolgozatomban már részletesen foglalkoztam. Ezek maradványairól ugyanis igen kevés adatot találtam a felhasznált paleolimnológiai irodalomban.

A Chydoridae család többi, a tó jelenéből és múltjából feljegyzett tagjait e sorozat további részeiben dolgozom fel. Akkor emlékezem meg *Bosmina*-maradványokról is. Így tavunk Cladocera faunájának alakulásáról talán kapunk legalább egy vázlatos képet.

I a Minden fajon ismertetem a récens előfordulás adatait, melyeket a negyvenes évek derekán a Balatoni Faunakatalóguson való munkálataim során vettem számba, kiegészítve újabb irodalmi adatokkal (Kottász, 1933, Entz—Kottász—Sebestyén, 1937, Meschkat, 1934, Ponyi, 1956, 1957, 1962, 1963, 1965, 1966, Sebestyén, 1947, 1948, 1949/50, 1957, 1959, 1965).

Tekintetbe vettem régebbi és újabb feljegyzéseimet, rajztanulmányaimat és újabban gyűjtött, még csak tájékozódás-szinten átnézett mintáim adatait is.

I b Egybevetettem a közismert európai irodalomból az előfordulásra vonatkozó adatokat (LILLJEBORG, 1901, K. BERG, 1929, WAGLER, 1937, SCOURFIELD és HARDING, 1958, FLÖSSNER, 1964), s utalok a Duna-delta és az Alduna árterületén levő sekély tavak adataira is (NEGREA, 1964, 1966).

I c A kérdéses fajon a külsőváz részeinek felületi üledékekből nyert adatait említem

II d—h A balatoni üledékekből feltárt szubfosszilis maradványok minőségi és mennyiségi adatainak értelmezése céljából áttanulmányoztam az újabb szakirodalomból azokat a dolgozatokat, melyek gerincét kladocera-maradványok tótörténeti értelmezése teszi. Ezt azért tartom szükségesnek, hogy felvilágosítást nyerjünk arról, hogy a kérdéses faj maradványai a különböző tavak üledékeiben mely rétegekből (kor) és milyen mennyiségben fordultak elő az idők folyamán, és minek lehet tulajdonítani az abundanciában beállott változásokat. Nagykiterjedésű tavunk üledékeiből ui. ezideig egyetlen furat kilenc mintája van — mennyiségileg is — kladocera-maradványokra elemezve.

Az irodalomban számos példa van arra, hogy a rétegeknek megfelelő adatsorozatból felállított „spektrumok" a populáció abundanciájának változásáról adnak képet. A változásból a pollenzónának megfelelő vagy azon belüli klímaváltozásra lehet következtetni, a fajok ekológiájának és az együtt tesek faji összetételének alapján (pl. GOULDEN, 1964: 40). Van példa arra, hogy a pollenzónák alapján megállapított klímaövek egybeesnek a Chydorida-együttesek összetételének változásával (MEGARD, 1967; DECOSTA, 1968). Egy trópusi tavon, ahol klímaváltozás kb. 4000 éve nem volt, a fajok abundanciájának és faji összetételének változása palynológiai eredmények közvetítésével az emberi behatás változásaira enged következtetni (COWGILL and HUTCHINSON, 1966: 126; GOULDEN, 1966 a: 118). Azt is láttuk, hogy a maradványok gyakorisága (egyedszám/cc) a tavak relatív termelékenységét látszik megvilágítani (FREY, 1960 a: 698; GOULDEN, 1966 b: 397).

A tavak morfometriájának mind nagyobb jelentőséget tulajdonít a limnológia. Ezért táblázatban közlöm az említendő tavak fontosabb adatait. *(1. táblázat.)*

II d = irodalmi adatok általában, maradványok ismertetése
II e = maradványok európai tavakból
II f = egy japán tóból
II g = hegyi tavakból
II h = közép-amerikai tavakból
II i = a Balatonból

E szempontok (**Ia—c**, **IId—i**) sorrendjében tárgyalom az egyes fajokat:

Sida crystallina O. F. MÜLLER 1785

I a DADAY (1897) a tó különböző részein hínárosban találta, legtömegesebben a parttól néhány m-nyi távolságban. Bekerül planktonmintába is pl HANKÓ gyűjtéseiben (PONYI, 1965). *Potamogeton*-állományban néha tömegével van (ENTZ B., 1947: „Almost exclusively on *Potamogeton*"; SEBESTYÉN több

feljegyzés). KOTTÁSZ planktonmintáiba is ilyen hely és nádas közelében került (KOTTÁSZ, 1933, E—K—S 48, 118 o. 12 B táblázat). Nádasokban is előfordul (MESCHKAT, 1934: 488; PONYI, 1962).

I b Üvegszerűen átlátszó szervezet, növényi mikroorganizmusokból álló táplálékát szűrve szerzi. Élettelen alzatra is rögzül (FLÖSSNER, 1964). Hínárral aquariumba kerülve csakhamar az üvegfalra tapad (tihanyi megfigyelés; WESENBERG—LUND, 1939: 469). Széltében elterjedt, tisztavizet kedvelő nyári forma. Lakustrin típusú vagy partközeli növényállományok lakója.

I c Maradványai előkerültek felszíni üledékmintákból (FREY, 1960a; MUELLER, 1964).

II d Általános rész: ZEMP, 1941: 57, 8 ábra; FREY, 1960a: 1—2 ábra, 1962b: 1138; GOULDEN, 1964, 10—11; MUELLER, 1964: 11—12 ábra.

II e Längsee, FREY, 1955, **C**
Wallensen, Alleröd, FREY, 1964: 44
Schleinsee, FREY, 1961
Herning, FREY, 1962 (l. GOULDEN, 1964; 14, 1, 3 táblázat)
Esthwaite water, GOULDEN, 1964, **C**, **P** és antennaizek. Első megjelenés az Allerödben, kevés. Úgy látszik, hogy a környezeti körülmények változását a maradványok mennyiségi változása nem indikálja.

II f Lake Nojiri, TSUKADA, 1967. A táblázatból a tó jégkorszaki és jégkorszak utáni fázisában sporadikus és alacsony abundanciával való jelenléte olvasható ki.

II g, h Hegyi tavakból és középamerikai tavakból nincs említve, ezek főként csak Chydoridákkal foglalkoznak.

II i A Balatonból (B 28) 3 jégkorszaki, 3 holocén (a felületi mintát is beleértve) és a jégkorszak és holocén határának megfelelő időből került elő összesen 21 maradvány: egyetlen antenna-íz kivételével, jól megőrzött, könnyen felismerhető postabdominális karom, — *1 a b ábra, 1. kép.*

Oxyurella tenuicaudis SARS 1862

E fajnak az *Alona* genusból való kiemelését a fejpajzs pórusrendszerének konfigurációja is igazolja (FREY, 1960a: 685, 695, 10—11 ábra. Lásd még 1958: 111 ábra).

I a Balatoni récens előfordulásáról egyetlen adatunk van MESCHKATtól, aki a balatonfüredi nádas zavarosvizű, kb. 40 m széles sávjától a partig terjedő „tiszta" (klar, clear) vizében találta zöldalgák és járulékos nádgyökérzet szövevényében, mint az ilyen területeket jellemző cönózis tagját (MESCHKAT, 1934: 489, 490, 491). DADAY a Magyar Faunakatalógusban (a továbbiakban MFK) nem sorolván fel konkrét lelőhelyeket, annyit mond e fajról *(Alona tenuicaudis* SARS), hogy az irodalom adatai szerint, úgy látszik, mindenütt gyakran előfordul.

I b Széltében elterjedt ritka faj (LILLJEBORG, K. BERG, WAGLER, SCOURFIELD és HARDING), hol nagyvizekben, hol kisvizekben gyakori. A víz detritusztartalma (FLÖSSNER), növényzet jelenléte is jellemzi lakóhelyét (K. BERG, FLÖSSNER, NEGREA). Sztenotopiára utaló balatoni előfordulás és az a körülmény, hogy az Angol-tóvidék tavaiból jelenléte nincs említve (SCOURFIELD és HARDING) ekológiai igényére vet némi fényt.

I c Felületi üledékekben talált maradványai alapján ki van mutatva Indiana két tavából (MUELLER, 1964: 25). — A Madison tavak üledékében talált fejpajzsok inkább az *Oxyurella longicauda* SARS külső vázához tartozhatnak (FREY, 1960a: 682, 695, 13—16 ábra).

II d Szubfosszilis előfordulásáról (**P S**) FREY informál először: Wallensen, Jégkorszak vége (Alleröd, fiatalabb tundrakor. (FREY, 1958: 243—244, 3—4 táblázat, 15—17, 111. ábra). A Schleinsee üledékében (VII—VIII—IX pollenzóna) kevés maradványa (**P S**) szórványos (FREY, 1961).

II g A Zeribar tó üledékében megállapított ugyancsak szórványos előfordulásból azt a következtetést vonja le a szerző, hogy három más fajhoz hasonlóan, a különböző korokban rövid életű populációkkal jellemezhető (MEGARD, 1967: 186).

II h A Laguna de Petenxil üledékében egyetlen utópotrohot talált GOULDEN. Egy másik hasonló lelet inkább az *O. longicaudata*nak tulajdonítható (GOULDEN, 1966a: 93, 11 ábra).

II i A Balaton üledékeiből is csupán **P**-t és **C**-t lehetett eddig feljegyezni a B 28 furat 80 sz. mintájából (ó h o l o c é n kevert tölgyeserdő fázis. Az éghajlat kontinentális jellege csökken. Atlanticus. (ZÓLYOMI, p. c.) és a 120. mintából (Határszint a p l e i s t o c e n és h o l o c e n között. Fiatalabb tundrakor és koraposztglaciális fenyő-nyír fázis (ZÓLYOMI, p. c.), egy-egy **P**. A 160 sz. mintából (p l e i s z t o c é n vége, késő glaciális idősebb tundrakor Ia veg. fázis [ZÓLYOMI, p. c.]) két **P** került elő. Az előzetes tájékozódásra átnézett Akali I furat két ú j h o l o c é n szintjéből (135, 173 cm) egy **P** és egy **C** van feljegyezve, mérettel (SEBESTYÉN, 1965 IB táblázat). — *2a, b ábra, 2. kép.*

Graptoleberis testudinaria FISCHER 1848

I a DADAY (1897) szerint RICHARD gyűjtötte először Keszthelyen, több lelőhelyet említ, ahonnan ezóta ismerjük: Badacsony, Tihany, Füred, Vörösberény, hínáros partokon, nem ritka.

A negyvenes években: Tihany, Kisöböl, 1948, július, fárnából (SEBESTYÉN, 1957: 172, 1959: 383). A Tihany előtti nyíltvíz iszapos növényzetnélküli tófenékről, sem a Kisöböl különböző ekológiai jellegű területeiről gyűjtött mintákba nem került (SEBESTYÉN, 1947, 1948).

A hatvanas évek felújított gyűjtései: Tihany, Kisöböl, 1963. VII. 16, neuston (SEBESTYÉN, 1965: 3. és IB táblázat); detritus, Tihany, Kisöböl, 1964. IX. 13.; Kerekedi-öböl közepe, fenékiszap, vízmélység 290 cm, 1964, IX. 18., sötét kékesszürke szapropel fölött keskeny világosdrapp iszapréteg. Legtöbb: *Pleuroxus uncinatus balatonicus*, chironomida lárvák, atkák, *Leptodora*. Kevesebb: (nem gyakorisági sorrendben) *Sida, Macrothrix laticornis, Acroperus harpae, Leydigia avcanthocercoides, Monospilus dispar*, Cyclopidák, *Corophium, Limnomysis benedeni*, egyetlen *Graptoleberis* példány.

A Sió *Myriophyllum*-állományából PONYI (1956: 105, 1957: 546) jegyezte fel.

I b Széltében elterjedt, közönséges nagy és kisvizekben, tőzeggödrökben is. Tavak parti övében benthikus. Kifejezetten fenéklakó. Kellő módon gyűjtve Dániában nagy mennyiségben gyűjthető (K. BERG, 1929). Kiterjedt makrofita-állományból sohasem hiányzik, leggyakoribb sűrű állományokban, faji válogatás nélkül, nem acidofil, nem jellemző oligotrof vizekre (FLÖSSNER, 1964).

Az Angol-tóvidék számos tavában él (SCOURFIELD és HARDING). Az Alpokban magasra hatol (LILLJEBORG).

I c A Madison tavak mindenik egységének és Indiana állam három tavának felületi üledékében ismertek maradványai (FREY, 1960a ill. MUELLER, 1964). Utóbbi vizekben a leggyakoribb tíz faj között van.

Longitudinális elterjedés: A Mississippi völgy északi szakaszában 10,4%-os gyakoriságban is előforduló *Graptoleberis* a szerző az északi fajok csoportjába helyezi. A Lake Chapman-ben (Ind.), ahol gyakorisága a legnagyobb értékű, a kladocerafauna összetétele: eurytop 32%, északi 67%, déli 1%.

II d Szubfosszilis maradványai: **S H C P.** *Zemp*, 1941: 64, 12 ábra **S**; FREY, 1958: 3—4 tábl, 252—253, 28—32 ábra, 1962b: 1140, 18, 36 ábra. GOULDEN 1964: 22. l. még DECOSTA 1967: 413.

II e Längsee FREY, 1955 **H S**: III. táblázat
Wallensen, FREY, 1958: 3. tábl. Legtöbb **S**. jégkorszak vége: Alleröd II, fiatalabb tundrakor III.

Schleinsee, FREY, 1961. VII—VIII—IX—X pollenzóna. Legtöbb maradvány a IX. végéből.

Herning, FREY 1962b. Az Eemi interglaciális legkorábbi rétegei kivételével, valamennyi mintában. **H S**

Esthwaite Water, GOULDEN, 1964. A legkorábbi rétegekben néhány maradvány. A VI-tól kezdődően, a Post Atlantic klímajavulással, a VII-en át, egyike a leggyakoribb Chydoridáknak.

II f Nojiri tó, TSUKADA. 1967. Táblázat alapján: Pleistocén végén és az ezutáni korokból származó mintákban megvan. A második hamueső után abundanciája emelkedik, noha fluktuál. Maximum kb. 15%. A kora jégkorszak utáni postglaciális rétegekben is elért közel 11%-ot.

II g Dead Man Lake. MEGARD (1964) csak egy furatból említi: 3900 évnél is valamivel fiatalabb rétegekből, kevés számban, folyamatos. Ma is tagja a tavi biotának.

Lake Zeribar (MEGARD, 1967): A tó feltárt történetében folyamatosan előfordult (C^{14} 22 600 ± 500 év B.P) 5 Chydorida között van.

Whimpy lake (DECOSTA, 1968): Jelenben növényzet között él, régebbi jelenlétéből a megfelelő kor növényzetben való gazdagságára lehet következtetni. Fosszilisan a Post Glacialis kezdetén jelenik meg a IIa Cydoridae zóna egyik „minor" elemeként, sporadikusan. A kiszáradás után (III zóna) előfordulása rendszeresebb.

II h A két közép-amerikai tóban a *Graptoleberis testudinaria* v. *occidentalis* van jelen. Ez synonim lehet a DADAY által Német Kelet Afrikából leírt v. *orientalis*-szal (GOULDEN, 1966a: 96)

II i Az analizált balatoni üledék-mintákból mindössze 8 maradvány van feljegyezve (4 **P**, 4 **S**), jóllehet a faj maradványai igen jellegzetesek. Felületi (1. sz.) és egy újholocén (40 sz.) mintából egy-egy **P**, az 100 sz.-ból (postglaciális mogyorófázis. Jelentkeznek a melegigényű lombosfák, V.) 1 héj. A két pleistocén mintából 3 **S** és egy **P**? van feljegyezve; két héj, minőségi készítményben. — *3—6 ábra, 3. kép*

Alonella rostrata KOCH 1841

I a Ezt a tavunkban közönséges fenéklakó Chydoridát a MFK említi a Balatonból (*Alona rostrata* KOCH) (DADAY, 1918, l. még 1897). Planktonmintába is bekerül (KOTTÁSZ, 1933, E.K.S. 1937: IB táblázat, SEBESTYÉN, 1964). Megtalálható neustonban és detrituszturzásokban (SEBESTYÉN, 1947, 1949/50, 1957, 1959, 1965). A Tihany—Balatonfüred szelvény mintáiban közönséges, Keszthelyről is ismert (hatvanas évek részben nem közölt adatai). Tavunk nyíltvízi és parti üledékében helyenként és az évszaknak megfelelően tömegesen él a *Monospilus*hoz hasonlóan, mely fajjal életmódja, életpályája több közös vonást tüntet fel. Ekológiai valenciája azonban szélesebb, bizonyos fokú szennyezettséget is eltűr (SEBESTYÉN, 1965: 207).

I b Széles körben elterjedt, holarktikus iliofil forma (NEGREA), homokon is megél, bár arra nem jellemző. Stagnáló sekélyvizű területeket néha egymagában népesít be (SCOURFIELD és HARDING). FLÖSSNER több tóra kiterjedt tervszerű vizsgálatai során növényzet között ritkán találta. Hogy alkalikus vizeket kedvelne (WAGLER), egyértelmű lehet azzal a megállapítással, hogy az Angol tóvidék számos tava közül csak egyből van jelentve (SCOURFIELD and HARDING). Melegkedvelésére récens és szubfosszilis adatok utalhatnak.

I c A külsőváz maradványai ismertek felületi üledékekből: Madison tavak két egységéből (FREY, 1960a), a három É-indianai tóból (MUELLER, 1964) és a Mississippi völgy különböző szélességében fekvő tavaiból, szórványosan (DECOSTA, 1964).

II d Szubfosszilis előfordulásáról kevés adat van. (Zemp, 1941: 64)
A fejpajzs leírását és rajzát FREY közli: 1959; 38, 1962a; 47—48 ábra, 1962b: 1141, 23, 46 ábra.

II e A Schleinsee postglaciális rétegeiből (VI—X) kevés maradvány került elő (FREY, 1961: 2. ábra).

Herning, FREY (1962b): 1141 **S H**. Eemi interglaciális és posteemi minták.

II f Lake Nojiri. TSUKADA táblázatából: A jégkorszak végi és Postglaciális mintákban végig vannak maradványok. A populációváltozás menete emlékeztet a *Monospilus*éra. Legnépesebb a tó történetének a két hamuesőközti szakaszában. A második hamueső kissé megtörte a változás irányának menetét. A tó felmelegedésével abundanciája emelkedik.

II g h Magashegyi tavakból és Közép-Amerikából nincs említés.

II i Balatoni üledékekből **S H** és néhány **P** van feljegyezve. Jégkorszakvégi mintákban előfordulása jelentéktelen és bizonytalan. Általában kevés a maradvány egészen a 80 **sz.** mintáig (208 cm, ± 4000 év B.P. Atlanticus, melegigényű lombosfák uralkodnak, ZÓLYOMI). *Pediastrum:* Cladocera arány, valamint a *Pediastrum simplex* clathrált varietásainak bőséges előfordulása magas vizet jelöl. (SEBESTYÉN, 1969). A minta arra utal, hogy a fenékfauna igen változatos lehetett e korban. Hasonlóan a *Monospilus*hoz, e maradványok a fenéküledék felületi rétegében (1. sz. minta) a leggyakoribbak. Meg kell azonban jegyezni, hogy a felületi és a következő minta (20 sz.) között mintegy 2500 év lehet (ZÓLYOMI). — 39 **H**, 32 **S**, 6 **P** — *7 a b ábra, 4—5 fénykép.*

Alonella nana BAIRD 1850

I a MFK és általában a magyar szakirodalom nem tesz említést e faj balatoni előfordulásáról *(Pleuroxus nanus* BAIRD), mely arról is nevezetes, hogy a legkisebb kladocera és egyben talán a legkisebb Arthropoda (SCOURFIELD—HARDING, MEUCHE). Nem közölt adataim között sem szerepel.

I b Gyakori nagyvizek (nagy tavak, folyók lassú folyású szakaszai) és kisvizek (pond) üledékeiben, növényzet között is. Tőzeggödrökben. Planktonmintába bekerül. Monocyclicus, egyes helyeken áttelel. A Duna-delta hét tava közül háromból ismert. Holarktikus, paleoarktikus jelleggel. (NEGREA; 1964, 1966)

I c A Madison tavak legkisebbikéből egyetlen **S** (FREY, 1960a). Indiana állam mindhárom tavában (MUELLER, 1964). A Mississippi völgy legészakibb szakaszának 12 adatából 8 volt pozitív. Délebbre eső tavakból nincs feljegyzés (DECOSTA, 1964: 75)

II d Eddigi szubfosszilis előfordulását és a maradványok leírását FREY ismerteti. 1958: 218, 263—255, 75—79 ábra. l. még FREY, 1962b: 1141
GOULDEN, 1964: 26—27.
A maradványokat (**S H**) kis méret, az **S** és **H** alakja és skulptúrája jellemzi (FREY, 1958: 254).

I e A Längsee (FREY, 1955: 153) üledékében (világosszürke mészgyttja) 23,8 m mély rétegben, a meromixis állapotot megelőző korban jelenlétét biztosan meg lehetett állapítani.

A Wallensenben (FREY, 1958: 254) jelenléte az Upper Allerödre (II veg. fázis) szorítkozik.

A Schleinsee (FREY, 1961) megvizsgált rétegeiben (VIII—X veg. fázis) kevés maradvány jelenléte folyamatos. A megelőző VII-ből egy adat.

Herning az Eemi-interglaciális és Posteemian csaknem valamennyi mintájában jelen vannak maradványai, az öt *Alonella* faj közül legnagyobb gyakorisággal (FREY, 1962b).

Esthwaite Water (GOULDEN, 1964). II—X. veg. fázis, leggyakoribb az Allerödben, a Postglaciálisban gyakorisága csökkenő, de végig megmaradt a közönséges fajok egyikének. **S H**

II g A Whimpy tó (DECOSTA, 1968). Üledékeiben megállapított I. Chydorida zónában és a II. zóna alján néhány más fajjal együtt jellemző csekély (minor) elem egy amerikai kis *Alona* és a *Chydorus sphaericus* nagy abundanciája mellett, ±300 cm mélység. Jégkorszak vége.

II i Balatoni üledékekből **S H P** került elő. A két pleistocén mintából 22 ill. 30 db, az óholocénből egy (100 sz. minta), az újholocénból (40 sz. minta) két db. A fejpajzs minden esetben hosszában két oldalról beköndörödött, h = 140—172 μ, 5 példány, 155,6 μ középérték, megközelíti a Wallensen anyag adatait. Az **S** és **H** jellemző bordái között hosszanti finom párhuzamos vonalak vannak. (10c. ábra). DECOSTA (1964: 80) valódi északi fajnak tartja, szubfosszilis leletek, a balatoni is, erre utal. Érdemes e faj után kutatni tavunkban, mert más északi kladocera fajok is élnek tavunkban. Algaszövedékben is kereshetjük, ahol MEUCHE (1939: 446) igen gyakorinak találta, s a Balaton lasion-kladocerafaunája még nincs felkutatva (VARGA, 1941: 296) — *10 a b c ábra, 6—7 kép.*

Alonella excisa FISCHER 1854

I a A balatoni récens előfordulásról kevés adatunk van. Első: a harmincas évek elején a tihanyi első mennyiségi planktonvizsgálatok során nyári hónapokban került merített planktonmintába (KOTTÁSZ, 1933, E—K—S, 1937: 12B táblázat). Fel van jegyezve a Remetebarlangok táján (Tihanyi-félsziget) húzódó nádasok szomszédságából is (KOTTÁSZ). A negyvenes évek derekán SEBESTYÉN jelentette a tihanyi Kisöbölből és a Gödrös nádasából (május—szeptember). Szeptember közepén efippiumos és petés ♀-ek (SEBESTYÉN, 1948, nem közölt rajzok). A Kisöbölből a hatvanas évek neustonmintáiban is megtaláltam (1965, IB táblázat).

I b Általában tavak szélén és kisvizekben, mocsarakban növényzet között, detritus tartalmú homokos vagy iszapos területeken honos. Planktonból FLÖSSNER említi. WAGLER acidofilnek tartja. A Duna-delta tavai közül négyből (NEGREA, 1964, 1966 2. tábl.), az Angol tóvidék sok tavából van említve (SCOURFIELD és HARDING).

I c Maradványok felületi üledékmintákból: Madison tavak (FREY, 1960a).
Indiana három tava (MUELLER, 1964)
Dead Man Lake, ritka (MEGARD, 1964 1. táblázat)
Ilyen vizsgálatok alapján DECOSTA az északi fajok csoportjába sorolja (DECOSTA, 1964: 81, 83).

II d Szubfosszilis előfordulás: ZEMP, 1941; 65, 9 ábra.
FREY, 1958: 3—4 tábl., 1959: **H** leírása, 1962a: 42. ábra, 1962 b: 1141, **S** és **H** leírása, 25, 35 ábra.
GOULDEN, 1964: 27

II e Längsee (FREY, 1955): **S H**
Schleinsee (FREY, 1961): Postglaciális: VII—X, a IX végén a legnagyobb gyakorisággal.
Herning (FREY, 1962b)- Late glacialis, Eemi interglacialis, és Postglacialis kezdeti rétegeiben **S H.**
Esthwaite Water, GOULDEN, 1964: II—VI—VIIb veg. f. Az Atlanticusban és Postatlanticusban a leggyakrabban előforduló fajok között. Paleolimnológiai adatokból úgy látszik, hogy eltűr hidegebb klímát, de nincs mindig jelen vagy kevéssé van képviselve ilyen klímának megfelelő üledékrétegekben. (GOULDEN, 1964. 27).

