A MAGYAR TUDOMÁNYOS AKADÉMIA
TIHANYI BIOLÓGIAI KUTATÓINTÉZETÉNEK ÉVKÖNYVE
(1970)
(VOL. XXXVII.)

ANNALES INSTITUTI BIOLOGICI (TIHANY) HUNGARICAE ACADEMIAE SCIENTIARUM

TIHANY, 1970

ANNAL. BIOL. TIHANY

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Szerkesztő:

S.-Rózsa Katalin

ANNAL. BIOL. TIHANY	37	3—15	HUNGARIA 1970

CYTO-TOPOGRAPHICAL STUDIES ON THE CENTRAL NERVOUS SYSTEM OF LYMNAEA STAGNALIS L.

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Received: February 2nd 1970

The first anatomical description of the central nervous system of *Lymnaea stagnalis* L. was written by DE LACAZE DUTHIERS in 1872. Later works (PELSENEER, 1901; ELO, 1938; CARRIKER, 1946; MOUSSA, 1950; HEKSTRA and LEVER, 1960; JOOSSE, 1964) have furnished further valuable data on the anatomy and histology of the nervous system of the pond snail *Lymnaea stagnalis* L. There are, however, unclarified question in this field such as the denomination of the different ganglia, which is not uniform even in most recent works (JAZMIZINA et al., 1968; KARNAUKOV et al., 1968). The nervous nature of the cells of the mediodorsal bodies first described about 70 years ago (PELSENEER, 1901) has been the subject of ample discussions (CARRIKER, 1946; MOUSSA, 1950; JOOSSE, 1964) but the role of these cells has remained unelucidated up to the present (BOER, 1965; BULLOCK and HORRIDGE, 1965; TAUC, 1966).

The purpose of the present work was to perform a cytological study on the central nervous system of *Lymnaea stagnalis* L. with special regard to the location of large neurons within the ganglia.

Material and method

For the investigations medium-sized specimens of *Lymnaea stagnalis* L. weighing 14—16 g were used. After dissection the central nervous system was fixed in CARNOY solution and in 8% formol. Embedding in paraffin was preceded by a careful orientation of the ganglia. Serial sections were cut at 7—8 μ thickness. Sectioning was made in the parallel plane of the caudal surface of the central nervous system. For staining a mixture of pyronine (GT Gurr, England) and malachite green (Edard Gurr, No 315) was employed, according to the method of BAKER and WILLIAMS (1965). From the serial sections light micrographs were made on which the largest diameter of each neuron was measured. According to their size the nerve cells were classified in five groups:

1. Neurons smaller than 50 μ
2. ,, from 50 to 100 μ
3. ,, from 100 to 150 μ
4. ,, from 150 to 200 μ
5. ,, larger than 200 μ

The nerve cells of various sizes were counted and determined. Determination of cells over 50 μ was made with precision while small cells were determined with a deviation of $\pm 5\%$. The micrographs made from serial sections of a ganglion were divided in 3 equal zones:

Caudal third of the ganglion (zone I)
Median third ,, ,, ,, (zone II)
Oral third ,, ,, ,, (zone III)

By this division the demonstration of the neurons in three zones of depth was rendered possible.

For the investigations we have used the central nervous systems of three medium-sized specimens of *Lymnaea stagnalis* L. for cell counts and 5—7 ganglia from each ganglion type for the determination of the distribution within the ganglion of cells exceeding 100 μ in diameter.

Results

The number of neurons found in the central nervous system of *Lymnaea stagnalis* L. and their distribution according to size are shown in *Tables 1—3.* As can be seen from the *Tables*, the central nervous systems of snails of about the same size and weight differ as regards the number of cells they contain. The distribution of nerve cells in the different ganglia displayed a certain regularity. The proportion of cells of different sizes was more or less similar in all the three central nervous systems.

Buccal ganglion

Of all paired ganglia of the central nervous system of *Lymnaea stagnalis* L. the buccal ganglia are the smallest in size connected by the longest connectives (cerebro-buccal connective) to the cerebral ganglia and at the same time they contain the smallest number of nerve cells. Most neurons larger than 100 μ in diameter were found in zone I and no cells over 150 μ were present in zone III. Cells over 200 μ in size were not seen in any of the three zones. Most neurons over 100 μ in size were located in the part of the ganglion facing the cerebral ganglion, in the area between the issue of the cerebro-buccal connective and bucco-buccal commissure *(Fig. 1)*. The number of neurons comprised in the right and left ganglia was nearly identical *(Tables 1—3)*. The n. gastricus anterior was found to be surrounded in a ring-like manner by 60—80 small nerve cells forming a marked bulging *(Fig. 2)* in the ganglion. The picture is similar to that displayed by the group of bag cells in *Aplysia* (Toevs and Brackenbury, 1969).

Cerebral ganglion

The small cells of the mediodorsal and laterodorsal bodies were not counted among the cells of the cerebral ganglion as they differ from the cells of the central nervous system in their morphology and physiology. The cerebral ganglion was found to contain 1700—2200 nerve cells. According to our data there is no essential difference between the right and left cerebral ganglia as

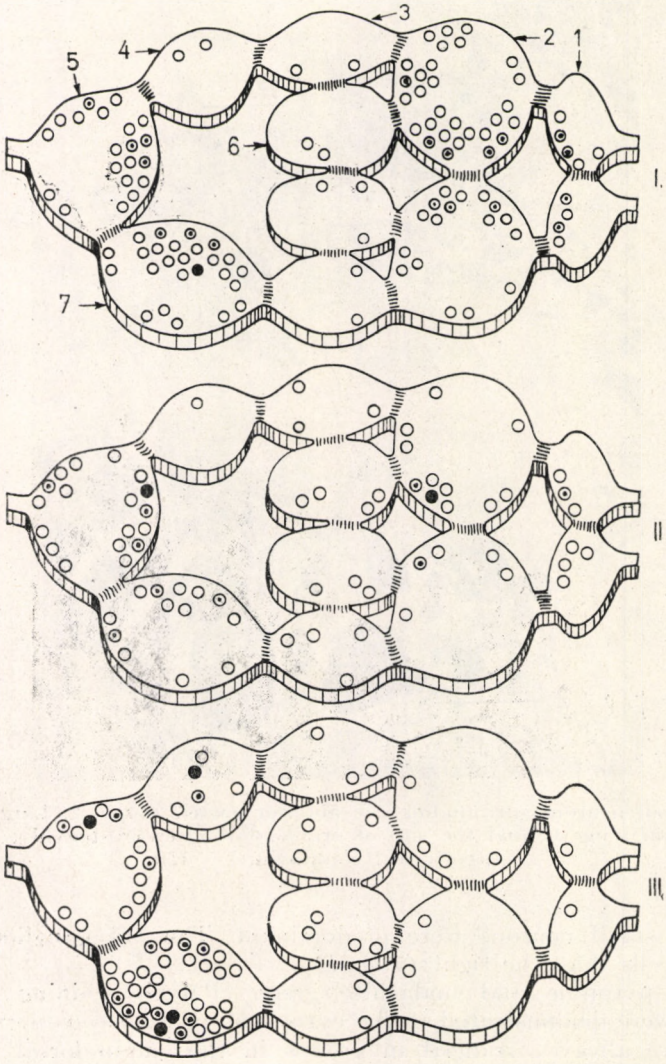

Fig. 1. Distribution of large (exceeding 100 μ in diameter) neurons in the central nervous system of *Lymnaea stagnalis* L.
zone I = caudal third; zone II = median third; zone III = oral third (basal part)
1. Left buccal ganglion; 2. Left cerebral g.; 3. Left pleural g.; 4. Left parietal g.; 5. abdominal g.; 6. left pedal g.; 7. right parietal g. (parieto-abdominal ganglion)
⊙ = neurons 100—150 μ in diameter; ○ = neurons 150—200 μ in diameter;
● = neurons larger than 200 μ in diameter

regards the number of cells. Neurons over 200 μ in diameter were rarely observed. The majority of neurons over 100 μ in diameter were localized, similarly to those in the buccal ganglion, in the caudal part (zone I), while in zone III only 3—4 such neurons were found. Large cells occurred mainly around the cerebro-cerebral commissure (Ccc) and in the anterior lobe. In the