II g Whimpy lake, DECOSTA, 1968: 419. Csak a jégkorszak végén, **S H**, kevés. II zóna második felében gyakoribb.

II h Laguna de Petenxil (GOULDEN, 1966a, lásd 104 o.)

II i Balaton: újholocén mintában (Akali 1. furat, 135 cm) **S** (SEBESTYÉN, 1965, IB táblázat). A B 28 furat mintái közül csupán a két jégkorszaki mintában volt 15 maradvány, **S H**. A héjon és a fejpajzson jól kivehető a jellemző finom struktúra (FREY, 1962b: 1144, 1962a: 42 ábra, 1959: 38). A fejpajzson friss készítményben (polyvinil lactophenol-lignin pink) a finom vonalkázottság mellett egy az *Alona exigua* fejpajzsára emlékeztető durvább retikuláltság is feltűnt (FREY, 1962a: 41 ábra), később csak a finom vonalkázottság volt kivehető. Hiánya a B 28 postglaciális mintáiban gyér populációra utalhat, mely jelenség ma is jellemzőnek látszik a Balatonra. — *8 a b, 9 ábra, 8 a b, 9 kép*

Chydorus globosus BAIRD 1850

I a DADAY a Kis-Balaton saját fajai között sorolja fel, a tó közepéről, növényzetből (1897). Az első balatoni adat *Potamogeton*-állomány közelében merített planktonmintából való. (KOTTÁSZ, 1933, E—K—S. 1937: 48. 118. 12B tábl.) Ismerjük nádasok *Scirpeto-Phragmitetum-fontinalosum* cenozisából (PONYI, 1962: 137), partközeli nádas és hínáros vizéből (Keszthely, 1964, IX. 16), valamint a tihanyi Kisöbölből, 1965. VII. 31. Az utóbbi példányok héján megvan a sötétbarna folt (SEBESTYÉN, nem közölt). A keszthelyi nádas szélén vett minta Cladocera-együttesének tagjai: *Eurycercus lamellatus, Camptocercus rectirostris, Pleuroxus uncinatus, Chydorus sphaericus, Ch. globosus* (nem gyakorisági rend). A Kisöböl sekély vizében gyűjtött mintában: *Alonella rostrata, Monospilus, Alona* sp, *Pleuroxus uncinatus, Macrothrix laticornis, Chydorus globosus* (fogyó sorrend).

I b Széltében elterjedt, főleg növényzet között *(Myriophyllum*, FLÖSSNER), szabad vízben. Sáros fenéken néha meglehetősen nagy számban (K. BERG). Közönséges (WAGLER). Meglehetősen ritka (SCOURFIELD és HARDING). Tavak parti övében, nagyobb „pond"-okban. A Duna-delta hét tava közül háromban. Hegyi tavakban, halastavakban is. (NEGREA, 1964 ill. 1966).

I c Madison tavakban, a Monona kivételével (FREY, 1960a).
Indiana: mindhárom tóban, kevés (MUELLER, 1964).
A Mississippi völgy északi részén csaknem mindenik tóban, az északibb fekvésűekben sporadikusan. Legnagyobb gyakoriságban az Otter pond-ban, pH = 6,5. A szerző az eurytop fajok csoportjába helyezi. (DECOSTA, 1964, 4, 6 tábl.)

II d Szubfosszilis előfordulás általában:
(ZEMP. 1941: 66 **S**
FREY, 1958: 263—266, 3—4 táblázat, 105—107 ábra; 1962b: 1142, 25—56 ábra. (GOULDEN, 1964 : 29).

II e Wallensen, FREY, 1958: Fiatalabb tundrakor. Egyetlen példány, **S P C**, méret.
Herning, FREY, 1962b: Eemi interglaciális, 11 maradvány.
Esthwaite Water, GOULDEN, 1964: Első megjelenése az Atlanticusban és Postatlanticusban, más Chydorida fajokkal együtt, a klíma meliorációjára közvetlenül utal (p. 43). Szórványos. **S H**

II g Dead Man Lake, MEGARD, 1964: 532, 537: Jégkorszaki üledék felső rétegében (C^{14} 3.900 $\pm$ 515 év) változatos Chydoridae fauna tagja. Vázrészei a mai üledékekben is megvannak.

II i A Balaton üledékeiben biztosan csak a 80. sz. mintában lehetett kimutatni: két **P** ♂ (e minta jellemzését l. az *Alona rostrata* részben).

A Balatonból felsorolt kevés adat: e feltűnő küllemű ritka faj előfordulása tavunk jelenében, valamint maradványainak száma beleillik abba a rövid jellemzésbe, melyet már paleolimnológiai megállapítások alapján lehetett felvázolni: „E faj populációja soha sem volt nagy (The species was never abundant), de ez tipikus a *Ch. globosus*ra. Az egész holarktikus területen széltében elterjedt" (GOULDEN, 1964: 29). — *11. ábra. 12. kép.*

Chydorus sphaericus O. F. MÜLLER 1785

I a DADAY (1897): A Balatonban hínáros partokon, homokon kevésbé. Tömegesen a Kis-Balatonban. Nyáron néhány példány merített plankton-mintában, nádas szomszédságában és homokos hínáros partok közelében is (KOTTÁSZ, 1933, E—K—S, 1937: 48, 119, 12B táblázat). Nádasok „tisztavizű" területein, ahol a nádra zöldmoszatok rögzülnek, gyakori (MESCHKAT, 1934: 490). Nyíltvízfelőli zónákból is le van írva (PONYI, 1962: 137). További adatok: Gödrös nádasának partközeli része 1945. XI. 6., tihanyi Kisöböl, csak növények között, a móló védett *Myriophyllum*-állományában, sok, 1945. XI. 6. Utóbbi helyen télen üledékben 1964. I. 10. (SEBESTYÉN 1958, 1959: 382 és nem közölt adatok).

I b Egész Európában közönséges, a legkülönbözőbb jellegű vizekben, parton, fenéken, planktonban. SCOURFIELD és HARDING nem is említ lelőhelyeket. A vizsgált tavakban ubikvista (FLÖSSNER). A Duna-delta három tavában (NEGREA, 1964).

I c A Madison tavakban rendkívül gyakori planktonban, maradványai felületi üledékekben (FREY, 1960a: 690, 1960b: 920).
Indiana mindhárom tavában a leggyakoribb Chydorida (MUELLER, 1964).
A Mississippi völgy 45 tavának mindenikében igen nagy gyakoriságban (DECOSTA, 1964: 74—75).

II d Szubfosszilis maradványok, általában: ZEMP, 1941: 66, S.
FREY, 1958: 260—263, 3—4 táblázat, 97—104 ábra; 1960a: 690—691, 693, 698, 26—27 ábra; 1962b: 1141—42, 27, 29, 45 ábra; 1962a: 63—64 ábra.
GOULDEN, 1964: 29—30.

II e Längsee (FREY, 1955 **S**)
Wallensen (FREY, 1958): Alsó és felső Alleröd: **S H comb.**
Schleinsee (FREY, 1961): 2. ábra 1. táblázat. A VII pollenzóna végétől csaknem valamennyi mintában, nem nagyon gyakori.
Esthwaite Water (GOULDEN, 1964): A tó korai fejlődése idején az egyidejűleg előforduló Chydoridákét felülmúló abundanciáját a hidegebb klímának lehet tulajdonítani. A hidegebb klímájú III. három Chydoridája közül a leggyakoribb (p. 42). Csökkenése a borealtól kékalgák hiányára utalhat. (p. 30).

II f Lake Nojiri (TSUKADA, 1967: 123 és tábl.) E tóban 12 000—8500 B. P. északi fajok domináltak, 8500—6000 B. P. között a déli fajok dominanciája emelkedik az északiak visszahúzódásával párhuzamosan. Ez a tó felmelegedésére utal. A Postglacialis II. végére eső hamuesők megzavarták némileg a Chydorida-fauna alakulását. — *Chydorus*

sphaericus megvan a Late Glacialis 2 mintájában, alacsony % (egyedszám per cc nedves üledék). A két hamueső közötti időszakban alacsony %-ban, a III.-ban fokozatosan majd erősebben növekedve, elér 15%-ot.

II g Dead Man Lake (MEGARD, 1964): A Late Pleistocene-üledék öt Chydorida zónájában — a IV kivételével — jelen van. Az I-ben „with overwhelming abundance against *Leydigia leydigi*" (csak e két faj van jelen). II-ben nagy *Alonák*kal (e zóna tetejének kora C^{14} 28 000 év), III-ban és V-ben trópusi kis *Alona*kkal. — A Post Pleistocén rétegben, a korábbi rétegekkel szemben, ahol csak 2—4 faj szerepelt, 12 faj maradványai találhatók. Ezek ma is élnek a tóban, s *Chydorus sphaericus* a legszembeötlőbb.

Lake Zeribar (MEGARD, 1967). A furatban végig megvan. A zónában kis *Alona*k dominanciáját követően a *Ch. sphaericus* dominál, B-ben már déli fajok lépnek fel, melyek a C-ban átveszik az uralmat.

Whimpy Lake (DECOSTA, 1968). E faj azon Chydoridák között van, melyek a különböző zónákban mindig domináltak. I zónában, jégkorszaki szigorú tavi környezetben északi és eurytop formák, II zónában a két domináló *Chydorus* fajok egyike, később (II b) mindkét *Chydorus* faj populációja valamennyire csökken. A tó kiszáradását követően ismét dominálnak.

II i Balaton. Tavunk üledékéből 25 maradvány került elő: **S** 22, **H** 2, **P** 1. Ennek 4/5 része két jégkorszakvégi mintából. — *12—16 ábra, 10. kép.*

A paleolimnológiai vizsgálatok megállapításai ráterelik a figyelmet erre a széltében elterjedt, a legkülönbözőbb vizekben élő gyakori Chydoridára *Ch. sphaericus* eurytop és ubikvista. Számszerű adatok mutatnak hidegtűrésére. E tulajdonságnak jelentősége van oly területeken, ahol a klíma limitálja a fajok számát (DECOSTA, 1968: 412). Hideg toleranciájára utal balatoni vonatkozásban a két jégkorszaki mintában való gyakoriság a néhány holocén mintáéval szemben, valamint az is, hogy üledékmintából korunkban télen is fel van jegyezve. Hogy a legtöbb Chydoridától eltérően nem „substratfaj", megmagyarázza bőségét egyes vizek planktonjában (FREY, 1960b: 690, 698).

A Mendota tó nyári planktonjában kékmoszatokkal egyidejű elszaporodása arra utal, hogy valami összefüggés lehet a rák és a moszat között (FREY, 1960b: 920). Ez viszont alapot nyújt arra, hogy maradványainak bőséges jelenlétéből a környezet kékmoszatokban való gazdagságára lehessen következtetni (GOULDEN, 1964: 30, DECOSTA, 1968: 419). Hogy gyökerező alámerült növényzet jelenlétét is jelezhetik a maradványok, nem zárja ki azt, hogy a populáció részben planktonikus lehetett (DECOSTA, 1968: 420).

Figyelmet érdemel gyakorisága a balatoni nádasok „tisztavizű" területein, zavarosvizű területekkel szemben, s az a körülmény, hogy a lasionban szabadon mozgó, lebegő életmódot folytathat, planktonikus (MESCHKAT, 1934: 490). A lasion a *Chydorus sphaericus* helyváltoztató mozgásának megfelelő környezet MEUCHE megítélésében is (1939: 448, II. táblázat), aki az algaszövedék állatvilágában a leggyakoribb kladocerának találta (1939: 447). Az Alduna-árterület sekély tavainak planktonjában minden mélységben él, különösen ahol dús az algavegetáció. Ez az alapja a gyökerező vízinövények és fonalasmoszatok között való tenyészésének (NEGREA, 1966: 149). A tihanyi móló közelében levő *Myriophyllum*-„bokrokat" egészen átszövik fonalasalgák, és itt nagy népességben él. A fonalasalgákkal való asszociációjának eszerint kettős jelentősége lehet: a szövedék alkalmas e pelágikus Chydorida tartózkodására és — közvetve — táplálékellátására is.

Hogy DADAY kiemeli a Kis-Balatonban való gyakoriságát, s hogy a Balatonban korunkban nádasok belsejében „tisztavizű" környezetben gya-

kori, arra utal, hogy zavaros vizű nyugtalan környezet nem felel meg ekológiai igényének. Ez is oly momentum, mely egyfelől a *Ch. sphaericus* ekológiai valenciájának elemzéséhez nyújthat adatot, részben pedig tavunk sajátos limnológiai képének bizonyos vonását világítja meg.

Chydorus piger SARS 1896

I a DADAY nem említ balatoni előfordulást. Egyetlen közlés récens előfordulásáról: Keszthely, Myriophyllum-állomány (PONYI, 1956: 110, 1957: 115).

I b Ritka (WAGLER) és széltében elterjedt (SCOURFIELD és HARDING), csak tavakban, partközelben, sporadikusan. Benthikus, specifikusan üledéklakó, hasonlóan a *Monospilus*hoz (LILLJEBORG, FLÖSSNER). Homokos talajon, onnan behatol algabevonatba, sűrű makrovegetációs területekre (FLÖSSNER). Az Angol-tóvidék sok tavából fel van jegyezve.

I c Indiana 3 tava (MUELLER, 1964): egyetlen maradvány Lake Vinonából, ahol a legtöbb mintát vett a szerző. Elevent nem talált.

II d A felhasznált irodalomban említett szubfosszilis leletek mind Európából származnak. FREY: 1958: 3—4 táblázat, 1959: 40; 1962a: 61—62 ábra; 1962b: 1142, 30, 53 ábra; 1964: 44.

GOULDEN, 1964: 30—31.

II e Längsee, FREY, 1955 **P**
Schleinsee, FREY, 1961: kevés, szórványos
Herning, FREY, 1962: **S H** Eemi interglaciális végén. 1. táblázat, 3. ábra.
Esthwaite water, GOULDEN 1964: **S H** A Late Borealben lesz gyakori, s ekkor a leggazdagabb Chydorida, jól fejlett populációja jellemző.

II i Balatoni üledékekből: jégkorszak végéről mindkét mintában 2 ill. 4 **H**, pleistocén és holocén határán egy **H**, három holocén mintában: 80., 60., 1., Utóbbiban 3 **S** és 2 **H**, a héjak közül kettő qualitativ mintából. Érdemes tovább keresni tavunkban.

A fentiekből kitűnik — mint említettük —, hogy a *Ch. piger* maradványok mind európai tavakból valók, ahol — GOULDEN megállapításában — hideg stenothermikus forma. FREY szerint Észak-Amerika savanyú lápvizeiben sok (abundant) (GOULDEN, 1964: 31). Dystrof, lágy és savanyú vizekben közönséges (FREY, 1962: 1145). Evvel a közléssel összhangban van az, hogy az Angol-tóvidék sok tavából van jelentve. Az említett hideg stenothermiából GOULDEN arra következtet, hogy a klíma akkor (Late Boreal) hűvösebb lehetett, mint ma az Angol-tóvidéken, ahol az Esthwaite water fekszik, viszont melegebb volt mint a megelőző korszakokban (GOULDEN, 1964: 42). — *17—18 ábra, 11. kép.*

Tavunkból nagyon kevés adat van mind récens, mind subfosszilis előfordulására, a paleolimnológiai irodalomban is kevés az adat. A balatoni adatokból azt lehetne következtetni, hogy tavunk jelenében s múltjában is populációja nem volt ill. nem lehetett nagy. Remélhetőleg gondos kereséssel lehetséges lesz valódi habitatját tavunkban körvonalazni.

Monospilus dispar SARS 1861

I a MFK *(M. tenuirostris* SARS) a Balatonon kívül még csak két lelőhelyet említ az országból. DADAY tavunk parti területein inkább homokon mint hínáron találta, planktonba is bejut (1897: Siófok, Keszthely). Nagyon

gyakori (DADAY, 1888: 86). Merített planktonmintából KOTTÁSZ (1933, E—K—S, 1937, 12B táblázat), szűrt vízoszlop mintákból SEBESTYÉN (1964: 2—3 táblázat) említi. Hínárosok krustáceáinak vizsgálata során a tihanyi Kút *Myriophyllum*-állományából, a Keszthelyi-öböl üledékéből s a tó egész területén vett üledékminták legtöbbjéből fel van jegyezve (PONYI, 1956, 1963 ill. 1966). Magam a negyvenes években a Kisöbölben, Gödrösben, Aszófői-öbölben fenéküledékben, neustonban és detritusz-túrásokban találtam (SEBESTYÉN, 1947, 1949/50: 59, 1957: 172, 1959: 241, 1965). A hatvanas évek üledékmintáinak csaknem mindenikében jelen van. Gyakorisága az egyes mintákban, a többi kladocerákhoz viszonyítva, általában magas, néha *Alonella rostrata*val együtt a leggyakoribb elem (SEBESTYÉN, 1965: 210).

I b Iszaplakó, inkább nagyvizekben, halastavakban, „pond"-okban is, iszapos, homokos fenéken él (NEGREA, 1966: 150). Svédországban lehatol 10—12 fonalnyi mélységbe (1 fonal = 1.829 m). FLÖSSNER megfogalmazása: szórványos, egyes helyeken tömegesen, a balatoni populációra is illik.

I c FREY (1960b) a Madison tavak egyikéből említi vázrészeit. A Mississippi völgy északi szakaszának tavai közül csak kettőből van említve (DECOSTA, 1964).

II d Szubfosszilis adat kevés van. (FREY, 1958: 4. táblázat; 1962b: 1142, 28, 47, 49 ábra; 41; 1962a: 50—51 ábra).

II e Schleinsee (FREY, 1961: 1. táblázat). A furat felső részében igen szórványosan, kevés.

Herning (FREY, 1962): Az Eemi interglaciális és Posteemi rétegekből feltárt maradványok (**H S**) spektrumot adnak. A *Chydorus sphaericus* után a legtöbb maradvány ehhez a fajhoz tartozik.

II f Lake Nojiri (TSUKADA, 1967). A táblázat szerint a furatban (Late glacial, Postglacial) végig megvan. A tó történetének melegvízi szakaszában abundanciája meghaladta a 60%-ot. A spektrum menete emlékeztet az *Alonella rostrata*éra.

II i Balatoni szubfosszilis anyagban leggyakoribb a héj meg a fejpajzs, néhány **P** is fel van jegyezve (maradványok leírását l. SEBESTYÉN, 1965: 211). Többsoros héjat kezeletlen mintában találtam.

A B 28 furatban, a két pleisztocén mintát kivéve, mindenikben megvan, feltűnő sok a felületi rétegekben. Ebben is emlékeztet *Alonella rostrata*ra. Az említett gyakoriság arra mutathat, hogy e fajok maradványai talán aránylag hamar lebomlanak, bár több a valószínűsége annak, hogy a tó újabb állapotában nőtt meg állománya. — *19—20 ábra.*

A balatoni Chydoridae fauna időbeli alakulásáról akkor kaphatnánk vázlatos képet, ha a különböző geológiai stb. koroknak megfelelő rétegekből (furatminták) feltárt összes maradványok adatait egybevetnők a szakirodalom megfelelő megállapításaival. Az itt tárgyalt tíz Cladocera faj adatait táblázatba rendezve mégis úgy tűnik fel, hogy bizonyos megállapítások máris rögzíthetők. (2 tábl.)

1. Ma a tó leggyakoribb Chydoridái közé tartozó *Alonella rostrata* és *Monospilus dispar* a jégkorszak végétől, kb. 10 000 év óta, mondhatnók folyamatosan, a tó biótájának tagja. E két faj spektruma vagyis gyakoriságának egymásutánja a különböző korokban hasonló (TSUKADA táblázata, Nojiri tó).

2. táblázat

B 28 furat kilenc mintájából feltárt és e dolgozatban
Frequencies of cladoceran remains of core B 28, Lake Balaton, discussed

Minta — Sample Mélység — Depth — cm Pollenzona — Pollenzone Faj — Species — Specimen	1 0—3 X		20 60 IX		40 123 VIII	
	db	%	db	%	db	%
Sida crystallina	2					
Oxyurella tenuicaudis						
Graptoleberis testudinaria	1	*0.13*			*	
Alonella rostrata	38	*5.96*	8	*2.93*	12	*2.69*
Alonella nana	?				2	*0.45*
Alonella excisa						
Chydorus globosus	?					
Chydorus sphaericus						
Chydorus piger	3	*0.47*				
Monospilus dispar	116	*18.18*	40	*14.65*	29	*6.50*
*Összes Cladocera (*Sida nélkül)	638		273		446	
**Maradvány per g száraz üledék (105 :C)	8 792		6 072		9 071	

* Adatok az összes Cladocerákra nemcsak e tíz fajra vonatkoznak és a meg nem határozható töredékeket is magukban foglalják.
Number of specimens of all cladoceran remains encountered per sample (*Sida* omitted) including those not being identified.

** Minőségi készítményekből.
Only in qualitative slides.

2. A ma ritkának ítélt fajok állománya a múltban sem lehetett népes: *Oxyurella tenuicaudis*, *Graptoleberis testudinaria*, *Alonella excisa*, *Chydorus piger*.

3. DECOSTA északi fajai közül a *Graptoleberis*, *Alonella nana* és *A. excisa* megvan a két pleisztocén végi mintában: idősebb tundrakor, 160 és 140. sz. minta, utóbbi a Balaton rétláp állapotát megelőző rétegből. (Szubarktikus klíma.)

4. *Graptoleberis* az Esthwaite water üledékeiben az Atlanticus (VI) klímajavulásával a Postatlanticuson át egyike a leggyakoribb Chydoridáknak (GOULDEN). A Zeribar magashegyi tóban azon öt faj között van, melyek a tó feltárt történetében (23 000 év) folyamatosan jelen vannak (MEGARD). E fajon a balatoni pleistocén előfordulást talán hidegtűréssel lehetne magyarázni, ahogy az megállapítható volt *Alonella excisa*ról, mely GOULDEN szerint eltűr hidegebb klímát, de nincs mindig jelen ilyen körülmények között. Az ubikvista és széltében elterjedt *Chydorus sphaericus* is, ez az eurytopnak minősített faj az Esthwaite tóban (GOULDEN) a leghidegebb pollenzónában (III) jelenlevő három Chydorida között a leggyakoribb volt.

5. A 80. sz. minta adatai e részletben is mutatják a kedvező klíma (atlanticus) hatását. E minta mikrofosszilia-anyaga nemcsak kladocera vonatkozásban, de általában is — mennyiségre és minőségre — kitűnt gazdagságával, változatosságával. A klíma meliorációjának hatása nyomot hagyott az *Alonella rostrata*- és *Monospilus dispar*-állomány alakulásán is. A *Chydorus globosus* maradványait ebből az egyetlen mintából sikerült fel-

tárgyalt kladoceramaradványok gyakorisága
in this paper. For explanation of headlines see English text p. 3.

60 170 VII		80 208 VI		100 250 V		120 290 III—IV		140 ; 330 Ib		160 370 Ia	
db	%	db	%	db	%	db	%	db	%	db	%
		5		4		*		5		1	
		2	*0.36*			1	*0.25*				
				1	*0.36*			2	*0.93*	2	*0.47*
5	*1.51*	15	*2.75*	1	*0.36*	*		1	*0.46*	?	
				1	*0.36*			22	*10.23*	30	*7.19*
								9	*4.18*	6	*1.43*
		2	*0.36*								
1	*0.30*	2	*0.36*	1	*0.36*	*		7	*3.25*	13	*3.11*
2	*0.60*	1	*0.18*			1	*0.25*	4	*1.86*	2	*0.47*
10	*3.03*	16	*2.94*	11	*3.97*	6	*1.50*				
329		544		277		391		215		417	
4 281		10 945		2 815		1 982		2 589		15 315	

tárni, mely faj populációja, GOULDEN tótörténeti értelmezése szerint, sohasem lehetett nagy.

6. A *Sida* szórványos adataiból legfentebb csak azt lehet kiolvasni, hogy tavunk őslakója. Tudjuk, hogy maradványai nem képeznek spektrumot.

Általában azt mondhatjuk, hogy a balatoni szubfosszilis adatok harmóniában látszanak lenni a jelen és a múlt — bár még hiányosan ismert — állapotával és — ugyancsak általában — beillszethetők a szakirodalom megfelelő megállapításaiba.

Összefoglalás

A dolgozat első tagja a folyamatban levő balatoni kladocera-tanulmánysorozat három részletben közlendő IV. fejezetének, melynek tárgya a tavi üledékekből feltárt kladocera-maradványok tótörténeti értelmezése, anyaga egy reprezentatív balatoni furat kilenc mintájában előforduló maradványok minőségi és mennyiségi adatai. Ez a részlet a *Sida*val és kilenc chydoridával foglalkozik.

Szerző megkísérli egybevetni a fent említett maradványok adatait, a mai ismeretek tekintetbevételével, különböző tavak tótörténeti kutatások során nyert megfelelő adataival (1. táblázat) és azok értelmezésével.

Noha a balatoni maradvány-adat aránylag kevés, azok táblázatba rendezésével *(2. táblázat)* mégis kiolvasni véljük a következőket:

1. A tó jelenében gyakorinak ítélhető fajok *(Alonella rostrata, Monospilus dispar)* a jégkorszak utáni idők óta lakják a tavat folyamatosan növekvő állományban.

2. „Ritka" fajok *(Oxyurella tenuicaudis, Graptoleberis testudinaria, Alonella excisa)* a múltban sem alkothattak nagy állományokat, éppen mint a tavunk jelenéből még föl nem jegyzett *Alonella nana* sem.