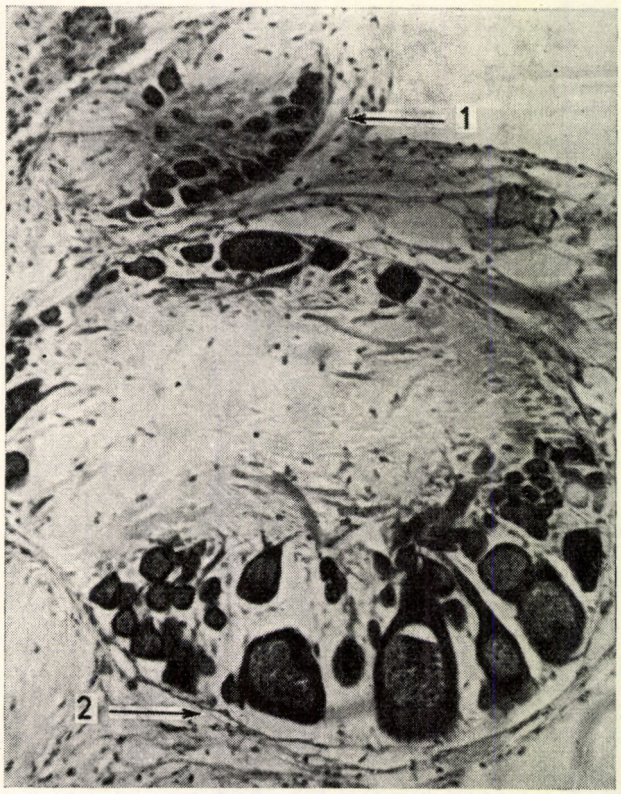

Fig. 2. 1. Small neurons surrounding the anterior gastric nerve. 2. Large cells of the buccal ganglion lying around the site of origin of the cerebro-buccal connective and bucco-buccal commissure. ×150

lateral lobe small neurons were predominant. The left ganglion contained more large cells than the right *(Fig. 1)*.

Using pyronine and malachite green (PMaG) staining disk-shaped formations were demonstrated in the cytoplasm of numerous nerve cells. The neurons were always arranged in groups in the caudo-dorsal area of the ganglion around the site of origin of the cerebro-cerebral commissure. Their number varied from 25 to 70. In the right cerebral ganglion the number of such cells was always higher (50—70 cells). The largest diameter of cells ranged from 70 to 140 μ.

Pleural ganglion

About 90—93 per cent of the neurons are small, under 50 μ in diameter. No cells larger than 150 μ occurred in any of the ganglia examined. The number of cells ranged from 800 to 1000. In all the specimens of *Lymnaea stagnalis* the cells of the right ganglion numbered about 100 more than those

TABLE 1

Cell number and size distribution of neurons in the different ganglia of Lymnaea stagnalis L. *weighing 14 g*

Ganglion	under 50 μ		50—100 μ		100—150 μ		150—200 μ		over 200 μ		Total number
	number	%	number	%	number	%	number	%	number	%	
Buccal (left)	470	87.2	59	10.9	7	1.3	3	0.6	—	—	539
Buccal (right)	432	85.5	60	11.9	11	2.2	2	0.4	—	—	505
Cerebral (left)	1512	90.5	111	6.7	35	2.1	12	0.7	—	—	1670
Crebral (right)	1460	88.0	180	10.8	18	1.1	2	0.1	—	—	1660
Pleural (left)	650	91.9	48	6.8	9	1.3	—	—	—	—	707
Pleural (right)	711	89.9	69	8.7	11	1.4	—	—	—	—	791
Parietal (left)	457	93.1	26	5.3	3	0.6	1	0.2	4	0.8	491
Parietal (right)	1265	83.2	189	13.3	49	3.5	12	0.8	5	0.4	1520
Pedal (left)	1775	89.4	202	10.2	9	0.4	—	—	—	—	1986
Pedal (right)	1893	88.8	225	10.5	12	0.6	1	0.05	—	—	2131
Abdominal	840	78.9	176	16.5	39	3.7	8	0.7	2	0.2	1065
Total number of cells in the central nervous system	11465	87.7	1345	10.3	203	1.6	41	0.3	11	0.08	13065

TABLE 2

Cell number and size distribution of neurons in the different ganglia of Lymnaea stagnalis L. *weighing 16 g*

Ganglion	under 50 μ		50—100 μ		100—150		150—200 μ		over 200 μ		Total number
	number	%	number	%	number	%	number	%	number	%	
Buccal (left)	438	89.8	42	8.6	8	1.6	—	—	—	—	488
Buccal (right)	432	89.6	43	8.9	6	1.3	1	0.2	—	—	482
Cerebral (left)	1514	84.8	241	13.5	28	1.5	3	0.2	—	—	1786
Cerebral (right)	1455	85.3	214	12.5	33	1.9	5	0.3	—	—	1707
Pleural (left)	899	90.6	81	8.3	11	1.1	—	—	—	—	981
Pleural (right)	965	92.6	71	6.8	5	0.5	1	0.1	—	—	1042
Parietal (left)	465	87.8	58	10.9	9	1.7	2	0.3	—	—	534
Parietal (right)	1510	78.0	335	17.3	67	3.4	23	1.2	1	0.05	1936
Pedal (left)	1866	87.0	267	12.4	13	0.6	—	—	—	—	2146
Pedal (right)	1974	88.3	249	11.1	11	0.5	1	0.04	—	—	2235
Abdominal	836	76.2	197	18.0	45	4.1	16	1.4	3	0.3	1097
Total number of cells of the central nervous system	12344	85.5	1798	12.4	236	1.6	52	0.4	4	0.03	14434

of the left ganglion *(Tables 1—3)*. Cells larger than 100 μ in size were few in number and scattered in the ganglion. Two or three of such cells were localized in each of the three zones *(Fig. 1)*.

Parietal ganglion

The number of cells in the two ganglia is not identic, the right ganglion containing about three times more nerve cells than the left *(Tables 1—3)*.

About 90—93% of the neurons in the left parietal ganglion are small (under 50 μ in diameter). Nearly all large cells are located in zone III. While

TABLE 3

Cell number and size distribution of neurons is the different ganglia of Lymnaea stagnalis L. *weighing 16 g*

Ganglion	under 50 μ		50—10 μ		100—150 μ		150—200 μ		over 200 μ		Total number
	number	%	number	%	number	%	number	%	number	%	
Buccal (left)	509	91.2	45	8.1	4	0.7	—	—	—	—	558
Buccal (right)	544	92.0	43	7.3	4	0.7	—	—	—	—	591
Cerebral (left)	2056	91.3	189	8.3	8	0.4	—	—	—	—	2253
Cerebral (right)	2107	90.9	205	8.8	5	0.2	1	0.05	—	—	2318
Pleural (left)	916	94.7	47	4.8	5	0.5	—	—	—	—	967
Pleural (right)	1015	92.8	74	6.7	5	0.5	—	—	—	—	1094
Parietal (left)	597	94.5	32	5.1	2	0.3	1	0.1	—	—	632
Parietal (right)	1425	83.6	225	12.5	43	2.4	9	0.5	2	0.1	1704
Pedal (left)	2086	88.1	274	11.6	8	0.3	—	—	—	—	2368
Pedal (right)	2127	87.8	289	11.9	6	0.2	1	0.04	—	—	2423
Abdominal	810	78.2	183	17.6	36	3.5	4	0.4	3	0.3	1036
Total number of cells in the central nervous system	14192	89.0	1605	10.0	126	0.8	16	0.1	5	0.03	15944