3. Északi fajok (DeCosta) közül *Graptoleberis testudinaria, Alonella nana, A. excisa* valamint a „ritka" (stenotop?) *Chydorus piger* a jégkorszak végén a tó biotájának tagjai voltak, valószínűen a mainál nagyobb populációval.

4. Balatoni adatok támogatni látszanak a szakirodalomnak azt a paleolimnológiai alapon nyert megállapítását, hogy a *Graptoleberis testudinaria* és az ubikvista *Chydorus sphaericus* hidegtoleranciája magas fokú.

5. Az atlanticus (80. sz. minta) kedvező klímájának kihatása balatoni anyagon is visszatükröződik.

6. Úgy látszik, hogy a balatoni mikrofosszilia -adatok beilleszthetők a szakirodalomban közölt megállapítások keretébe.

Bevezetőben szerző felveti az eprofundális jellegű tófenék kladocera-együttese eredetének problémáját.

A tó nagy kiterjedésének és hosszú múltjának megfelelően az aránylag kevés adatot feldolgozó tanulmány eredményei csak tájékoztató jellegűek lehetnek.

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CLADOCERA STUDIES IN LAKE BALATON IV.
SUBFOSSIL REMAINS IN THE SEDIMENTS OF LAKE BALATON I.

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Results of paleolimnological study on cladocerans at a preparatory level were published in 1965.

The paper presented here is the first part of a study on both recent and subfossil cladocerans known at present from the lake as well as remains of which have been recovered from the sediments (nine samples of core B 28, see SEBESTYÉN 1968: 203, 1969).

The aim of this study is to gain some insight into the history of the cladoceran fauna especially that of chydorids in this lake.

Both littoral and open water territories (zones) are extensive in Lake Balaton which is a shallow water-body of large extent and long shores *(Table 1)*. Nowadays length of the shore line measures but 180 Km. Formerly it was established as 225 Km (CHOLNOKY). On account of the regression of this scenescent lake open water territories assume here and there a littoral nature on a larger scale than before as a consequence of the present regulation of the waterstand (SEBESTYÉN, 1943).

The plankton of Lake Balaton is fairly rich in nonchydorid cladocerans

(Daphnia, Diaphanosoma, Leptodora). These leave nearly no remains in the sediments of this lake, with the exception of *Bosmina*, the population which is, however, rather small.

In the quantitive slides 3500 cladoceran remains have been tallied of which *Bosmina* 91 specimens = 2,6 per cent
Sida 16 = 0.45 per cent
filtercomb, *Daphnia* 2 (+ 2 from qualitative slides),
all the rest are of chydorids.

This paper deals with *Sida crystallina* and the following chydorids: *Oxyurella tenuicaudis, Graptoleberis testudinaria, Alonella rostrata, A. nana, A. excisa, Chydorus globosus, Ch. sphaericus, Ch. piger* and *Monospilus dispar*.

In order to get better acquainted with the non-planktonic cladoceran members of the present biota of the lake, sample series were taken in this decade between Balatonfüred and Tihany including open water sites where the water column exceeds 300 cm and at various habitats at both ends of this profile, omitting, however, dense growth of rooted aquatics and reed stands. This material, as yet, has not been fully analysed.

Nature of the bottom corresponding with the open water is that of the eprofundal (sensu LENZ = sublittoral, sensu WESENBERG-LUND), having no rooted vegetation. The cladoceran fauna of the bottom of the open water seems to be rather restricted in comparison with the various littoral habitats (SEBESTYÉN, 1943, 1947, 1948, 1965). Origin of the cladoceran community of the eprofundal benthos in Lake Balaton might be traced back to the limnological past of the lake in the Pleistocene, being perhaps—at least partly—a pauperated remain of a diverse littoral community of that age.

Table 2 includes numerical data on the subfossil remains of those cladocerans which are the objects of this part of the study. It should be kept in mind that core B 28 is a representative one among those numerous ones which have been studied palynologicaly (ZÓLYOMI, p.c.). Although the samples of this core represent layers corresponding to a wide series of pollen zones, the time interval of subsequent samples analysed may be estimated at several thousand years. Table 2 includes the actual numbers of remains of the ten species and the structure of the assemblies in per cent of the total cladoceran remains found in the quantitative slides. Bottom lines of this table include number of specimens of all cladoceran remains encountered per sample including those not being identified as well as the calculated values of the same per gram dry weight (105 °C) of sediment.

In the Hungarian text discussing the ten species the following items have been considered (see p. 9—33):

Recent situation **I a-c**

I a Present status of our knowledge on the occurrence in Lake Balaton based upon pertinent literature, unpublished data of the author being added.

I b General distribution of the species including some suggestions on their ecology based upon selected literature commonly used in Europe (LILLJEBORG, K. BERG, WAGLER, SCOURFIELD and HARDING, some works of both FLÖSSNER and NEGREA).

I c Occurrence of exoskeletal remains recovered from the surficial sediments in some North-American waters (FREY, 1960 a, b; DECOSTA, 1964; MEGARD, 1964; MUELLER, 1964).

Subfossil remains **II d-i**

Results of pioneer resp. basic investigations had been studied in general (ROSSOLIMO, 1924; ZEMP, 1941; DEEVEY, 1942).

II d Bibliographic data of description and figures of remains
II e Occurrence of remains in modern and extinct lakes in Europe
II f Lake Nojiri, Japan
II g Mountain lakes of high altitudes
II h Small tropical lakes, Central America
II i Lake Balaton

*

Various data concerning some lakes investigated lately (1955—1968) from a paleolimnological point of view are given in *Table I.* Headlines:

1 = name of lake
2 = geographic position
3 = morphometry
4 = type of lake
5—6 = core, geological age and pollenzone of layers where the samples originate from
7 = remarks
8 = author

These data my be informative for readers to whom the related literature is not available.

In these works (column 8, *Table 1* and References) the numerous data on subfossil remains from a series of subsequent layers could be arranged in spectra. This way the changes taking place in the cladoceran assemblies throughout the ages might be enlightened, and connections with the climatic changes as well as the effects of other external factors may be established or looked for. Subfossil data from Lake Balaton at hand would not permit to attempt such arrangement, because merely every 20th sample of a 410 cm long core was analysed as far. However from the tabular setting of the data as it is seen in *Table 2* some interpretations might be made especially when the present distribution of the species is known.

In the followings an attempt is made in order to interpret the Balaton data both recent and subfossil in reference with the findings on lakes in other lands.

1. Remains of species inhabiting at present Lake Balaton in large populations: *Alonella rostrata* and *Monospilus dispar*, are well represented in the Post Pleistocene samples. They very likely inhabit our lake continuously (?) for the last ± 10,000 years in increasing populations.

These iliophilic forms live, at least in certain parts of the lake, side by side, *Monospilus* being, in general, more frequent. (For the structure of cladoceran community at the end of July including both species see SEBESTYÉN, 1965: 210).

Ecological valence of *Alonella rostrata* seems to be wider: it does not mind stagnant water. At the end of summer when shallow lagoons may be formed

along the shores, *Alonella rostrata* inhabits them in dense population, being sometimes the only cladoceran in such environment (SEBESTYÉN, 1965: 207, 220, SCOURFIELD and HARDING).

Courses of the spectra of these forms run fairly in a similar way in the sediments of Lake Nojiri. Their populations increase along the warming up of this lake (TSUKADA, 1967). There are but few other occurrences recorded: both were recovered from the sediments of Schleinsee and that of the extinct lake at Herning, in the Eeemian interglacial and Post-Eemian, *Monospilus* being the most frequent here. Occurrence in Schleinsee is scarce. (FREY, 1961, 1962 b) — *Figures 7a, b*, 19—20, microphoto 3—4).

2. *Chydorus sphaericus* is very frequent in our lake too, it seems to be present in various habitats. Size of its population however does not seem to reach that of the two species mentioned above.

Extensive and thorough studies of MESCHKAT (1934) and MEUCHE (1939) suggest that *Chydorus sphaericus* occur in considerable number in the lasion of filamentous algae. In Lake Balaton it is frequent in the horizontal clear-water section of the littoral reed-growths (MESCHKAT, 1934: 490). It is the most frequent cladoceran in the lasion of several lakes in Germany (MEUCHE, 1939: 448, *Table 11)*. In the opinion of these authors the structure of the lasion provides suitable environment for this semipelagic chydorid. NEGREA (1966: 149) points out the significance of algae in the life-method of this species. The fairly dense occurrence in some protected *Myriophyllum*-growth in Lake Balaton suggests that it minds turbid water. Its frequent occurrence in Kis-Balaton in DADAY's time, a water-body the bottom of which is totally covered with submerse aquatics, suggests the same, as well as MESCHKAT's finding mentioned above.

There are many records on the occurrence of subfossil remains of *Chydorus sphaericus* in the various lakes with the exception of the tropical ones. It seems to have an important role in the past history of mountain lakes (MEGARD, 1964, 1967; DECOSTA, 1968). From its subfossil occurrence in the various climatic situations the conclusion is drawn that it is an eurytopic, most ubiquitous cold-tolerant form (GOULDEN, 1964: 42, 47; DECOSTA, 1968: 412). Its presence suggests very likely a simultaneous bloom of bluegreen algae (FREY, 1960a: 690, 1960b: 920; GOULDEN, 1964: 30).

The rather scarce occurrence of its remains in the Balaton sediments — with the exception of the two Late-Pleistocene — may suggest that its population had never benn abundant in our lake in the Post-Pleistocene. The fact that 4/5 parts of the recovered 25 remains (mostly shells) were found in Late-Pleistocene layers supports the cold-tolerance of this species. Later on, however, this lake as a whole could not offer favourable conditions of existence for this species. Perhaps the agitated and turbid water in most part of the lake could not offer suitable environment for this semipelagic form. — *Figures 12—16*, microphoto 10.

3. There are but very few records on the recent occurrences of the following chydorids in Lake Balaton: *Oxyurella tenuicaudis, Graptoleberis testudinaria, Alonella excisa, Chydorus globosus* and *Chydorus piger.*

Graptoleberis testudinaria is the only one known from the lake in DADAY's time. He mentioned it from five localities, it has been reported lately but from two of those. *Chydorus globosus* was known by DADAY only from Kis-Balaton. It is noted lately from four localities, all of them being stands of macrovegetation (see Hungarian text p. 241, including the structure of two communities

with this species). Both *Alonella excisa* and *Oxyurella tenuicaudis* are in DADAY's list (1904: 94), enumerating species not known at that time from Lake Balaton but only from neighbouring small ponds and bogs. At present we know *Alonella excisa* from five localities, there is but one record from *Oxyurella tenuicaudis* and *Chydorus piger*. *Alonella nana* is known, as yet, only in subfossil condition.

Graptoleberis testudinaria, *Alonella excisa* and *A. nana* are considered as norther forms based upon the results of an extensive investigation aiming at the longitudinal distribution of chydorid cladocerans (DECOSTA, 1964). K. BERG (1929: 86) calls attention to the importance of the mode of sampling for this strictly benthic form. Morphologies of both the rostrum and postabdomen may suggest a specific mode of life in comparison with other limicol chydorids (KURZ).

In Europe *Graptoleberis* enjoys a wide distribution. Paleolimnological investigations call attention to the significance of its Southern-European occurrences. This seems to be in harmony with the increase of its population along with the amelioration of the climate (Atlanticus, Post-Atlanticus) (Esthwaite water, GOULDEN, 1964: 22, *Tables 5—6)*. It can endure climatic changes in a larger scale as shown in the case of Lake Zeribar and Dead Man Lake (MEGARD, 1964, 1967).

This species seems to be substituted by v. *occidentalis* in tropical lakes *(Goulden*, 1966 a, b).

There are but seven remains recovered as far from Lake Balaton sediments, in quantitative slides. Four records in the Late-Pleistocene demonstrate, perhaps, the cold-tolerance of this species.

Subfossil occurrence indicates weedy environment (DECOSTA, 1968: 419-420). In both samples of the Late-Pleistocene, Lake Balaton such remains were well represented.

Remains of *Alonella nana* are very frequent in the Late-Pleistocene samples from Lake Balaton but there are only three records from the Post-Pleistocene period. On the subfossil shell fine parallel lines could be discerned between the longitudinal striae *(Fig. 10c)*. All the headshields were laterally folded (length = 140—172 μ, 5 specimens, mean 155.6 μ, being somewhat less then the Wallensen data (FREY, 1958: 254).

Alonella nana was found by MEUCHE as being the second frequent cladoceran in the lasion (1939: 446). In NEGREA's consideration it is a Holarctic species.

Remains of *Alonella nana* are reported from the sediments of all the European lakes considered in this paper. It is the most frequent *Alonella* in the Eemian interglacial at Herning (FREY, 1962b). In Whimpy lake at the end of the Pleistocene it formed a characteristic minor element against the very abundant *Chydorus sphaericus* and a tropical small *Alona* (DECOSTA, 1968: Chydorid zone I and at the bottom of zone II). It would be interesting to find out the significance of the minute size of this chydorid in a contemporary community.

Remains of *Alonella excisa* have been encountered only in the Late-Pleistocene samples in Lake Balaton. On the subfossil headshield beside the characteristic fine scretch marks depicted by FREY (1962a: Fig. 42) a coarse reticulation known from the headshield of *Alonella exigua* (FREY, 1962a: Fig. 41) could be discerned on fresh slide (polyvinil lactophenol-ligninpink), however this coarse pattern soon disappeared. — *Figures 6 a, b, c*, Microphoto:

8 a, b, 9). (My old notes and sketches on the Balaton specimens of this species would suggest study on its morphology).

In spite of the only one recent record of *Chydorus piger* in Lake Balaton there are a few records from various periods of the Post-Pleistocene layers. This latter find is may be explained by the distinct morphologies of the headshield and shell.

All subfossil records from other lakes are from Europe. It was the most frequent chydorid in the Late-Boreal in Esthwaite water (GOULDEN, 1964). In the opinion of this author, it is a cold stenothermous form in Europe. FREY's data would suggest acidophily (FREY, 1962b: 1145; GOULDEN, 1964: 31). Its presence in many lakes in the English Lake District (SCOURFIELD and HARDING) seems to be in harmony with FREY's data.

Oxyurella tenuicaudis is an other species of which there is as yet only one date concerning its occurrence in Lake Balaton. MESCHKAT found it in the clear-water section of the reed-growth off Balatonfüred among filamentuous greenalgae being epiphytic on reed stem, as well as at the same place among the adventive roots of the reed. This author considers it as a characteristic member of the community living in such microhabitat (MESCHKAT, 1934:489, 490, 491). This occurrence may suggest stenotopy of some kind. It is not mentioned from the English Lake District by SCOURFIELD and HORDING.

The first record of its subfossil remains is from Wallensen in the Late-Postglacial (ALLERÖD, younger DRYAS, FREY, 1958). It is reported from Schleinsee (FREY, 1961). From its sporadic occurrence in Lake Zeribar the conclusion was drawn by MEGARD (1967: 186), that this species with three other chydorids "... developed only short-lasting populations at various times". From the Laguna de Petenxil only one postabdomen has been mentioned (GOULDEN, 1966 a).

But a few remains (**P C**) were found in the sediments of Lake Balaton. *Figures 2 a, b, Microphoto 2.*

There are but few records on the recent occurrence of *Chydorus globosus* in Lake Balaton, all from weedy littoral habitats. The Balaton population has the brown spots on the shell. Two male postabdomens have been recovered (sample 80, Atlanticus).

Subfossil remains of this species have been found, sporadically, in three European and one American lakes. Its first appearance together with some other chydorids suggests the amelioration of the climate (Atlanticus, Post-Atlanticus) (Esthwaite Water, GOULDEN, 1964; 43). In Dead Man Lake being member of the present biota there remains have been recovered with other chydorids from the upper layers of Pleistocene sediments (C^{14} 3,900 $\pm$ 515 years) (MEGARD, 1964: 532, 537). "This species was never abundant, but this is typical for *Chydorus globosus*" (GOULDEN, 1964: 29).

4. *Sida crystallina* is quite frequent in the littoral of Lake Balaton favorizing, seemingly, *Potamogeton perfoliatus* for substrate. Nearly every sample contains few postabdominal claws, partly fragments. These give evidences that *Sida* belongs to the biota of the lake since its formation.

5. Data of sample 80 in *Table 2* seem to suggest the favourable effects of the amelioration of the climate in the Atlanticus, in general. The microfossil assembly in this sample is the most various among all the samples analysed and rich quantitatively too.

Part 3 of this series deals with the several *Alona* species and, part two with all the other chydorids of Lake Balaton and *Bosmina*.

ИЗУЧЕНИЕ КЛАДОЦЕР ОЗЕРА БАЛАТОН. IV. СУПФОССИЛЬНЫЕ ОСТАТКИ В ОСАДКАХ БАЛАТОНА I.

О. Шебештьен

Данная статья является первой из серии, излагающей результаты исследования кладоцер осадков озера Балатона, в этой серии статей автор дает исторический подход к открытым в осадках озера остаткам ракообразных и одновременно анализирует количественные и качественные данные девяти вертикальных проб осадков. Данная статья приводит данные о *Sida* и Chydoridae.

Автор сопоставляет свои результаты относящиеся остаткам данного вида тепернешним нахождением этого же вида и теми данными которые были получены при изучении других озер (таблица № 1).

Несмотря на то, что известны относительно немногочисленные данные, касающихся остатки озера Балатон, все же на основе табл. 2. можно придти к следующим выводам.

1. Те виды, которые находятся в озере в настоящее время *(Alonella rostrata, Monospilus dispar)* обитают озеро с ледьяного эпоха и их число постепенно возрастает.

2. Редкие виды *(Oxyurella tenuicaudis, Grapto leberis testudinaria, Alonella excisa)* никогда не были представлены высокой численностью и то же самое характерно для *Alonella nana*, который в настоящее время не найден в озере.

3. Среди северных видов (DeCosta) *Graptoleberis testudinaria, Alonella nana, A. excisa* и редко встречающийся (стенотопный?) *Chydorus piger* участвовали в биотопе озера в конце льядного эпоха, по всей вероятности, их популяция превосходила тепернейший.

4. Полученные данные поддерживают те палеолимнологические результаты согласно которым холодно-выносливость *Graptoleberis testudinaria* и *Chydorus sphaericus* высокая и в озере Балатон.

5. Благополучное воздействие Атлантического океана (образец № 80) обнаруживается и на материале, собранном с Балатона.

6. Наши данные в отношении микрофоссилии хорошо согласуются с известными литературными данными.

Автор обсуждает вопрос о характере дна озера и о происхождении осадка ракообразных.

Так как размеры озера велики данные приведенные здесь носят только ориентировочный характер.

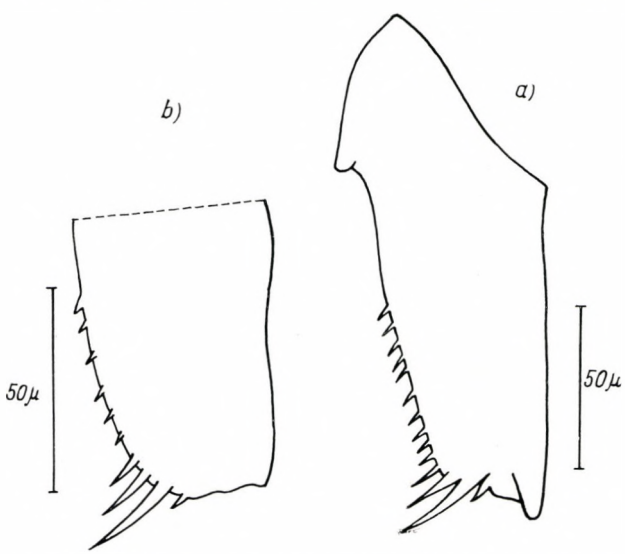
Fosszilis példányoknál adva a minta száma, r = récens példány Cam. luc. rajzok

sf = subfossil remains **r** = recent material

Explanation of Figures
(Camera lucida drawings)

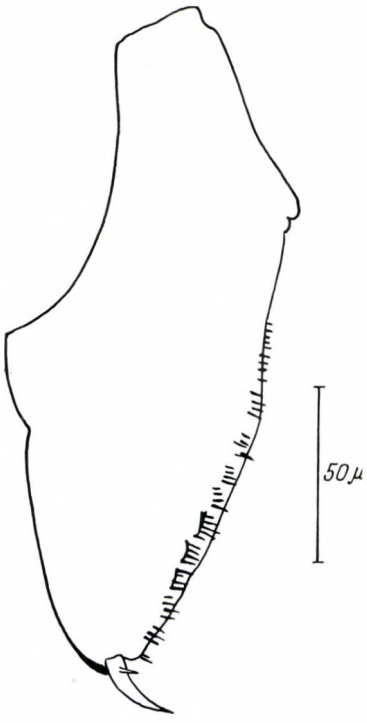
1a *Sida crystallina* karom h = 243 μ B28/140 No 1037
1b *Sida crystallina* karom-töredék h = 210 μ B28/80 No 501a

1a. *Sida crystallina* O. F. MÜLLER, claw l= 234 μ Sample 140. No 1037 **sf**
1b. *Sida crystallina* O. F. MÜLLER. Fragment of claw l= 210 μ Sample 80 No 501a **sf**



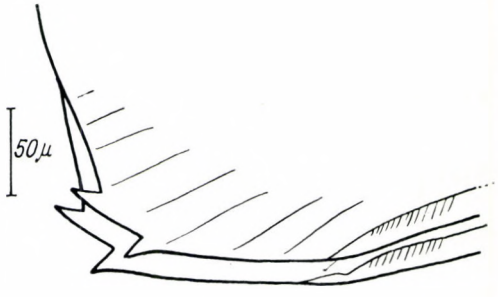
2a *Oxyurella tenuicaudis* utópotroh B28/120 No 909
2b *Oxyurella tenuicaudis* utópotroh-töredék B28/80 No 593

2a. *Oxyurella tenuicaudis* SARS Postabdomen. Sample 120 No 909 **sf**
2b. *Oxyurella tenuicaudis* SARS. Fragment of postabdomen. Sample 80. No 593 **sf**



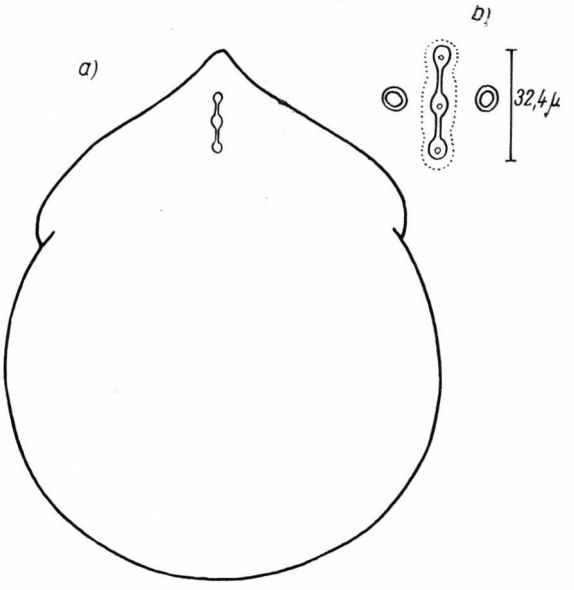
3 *Graptoleberis testudinaria* utópotroh Tihany 1964 XI/13 Pr 145 r

3. *Graptoleberis testudinaria* FISCHER. Postabdomen 13. XI. 1964. Tihany Slide 145



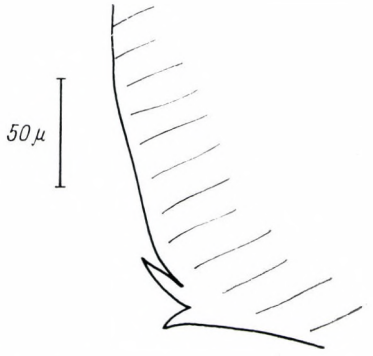
4 *Graptoleberis testudinaria* héjpár posterior-ventralis sarok B. Füred 1963 IX/18 Pr 103 r

4. *Graptoleberis testudinaria* FISCHER Posterior-ventral angle of shell 18. IX. 1963 Balafonfüred Slide 103 **r**



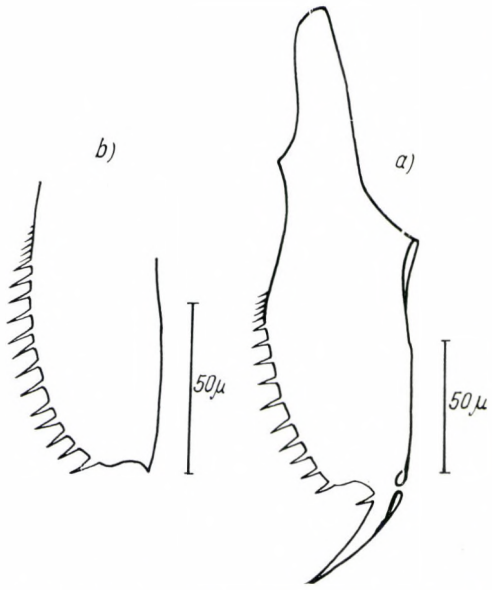
5a *G.t.* előbbi példány fejpajzsa (körvonal) h = 275 μ
5b u.a. pórusrendszer erősebb nagyításban

5a. Headshield of No 4. specimen (outline) l = 275 μ
5b. Configuration of pores of headshield of No 5a in larger magnification



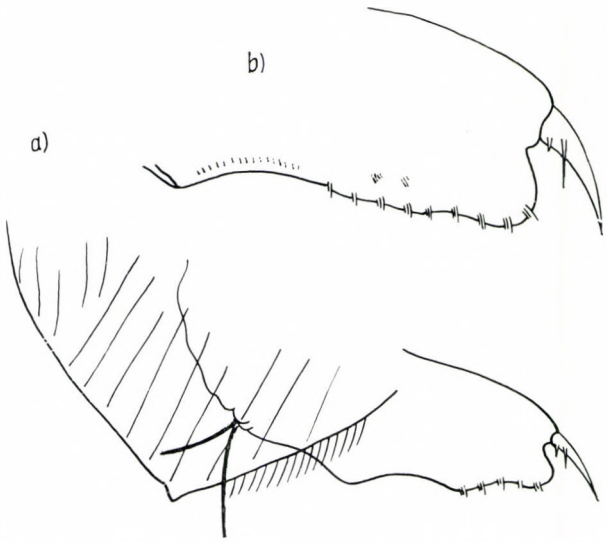
6 *G.t.* héj posterior-ventrális sarok B28/100 No 881a

6. *Graptoleberis testudinaria* FISCHER Posterior-vental angle of shell Sample 100 No 881a **sf**



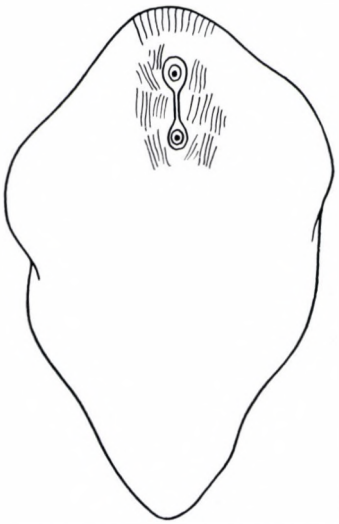
7a *Alonella rostrata* utópotroh, Tihany 1963 IX 24 Pr 105 r
7b *Alonella rostrata* utópotroh-töredék B28/40 No 329

7a. *Alonella rostrata* Koch Postabdomen 24. IX. 1963 Tihany Slide 105 r
7b. *Alonella rostrata*. Fragment of Postabdomen. Sample 40. No 329 **sf**



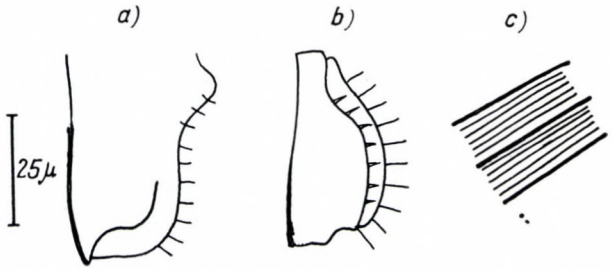
8a *Alonella excisa* héj posterior-ventralis sarok és utópotroh, Tihany Gödrös 1945 VII/11 (Teljes példány hossza 280 μ) r
8b ua. utópotroh erősebb nagyításban

8a. *Alonella excisa* Fischer. Posterior-ventral angle of shell and postandoben 11. VII. 1945. Tihany, intact specimen l = 280 μ **r**
8b. The same postabdomen in larger magnification



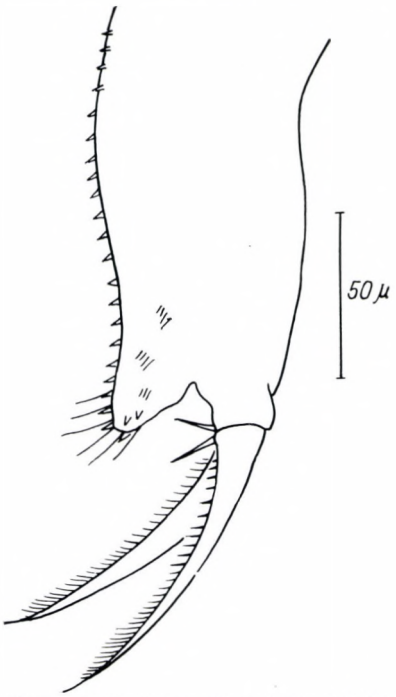
9 *Alonella excisa* fejpajzs h = 237 μ B28/160 No 1114 felületi mintázat csak részben ábrázolva (v.ö. 9. sz. képpel)

9. *Alonella excisa* FISCHER. Headshield l = 237 μ Sample 160 No 1114 **sf**



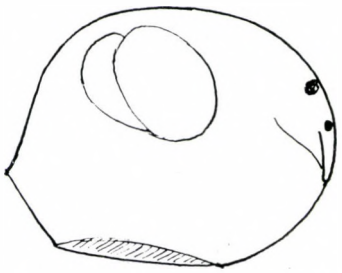
10a *Alonella nana* (?) utópotroh h=43 μ B28/40 No 407
10b *Alonella nana* (?) utópotroh h=50 μ B28/40 No 366
10c *Alonella nana* héjmintázat szabadkézi vázlat szubfoszszilis példányról

10a. *Alonella nana* BAIRD (?) Postabdomen l = 43 μ Sample 40 No 407 **sf**
10b. *Alonella nana* BAIRD (?) Postabdomen l = 50 μ Sample 40 No 366 **sf**
10c. *Alonella nana* BAIRD pattern of the surface of shell (free hand sketch) **sf**



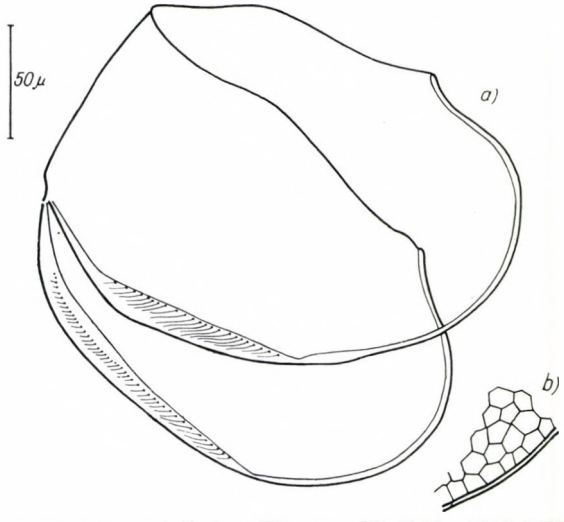
11 *Chydorus globosus* utópotroh, Keszthely 1966 V/5 Pr 227 r

11. *Chydorus globosus* BAIRD. Postabdomen 5.V.1966. Keszthely Slide 227 **r**



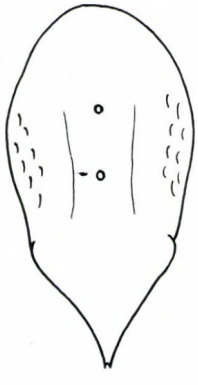
12 *Chydorus sphaericus* ♀ balatoni aquariumból (Potamogeton perf.) 1946 VII/16 r

12. *Chydorus sphaericus* O. F. MÜLLER ♀ aquarium specimen from Lake Balaton. 16.VII. 1946. **r**



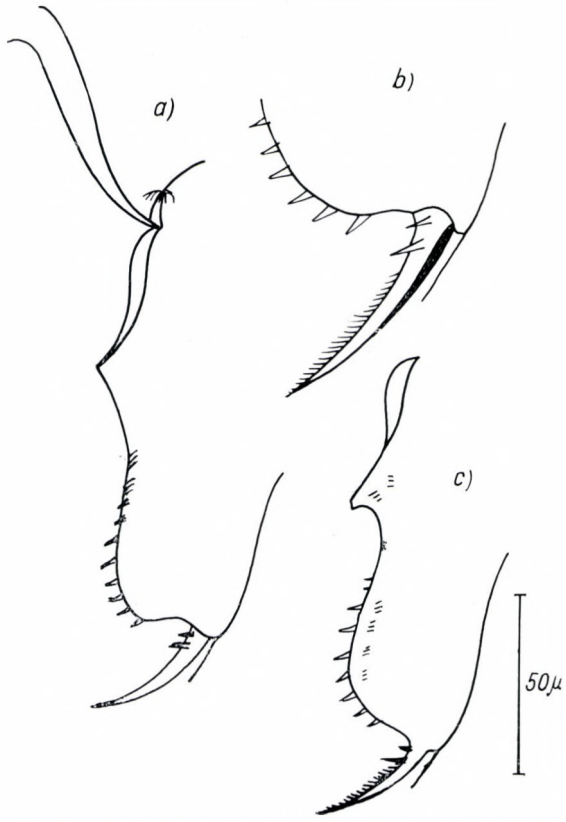
13a *Chydorus sphaericus* héjpár, Tihany Kisöböl 1965 VIII/3 Pr 225 r
13b ua elülső ventrális sarok részlete: hálózatos struktúra

13a. *Chydorus sphaericus* Shell 3.VIII.1965. Tihany Slide 225 **r**
13b. Reticulation of the inferior ventral angle of shell, Fig. 13a



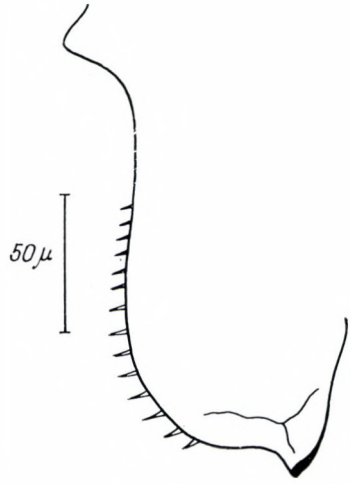
14 *Chydorus sphaericus* fejpajzs h = 415,4 μ Tihany 1963 VII/26 neuston Pr 5/3 r

14. *Chydorus sphaericus* Headshield 26.VII.1963. Tihany Neustonl 415,4 μ Slide 5/3 **r**



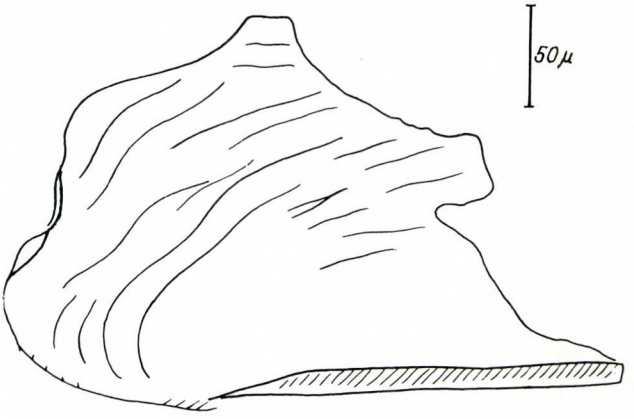
15a *Chydorus sphericus* ♀ utópotroh, Tihany 1946 IV/18 r
15b Előbbi distális vége, erősebb nagyítás
15c *Chydorus sphaericus* utópotroh, Gödrös 1946 V/28 r

15a. *Chydorus sphaericus* ♀ Postabdomen 18.IV. 1946 **r**
15b. Distal end of specimen *Fig. 15a.* in larger magnification
15c. *Chydorus sphaericus* Postabdomen 28.V.1946. Tihany, Gödrös, **r**



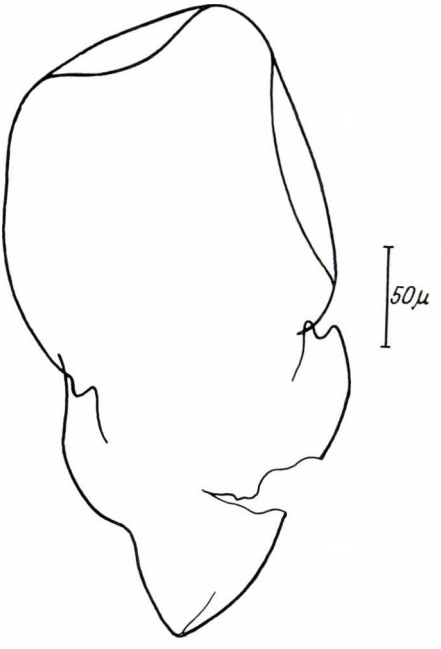
16 *Chydorus sphaericus* ? utópotroh B/28/80 No 448

16. *Chydorus sphericus* (?) Postabdomen Sample 80 No 448 **sf**



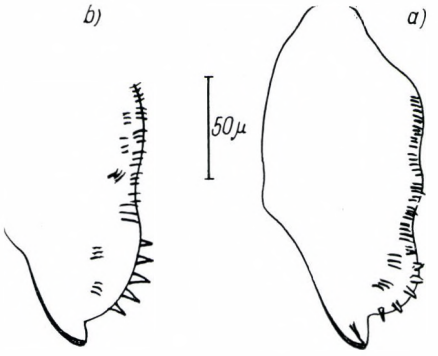
17 *Chydorus piger* (?) héj-töredék B28/1 No 595 a

17. *Chydorus piger* Sars (?) Fragment of shell Sample 1 No 595a **sf**



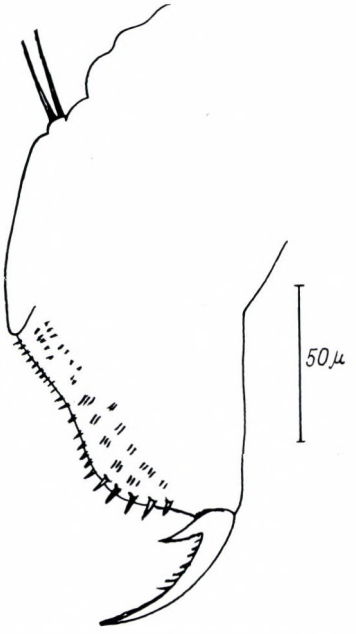
18 *Chydorus piger* fejpajzs körvonala B28/40 No 1045

18. *Chydorus piger* Headshield, outline Sample 40 No 1045 **sf**



19a *Monospilus dispar* ♀ utópotroh. 19b u.annak distális vége erősebb nagyításban B28/40 No 398

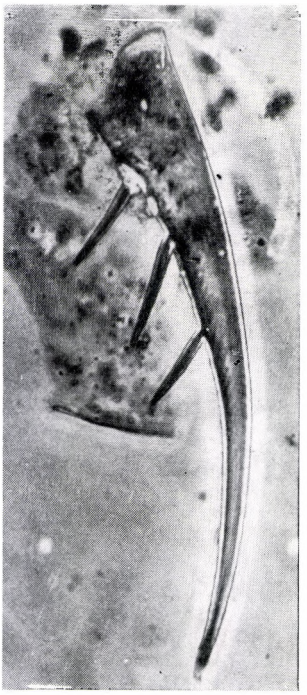
19a. *Monospilus dispar* ♀ Postabdomen Sample 40 No 398 **sf**
19b. Distal end of specimen *figured* in *19a* in larger magnification



20 *Monospilus dispar*, vedlő ♀ utópotroh, Tihany 1963 IX/24 Pr 105 r
e példány méretei μ-ban
S = 350×315 (három v. négysoros)
H = 205 4 rostrum hegye ötkarélyos
C = 55 basalis karom = 21,6
Md = 113,5

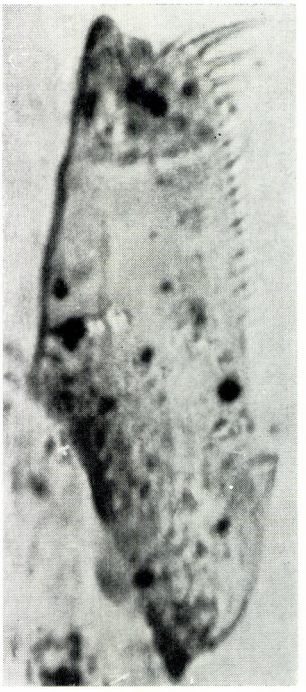
20. *Monospilus dispar* ♀ (in the act of molting). Postabdomen 24. IX. 1963. Slide 105 **r**
Measurements of this specimen: in μ S = 350×315
H = 205,4, C = 55, basal spine = 21,6, Md = 113,5

Mikrofelvételek
Explanation of microphotograms



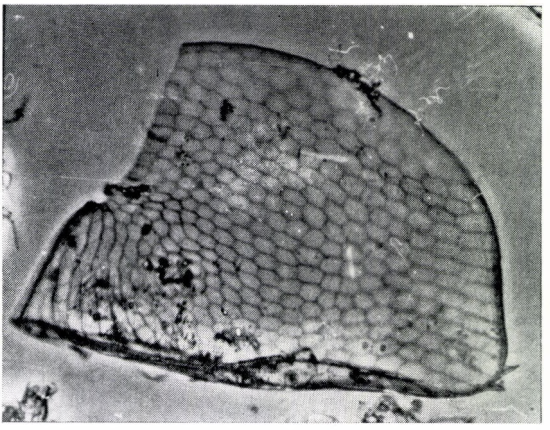
1. *kép: Sida crystallina*, karom 80. minta h = 210 μ No 501

1. *Sida crystallina* O. F. MÜLLER. Postabdomen l = 210 μ. Sample 80. No 501



2. *kép: Oxyurella tenuicaudis*, u. potr. 129. minta h = 143,7 μ No 909

2. *Oxyurella tenuicaudis* SARS. Postabdomen l = 143,7 μ. Sample 120. No 909

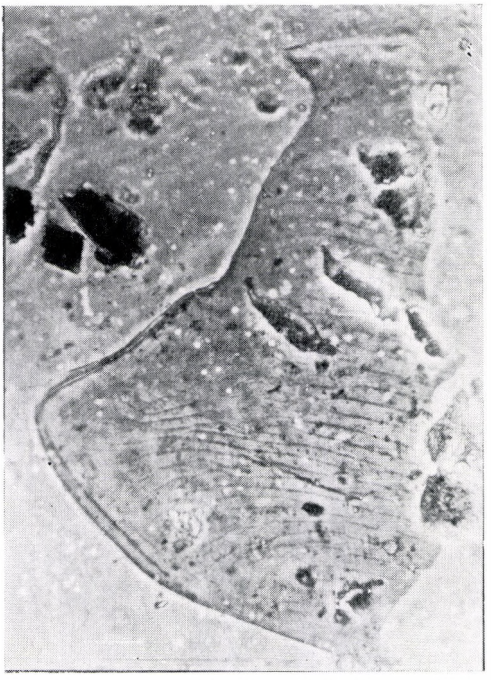


3. *kép: Graptoleberis testudinaria*, héj 160. minta h = 315 μ No 1167

3. *Graptoleberis testudinaria* FISCHER. Shell l = 315 μ. Sample 160 No 1167

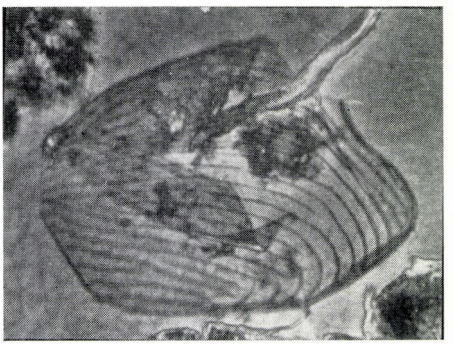
4. kép: Alonella rostrata fejpajzs 40. minta h = 156 μ

4. *Alonella rostrata* KOCH. Headshield l = 156 μ. Sample 40.



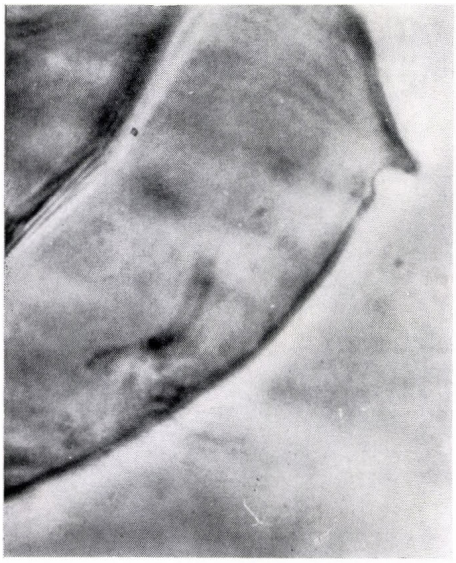
5. kép: Alonella rostrata, héj 80. minta h = 520 μ No 581

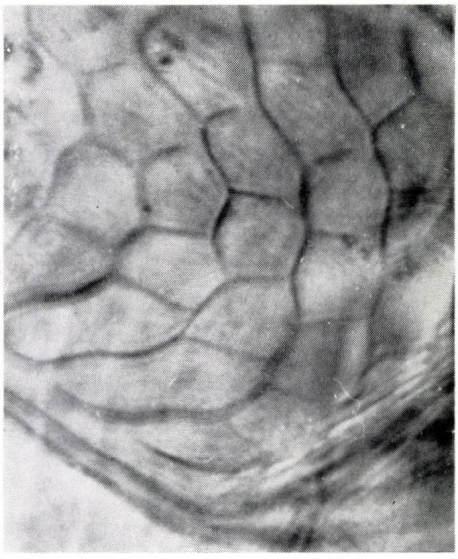
5. *Alonella rostrata* KOCH. Shell. l = 520 μ Sample 80. No 581



6. *kép: Alonella nana*, héjpár 140. minta h = 190 μ No 1146
6. *Alonella nana* BAIRD. Shell l = 190 μ. Sample 140. No 1146

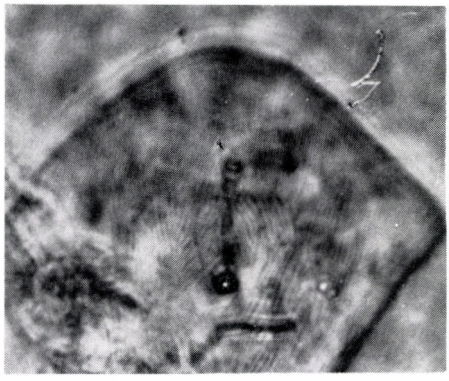
7. *kép: Alonella nana*, fejpajzs 140. minta h = 158 μ No 1144
7. *Alonella nana* BAIRD, Headshield l = 158 μ. Sample 140. No 1144





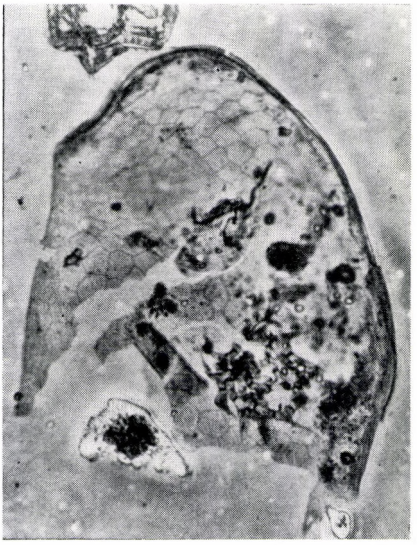
8a. kép: Alonella excisa, héj posteoventralis sarok 140. minta No 1006
8b. kép: Alonella excisa, előbbi héj részlete erősebb nagyítással, egész héj h = 232 μ

8a. *Alonella excisa* FISCHER. Posterior-ventral angle of shell. (whole length of shell = 230 μ) Sample 140. No 1006
8b. Detail of same shell showing structure in larger magnification



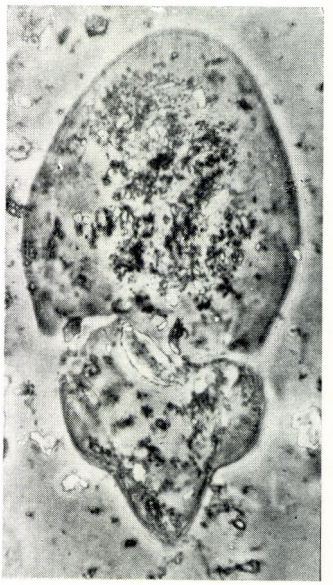
9. kép: Alonella excisa, fejpajzs részlete 160. minta h = 237 μ No 1114

9. *Alonella excisa* FISCHER. Detail of headshield (whole length = 237 μ.) Sample 160 No 1114



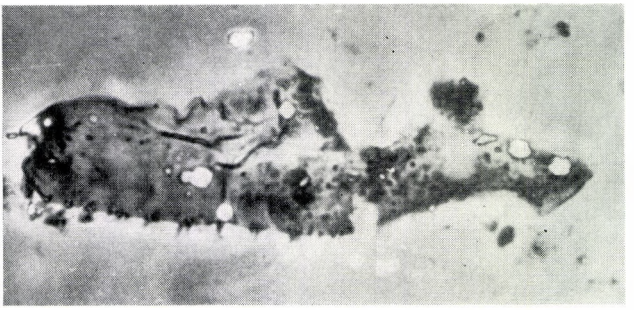
10. kép: Chydorus sphaericus? héj 80. minta sz = 233 μ No 471

10. *Chydorus sphaericus* O. F. MÜLLER (?) Shell, fragment. Width = 233 μ. Sample 80. No 471 (in some of the reticulae fine striae are shown, being similar to that of an *Alonella excisa)*



11. kép: Chydorus piger fej pajzs 120. minta h = 384 μ No 936

11. *Chydorus piger* SARS. Head shield l = 384 μ. Sample 120 No 936



12. kép: Chydorus globosus, autópotroh 80. minta h = 138 μ No 506
A mikrofelvételek quantitativ lemezek anyagán készültek.
polyvinil lactophenol — ligninpink: 1, 2, 4, 5, 7, 10, 12 kép,
gentianaviolet — glycerin gelatin: 8a 8b
picrinsav — glycerin: 9
ZS.-NAGY IMRE M. D. felv.