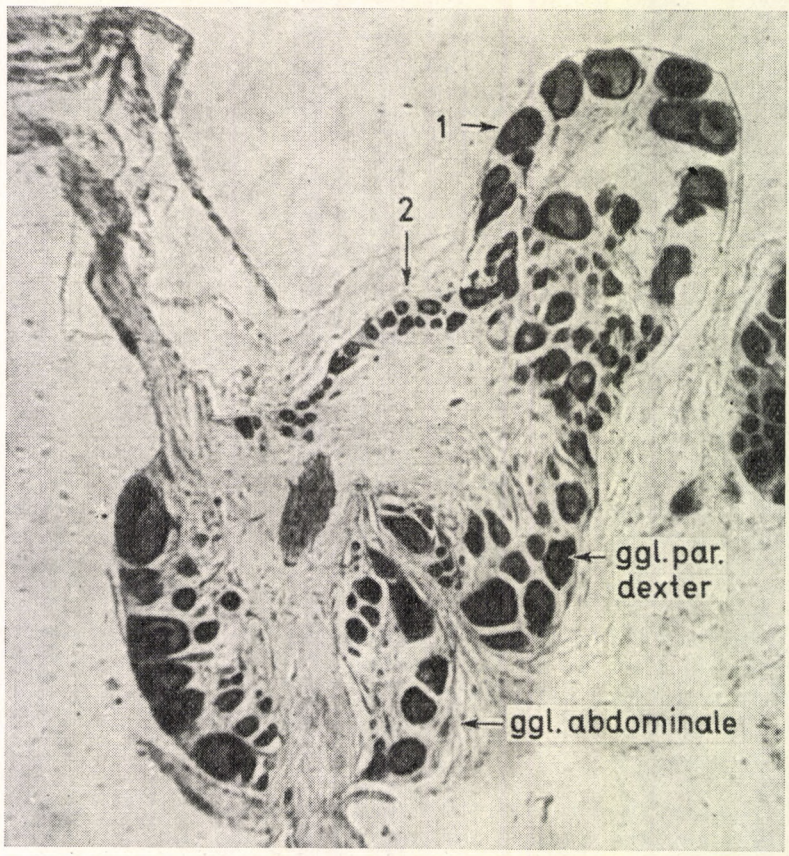

Fig. 3. Micrograph of the oral part of the right parietal ganglion. 1. Lateral part; 2. Medial part ×40

in zone I and II there are but one or two large cells, their number rises to 4—8 in zone III, some of them exceeding 200 μ in size. In our material consisting of 6 left parietal ganglia we have invariably found two neurons over 150 μ in the basal part (zone III) of the ganglion.

In addition to the difference in cell number between the two ganglia, the right parietal ganglion differs from the left also in structure and distribu-

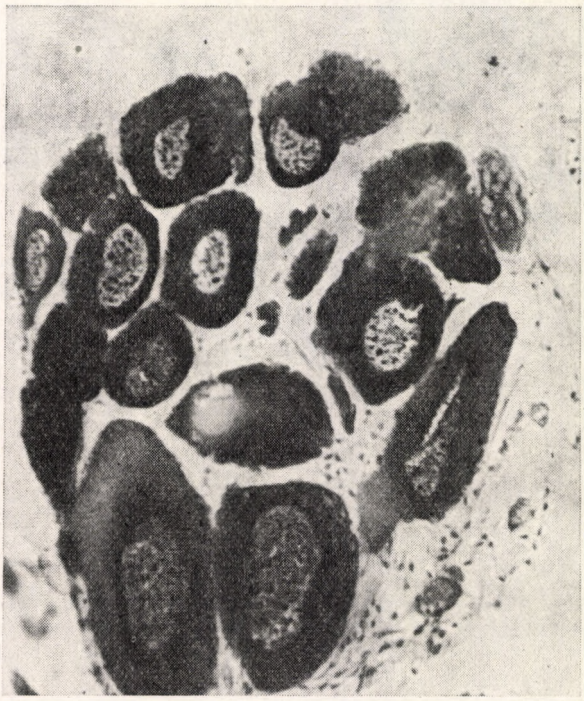

Fig. 4. Large cells in the basal part (zone III) of the right parietal ganglion not surrounded by small cells. ×200

tion of cells of various sizes. It may be divided into a medial and a lateral part. In the latter area of the ganglion a marked protuberance is present which is even more conspicuous in histologic section *(Fig. 3)*. Such a protuberance is always absent in the left ganglion. A further difference between the two ganglia is the strikingly high number of large cells (among them one or two exceeding 200 μ in size) in the caudal part of the right parietal ganglion. The median part (zone II) consists mainly of small cells. The large cells present in this zone are usually surrounded by numerous small cells under 50 μ in diameter. Similarly to the left ganglion, the oral part (zone III) of the right one contains many large cells, most of them located in the lateral part of the ganglion. The large cells in this zone are rarely surrounded by small cells *(Fig. 4)*. In some specimens the examination disclosed nerve cells outside the ganglion, in the immediate vicinity of the connective tissue. In one case the larger part of the neuron was lying in the connective tissue between the abdominal and the right parietal ganglion, while its minor part was within the ganglion *(Fig. 5)*.