12. *Chydorus globosus* BAIRD. Postabdomen = 138 μ. Sample 80. No 506
All microphotos from quantitativ slides. 1, 2, 4, 5, 7, 10, 12 polyvinil lactophenollignin pink- 8 a b glycerine gelatine-gentianaviolet- 9 glycerine-picric acid
Photo I. ZS.-NAGY M. D.

ANNAL. BIOL. TIHANY	36	257–292	HUNGARIA 1969

HORIZONTAL PLANKTON INVESTIGATIONS IN LAKE BALATON VII. ON THE PHYTOPLANKTON OF LAKE BALATON, BASED ON SCOOPED SAMPLES AND FILTRATES TAKEN IN 1966

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Received: 21st February, 1969

The present paper is a sequence to those earlier publications containing data on the horizontal distribution of plankton organisms in Lake Balaton (SEBESTYÉN, 1960, 1964; TAMÁS, 1961, 1965, 1967; P.-ZÁNKAI and KERTÉSZ, 1967; PONYI, 1968).

The object of the investigations was twofold:

a) to obtain further data, practically in the same time (within 2 days), on the quantitative composition and variation of the phytoplankton of the entire lake in the warm season;

b) to gain further information on the lacustrine distribution of the phytoplankton, based on a series of samples taken from the transverse sections.

Data of sampling and methods of processing

The series of plankton samples have been collected once in a monthly (May-November, 1966) in three localities each in the three south-western and the two north-eastern transverse sections (M, K, G, A, E) of the lake. A map of the localities, and some other data on the collecting sites are given in *Table 1*, TAMÁS, 1967, p. 234–235.

The series of quantitative water samples have been taken by a Friedinger apparatus, the filtrates by nets No. 6 and No. 25. The quantitative composition

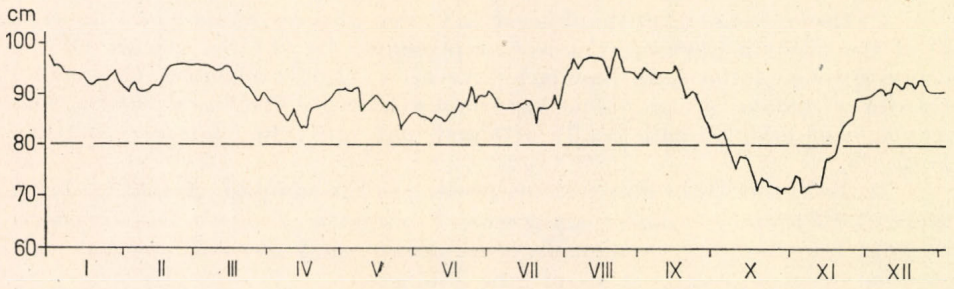

Fig. 1. The annual variation of the water-level of Lake Balaton in 1966, based on the data measured at Tihany [0 point = 104 075 m a. s. l. (Adriatic)]

of the phytoplankton present in the water samples was studied and counted under an Utermöhl plankton microscope (TAMÁS, 1967, pp. 191—193).

Fig. 1 shows the water-level changes of Lake Balaton in 1966, while *Fig. 2* illustrates the annual fluctuation of temperature in the water.

Concurrently with the collecting of the water sample series, the chemical composition of the water was studied by the researchers of "KÖJÁL", Com. Veszprém (ORSÓS, 1968).

For the identification of microorganisms we used the taxonomic works listed in References.

Fig. 2. The annual fluctuation of water temperature of Lake Balaton in 1966, in the small bay at Tihany (measured at a depth of ±30 cm)

Results

413 scooped and 112 filtrated plankton samples, taken from 15 localities of the 5 transverse sections of the Lake during May—November, 1966, have been studied. The identified microscopic plants and the aquatic fungi belonged to 6 large systematic phyla. The order of frequency is as follows:

	Species	Variety	Form
Chrysophyta	71	5	2
Chlorophyta	57	12	1
Cyanophyta	15	—	—
Euglenophyta	14	—	—
Pyrrophyta	5	—	—
Mycophyta	3	—	—
Total:	165	17	3

All three classes (Xanthophyceae 2, Chrysophyceae 8, Bacillariophyceae 68) of the phylum Chrysophyta were represented by several species. Of the pelagic species of the class Bacillariophyceae, *Cyclotella bodanica*, *C. ocellata*, *Melosira granulata*, *M. granulata* var. *angustissima*, *Nitzschia acicularis*, and *Stenopterobia pelagica* had locally attained high individual numbers in 1966 *(Table 1)*.

Of the tychoplanktonic diatoms present in the samples, *Amphora ovalis*, *Diploneis elliptica*, *Fragilaria construens*, *F. pinnata*, *Navicula cryptocephala*, *Nitzschia amphibia*, *N. tryblionella* var. *debilis*, and *Surirella robusta* var. *splendida* occurred locally in significant numbers.

The species *Cocconeis placentula* , *C. placentula* var. *euglypta*, and *Synedra parasitica* engaged in ecesis, in this year, too, the specimens of *Cymatopleura*

elliptica , *C. solea* and *Nitzschia sigmoidea* almost invariably present in the open water of Lake Balaton.

Botryococcus braunii and *Planktonema lauterborni*, belonging to class Xanthophyceae, reached high individual numbers only locally.

Among the species of class Chrysophyceae, the high individual values of *Dinobryon divergens* were locally significant. *Dinobryon sertularia* and *D. sociale* occurred, together with *Salpingoeca frequentissima*, only in the filtrate samples (see *Table 1).*

The percentual local frequency of the phylum varied between 2—97% during the period May—November considering scooped samples. The phylum constitutes 42% of the total algal species.

Phylum Chlorophyta stands in the second place as regards frequency. The majority of its 70 species belongs to order Chlorococcales.

Of the pelagic species, the individual numbers of *Dictyosphaerium pulchellum*, and the *Ankistrodesmus*, *Oocystis*, and *Scenedesmus* taxa were significant. Among the Desmidiaceae, *Closterium aciculare* reached the highest value, similarly to the data of the previous year, in the late summer samples of section Balatonalmádi—Balatonvilágos. Besides the *Closterium* species, *Staurastrum gracile* and *S. paradoxum* were yet frequent in the summer samples.

The percentual occurrence of the species in the phylum varied, on the basis of scooped samples, between 0.1—27% during the period May—November. The phylum constitutes 38% of the total algal species.

The 15 species of the phylum Cyanophyta were divided between two orders (Chroococcales 6, Oscillatoriales 9), however the majority of taxa was present in very low individual numbers in all samples. *Lyngbya circumcreta* reached its highest value, 6300/liter, in section Szigliget-Balatonmária in August. And the highest value of the individual numbers of *Lyngbya limnetica*, 4900/liter, was found in the September sample deriving from section Balatonfüred—Zamárdi. In Keszthely-bay, the water-bloom caused by *Aphanizomenon flos-aquae*, attaining immense proportions during the summer months, was unique in the history and life of Lake Balaton (HORTOBÁGYI and KÁRPÁTI, 1966, 1967).

The percentual occurrence of the phylum in the investigated localities varied, by the scooped samples, between 0.3—96.4% during the period May—November. The phylum constituted 8% of the total algal species.

Next in the order of frequency is phylum Euglenophyta; of its 14 species 11 belongs to order Euglena, and 3 to order Colaciales, *Euglena acus*, *E. ehrenbergii*, *E. klebsii*, *E. oxyuris* and *Phacus acuminatus* were present in locally high individual numbers.

Similarly to the situation in earlier years, the species *Colacium cyclopicola*, *C. simplex*, and *C. vesiculosum*, known as epibionts of Rotatoria and planktonic Crustacea, were frequent in the filtrate samples (TAMÁS, 1964, p. 248; 1965, p. 234; 1966).

The percentual occurrence of the phylum in the investigated localities varied, by the scooped samples, between 0—3.4% during the period May—November. The phylum constituted 7% of the total algal species.

The 5 representatives of the two classes (Chryptophyceae 1, Peridineae 4) of phylum Pyrrophyta occurred in all samples. The species *Ceratium hirundinella* reached its highest value, 122380/liter, in the September sample taken in

Table 1

A quantitative analysis of the phytoplankton of Lake Balaton on the basis of collections in 1966 (ind./l = 1000 individuals per litre; + = filtrated plankton (Nr. 25 net); N = evening filtration)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
CYANOPHYTA *Chroococcales*											
Aphanocapsa delicatissima W. et G. S. West	V.	—	+	—	+	—		—		—	
	VI.	—	+ N	—	+	—	+	—		—	+
	VII.	—	N	—		—		0.90	+ N	—	+ N
	VIII.	—	+ N	0.20		0.10		0.30	+ N	0,08	+ N
	IX.	—	+	—	+	0.30	+	0.60	+ N	—	+ N
	X.	—	+	—	+	—	+	—	+ N	—	+ N
	XI.	—		—		—	+	—	+	—	+
Aphanothece clathrata var. *brevis* Bachm.	V.	0.13	+ N	—		—		—		—	
	VI.	—	+ N	—	+	—		—		—	
	VII.	0.27		—		—		1.00	+ N	0.08	+
	VIII.	—		1.20		0.20		0.20	N	—	
	IX.	—		—		0.10	+	0.20	+ N	—	+ N
	X.	—	+	—	+	—	+	—	+ N	—	+ N
	XI.	—		—		—	+	—	+	—	+
Chroococcus limneticus Lemm.	V.	—		—	+	—		—	+	0.04	+
	VI.	0.27	+ N	0.10	+	0.20	+	—	+ N	—	
	VII.	—	N	—		0.20		0.10	+ N	—	+ N
	VIII.	—	+	—		—		0.20	+ N	—	+ N
	IX.	0.13	N	—		—	+	0.40	+ N	0.08	+ N
	X.	—		0.02		0.05		0.04	+ N	—	+ N
	XI.	—	+	0.02	+	—	+	—	+	—	+
Chroococcus minimus (Keissl.) Lemm.	V.	—		—		—		—		—	N

Coelosphaerium kützingianum Naeg.	V.	0.20		—	+	—		—	+	N	0.24	N
	VI.	—	N	—	+	—	+	—	+	N	—	+ N
	VII.	0.27		—	+	0.20	+	0.20	+	N	—	+ N
	VIII.	—	+	—		—	+	0.10	+	N	—	+ N
	IX.	—	N	—		0.20	+	—	+	N	0.08	+ N
	X.	—	+	—	+	0.05	+	0.07	+	N	—	+ N
	XI.	0.07	+	—	+	0.05	+	0.05	+		—	+
Gomphosphaeria lacustris Chod.	V.	—		—		—	+	—	+	N	—	N
	VI.	—		0.10	+	—	+	—		N	—	+ N
	VII.	—		—		—	+	—	+	N	—	+ N
	VIII.	—		0.40		—	+	0.10	+	N	—	+ N
	IX.	—	N	—		0.10	+	0.10	+	N	—	+ N
	X.	—		0.05	+	—	+	0.10			—	+
	XI.	—	N	—		—		—			—	
Merismopedia glauca (Ehr.) Naeg.	VI.	—		3.20		—		—			—	
	VII.	—		—		—		0.80			—	
	IX.	—		—		0.80	+	6.40			—	
	X.	—		—	+	0.50		—			—	+
Merismopedia tenuissima Lemm.	VI.	—	+ N	6.40		—		—			—	
	VII.	—	N	—		—		—			—	
	VIII.	—		—		—		1.60			—	
	IX.	—		—		1.60		1.00			—	+
	XI.	—		—		—		—	+		—	
Microcystis flos-aquae (Wittr.) Kirchn.	V.	—		—	+	—	+	—	+	N	—	+ N
	VI.	—	+	—		—	+	—	+	N	—	+ N
	VII.	—		—		—	+	0.80	+	N	0.08	+ N
	VIII.	8.54	+	0.40		—	+	0.70	+	N	0.08	+ N
	IX.	—		—	+	1.10	+	1.00	+	N	0.32	+ N
	X.	—	+	—	+	—	+	—		N	—	+ N
	XI.	—		—		—		—	+		—	+
Oscillatoriales												
Anabaena spiroides Kleb.	VII.	0.07		0.05		0.05		—			—	
	VIII.	0.50	+	0.10		—		—			—	
	IX.	—		0.10		—		—			—	

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i/l*	No. 25	*i/l*	N.o 25	*i/l*	No. 25	*i/l*	No. 25
Aphanizomenon flos-aquae var. *klebahnii* ELENK.	V.	—		0.30		0.80		0.70	N	0.56	
	VI.	0.93	+ N	0.30		0.90	+	0.20	+ N	0.90	+ N
	VII.	27.33	+ N	15.00	+	200.40	+	5.83	+ N	6.27	+ N
	VIII.	1112.50	+ N	1471.43	+	75.00	+	11.00	+ N	27.40	+ N
	IX.	695.20	+ N	978.00	+	16.00	+	9.80	+ N	35.20	+ N
	X.	1.41	+	0.51	+	0.57	+	0.22	+	0.14	+ N
	XI.	0.22	N	0.20		0.17		0.12		0.14	
Lyngbya circumcreta G. S. WEST	V.	0.40	+	—	+	0.40	+	0.47	+ N	0.39	+ N
	VI.	0.40	N	0.70	+	0.70	+	0.95	+ N	0.20	+ N
	VII.	0.47		0.65	+	4.45	+	2.97	+ N	2.74	+ N
	VIII.	0.17	+	2.00	+	6.35	+	6.30	+ N	5.48	+ N
	IX.	—	+ N	0.35	+	2.70	+	2.87	+ N	2.82	+ N
	X.	—		0.09	+	0.10	+	0.17	+ N	—	+ N
	XI.	0.13	+ N	0.17	+	0.17	+	0.10	+	0.12	+
Lyngbya limnetica LEMM.	V.	0,53	N	0.30		0.60	+	0.35	+ N	0.22	N
	VI.	—		0.10	+	1.60	+	0.50	+ N	0.70	+
	VII.	—	+	0.40		0.80	+	1.10	+ N	0.96	+ N
	VIII.	—		—		0.50	+	2.60	+ N	4.36	+ N
	IX.	0.13		—		1.80	+	4.90	+ N	0.96	+ N
	X.	0.07	+	0.10		0.07	+	0.10	+ N	0.12	+ N
	XI.	0.05	N	0.10	+	0.07	+	0.10	+	0.10	+
Oscillatoria tenuis AG.	XI.	—	+	—		—		—		—	+
Pseudanabaena catenata LAUT.	X.	—		—	+	—		—	+	—	
	XI.	—	+	—		—	+	—	+	—	

EUGLENOPHYTA *Euglenophyceae* *Euglenales*												
Euglena acus EHR.	V.	—		—		—		0.05		—		
	VI.	—	N	0.20	+	—		0.02		—		
	VII.	—		0.37	+	1.85		1.39	+	0.13		
	VIII.	—		0.80		0.40	+	0.02		—		
	IX.	—		—		—		0.05		—		
	X.	—		0.07		0.05	+	0.10		0.18	+	
	XI.	—		—		0.02		—		—		
Euglena ehrenbergii KLEBS	V.	0.08		0.13		0.19		0.09	N	—		
	VI.	0.16		0.57		0.64		0.26	+	—		
	VII.	0.40		0.07	+	0.14	+	0.10		0.28		
	VIII.	0.10		1.27		0.16	+	0.14		0.03		N
	IX.	0.03		0.18	+	0.06		0.06		0.14		
	X.	0.03		0.12		0.07		0.07		0.08	+	
	XI.	0.10		0.05		0.10		0.10		0.08		
Euglena klebsii (LEMM.) MAINX	V.	0.02		0.10		0.22		0.30		—		
	VI.	0.40		0.65	+	0.07		—		—		
	VII.	0.20		0.20		0.15	+	0.32	+	1.02		
	VIII.	1.75	+	2.80		0.77		0.30		0.08		N
	IX.	0.17		—		0.24	+	0.27	+	0.48		
	X.	0.13		0.15		0.10	+	0.10	+ N	0.10	+	N
	XI.	0.07		0.10		0.10		0.10		0.10		
Euglena limnophila LEMM.	V.	0.27		—	+	—		—		—		
	VI.	—		0.10		—		—		—		
	VIII.	—		1.10		0.35		—	+	—		
	IX.	—		—	+	0.03		—		—		
	X.	0.10		0.07		—		—	+	—		
	XI.	0.10		0.07		0.10		—		—		
Euglena oxyuris SCHMARDA	V.	0.10		0.06		0.05	+	0.11		0.01		
	VI.	0.52	+	0.35	+	0.19	+	0.07	+ N	—		
	VII.	0.53		0.21	+	0.94	+	1.73	+ N	1.42	+	
	VIII.	0.58		4.60	+	0.91	+	0.28	+	0.35	+	N
	IX.	—	N	0.12	+	0.39		0.82	+ N	0.49	+	N
	X.	—	+	0.07		0.07	+	0.10	+ N	0.10	+	N
	XI.	0.10		0.10	+	0.07		0.05		0.10		

Table 1 (continued)

Species	Period	Local ity									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Phacus acuminatus STOKES	V.	—		—		—	+	0.05	+	0.02	
	VI.	0.27	+	—	+	0.02	+	—	+	—	+
	VII.	0.27		0.02	+	0.40	+	0.70	+	—	N
	VIII.	0.64	+	0.53		0.40	+	—	+	0.04	
	IX.	—		—	+	—		0.06	+ N	0.16	
	X.	—		—		0.02	+	0.05	+ N	0.06	+
	XI.	0.07	+	—		—		0.05		0.08	
Phacus longicauda (EHR.) DUJ.	VIII.	—		—		—	+	—		—	
Phacus tortuosus ROLL	V.	0.07		—		—		—		—	
	VIII.	0.27		1.50		—		—		—	
	X.	0.03		—		—		—		—	
Phacus trypanon POCHM.	VIII.	—		0.60	+	0.10		—		—	
Phacus sp.	VI.	—		—		—		0.10	+	—	
	VIII.	0.27		—		—		—		—	
Trachelomonas volvocina EHR.	VII.	—	N	—		—		—		—	
Colaciales											
Colacium cyclopicola (GICKLH.) BOURR.	V.	—	+ N	—	+	—	+	—		—	
	VI.	—	N	—		—		—		—	
	VII.	—	N	—		—	+	—	+	—	
	VIII.	—	N	—		—		—		—	
Colacium simplex HUBER—PESTALOZZI	V.	—	+ N	—	+	—	+	—		—	
	VI.	—	N	—		—		—		—	
	VII.	—	N	—		—	+	—		—	
	VIII.	—	N	—		—		—		—	

Colacium vesiculosum Ehr.	V.	—	N	—	+	—	+	—	+ N	—	+ N
	VI.	0.13	+ N	—	+	—	+	—	+ N	—	+ N
	VII.	—	+ N	—	+	0.40	+	0.30	+ N	0.16	+ N
	VIII.	2.67	+ N	—	+	0.40	+	—	+ N	—	N
	IX.	0.13	N	—		0.10	+	—	N	—	+ N
	X.	0.10	+	—	+	0.05	+	0.10	+ N	0.04	+ N
	XI.	0.10	+ N	—	+	0.07	+	0.10	+	—	+
PYRROPHYTA *CHRYPTOPHYCEAE*											
Cryptomonas erosa Ehr.	V.	—		—		—		—		—	+ N
	VI.	—		—		—	+	—		—	
	VII.	—		—		—		—		—	+
PERIDINEAE											
Ceratium hirundinella (O. F. Müll.) Schrank	V.	0.08	+ N	1.16	+	3.75	+	4.22	+ N	3.92	+ N
	VI.	21.15	+ N	14.63	+	9.32	+	9.07	+ N	11.98	+ N
	VII.	25.34	+ N	52.22	+	27.27	+	8.04	+ N	18.88	+ N
	VIII.	17.23	+ N	122.38	+	25.73	+	10.57	+ N	24.43	+ N
	IX.	4.09	+ N	14.26	+	9.84	+	5.39	+ N	16.10	+ N
	X.	0.07	+	0.11	+	0.05	+	0.10	+ N	0.06	+ N
	XI.	0.03	+ N	0.04	+	0.02		0.02	+	0.02	
Diplopsalis acuta Entz	V.	—		—		0.10	+	0.09	+ N	0.08	N
	VI.	0.08	+	0.10	+	0.40	+	0.03	+ N	0.12	+ N
	VII.	1.48	+ N	0.87	+	0.65	+	0.36	+ N	0.33	+ N
	VIII.	1.73	+	1.17	+	0.36	+	0.12	+ N	0.22	+ N
	IX.	0.02		0.06	+	0.01	+	0.11	+ N	0.25	+ N
	X.	0.01		0.05	+	0.04	+	0.03	+ N	0.02	+ N
	XI.	0.01		0.01		0.01		0.01	+	0.02	
Glenodinium gymnodinium Penard	VI.	—		0.03		0.07		0.02		0.02	
	VII.	0.02		0.03		0.10		—		0.08	
	VIII.	0.09		0.03		0.04		0.03		0.01	
Gonyaulax apiculata (Penard) Entz	V.	—		—		0.08	+	0.04	N	0.05	N
	VI.	0.04	N	0.08		0.11		0.05	+	0.08	
	VII.	0.28		0.20	+	0.75	+	0.25	+ N	0.33	N
	VIII.	1.90	+ N	1.03		0.64		0.12	+ N	0.40	N
	IX.	0.07	+ N	0.08	+	0.11	+	0.10	+ N	0.14	+ N
	X.	—		0.02		0.01		0.01		0.02	

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
CHRYSOPHYTA *XANTOPHYCEAE*											
Botryococcus braunii KÜTZ.	V.	—		—	+	—	+	—		0.04	
	VI.	—	+	—	+	—		—	+ N	—	+ N
	VII.	—	+ N	—		1.60	+	2.60	+ N	0.43	+ N
	VIII.	1.33	+	0.47	+	0.50	+	0.80	+ N	0.16	+ N
	IX.	—	+ N	0.13	+	1.60	+	0.75	+ N	2.96	+ N
	X.	—	+	0.50	+	0.17	+	0.10	+ N	0.08	+ N
	XI.	—	+	0.10	+	0.10	+	0.10	+	0.10	
Planktonema lauterborni SCHMIDLE	V.	4.00	+ N	6.50	+	9.30	+	5.15	+ N	1.16	+ N
	VI.	3.87	+ N	16.10	+	3.50	+	0.20	+ N	1.90	+ N
	VII.	1.13	N	0.80	+	4.00	+	1.60	+ N	1.28	+ N
	VIII.	—		0.60		3.12	+	0.30	+ N	0.16	N
	IX.	—		0.10		2.20	+	0.90	+ N	0.08	+ N
	X.	—		0.20	+	0.10	+	0.15	N	0.08	+ N
	XI.	0.10		0.10		0.12	+	0.10	+	0.12	+
CHRYSOPHYCEAE											
Dinobryon divergens IMH.	V.	—		—		0.10	+	0.20	+ N	—	+ N
	VI.	0.27	+ N	1.40	+	0.80	+	—	+ N	—	+ N
	VII.	0.27	+ N	—	+	—	+	1.90	+ N	0.72	+ N
	VIII.	—	+	—		—	+	0.40	+ N	0.08	+ N
	IX.	—		—		—	+	—	+ N	—	N
	X.	—	+	—	+	—	+	—	+ N	—	+ N
	XI.	—	+	—	+	—	+	—	+	—	+
Dinobryon sertularia EHR.	V.	—		—		—		—	N	—	
Dinobryon sociale EHR.	V.	—		—		—		—	+	—	N
	VI.	—		—	+	—		—		—	
	VII.	—		—		—	+	—	+	—	
	X.	—		—		—		—	+ N	—	+ N
	XI.	—		—	+	—	+	—		—	

Mallomonas acaroides PERTY	V.	—	+	—		—		—		—		
	VIII.	4.53		—		—		—		—		
	IX.	2.56		0.10		—		—		—		
Mallomonas elongata REVERDIN	VIII.	0.80		—		—		—		—		
	IX.	0.16		—		—		—		—		
Mallomonas tonsurata TEILING	V.	—	+	—		—		—		—		
	VIII.	9.07		—		—		—		—		
	IX.	2.32		—		—		—		—		
Salpingoeca frequentissima (ZACH.) LEMM.	V.	—	N	—		—		—		—		
	VI.	—	N	—		—		—		—		
	VII.	—	N	—		—	+	—	N	—	+	N
	VIII.	—	+ N	—	+	—		—	+ N	—		N
	IX.	—		—		—		—	N	—	+	N
	X.	—		—	+	—	+	—		—		
	XI.	—	+	—		—		—	+	—		
Synura uvella EHR.	V.	—	+	—		—		—		—		
	VI.	—	N	—		—		—		—		
	VII.	—	N	—		—		—		—		
	VIII.	1.33		1.00		—		—		—		
	XI.	—	+	—		—		—		—		
Bacillariophyceae Centrales												
Attheya zachariasi J. BRAUN	V.	—		—		—	+	—	+	—		N
	VII.	—		—		—	+	—	+ N	—	+	N
	VIII.	—		—		—	+	—	+ N	—	+	N
	IX.	—		—		—	+	—	+	—	+	N
	X.	—		—		—		—	+ N	—	+	N
	XI.	—		—	+	—		—		—		
Cyclotella bodanica EULENST.	V.	66.60	+ N	80.50	+	44.50	+	28.00	+ N	5.08	+	
	VI.	68.00	+ N	71.00	+	75.00	+	32.00	+ N	39.00	+	N
	VII.	44.00	+ N	19.00	+	44.00		22.00	+ N	11.20	+	N
	VIII.	41.30	+	27.67		6.00	+	4.50	N	2.08	+	N
	IX.	3.20	N	3.00	+	3.80	+	4.70	+ N	6.40	+	N
	X.	12.00	+	3.75	+	2.12	+	3.12	+ N	4.00	+	N
	XI.	4.00	N	3.37	+	3.25	+	6.12	+	4.60	+	

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Cyclotella meneghiniana Kütz.	V.	—	N	—		—		—		—	
	VI.	—	+	—		—		—		—	
Cyclotella ocellata Pant.	V.	82.67	+ N	77.50	+	47.00		37.75	+ N	5.40	+ N
	VI.	97.33	+ N	111.50	+	110.00	+	32.00	+ N	58.00	+ N
	VII.	74.67	+ N	37.00	+	62.00		42.00	N	20.00	+ N
	VIII.	74.67	+	42.33		10.50	+	9.00	N	3.96	+ N
	IX.	6.80	N	6.00	+	6.80	+	8.50	+ N	12.00	+ N
	X.	36.00	+	6.25	+	5.87	+	6.25	+ N	6.40	+ N
	XI.	7.67	N	6.25	+	7.37	+	6.62	N	7.60	+
Melosira granulata (Ehr.) Ralfs	V.	8.00	+ N	4.20		0.20	+	—	+ N	—	+ N
	VI.	48.00	+ N	18.00	+	—	+	2.20	+ N	0.50	+ N
	VII.	24.53	+ N	8.00	+	9.00	+	13.30	+ N	9.80	+ N
	VIII.	4.87	+ N	5.80		3.50	+	10.50	+ N	12.40	+ N
	IX.	4.00	N	9.00	+	—	+	4.25	+ N	1.94	+ N
	X.	4.00	+	5.37	+	7.75	+	7.25	+ N	5.40	+ N
	XI.	8.00	+ N	9.50	+	11.75	+	5.75	+	1.80	
Melosira granulata var. *angustissima* O. Müll.	V.	7.33	+ N	1.00	+	—		0.25	+ N	—	N
	VI.	53.33	+ N	2.00	+	—		1.00		—	
	VII.	—	N	7.00		1.00	+	1.00	+ N	—	+ N
	VIII.	2.00		0.40		—	+	—	+	0.28	+ N
	IX.	—		—	+	—	+	—	N	1.20	+ N
	X.	2.67	+	2.00	+	—		0.37	+ N	0.20	+ N
	XI.	0.33	N	0.50	+	0.50	+	0.25	+	0.06	+
Melosira granulata var. *angustissima* f. *spirale* Müll.	VI.	—	N	—		—		—		—	
	XI.	—	N	—		—	+	—		—	
Melosira varians C. A. Ag.	IX.	—	N	—		—		—		—	
	X.	—		—	+	—	+	—		—	
	XI.	—	N	—		—		—		—	

Stephanodiscus dubius (Fricke) Hust.	X.	—		—		—		—		—	+
Pennales											
Amphora ovalis Kütz.	V.	0.47	N	—		0.10		0.80	+ N	—	+
	VI.	0.13	N	—		0.30	+	1.50	+	0.30	+
	VII.	0.53	+ N	0.40	+	2.40	+	0.87	+ N	0.24	
	VIII.	0.27		0.93	+	1.82	+	1.42	+ N	0.64	+ N
	IX.	0.27	N	0.20	+	0.20		0.41	N	—	
	X.	2.13	+	2.21	+	0.30	+	0.46	+ N	0.70	+
	XI.	0.23	+ N	0.65	+	8.00	+	0.40	+	0.50	+
Amphora ovalis var. *pediculus* Kütz.	V.	1.20	N	0.10		—		0.10		—	
	VII.	—	N	0.20		—	+	—		—	
	VIII.	—		0.20		—		—		—	
	X.	0.10	+	0.10	+	—		—		—	
	XI.	—	N	—		—		—		—	
Asterionella formosa Hassal	V.	—	N	—	+	—	+	—	+	—	N
	VI.	0.13	+	—	+	—		—		—	
	X.	—	+	—	+	—	+	—		—	
	XI.	—	+	—	+	—	+	—		—	
Caloneis schumanniana var. *biconstricta* Grun.	V.	—		—		—		—	N	—	
	VI.	—		—		0.10		0.12	N	—	
	VII.	—	+	—		—		—		—	
	VIII.	—		—		—		0.21		—	
	IX.	—		—		—		—	N	—	
	X.	0.07		0.15		—		—		—	
Campylodiscus noricus var. *hibernica* (Ehr.) Grun.	V.	—		—		—		—	N	0.15	
	VI.	—		—		—		0.10		—	
	X.	—		—		—		0.07		—	
Cocconeis placentula Ehr.	V.	—		—		—		—		—	+
	VI.	—		—		—		—	+ N	—	
	VII.	—		—		—		—		1.76	

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Cymatopleura elliptica (Bréb.) W. Smith	V.	0.15	+ N	—		—	+	0.04	+ N	0.05	+ N
	VI.	—		0.01	+	0.10	+	0.53	+ N	—	+ N
	VII.	0.80	+	0.66	+	0.60	+	0.16	+	0.06	+ N
	VIII.	0.25	+	0.20	+	0.28	+	0.42	+ N	0.24	+ N
	IX.	0.10		0.03	+	0.04		0.02	+ N	0.02	N
	X.	0.40	+	0.25	+	0.20	+	0.10	+ N	0.10	+ N
	XI.	0.03	+ N	0.10	+	0.05	+	0.07	+	0.10	+
Cymatopleura solea (Bréb.) W. Smith	V.	0.03		—		—		—	N	—	+
	VI.	—		—		—		0.69	+ N	—	+
	VII.	—	+	0.05		0.31	+	—		0.02	
	VIII.	—		0.05		0.05	+	0.15	+	0.28	+ N
	IX.	—		—		—		0.10	+	0.04	
	X.	—	+	0.05		0.07	+	0.05		0.08	N
	XI.	0.03	N	0.05		—	+	0.05	+	0.02	
Cymatopleura solea var. *regula* (Ehr.) Grun.	V.	—	N	—		—		—		—	
Cymbella cymbiformis (Kütz.) V. Heurck	VIII.	—		0.20		—		0.10		—	
Cymbella ehrenbergii Kütz.	V.	—		—		—		—	N	—	
	X.	—	+	—		—		—		—	
Cymbella lanceolata (Kütz.) V. Heurck	V.	—		—		—		—		0.03	
	VIII.	—		—		—		0.10		—	
Cymbella prostata (Berk.) Cleve	V.	—	N	—		—		—		—	
	XI.	—	N	—		—		—		—	
Diploneis domblittensis (Grun.) Cleve	X.	—		—		—		—	+	—	
	XI.	—		—		—		—	+	—	

Diploneis elliptica (Kütz.) Cleve	V.	—	N	—		—		0.40	N	0.06		
	VI.	—		—		—		1.10	+	0.20		N
	VII.	0.27	+	0.40		—		1.40	N	—		
	VIII.	—		—		—		1.30	+	0.28		+ N
	IX.	—		—		—		0.20	N	0.08		+
	X.	0.80		—		—		0.15	+	0.20		+ N
	XI.	—		0.10		—		—		—		
Diploneis puella (Schum.) Cleve	V.	—		—		—		0.15		—		
	VII.	—		—		0.20		—		—		
	VIII.	—		—		—		—		—		+
	IX.	—		—		—		—		0.08		
	XI.	—		—		0.07		—		—		
Epithemia hyndmanni W. Smith	VI.	—		—		—		0.10		—		
	VIII.	—		—		—		0.02		—		
Epithemia sorex Kütz.	VIII.	—		0.20		—	+	—		—		
	XI.	—		—		—		—	+	—		
Epithemia turgida (Ehr.) Kütz.	VI.	—		—		—		—	N	—		
Fragilaria construens (Ehr.) Grun.	V.	5.33	N	—		—		1.25	+ N	0.76		+
	VI.	—		1.00	+	6.00	+	14.00	+ N	—		+
	VII.	16.80	+ N	6.00		3.00	+	4.30		—		
	VIII.	2.67	+	7.00	+	1.00		11.00	+ N	0.28		
	IX.	2.40	+	—	+	0.10		0.50	N	—		
	X.	16.00	+	3.19		0.20		0.10		0.20		
	XI.	—		0.62	+	—		0.10	+	—		
Fragilaria pinnata Ehr.	V.	3.00	N	—		—		—		—		
	VI.	—		—		1.50		2.00		—		
	VII.	—		—		—		—		6.40		
	VIII.	—		4.00		0.50		4.50		0.08		N
	IX.	0.67		—		—		1.00		—		
	X.	10.13	+	0.75		—		—		—		
	XI.	1.33	+ N	1.00		0.87		1.50		1.00		
Gomphonema acuminatum Ehr.	VII.	0.27		—		—		—		—		

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Gomphonema intricatum var. *vibrio* (Ehr.) Cleve	V.	—		—		—		—		0.10	
Gomphonema olivaceum (Lyngb.) Kütz.	X.	—	+	—		—		—		—	
Gyrosigma acuminatum (Kütz.) Rabh.	V.	—		—		—		—		0.04	
	VI.	—		—		—	+	—		—	
	X.	—		—		0.05		—		—	
	XI.	—		—		—		0.07		0.06	
Gyrosigma attenuatum (Kütz.) Rabh.	V.	—	N	—		—		—		—	+
	VI.	—		—		—		0.07		—	
	VII.	—		—	+	—		—		—	
	VIII.	—		—		—		—	+ N	0.08	
	IX.	—		—		—		—		—	+
Gyrosigma distortum var. *parkeri* Harris	V.	0.07		—		—		0.05		—	
	VI.	—		—		—		0.10		—	
	VII.	—		—		—		0.02		—	
	VIII.	—		—		—		0.10	N	—	
	IX.	—	N	—		—		—		—	
	X.	0.27	+	0.03		—		—		—	
Gyrosigma kützingii (Grun.) Cleve	V.	0.07	N	—		—		0.15	+ N	—	
	VI.	—	N	—		—		0.60		—	N
	VII.	—	N	—		0.20		0.30		0.16	N
	VIII.	0.13	N	0.40		—	+	0.30	N	0.02	N
	IX.	—		—		—		0.07		0.08	
	X.	—	+	0.05	+	—		0.12		0.10	+ N
	XI.	0.05	+ N	0.07		—		—		—	
Gyrosigma prolongatum (W. Smith) Cleve	V.	—		—		—		—	N	—	
	VII.	—	N	—		—		—		—	
	XI.	—	+ N	—		—		—		—	

Navicula costulata Grun.	V.	—	N	—		—		—	N	—		
Navicula cryptocephala Kütz.	V.	0.67	N	—		—		—		—		
	VI.	—		0.20		—		0.50	+	0.10		
	VII.	—		0.60		2.20		0.10		0.16		
	VIII.	—		0.20		0.40		0.50	+	0.28	+	
	IX.	—		—		—		0.10		0.08		
	X.	—	+	0.50		—		0.20		0.20		
	XI.	—		—		—	+	—		—		
Navicula dicephala (Ehr.) W. Smith	XI.	—		—		—		—	+	—		
Navicula gracilis Ehr.	V.	—	N	—		—		0.15		—		
	VI.	—	N	—		—		0.20	N	—		N
	VII.	—	N	0.20		—		0.10	+	—		
	VIII.	—		0.30		0.40	+	0.30	N	0.16		
	X.	0.27	+	0.20		0.10	+	0.20	+	—		
	XI.	—	N	0.15	+	—		0.10		0.10	+	
Navicula hungarica var. *capitata* (Ehr.) Cleve	V.	0.13	+ N	—		—		—	N	—		
	VI.	—		—		—		0.20		—		
	VII.	—		—		0.40		0.10		—		
	VIII.	—		0.47		—		0.20		—		
	IX.	—		—		0.10		—		—		
	X.	—		0.10	+	—	+	0.05		—		
	XI.	—		—		—		0.05	+	—		
Navicula placentula (Ehr.) Grun.	V.	—	N	—		—		—		—		
	VI.	—		—		—		0.20	+	—		N
	VII.	—	+ N	—	+	0.20		0.40		—		
	VIII.	—		—		—		0.10	N	—		
Navicula placentula f. *rostrata* A. Mayer	V.	—		—		—		—	N	—		
Navicula pupula Kütz.	V.	—	N	—		—		—		—		
Navicula reinhardtii Grun.	IX.	—	+	—		—		—		—		
	XI.	—	N	—		—		—		—		

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Navicula scutelloides W. SMITH	VIII.	—		—		—		0.10		—	
Nacicula tuscula (EHR.) GRUN.	VIII.	—		—		—		0.10		—	
	IX.	—		—		—		—	+	—	
Nitzschia acicularis W. SMITH	V.	0.07	+ N	—		0.30	+	2.45	+	—	
	VI.	—	N	—	+	0.10	+	0.50	+	0.20	+ N
	VII.	—		1.60		1.80		1.90	N	0.48	N
	VIII.	—		0.47		1.60		4.90	+ N	0.88	+ N
	IX.	—		—		0.40		0.86	+	0.96	+ N
	X.	0.27	+	0.65	+	0.50	+	0.60	+ N	0.80	+ N
	XI.	—	N	—		0.10	+	0.10		—	
Nitzschia amphibia GRUN.	V.	0.33	+ N	—		—		0.20		0.08	+
	VI.	0.27	N	0.30		0.30	+	1.80	N	0.20	
	VII.	—	+	3.40		3.20		0.20		0.16	N
	VIII.	—		0.80		2.50		4.80	+	1.60	N
	IX.	—		—		0.20		0.20	+	0.24	+
	X.	0.27	+	1.50	+	1.00	+	0.30	+	0.24	+
	XI.	0.20	+ N	0.50	+	0.75	+	—		0.28	+
Nitzschia sigmoidea (EHR.) W. SMITH	V.	0.33	N	—		—		0.40	+ N	0.06	+ N
	VI.	—	N	—		—		0.71	+ N	0.10	+ N
	VII.	0.60	N	0.49	+	1.00	+	0.40		0.02	
	VIII.	—	N	0.40		0.30	+	0.30	+ N	0.41	+ N
	IX.	—		—	+	—		0.20	+ N	0.08	
	X.	—	+	1.10	+	0.20	+	0.20	+ N	0.30	+ N
	XI.	0.10	N	—		0.25	+	0.15	+	0.20	+
Nitzschia tryblionella var. *debilis* (ARNOTT) A. MAYER	V.	—		—	+	—		0.05		—	
	VI.	—		—		—		0.30		—	N
	VII.	0.27	+	0.80		0.60		0.30		—	
	VIII.	0.27		1.33	+	—		0.50	+ N	0.12	
	IX.	0.13		—		0.10		0.10	+	0.08	
	X.	0.80	+	0.30	+	—		0.10	+	—	
	XI.	—	N	—		—		—		—	

Nitzschia tryblionella var. *victoriae* GRUN	VIII.	—		—		0.10		—		—					
Opephora martyi HÉRIBAUD	V.	0.13	N	—		—		—	N	—					
	VI.	—		—		—		0.20		—					
	VII.	—		—	+	—		—		—					
	VIII.	—		—		—		0.50		0.08					
	IX.	—		0.10		—		—		—					
Rhoicosphenia curvata (KÜTZ.) GRUN.	V.	—	N	—		—		—		—					
	X.	0.27		—		—		—		—					
	XI.	—	N	—		—		—		—					
Rhopalodia gibba (EHR.) O. MÜLL.	VI.	—		—		—		0.20		—					
Stauroneis smithii var. *incisa* PANT.	X.	—		—	+	—		—		—					
Stenopterobia pelagica HUST.	VI.	—		—		—		0.02	N	—					
	VII.	0.03		0.26		0.14	+	—		0.16					
	VIII.	—		—		0.13	+	0.15	+	0.04					
	IX.	—		—		—		0.10		—					
	X.	—	+	0.55	+	—	+	—		—					
	XI.	—		—		—		—	+	—					
Surirella biseriata BRÉB.	V.	—	N	—		—		—		—					
	VI.	—		—		—		—		—		N			
	VII.	—	N	—		—		—		—					
Surirella robusta var. *splendida* (EHR.) V. HEURCK	V.	0.02	+ N	—	+	—		0.05	+ N	—		N			
	VI.	—	N	—		—		0.56	+ N	—		+ N			
	VII.	0.93	+ N	0.67	+	0.16	+	0.04	+	—					
	VIII.	0.08	+	0.10	+	0.14	+	0.71	+ N	0.22		+ N			
	IX.	—	+	0.03	+	0.03		0.05	+ N	—		+ N			
	X.	0.13	+	0.76	+	—	+	—	+ N	—		+ N			
	XI.	—	N	—	+	—		—	+	—		+			
Surirella tenera GREG.	VIII.	—		—		—		—		—		N			
	XI.	—	N	—		—		—		—					
Surirella turgida W. SMITH	V.	—		—		—		—		—		N			
	VII.	—		—		0.10		—		—					
	VIII.	—		—		0.05		0.07	+	0.08					
	IX.	—		—		—		—	N	—					
	X.	—		—		—		—	+	—					

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Synedra acus var. *angustissima* Grun.	V.	0.07	N	—		—		—	N	—	
	VI.	—	+	—		—		0.10	N	—	+
	VII.	0.27		—		—		—	N	—	
	VIII.	—		—		—	+	—		0.08	
	IX.	—		—		—	+	—	N	—	
	X.	—		0.20		—	+	0.05	+ N	0.06	+ N
	XI.	—		—		—	+	—	+	—	
Synedra capitata Ehr.	VI.	—		—		—		—	+	—	
	VII.	—	N	—		—		—		—	
Synedra parasitica (W. Smith) Hust.	VII.	—		—		—		0.20		—	
	VIII.	—		—		—		0.10		—	
	XI.	—		—		—		—	+	—	
Synedra ulna var. *biceps* (Kütz.) V. Schönf.	V.	—	N	—		—		—		—	
Synedra ulna var. *spathulifera* Grun.	XI.	—	N	—		—		—		—	
CHLOROPHYTA *Chlorophyceae Volvocales*											
**Chlamydomonas intermedia* Chodat	VI.	—	+	—	+	—		—		—	+
	VII.	—	N	—		—		—		—	
Chlamydomonas sp.	V.	—	+ N	—		—		—	N	—	
	VI.	—	+ N	—		—	+	—		—	N
	VII.	—		—		—		—	+ N	—	N
Pandorina morum (Müller) Bory	VI.	—	+	—		—		—		—	
	VII.	0.80		—		—		—		—	

TETRASPORALES												
Stylosphaeridium stipitatum GEITLER	V.	—	+	—		—		—		—		
CHLOROCOCCALES												
Actinastrum hantzschii LAGERH.	V.	0.07		—		—		—		—		
	VI.	—	+ N	—		—		—		—		
	VIII.	—		—		—		—		0.04		
Ankistrodesmus braunii (NAEG.) BRUNNTH.	IX.	—		—		—	+	—		—		
Ankistrodesmus falcatus (CORDA) RALFS	V.	—	+	0.10		0.30		0.05	+ N	0.04		
	VI.	0.13	N	—		1.30	+	0.20		0.10		N
	VII.	—	N	0.40		1.20		0.42	N	0.80		
	VIII.	0.27		—		—	+	0.10		0.24		
	IX.	—		—		0.50		0.10		0.16		
	X.	—		0.10		0.10		0.05	N	0.08		N
	XI.	—		0.05		0.07		0.05		0.04	+	
Ankistrodesmus falcatus var. *acicularis* (A. BRAUN) G. S. WEST	V.	0.13	+ N	—	+	0.10		0.25		0.08		
	VI.	0.40		0.07		0.40		0.20	+	1.50		
	VII.	—		0.40		1.00		0.66		1.41	+	
	VIII.	—		0.53		0.40		0.50	N	1.76	+	
	IX.	—		0.80		4.80		2.62		1.12		N
	X.	0.13		0.10		0.15	+	0.10	+ N	0.10	+ N	
	XI.	—		—		0.07	+	0.07	+	0.05		
Abkistrodesmus falcatus var. *mirabile* W. et G. S. WEST	V.	—		0.10		—	+	—		0.04		
	VI.	—		—		—		—	+	—		
	VII.	—		0.20		—		0.10		—		N
	VIII.	—		0.50		3.07	+	4.40	+ N	1.36		
	IX.	—		0.30	+	6.30		0.81		1.46	+	
	X.	—		—		0.10	+	—		0.12	+	
Ankistrodesmus falcatus var. *spirilliformis* G. S. WEST	V.	0.67	+ N	—		—		—		—	+	
	VI.	—		—		0.10	+	—		—	+	
	VII.	—	N	—		0.40	+	—	N	0.08		
	IX.	—		—		—		0.05	+	—		
	X.	—		—		—		0.05	+ N	0.08	+	
	XI.	—		—		—		0.05	+	0.06	+	

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Ankistrodesmus lacustris (Chod.) Ostenf.	V.	0.40	N	—		0.80		0.40	N	1.22	
	VI.	0.13	+	0.40	+	0.20		—		0,50	+ N
	VII.	—		—		2.80		0.70	N	0.72	N
	VIII.	1.07		0.80		—		0.60		0.08	
	IX.	—		—		1.20		0.30	N	0.32	+
	X.	—		—		0.10		0.10		0.10	
	XI.	0.10		0.20		0.17		0.20		0.16	
Ankistrodesmus longissimus (Lemm.) Wille	IX.	—		—		—		—		0.48	
Chodatella balatonica Scherffel	V.	—	+ N	—		—		—		—	
	VI.	—		—		—	+	—		—	
Chodatella quadriseta Lemm.	VI.	—	N	—		—		—		—	
Coelastrum microporum Naeg.	V.	—		—		—	+	—	+ N	0.04	
	VI.	—	+	—		—	+	—	N	0.10	
	VII.	—	N	—		—	+	0.10	+ N	0.02	N
	VIII.	0.20	+	0.50		—		0.02		—	N
	IX.	—		—		—		0.02	N	—	
	X.	—		0.05	+	0.06	+	0.07	+	0.04	+ N
	XI.	—		—		—	+	—	+	—	
Crucigenia quadrata var. *octogona* Schmidle	V.	0.27	+	0.80	+	4.00		2.35	+ N	1.68	+
	VI.	—		1.60		3.60	+	—	+	4.20	+ N
	VII.	2.93	+	2.40	+	1.70		1.70	+ N	1.28	N
	VIII.	0.27		1.20		1.90	+	0.40	+ N	0.96	+ N
	IX.	—		0.10		1.30		0.20		0.50	+
	X.	0.10		—		0.15	+	0.12	+	0.16	+ N
	XI.	0.17		—		0.15	+	0.20	+	0.24	+
Crucigenia tetrapedia (Kirchner) W. et G. S. West	X	—		—		—		—		—	+
	XI.	—	+	—		—	+	—	+	—	

Dictyosphaerium pulchellum Wood.	V.	0.40	+ N	2.00	+	0.50	+	0.25	+ N	0.36	+ N
	VI.	3.13	+ N	1.55	+	1.40	+	—	+ N	0.60	+
	VII.	0.80	+	—	+	1.80	+	2.20	+ N	1.41	+ N
	VIII.	0.27	+	0.73	+	1.20	+	2.50	+ N	1.32	+ N
	IX.	0.20	N	0.30	+	0.40	+	2.60	+ N	0.72	+ N
	X.	0.17	+	0.20	+	0.20	+	0.20	+ N	0.50	+ N
	XI.	0.10	+	0.22	+	0.15	+	0.20	+	0.50	+
Kirchneriella lunaris (Kirchn.) Moebius	V.	—	+	—		—		—		—	
	VI.	—		—		0.20		—		—	
	VIII.	—		—		—		—		—	N
	IX.	—		—		—		—		0.06	
Kirchneriella obesa (W. West) Schmidle	V.	—	+	—		0.10		—		—	
	VI.	—	N	—	+	—		—		—	N
	VII.	—		—		—	+	0.10	N	—	+
	VIII.	—		—		0.10		—	N	—	
	IX.	—		—		—		—	+ N	—	
	X.	—		—		—		—		0.06	+ N
	XI.	—		—		0.05	+	—		—	
Oocystis elliptica f. *minor* W. West	V.	—		—		—	+	—		0.08	
	VI.	0.13		—	+	—	+	—		—	
	VIII.	—		—		—		—	+	0.04	
	X.	—		—		—		—		0.04	+
	XI.	—		—		—		—	+	—	
Oocystis novae semliae f. *major* Wille	VI.	—		—		—		—	N	—	
Oocystis rupestris Kirchn.	VI.	—		—		—		—	N	—	
Oocystis solitaria Wittr.	V.	0.13	+	0.40	+	0.60	+	0.55	+ N	0.48	+
	VI.	1.07	+ N	1.30	+	0.60	+	0.20	+ N	1.00	+ N
	VII.	1.33	N	—		1.00	+	0.50	N	0.40	N
	VIII.	0.80	+ N	0.47		0.72	+	0.70	+ N	0.76	N
	IX.	0.20	N	—		0.80	+	0.70	+ N	1.12	+ N
	X.	—		—		—		0.30	+	0.30	+ N
	XI.	0.17	+ N	0.15	+	—		0.25	+	0.30	+

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Oocystis solitaria f. *wittrockiana* PRINTZ	V.	0.07	+	—	+	0.20	+	—	+	0.18	+
	VI.	0.27	+	—	+	—		—	N	0.08	
	VII.	—		—		1.00		1.30	N	1.60	+ N
	VIII.	—		0.60		0.40		0.30	+	0.32	N
	IX.	—		—		0.20	+	0.04	N	0.72	+ N
	X.	—		—		0.10	+	0.15	+ N	0.20	+ N
	XI.	0.07		—		—		0.10	+	0.20	+
Oocystis submarina LAGERH.	V.	1.47	N	0.30	+	0.30	+	—	+ N	0.42	
	VI.	0.53		0.10	+	—	+	—	+ N	0.80	+
	VII.	—		—		0.20		0.40	N	0.40	N
	VIII.	0.27		0.20		0.20		—	+	0.04	
	IX.	—		—		0.10		—		—	
	X.	—		—		—		0.30		0.30	
	XI.	—		0.20	+	—		—		0.40	
Pediastrum boryanum (TURP.) MENEGH.	V.	—	+ N	—		—	+	—	+ N	—	
	VI.	0.13	+ N	—		—		—	+ N	—	
	VII.	0.07	N	—		—	+	—		—	
	VIII.	0.22	+	—		—		0.02	+	—	N
	IX.	—		0.01	+	—	+	—	+	—	
	X.	—	+	0.10	+	—		—	N	—	
	XI.	0.10	+ N	—	+	0.07	+	0.10	+	0.06	
Pediastrum clathratum (SCHROET.) LEMM.	V.	—	+ N	—	+	0.02	+	—	+	0.03	+ N
	VI.	0.13	+ N	0.10	+	0.05	+	0.02	+ N	—	+ N
	VII.	0.03	+ N	0.15	+	0.24	+	0.25	+ N	0.14	+ N
	VIII.	0.25	+	0.18		0.13	+	0.07	+ N	0.10	+ N
	IX.	—	+	0.07		0.04	+	0.02	+ N	0.04	+ N
	X.	—	+	0.10	+	0.10	+	0.05	+ N	0.06	+ N
	XI.	—	N	0.05	+	0.05	+	0.07	+	0.18	+

Pediastrum duplex var. *genuinum* A. Braun.	V.	—	+ N	—		—		—	+	—		
	VI.	—	N	—		—	+	—	N	—		N
	VII.	—	N	—		—		—		—		+
	VIII.	—	N	—		0.02		0.07	N	—		
	IX.	—	N	—		—	+	—	N	—		+
	X.	—		—		—	+	—		—		
	XI.	—	N	—	+	—	+	—		—		
Pediastrum duplex var. *reticulatum* Lagerh.	V.	0.17	N	0.04	+	0.15	+	0.12	+ N	0.04		+ N
	VI.	0.28	+ N	0.20	+	0.06	+	0.11	+ N	—		+ N
	VII.	0.07	+ N	0.04	+	0.16	+	0.18	+ N	0.08		+ N
	VIII.	0.59	+ N	0.55	+	0.09	+	0.10	+ N	0.02		+ N
	IX.	0.03	+ N	0.11	+	0.04	+	0.08	+ N	0.05		+ N
	X.	—	+	0.11	+	0.07	+	0.10	+ N	0.14		+ N
	XI.	0.07	+ N	0.10	+	0.17	+	0.09	+	0.10		+
Pediastrum simplex Ralfs	V.	—		—		—	+	—		—		
	VII.	—		0.02		0.02	+	0.04	N	0.02		N
	VIII.	—	+	—		0.10	+	—	+ N	0.04		N
	IX.	—	N	0.08	+	0.04		0.02	+	0.02		+ N
	X.	—	+	—	+	—	+	—		—		+ N
	XI.	—	N	—		—		—		—		+
Pediastrum tetras (Ehr.) Ralfs	VIII.	0.27		—		—		—		—		
Quadrigula lacustris (Chod.) G. M. Smith	V.	—		—		0.10		0.60		—		
	VII.	—		—		—		0.40		0.08		
	VIII.	—		0.20		0.10		—		0.04		
	IX.	—		—		—		0.20		0.08		
	X.	—		—		—		—		—		N
	XI.	—		—		—	+	—		—		
Rhopalosolen sebestyenae Fott	V.	—		—		—		—		—		N
	VI.	—		—		—		—		—		+
	VII.	—		—		—		—	N	—		
Scenedesmus acuminatus (Lagerh.) Chod.	VII.	0.27		—		—		—		—		
	VIII.	—		0.20		—		—		—		
	X.	—		—	+	—		—		—		
	XI.	—		—		—	+	—		—		

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Scenedesmus acutus (MEYEN) CHOD.	VII.	—	N	—		—		—		—	
**Scenedesmus anomalus* (G. M. SMITH) TIFF.	VIII.	—		—	+	—		—		—	
Scenedesmus arcuatus LEMM. forma UHERKOV.	V.	—		—		—		—		—	N
	VI.	0.13		—		—		—		—	N
	VII.	—		—		—	+	—	N	—	+
	VIII.	0.53		—		—		—	N	—	
	IX.	—		—		—		—	N	—	+ N
	XI.	—		—	+	—	+	—	+	—	
Scenedesmus balatonicus HORTOB.	V.	—		—		—		0.02		0.03	
	VI.	—		0.02		0.11		0.04		—	
	VII.	—		—		0.02		0.14		0.04	+
	VIII.	—		0.20		0.17		0.05	+ N	0.03	
	IX.	—		0.01	+	0.10	+	0.24		0.13	
	X.	—		—		—		—		0.12	+
Scenedesmus bicaudatus var. *brevicaudatus* HORTOB.	VI.	—	+	—		—		—		—	
Scenedesmus ecornis (RALFS) CHOD.	V.	—	+	—		—		0.10	+	0.26	
	VI.	—	+ N	—	+	—		0.10	N	—	+ N
	VII.	0.27		—	+	0.20	+	0.40	+ N	0.24	N
	VIII.	0.27	+	0.20		0.40	+	—		0.08	+
	IX.	—		—		—		0.10		0.40	+
	X.	0.10	+	—	+	0.07		0.10		0.16	+ N
	XI.	—	+	—		—	+	0.10	+	—	
Scenedesmus ecornis var. *disciformis* CHOD.	VIII.	—	+	—		—		—		—	
	IX.	—		0.10		—		—		—	

Scenedesmus intermedius CHOD.	V.	—		—		—		0.05		—						
	VI.	—	N	—		—		—		—						
	VIII.	—	+	—		—		—		—						
	IX.	—		—		—		—		—		N				
Scenedesmus intermedius var. *acaudatus* HORTOB.	V.	0.07	+ N	—		—		—		—						
Scenedesmus intermedius var. *balatonicus* HORTOB.	V.	0.07	+ N	—		—		—		—						
	VI.	—	N	—	+	—		—		—						
	VII.	—	N	—		—		—		—						
	XI.	—		—	+	—		—		—						
Scenedesmus intermedius var. *bicaudatus* HORTOB.	V.	—		—	+	—		—		0.04						
	X.	—		0.05	+	—		—		—						
Scenedesmus quadricauda (TURP.) BRÉB.	V.	—	+ N	—		0.20		0.30	+	0.06						
	VI.	0.27	+ N	0.50	+	0.40	+	—		—						
	VII.	1.07	+ N	—		0.80		0.50	+ N	0.40		N				
	VIII.	2.13	+ N	0.40		0.10		—	+	0.08						
	IX.	0.05		—	+	0.30		—	+	—						
	X.	—	+	0.20	+	0.10	+	0.15	+ N	0.16		+ N				
	XI.	0.07	+ N	0.10	+	0.17	+	0.20	+	0.10		+				
Scenedesmus quadricauda var. *longispina* (CHOD.) G. M. SMITH	VIII.	0.53		—		—		—		—						
Scenedesmus quadricauda var. *maximus* W. et G. S. WEST	VIII.	—	+	—		—		—		—						
Scenedesmus spinosus CHOD.	VI.	—	N	—		—		—		—						
Scenedesmus sp.	V.	—	N	—		—		—		—						
Schroederia setigera LEMM.	V.	0.33	+	—		—		—		0.12						
	VI.	—		0.12		—		—		—						
	VII.	—		—		—		—	N	—						
	IX.	—		—		—		—		0.16						
Selenastrum gracile REINSCH	V.	—	N	—		—		—		—						
	VI.	—	+ N	—		—	+	—		—		+				
	VII.	—		—		—		—	N	—						

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25	*i*/l	No. 25
Tetraëdron trigonum	VII.	—		—		—		0.10		—	
(NAEG.) HANSG.	VIII.	—		0.20		—		—		—	
Tetrastrum staurogeniaeforme	V.	—	+	—		—		0.05		—	
(SCHROEDER) LEMM.	VI.	0.13		—		—		—		—	
	VIII.	0.53		—		—		—		—	
	XI.	—	+	—		—		—		—	
Zygnematales											
Closterium acerosum (SCHRANK.) EHR.	V.	—		—		0.50		—		—	
	VI.	—	+	—		—	+	—		—	
	VII.	—		0.09		0.41		0.10		0.04	
	IX.	—		0.02		—		—		—	
Closterium acerosum var. *elongatum*	VII.	—		0.05		0.02	+	—		—	
BRÉB.	VIII.	—		—		—	+	—		—	
	XI.	—		—		—		—	+	—	
Closterium aciculare WEST	V.	—		0.10		—		—	+	0.08	N
	VI.	0.52		0.60		1.50		0.17	+	—	+
	VII.	—		—		2.20	+	0.34	+ N	0.63	+ N
	VIII.	0.07		0.40		0.35	+	0.33	+ N	0.47	+ N
	IX.	0.13		—		0.28	+	1.20	+ N	2.21	+ N
	X.	—		0.06		0.20	+	0.10	+ N	0.20	+ N
	XI.	—		0.10	+	0.25	+	0.17	+	0.30	+
Closterium attenuatum EHR.	X.	—		—		—	+	—	+	—	
	XI.	—		—		—	+	—	+	—	
Closterium parvulum NAEG.	VI.	0.40		—		—		—		—	
	VIII.	—		—		—	+	0.02		—	+
	IX.	—		—		0.02		0.12		—	
	X.	—		—		—	+	—	+	—	
Closterium parvulum var. *angustum*	VIII.	—		—		0.40		—		—	
W. et G. S. WEST	IX.	—		—	+	—		0.20	+	—	

Closterium polystictum NYGAARD	V.	—		—		—		—		0.02		
	VI.	—		—		—	+	0.12	+	—		
	VII.	—		—		0.02		—		—		
	VIII.	—		—		—		0.05		—		
	X.	—		—		—		—		—	+	
Closterium praelongum BRÉB.	X.	—		0.15		—		—		—		
Closterium pronum BRÉB.	VII.	—		—		—		0.10		0.12		
	IX.	—		—		—		0.10		0.08		
Closterium strigosum BRÉB.	V.	—		—		—		0.09		0.06		
	VI.	0.27		—		—		—		—		
	VII.	0.07		0.05		—		0.10		—		
	VIII.	—		—		—		—		0.09		
	IX.	0.03		0.08		0.01		—	N	—	+	
	X.	—		0.02		—		0.10		0.10		
									+		+	
Closterium venus KÜTZ.	XI.	—		—		—		—		—		
**Cosmarium bioculatum* BRÉB.	VII.	—		—		—		—		—		N
	IX.	—		—		—		0.10		0.08		
Cosmarium botrytis MENEGH.	VII.	—		—		—		—		—		N
Staurastrum gracile RALFS	V.	—	+ N	—	+	0.20	+	0.10	+ N	0.16		N
	VI.	0.27	+ N	0.10	+	0.41	+	0.21	+ N	0.80		N
	VII.	—		0.09		1.51	+	0.62	+ N	0.45	+ N	
	VIII.	0.07		0.40		2.40	+	1.50	+ N	0.92	+ N	
	IX.	—	+	0.40	+	1.80	+	0.56	+ N	1.09	+ N	
	X.	—	+	0.15	+	0.15	+	0.20	N	0.30	+ N	
	XI.	0.10		0.10	+	0.10	+	0.20	+	0.14	+	
Staurastrum paradoxum MEYEN	V.	—	N	0.05	+	0.02	+	—	+ N	0.01		N
	VI.	0.07	+ N	—	+	0.20	+	—	+ N	—		N
	VII.	—	+ N	0.07	+	2.60	+	0.67	+ N	0.24	+ N	
	VIII.	0.07	+	0.50	+	1.20	+	1.10	+ N	0.80	+ N	
	IX.	0.16	N	0.10	+	0.40	+	0.76	+ N	1.12	+ N	
	X.	0.10	+	0.15	+	0.10	+	0.12	+ N	0.10	+ N	
	XI.	0.10	+ N	0.10	+	0.09	+	0.10	+	0.12	+	

Table 1 (continued)

Species	Period	Locality									
		M		*K*		*G*		*A*		*E*	
		i/1	No. 25	*i*/1	No. 25	*i*/1	No. 25	*i*/1	No. 25	*i*/1	No. 25
MYCOPHYTA *Phycomycetes*											
**Phlyctidium globosum* Skuja	VIII.	—	+	—	+	—		—		—	
	IX.	—	N	—		—		—		—	
Dactylosporium sp.	V.	—		—		—		—		0.12	
	VI.	—		0.30		—		0.10		—	
	VII.	—		—		—		—		0.08	
	VIII.	—		1.20		—	+	0.02		—	
	IX.	0.03		—		—		—		—	
	X.	—		—		—	+	—		—	
Asterothrix rhaphidioides (Reinsch) Printz	V.	—	+	—		—		—		—	
	VIII.	—		—	+	—		—		—	

section Szigliget—Balatonmária in this year. In the same section, we noted the high value 52220/liter already in July. The species *Diplopsalis acuta* and *Gonyaulax apiculata* approached in some localities 2000 individuals per liter.

The percentual occurrence of the phylum in the investigated localities varied, in the scooped samples between 0—33% during the period May—November. The phylum constituted 3% of the total algal species.

Phylum Mycophyta was represented by 3 species. Among these, *Asterothrix rhaphidioides* and a *Dactylosporium* sp. were already known from Lake Balaton (Hortobágyi, 1949; Tamás, 1965, 1967). The third species, *Phlyctidium globosum* Skuja (Skuja, 1956, p. 367, *Table 63, Figs. 1—5)* appeared as the parasite of *Aphanizomenon flos-aquae* during its water-bloom. At the time of the water-bloom, this fungus attached the spores of the *Aphanizomenon* filaments, and an infection of about 1% could be demonstrated in the population.

The percentual occurrence of phylum Mycophyta varied, on the basis of the scooped samples, between 0—0.4% during the period May—November. The phylum constituted 2% of the total algal species.

With respect to the number of species (63) and also individuals (1,718,820/liter), the August sample taken in section Szigliget—Balatonmária was the richest of all 15 localities. The poorest in number of species was the May sample in section Szigliget—Balatonmária (21),and in individual number the November sample taken between Balatonalmádi—Balatonvilágos (20250/liter).

The species *Ceratium hirundinella*, *Cyclotella bodanica*, and *C. ocellata* were present in all localities, during the entire period of investigation (May—November).

Phylum Chrysophyta reached the highest numbers and percentual values, occurring in 72—97.2% in the samples of Keszthely-bay, and in 54.1—96.6% in section Szigliget—Balatonmária (see *Tables 1, 2)*.

The highest individual number refers, contrary to our experience in the preceding year, to phylum Cyanophyta in the August sample of section Szigliget—Balatonmária (1,475,730/liter). This high individual value was the result of the *Aphanizomenon* water-bloom which extended in the entire width of Lake Balaton, from Keszthely-bay on even beyond section Szigliget—Balatonmária. The mass of blue algal filaments relegated the algae of the other phyla completely to the background (see *Table 1)*. The changes in its individual numbers are well traceable by the data of our collections deriving from 26 July, 23 August, and 21 September *(Table 1)*. On 26 July, the value was 200400/liter in section Ságpuszta—Balatonszemes, and no more than about 20000/liter in Keszthely-bay. Though the September values decreased to about half of those in August, they were still considerable (695200—978000/liter).

The organic pollution getting into the area of Keszthely-bay (Fenékpuszta, slaughter-house, ratting-pits, sewage-waters of Büdösárok) affected favourably the mass proliferation of *Aphanizomenon*. The development and decline of the water-bloom took two entire months. The temperature of the water was, aside of some very small fluctuations, above 20 °C beginning with the middle of June *(Figs. 1, 2)*, and a comparative calm reigned in this SW part of Lake Balaton from the end of July till the end of September.

All water-blooms, hitherto observed and published from Lake Balaton (Sebestyén, 1934; Entz and Sebestyén, 1946, p. 282; Hortobágyi, 1962), had ceased after some hours owing to sudden outbreaks of squalls or strong

Table 2

The distribution of specific numbers per algal phyla number of individuals per litre,

Systematic group	Period	*M*			*K*		
		Number of species	*i/l*	%	Number of species	*i/l*	%
CYANOPHYTA (15)	V.	4	1.26	0,7	2	0.60	0.3
	VI.	3	1.60	0.5	7	10.90	4.2
	VII.	5	28.41	12	4	16.10	10
	VIII.	4	1121.71	86.2	7	1475.73	86
	IX.	3	695.46	96.1	3	978.45	96.4
	X.	2	1.48	1.7	5	0.77	2
	XI.	4	0.47	2	4	0.49	2
EUGLENOPHYTA (14)	V.	5	0.54	0.3	3	0.29	0.2
	VI.	5	1.48	0.5	5	1.87	0.7
	VII.	4	1.40	0.6	5	0.87	0.5
	VIII.	6	6.01	0.5	8	13.20	0.7
	IX.	3	0.33	0	2	0.30	0
	X.	5	0.39	0.4	5	0.48	1
	XI.	6	0.54	2.2	4	0.32	1
PYRROPHYTA (5)	V.	1	0.08	0.1	1	1.16	0.7
	VI.	3	21.27	7	4	14.84	6
	VII.	4	27.12	12	4	53.32	33
	VIII.	4	20.95	1.6	4	124.61	7.2
	IX.	3	4.18	0.8	3	14.40	1.4
	X.	2	0.08	0.1	3	0.18	1
	XI.	2	0.04	0.2	2	0.05	0
CHRYSOPHYTA (78)	V.	21	180.67	96.7	6	169.80	96.6
	VI.	9	271.33	89	10	221.51	86
	VII.	15	165.37	72	19	87.53	54.1
	VIII.	15	143.57	11	23	95.12	5.5
	IX.	11	22.61	3	10	18.69	2
	X.	18	86.58	97.2	25	30.71	91
	XI.	11	21.97	91.2	15	23.06	91
CHLOROPHYTA (70)	V.	13	4.25	2.2	9	3.89	2.2
	VI.	19	8.39	3	13	6.66	3
	VII.	11	7.71	3.4	12	3.96	2.4
	VIII.	19	8.68	0.7	20	8.96	0.5
	IX.	7	0.80	0.1	14	2.48	0.2
	X.	4	0.50	0.6	14	1.54	5
	XI.	10	1.05	4.4	11	1.37	6
MYCOPHYTA (3)	V.	—	—	—	—	—	—
	VI.	—	—	—	1	0.30	0.1
	VII.	—	—	—	—	—	—
	VIII.	—	—	—	1	1.20	0.1
	IX.	1	0.03	0	—	—	—
TOTAL (185)	V.	44	186.80	100	21	175.74	100
	VI.	39	304.07	100	40	256.08	100
	VII.	39	230.01	100	44	161.78	100
	VIII.	48	1300.92	100	63	1718.82	100
	IX.	28	723.41	100	32	1014.32	100
	X.	31	89.03	100	42	33.68	100
	XI.	33	24.07	100	36	25.29	100

and per cent, on the basis of collections in 1966 (ind./l. = 1000 individuals per litre)

Locality								
G			*A*			*E*		
Number of species	*i*/l	%	Number of species	*i*/l	%	Number of species	*i*/l	
3	1.80	1.6	3	1.52	1.6	5	1.45	6
4	3.40	1.5	3	1.65	1.5	3	1.80	1.4
6	206.10	52	9	13.70	10.2	5	10.13	10.4
5	82.15	52	10	23.10	22	5	37.40	38.3
10	24.70	35.3	10	27.27	40	6	39.46	41.2
6	1.34	6	6	0.70	3	2	0.26	1.1
4	0.46	1.3	4	0.37	1	3	0.36	1.7
3	0.46	0.4	5	0.60	0.6	2	0.03	0.1
4	0.92	0.4	4	0.45	0.4	—	—	—
6	3.88	1	6	4.54	3.4	5	3.01	3.2
8	3.49	2	4	0.74	0.7	4	0.50	0.5
5	0.82	1.1	5	1.26	2	4	1.27	1
6	0.36	1.6	6	0.52	2	6	0.56	2
6	0.46	1.3	5	0.40	2	4	0.36	1.7
3	3.93	3	3	5.35	6	3	4.05	17
4	9.90	4.4	4	9.17	9	4	12.20	10
4	28.77	7	3	8.65	6.4	4	19.62	20.3
4	26.77	17	4	10.84	10.2	4	25.06	25.7
3	9.96	14.2	3	5.60	8	3	16.49	17.2
3	0.10	0.4	3	0.14	1	3	0.10	0.4
2	0.03	0.1	2	0.03	0.1	2	0.04	0.1
7	101.50	88	19	77.59	86	13	13.01	53.7
11	197.70	89	30	93.80	88	10	100.50	80.9
22	138.11	35	23	95.19	71	17	53.05	55
19	32.89	21	33	58.45	55	26	24 98	25.7
13	15.67	22.4	20	23.21	34	16	26.32	28
14	18.63	84	21	19.99	84	17	19.14	82
13	33.18	93	16	21.53	87.9	14	16.54	82
16	8.09	7	15	5.28	5.8	23	5.53	22.8
15	10.53	4.7	10	1.37	1	10	9.68	7.7
21	19.30	5	25	12.12	9	22	10.60	11
20	13.45	8	19	12.83	12.1	22	9.59	9.8
19	18.63	27	22	11.04	16	22	12.12	12.6
16	1.76	8	18	2.36	10	22	3.42	14.5
13	1.56	4.3	16	2.15	9	16	2.95	14.5
—	—	—	—	—	—	1	0.12	0.4
—	—	—	1	0.10	0.1	—	—	—
—	—	—	—	—	—	1	0.08	0.1
—	—	—	1	0.02	0	—	—	—
—	—	—	—	—	—	—	—	—
32	115.78	100	45	90.34	100	47	24.19	100
38	222.45	100	52	106.54	100	27	124.18	100
59	396.16	100	66	134.20	100	54	96.49	100
56	158.75	100	71	105.98	100	61	97.53	100
50	69.78	100	60	68.38	100	51	95.66	100
45	22.19	100	54	23.71	100	50	23.48	100
38	35.69	100	43	24.48	100	39	20.25	100

winds. On 20 September, 1962, the water in front of the Biological Research Institute at Tihany turned to a greenish tinge owing to the mass occurrence of *Aphanizomenon flos-aquae* (150,000/liter) in the open water. The phenomenon lasted for 14 days. It was again repeated, though with smaller values (64,000/liter) in September, 1963, and lasted until the end of the month (TAMÁS, 1965. p. 100, *Table 1).* At that time, the temperature of the water was around 20 °C until 27 September, and calm, rainless weather reigned for several weeks.

In Keszthely-bay, the mass occurrence of *Dinobryon divergens* in July, 1965, was followed by the water-bloom, extending over all three sections of the south-western part of Lake Balaton, of *Asterionella formosa — Melosira granulata — M. granulata* var. *angustissima*, appearing in individual numbers running to a million per litre (TAMÁS, 1967, p. 222). The *Asterionella — Melosira* complex gave place to a sporadic *Microcystis—Aphanizomenon* water-bloom in September (TAMÁS, 1967, p. 222), with rather detrimental effects on the diatomaceous population living on the surface of the mud (TAMÁS, 1966, p. 197).

The four species as yet unpublished from Lake Balaton *(Chlamydomonas intermedia, Scenedesmus anomalus, Cosmarium bioculatum, Phlyctidium globosum)* are marked by ✳ in *Table 1.*

The over-proliferation of algae by the effects of organic pollution is a well-known phenomenon in literature (EDMONDSON, 1968). The problems of Keszthely-bay (FÜZESI and SÁGI, 1966) are further aggravated by the series of repeated water-blooms and discolorations which, owing to the disagreable smell of the decomposing algae and their slimy masses, render the water of the lake unsuitable for bathing.

Summary

The author studied 413 scooped and 112 filtrated plankton samples taken from 15 localities of the 5 standard transverse sections of Lake Balaton, in the period May—November, 1966. The 165 identified species, 17 varieties, and 3 forms belong, together with the aquatic fungi, to 6 systematic phyla, in the order of frequency of occurrence, to the Chrysophyta (78), Chlorophyta (70), Cyanophyta (15), Euglenophyta (14), Pyrrophyta (5), and Mycophyta (3).

Of the 15 localities, the August sample deriving from section Szigliget—Balatonmária was the richest both as to the number of species (63) and individuals (1,718,820/liter). The poorest in the number of species (21) was the May sample from Szigliget—Balatonmária, and in the number of individuals (20,250/liter) the November sample from Balatonalmádi—Balatonvilágos.

Deviating from the situation in the preceding year, it was phylum Cyanophyta which reached the highest individual numbers (1,475,730/liter) in the August sample of section Szigliget—Balatonmária. This high value referred to the water-bloom of *Aphanizomenon flos-aquae* var. *klebahnii*, extending from Keszthely-bay on even beyond section Szigliget — Balatonmária, in the entire width of Lake Balaton. The immense mass of this filamentous blue-green alga relegated all other algal species to the background. During the time of the water-bloom, the presence of the aquatic fungus, *Phlyctidium globosum* SKUJA, in the spores of *Aphanizomenon* was also significant, causing an infection of about 1 per cent.

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HORIZONTÁLIS PLANKTONVIZSGÁLATOK A BALATONON VII. A TÓ FITOPLANKTONJÁRÓL AZ 1966 ÉVI MERÍTETT MINTÁK ÉS HÁLÓSZÜREDÉK ALAPJÁN

Tamás Gizella

Összefoglalás

Szerző 1966 évben májustól novemberig a tó 5 harántszelvényének 15 gyüjtőhelyéről 413 merített és 112 hálószüredék mintát vizsgált. A meghatározott 165 faj, 17 változat, 3 forma a vizigombákkal együtt 6 rendszertani törzsbe tartozik, gyakorisági sorrendben: Chrysophyta 78, Chlorophyta 70, Cyanophyta 15, Euglenophyta 14, Pyrrophyta 5, Mycophyta 3.

A 15 gyüjtőhely közül fajszámban (63) és egyedszámban is (1,718.820/liter) a Szigliget-Balatonmária augusztusi mintája volt a leggazdagabb. A legszegényebb pedig fajszámban (21) a Szigliget-Balatonmária májusi, egyedszámban a Balatonalmádi-Balatonvilágos novemberi mintája (20.250/lit) volt.

A legmagasabb egyedszámot — a korábbi évtől eltérően — a Cyanophyta törzs érte el a Szigliget-Balatonmária közötti szelvény augusztusi (1,475.730/lit) mintájában Ez a magas szám az *Aphanizomenon flos aquae* var. *klebahnii* vízvirágzás következménye volt, mely a tó teljes szélességében a Keszthelyi-öböltől a Szigliget-Balatonmária harántszelvényen is túl terjedt. Más algatörzsekhez tartozó fajok jelenlétét háttérbe szorította ez a fonalas kékalga tömeg. A vizvirágzás idején jelentős volt az *Aphanizomenon* kitartósejtjeiben lévő *Phlyctidium globosum* SKUJA vizigomba. Az állományban mintegy 1%-os fertőzöttség volt kimutatható.

ГОРИЗОНТАЛЬНЫЕ ИССЛЕДОВАНИЯ ПЛАНКТОНА ОЗЕРА БАЛАТОН VII. О ФИТОПЛАНКТОНЕ ОЗЕРА ПО ОБРАЗЦУ САЧКА И ПОГРУЖЕННОЙ ПРОБЫ

Г. Тамаш

Было изучено 413 образцов погруженных сачков и 112 проб фильтрата сачка, собранных в 15 местах пяти поперечных сечений озера с мая по ноябрь 1966 года. В ходе исследования было определено 165 видов, 17 разновидностей, 3 формы и некоторые водяные грибы, которые относятся к 6 классам по следующему ряду: Chrysophyta 78, Chlorophyta 70, Cyanophyta 15, Euglenophyta 14, Pyrrophyta 5, Mycophyta 3. Из изученных образцов самым богатым оказался и по числу встречающихся видов (63) и по численности отдельных видов (1.718.820/л) образец, собранный в Сиглигет—Балатонмарии в августе. По числу видов (21) самым бедным оказался образец, собранный в мае в Сиглигет—Балатонмарии, а по численности отдельных видов наиболее бедный образец (20.250/литр) был найдён в ноябре в Балатонмарии—Балатонвилагош.

В отличии от результатов, полученных в предыдущих годах, в наивысщей численности был обнаружен вид Cyanophyta в образце Сиглигет—Балатонмарии, собранной в августе (1.475.730/литр). Обнаруженное высокое число являлось результатом массового распространения *Aphanizomenon flos aquae* var. *klebahnii* от Кестхейского залива до Сиглигет—Балатонмарии. Остальные виды водорослей были угнетены в этой огромной массой синезеленых водорослей. Во время массового появления *Aphanizomenon* были обнаружены и водяные грибы, *Phlyctidium globosum* SKUJA.. Приблизительно один процент от водорослей был заражен в этими грибами.

CHRONICLE

The 1968 year was the last one in a period of the 3 years' research plan. Two scientific departments continued investigations as it was scheduled.

1. *Department of Experimental Zoology* has carried out investigations on Pelecypoda and Gastropoda within the main topic "Physiological and morphological specificities of neurohumoral regulation on invertebrates", according to the following themes:
   a) Investigations of the microscopic and submicroscopic structures, elementary physiological processes and biochemical characteristics of the nervous system on cellular level;
   b) Investigations of rhytmic functioning of effector organs (close muscles) on glochidia and adult forms of the Anodonta.
   c) Investigations of the chemical agents taking part in the regulation of heart activity and their mode of action.
   d) Investigations of the complex regulatory function of the nervous system in ecological, functional-morphological and enzymo-chemical aspects.
2. Investigations of the *Department of Hydrobiology* were carried out within the main theme "Hydrobiological investigations of Lake Balaton" in the following aspects:
   a) Continuous and systematical investigations of plankton and benthos of Lake Balaton.
   b) Comparative investigations on the properties of proteolitic enzymes in several species of Crustacea.
   c) Studies on the fat metabolism of fish and other water-organisms.

Dr. Olga Sebestyén, the former head of the Department of Hydrobiology, now retired, continued her work on the theme "Paleolymnological investigations on microfossiles of Lake Balaton sediments".

Results of the work performed by the members of the two Departments were published partly in *Annal. Biol. Tihany* 35, and partly in different Hungarian and foreign journals (see *Annal. Biol. Tihany*, 1969. 36, p. 301).

The Institute's permanent staff was in 1968: 54 personals divided as follows: 19 scientific research workers, 14 technical assistants, 6 administrative and 15 other workers.

On the 1st of April Dr. I. Zs.-Nagy scientific research worker was granted Ph. D. for his dissertation entitled "Cytological investigations on the central nervous system of *Anodonta cygnea* L."

S. HERODEK obtained a title of the doctor of University on the basis of his work entitled "Investigations of tryglicerid synthesis on the fat tissue incubated with 1-^{14}C-palmitic acid."

The following changes took place in the scientific staff of the Institute: Dr. F. LUKACSOVICS scientific research worker left for Higher Technical School of Catering, Budapest, on the 16th of January. CS. CSABA biologist-geologist on the 1st of July 1968 was posted as an assistant scientific co-worker to the Department of Experimental Zoology.

Inland study trips

Dr. I. ZS.-NAGY scientific research worker visited the Institute of Pathological Anatomy of the Medical School in Pécs for one week and I. KISS assistant scientific co-worker spent a week at the Physiological Institute of Debrecen Medical School.

Travels abroad

J. OLÁH assistant scientific co-worker has finished his study trip on the 19th of March in Borok (Soviet Union) lasting for 6 months.

Dr. I. ZS.-NAGY scientific research worker worked, following an invitation, in the Institute of the Experimental Gerontology in Basel from the 16th of April to the 15th of June and attended the Fourth European Regional Conference held in Rome, in 1—7 September.

Dr. E. LÁBOS scientific research worker has finished his 8 months long study trip granted by ASTEF in Marseille on the 25th of June.

Dr. K. S.-RÓZSA senior scientific research worker following the invitation of the St. Andrews University worked in the Gatty Marine Laboratory (Scotland) from the 29th of June to the 31th of August.

Dr. J. SALÁNKI director attended XXIV International Physiological Congress, held in Washington from the 24th of August to the 9th of September; later on he was on a study trip in the GDR from the 6th of October to the 27th of the same month.

T. FARKAS scientific research worker attended the Third International Symposium on Drugs Affecting Lipid Metabolism, held in Milan in 7—16 September; further, he was on a study trip in the Soviet Union from the 23rd of September till 18th of November.

Dr. J. PONYI deputy head of the Department of Hydrobiology was on a study trip in Poland from the 29th of November till 18th of December.

I. VARANKA assistant scientific research worker visited the Soviet Union from the 24th of November till 2nd of December.

Dr. B. ENTZ deputy director has been working in Ghana in the whole year as an expert.

Visiting scientists from inland and abroad

Similarly to the previous years also in 1968 several investigators worked in the Institute:
Within the frame of cooperation visitors were as follows:

A. SAKHAROVA spent two weeks and D. A. SAKHAROV spent 3 months in the Institute from Moscow, Institute for Developmental Biology. They worked at the Zoological Department.

Professor G. MÜLLER spent one week, and his co-workers G. GASTNER and H. SCHÄFFER one month from Heidelberg (GFR),

Dr. R. KLEINE one month from Halle (GDR), and

Dr. G. HEDER worked one week on the Hydrobiological Department from Berlin (GDR).

Besides the above recounted a short visit was paid by the following scientists: Prof. A. A. ALBANESE (USA), Dr. E. HENTSCHEL (GDR), V. LUPEA (RPR), Prof. A. MENDIA (Italy), R. PISARSKA (Poland), Dr. M. REZK (UAR), Prof. L. RUDESCU (RPR), M. TELEGUT (RPR), Dr. M. VOLKENSTEIN (Soviet Union).

The following Hungarian scientists worked here as visiting research workers or visited the Institute by the aim of exchanging experiences;

From B u d a p e s t:

Prof. Dr. G. ÁDÁM, Eötvös Loránd University, Department of Comparative Physiology,

Dr. G. CSÁKVÁRI, Medical University, Institute of Pathophysiology,

Dr. E. DONÁSZY OMMI,

Dr. L. FELFÖLDY, G. KONTUR and R. VÁSÁRHELYI, VITUKI

Dr. G. GÁRDOS, Central Institute of Haematology and Blood Transfusion

Dr. H. KALÁSZ, Pharmacological Institute,

KOVÁCSNÉ Dr. É. MURAI, Dr. I. MATSKÁSI, Dr. I. MÉSZÁROS and F. SZIKLAI, ELTE, Zoological Collection,

B. MADARÁSZ postgraduate student, Eötvös Loránd University, Institute of Zoology

Dr. S. TÓTH, Medical University, Institute of Gerontology

Dr. I. TÖRŐ, Jr. Dr. Sz. VIRÁGH, Medical University, Institute of Histology and Embriology

B. TURCSÁNYI, Central Research Institute for Chemistry of the Hungarian Academy of Sciences

Dr. GY. RÓZSA, Director of the Library of the Hungarian Academy of Sciences and his co-workers M. SIMON and B-NÉ BÜKY paid two days visit to the Library of the Institute.

From D e b r e c e n:

Prof. Dr. G. BOT, Dr. M. CSORNAI and Dr. G. VEREB, Medical University, Institute of Medical Chemistry

J. SERFŐZŐ, Kossuth Lajos University, Department of Zoology,

Dr. K. VEZEKÉNYI, Medical University, Institute of the Dermatology.

From K a p o s v á r:

Dr. F. CSONTI, KÖJÁL.

From P é c s:

Dr. M. GARAMVÖLGYI, Biophysical Institute

From S z e g e d:

Prof. Dr. A. ÁBRAHÁM, Dr. F. BICZÓK, and Dr. A. STAMMER, József Attila University, Department of Zoology

Prof. Dr. B. CSILLIK, Medical University, Institute of Anatomy,

Dr. G. Uherkovich, Station for Tisza Research,

During the summer months 14 Hungarian and 3 foreign university students joined the Institute's work for 3—4 weeks.

In the 1968 year the Scientific Council of the Institute was recreated by the Biological Department of the Hungarian Academy of Sciences as consequence of the reorganization of special committees in 1967.

The Scientific Council of the Institute:

E. Dudich academician
I. Törő academician
G. Ádám D. Sci.
B. Csillik, M. D., D. Sci.
T. Hortobágyi, D. Sci.
B. Entz, Ph. D.
O. Fehér, M. D., Ph. D.
G. Gárdos, Ph. D.
J. Ponyi, Ph. D.
K. S.-Rózsa, Ph. D.
J. Salánki, Ph. D. Director of the Institute, head of the Council
J. Szegi, Ph. D.

Meetings

In the course of the year 1968 four meetings were held at the Institute:

1. Between the 7th and 13rd of June retraining course for the teachers of biology in the secondary schools was held with 20 participants by the Biological Department of the Hungarian Academy of Sciences.
2. From the 19th to the 23rd of June Summer School in the field of nuclear physics in the organization of the Central Research Institute of Physics of the Hungarian Academy of Sciences with the participation of 45 scientists.
3. Between the 17th—19th September a Colloquium entitled: "Biological membranes and their specialization" was held by the Biological Department of the Hungarian Academy of Sciences with some 50 scientists.
4. Between the 3rd—5th October "Hydrobiological Days" attended by 30 participants was organized by the Hydrobiological Society and the Department of Hydrobiology of the Institute.

Improvement in research facilities

The equipment park was improved among others by a TEKTRONIX oscilloscope Typ 555, two NFPK research microscopes, a refrigerator of great capacity.

At the end of 1968 the Library of the Institute registered 43709 volumes. Over 610 different periodicals are being received currently, among them 12 is in "Abstracts".

The Institute's year-book, *Annal. Biol. Tihany*, Vol. 35. (1968) was sent to 579 Institutions all over the world, in exchange the Library received 345 different journals and publications.

KRÓNIKA

Az 1968-as év a hároméves tervciklus utolsó éve volt. A két tudományos osztály tervének megfelelően dolgozott:

1. A *Kísérletes Állattani Osztály* „A neurohumorális szabályozás fiziológiai sajátosságainak vizsgálata gerinctelen állatokon" — című témán belül az alábbi résztémákat tanulmányozta Pelecypodák és Gastropodák vonatkozásában:

a) Az idegrendszer mikroszkópos és szubmikroszkópos szerkezetének, elemi élettani jelenségeinek, valamint biokémiai sajátosságainak kutatása,

b) Ritmikusan működő effektor szervek (záróizom) működésének vizsgálata lárvákon, illetve kifejlett kagylókon,

c) A szívműködés szabályozásában szerepet játszó kémiai ágensek és azok hatásmechanizmusának vizsgálata,

d) Az idegrendszer komplex regulációs működésének vizsgálata, ökológiai, élettani, funkcionális morfológiai és enzimkémiai szinten.

2. A *Hidrobiológiai Osztály* vizsgálatait a „Balaton hidrobiológiai kutatása" c. főtémán belül az alábbi résztémákban folytatta:

a) A Balaton folyamatos és rendszeres plankton, bentosz és nekton vizsgálata.

b) Néhány rákfaj proteolytikus enzimének tulajdonsága és összehasonlító vizsgálata,

c) Zsíranyagcsere vizsgálatok halakon és egyéb vízi szervezeteken.

Dr. Sebestyén Olga nyugalmazott osztályvezető továbbra is aktív tudományos tevékenységet fejtett ki az Intézetben. Munkáját „Paleolimnológiai vizsgálatok balatoni üledékekben" c. témakör keretében folytatta.

A két osztályon dolgozó kutatók tudományos tevékenységét tükröző dolgozatok részben az *Annal. Biol.* 35. kötetében, részben más hazai és külföldi folyóiratokban kerültek publikálásra (lásd *Annal. Biol. Tihany* 1969, 36, 301 oldal).

Az Intézet személyi állománya: 54 fő, ami a következőképpen oszlott meg, kutató: 19, kutatási segéderő: 14, adminisztratív: 6, egyéb: 15.

Dr. Zs.-Nagy Imre tudományos munkatárs április 1-én megvédte: „Citológiai vizsgálatok a tavi kagyló *(Anodonta cygnea* L.) központi idegrendszerében" c. kandidátusi disszertációját.

Herodek Sándor tudományos munkatárs egyetemi doktori címet nyert „A triglicerid szintézis vizsgálata 1-^{14}C-palmitinsavval inkubált zsírszövetben" c. munkája alapján.

Az Intézet kutatói állományában az alábbi változások történtek: dr. Lukacsovics Ferenc tudományos munkatárs január 16-ával a Felsőfokú Élelmiszeripari Technikumba (Budapest) távozott. Csukás Csaba biológia-földrajz szakos tanár 1968. július 1-vel tudományos segédmunkatársi kinevezéssel az Intézet Kísérleti Állattani Osztályára került.

Belföldi tanulmányutak:

Dr. Zs.-Nagy Imre tudományos munkatárs és Kiss István tudományos segédmunkatárs 1—1 hetet töltött a Pécsi Orvostudományi Egyetem Kórbonctani, illetve a Debreceni Orvostudományi Egyetem Élettani Intézetében.

Külföldi utak:

Oláh János tudományos segédmunkatárs 1968. március 19-vel fejezte be 1967-ben megkezdett 6 hónapos tanulmányútját Borokban (Szovjetunió).

Dr. Zs.-Nagy Imre tudományos munkatárs meghívásra a Bázeli Gerontológiai Intézetben dolgozott április 16—június 15-ig, továbbá részt vett a Rómában szeptember 1—8-ig megrendezett IV. Európai Regionális Elektronmikroszkópos Kongresszuson.

Dr. Lábos Elemér tudományos munkatárs június 25-vel fejezte be 1967-ben megkezdett, 8 hónapos ASTEF ösztöndíjas tanulmányútját Marseilleban.

Dr. S.-Rózsa Katalin tudományos főmunkatárs meghívásra június 29—augusztus 31-ig a St. Andrewsi Egyetem Gatty Marine Laboratóriumában dolgozott.

Dr. Salánki János igazgató augusztus 24—szeptember 9-e között részt vett a Washingtonban megrendezésre került XXIV. Nemzetközi Élettani Kongresszuson, továbbá október 6—27-ig terjedő időben tanulmányúton volt az NDK-ban.

Farkas Tibor tudományos munkatárs részt vett szeptember 7—16 között a „Third International Symposium on Drugs Affecting Lipid Metabolism" c. rendezvényen, Milánóban, majd szeptember 23—november 18-a között tanulmányúton volt a Szovjetunióban.

Dr. Ponyi Jenő a Hidrobiológiai Osztály mb. vezetője november 29—december 18-ig tanulmányúton volt Lengyelországban.

Varanka István tudományos segédmunkatárs november 24—december 22-ig a Szovjetunióban volt tanulmányúton.

Dr. Entz Béla igazgatóhelyettes egész év folyamán Ghanában dolgozott, szakértői minőségben.

Vendégkutatók

Az előző évekhez hasonlóan több külföldi és hazai kutató dolgozott az Intézetben.

Tudományos együttműködés keretében

Alla Sakharova 2 hetet, D. A. Sakharov (SzU, Moszkva) 3 hónapot dolgozott a Kísérletes Állattani Osztályon,

G. Müller professzor (NSzK, Heidelberg) 1 hetet, valamint két munkatársa, M. Gästner és H. Schäffer 1 hónapot,

Dr. R. Kleine (NDK, Halle) 1 hónapot,

Dr. G. Heder (NDK, Berlin) 1 hetet dolgozott a Hidrobiológiai Osztályon.

Fentieken kívül pár napos látogatást tettek az Intézetben Prof. A. A. Albanese (USA), Dr. E. Hentschel (NDK), V. Lupea (RPR), Prof. A. Mendia (Olaszország), R. Pisarska (Lengyelország), Dr. M. Rezk (Egyesült Arab Köztársaság), Prof. L. Rudescu (RPR), M. Telegut (RPR), Dr. M. Volkenstein (SzU).

Hazai kutatók közül vendégkutatóként dolgoztak, ill. tapasztalatcsere céljából keresték fel az Intézetet:

Budapestről

Dr. Ádám György (ELTE Állatélettani Tanszék)
Dr. Csákvári Gábor (BOTE Kórélettani Intézet)
Dr. Donászy Ernő (OMMI Vízélettani Osztálya)
Dr. Felföldy Lajos, Kontur György, Vásárhelyi Réka (VITUKI)
Dr. Garamvölgyi Miklós (Orvostovábbképző Intézet)
Dr. Gárdos György (Orsz. Vértranszfúziós Szolgálat Központi Kutató Intézete)
Kalász Huba (Gyógyszertani Intézet)
Kovácsné dr. Murai Éva, dr. Matskási István, dr. Mészáros István, Sziklai Ferenc (a Nemzeti Múzeum Állattárából)
Madarász Bálint egyetemi hallgató ELTE Általános Állattani Tanszéke
Dr. Tóth Sándor (BOTE Gerontológiai Intézet)
Ifj. dr. Törő Imre, dr. Virágh Szabolcs (BOTE Szövet- és Fejlődéstani Intézet)
Turcsányi Béla (MTA KKKI)
Dr. Rózsa György a MTA Könyvtárának igazgatója és két munkatársa Simon Mária és Büjy Béláné 2 napos látogatást tettek a Könyvtárban.

Debrecenből

Dr. Bot György, dr. Csornai Márta, dr. Vereb György az Orvostudományi Egyetem Orvosi Vegytani Intézetéből,
Serfőző József a Kossuth Lajos Tudományegyetem Állattani Intézetéből,
Dr. Vezekényi Klára az Orvostudományi Egyetem Bőrklinikájáról

Kaposvárról

Dr. Csonti Ferenc (KÖJÁL)

Pécsről

Dr. Garamvölgyi Miklós a Biofizikai Intézetből

Szegedről

Dr. Ábrahám Ambrus, dr. Biczók Ferenc, dr. Stammer Aranka a József Attila Tudományegyetem Állattani Intézetből,
Dr. Csillik Bertalan a Szegedi Orvostudományi Egyetem Anatómiai Intézetéből,
Dr. Uherkovich Gábor a Tiszakutató Állomástól.

A nyári hónapokban 14 hazai és 3 külföldi egyetemi hallgató kapcsolódott be az intézet munkájába 3—4 hétre.

Az 1968-as évben a Biológiai Tudományok Osztálya ismét létrehozta az Intézet Tudományos Tanácsát, minthogy a szakbizottságok 1967 folyamán történt átszervezése során nem alakult olyan Bizottság, amely az intézeti Tudományos Tanács feladatait elláthatná.

A Tudományos Tanács

elnöke: dr. Salánki János a biológiai tudományok kandidátusa, intézeti igazgató,
tagjai: Dudich Endre akadémikus
Tőrő Imre akadémikus
Ádám György a biológiai tudományok doktora
Csillik Bertalan az orvostudományok doktora
Hortobágyi Tibor a biológiai tudományok doktora
Entz Béla a biológiai tudományok kandidátusa
Fehér Ottó az orvostudományok kandidátusa
Gárdos György a biológiai tudományok kandidátusa
Ponyi Jenő a biológiai tudományok kandidátusa
S.-Rózsa Katalin a biológiai tudományok kandidátusa
Szegi József a biológiai tudományok kandidátusa

Rendezvények

1968 folyamán 4 nagyobb rendezvény került lebonyolításra az Intézetben:

1) június 7—13-ig Biológus tanárok továbbképzése a MTA Biológiai Tudományok Osztályának rendezésében. Résztvevők száma: 20
2) Június 19—23. Magfizikai Nyári Iskola a MTA Központi Fizikai Kutatóintézete rendezésében, 45 fő részvételével.
3) Szeptember 17—19. Biológiai membránok és specializációjuk c. kollokvium a MTA Biológiai Tudományok Osztályának rendezésében, 50 fő részvételével.
4) Október 3—5 között Hidrobiológus Napok a Hidrológiai Társaság és az Intézet Hidrobiológiai Osztálya rendezésében, 30 fő részvételével.

Kutatási feltételek fejlődése

Az év folyamán beszerzett jelentősebb műszerek: kétsugaras Tektronix oszcilloszkóp Typ 555
2 db NFPK kutató mikroszkóp
nagyteljesítményű hűtőgép

Az évvégi összesítés alapján az intézeti könyvtár állomány 43 709 egység. Az év folyamán összesen 610 különböző folyóirat és kiadvány járt az Intézetbe, köztük 12 referáló jellegű.

Az intézeti Évkönyv — *Annal. Biol. Tihany* 35. kötetét 579 címre küldtük meg, melyért cserébe 345 kiadvány érkezett.

LIST OF PAPERS PUBLISHED ELSEWHERE AS IN VOL. 35 OF OUR ANNALES

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Herodek S.: Temporal changes in the distribution of labeled palmitic acid in the different lipids of rat's adipose tissue, liver and diaphragm. — *Acta Biochem. et Biophys. Acad. Sci. Hung.* **3,** 227—237.

Lábos E.: On the mechanism of anisotropic properties of excitability for adductor-response of glochidia. — In: *Neurobiology of Invertebrates* (Ed.: J. Salánki) *Akadémiai Kiadó, Budapest and Plenum Press, New York,* 1968. pp. 293—301.

Lábos E., T. Pécsi, J. Salánki: On the synchrony control of the two adductors in the fresh-water mussel *Anodonta cygnea* L. — *Acta Biol. Acad. Sci. Hung.* **19,** 381—390.

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S.-Rózsa K.: Cyclic-3,5-AMP as a second messenger of excitatory influences on the heart of *Helix pomatia.* — In: *Neurobiology of Invertebrates* (Ed.: J. Salánki) *Akadémiai Kiadó, Budapest and Plenum Press, New York,* 1968. pp. 327—334.

Salánki J.: Role of cerebral ganglia in the regulation of activity in freshwater mussel *(Anodonta cygnea* L.). — In: *Neurobiology of Invertebrates* (Ed.: J. Salánki) *Akadémiai Kiadó, Budapest and Plenum Press, New York,* 1968. pp. 493—501.

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1. ZOOLOGY — ÁLLATTAN — ЗООЛОГИЯ

2. HIDROBIOLOGY — HIDROBIOLÓGIA — ГИДРОБИОЛОГИЯ

A kiadásért felel az Akadémiai Kiadó igazgatója Műszaki szerkesztő: Merkly László

A kézirat nyomdába érkezett: 1969. V. 28 Példányszám: 900 Terjedelem: 26,95 (A/5) ív + 3,5 (A/5) iv műmelléklet

69.67732 Akadémiai Nyomda, Budapest — Felelős vezető: Bernát György