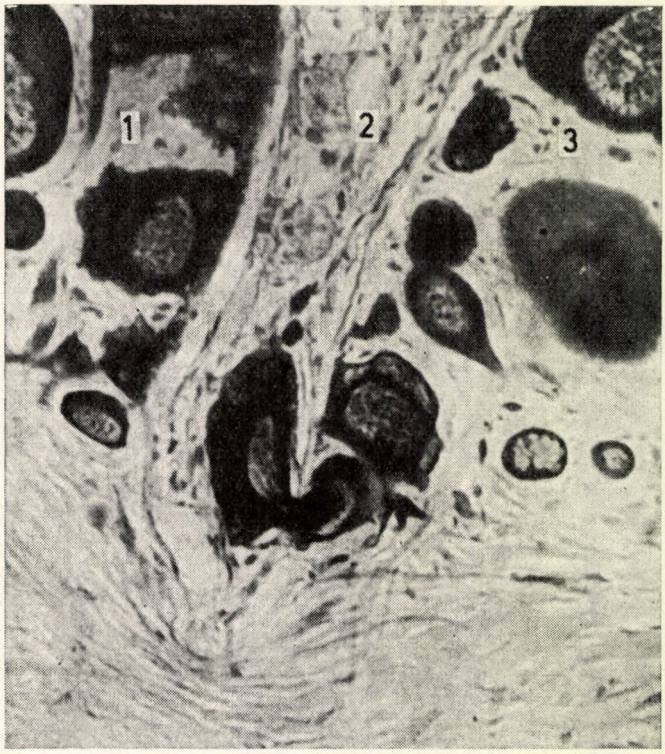

Fig. 5. Neuron lying in the connective tissue between the abdominal and right parietal ganglion. A minor part of the cell is located in the right parietal ganglion.
1. abdominal ganglion; 2. connective tissue between the ganglia; 3. parieto-abdominal ganglion. ×200

Abdominal ganglion

The percentage ratio of small cells is only about 76—78 in this ganglion. In the central nervous system the abdominal ganglion is the richest in cells exceeding 100 μ in diameter. Cells over 150 μ in size were also found to be relatively numerous and large cells over 200 μ in size were found in all the specimens examined. Numerical distribution of large cells was nearly identic in all three zones. Most of them were located in the area facing the cerebral ganglion, between the sites of origin of the two parieto-abdominal connectives *(Fig. 1)*. The large cells located here were usually close to each other and in most cases they were not surrounded by small cells.

Pedal ganglion

Of all the ganglia the pedal ganglion contains the highest number of neurons, about 4500—5000, which is about 30—31% of all the cells of the central nervous system. About 88—89% of the cells are small in size (under 50 μ). Cells exceeding 150 μ in diameter were only observed occasionally, and cells over 200 μ were never encountered in the specimens examined. While

1.4% of all the cells of the central nervous system are about 100—150 μ in diameter, this percentage ratio in the pedal ganglion is but 0.4—0.5 *(Tables 1—3)*.

Neurons larger than 100 μ in size are scattered in the ganglion, each zone containing about 3—5 such cells *(Fig. 1)*.

Discussion

From our results it appears that the central nervous system of *Lymnaea stagnalis* L. weighing 14—16 g contains about 13000—16000 nerve cells. The differences between certain specimens are so great that they cannot be attributed to errors due to calculation. The data shown in *Table 1* refer to specimens weighing 14 g and those shown in *Tables 2* and *3* to snails weighing 16 g. The difference in cell number is probably due to individual variations. According to KUNZE (1917) there is an individual difference in size in the large cells of the central nervous system of *Helix pomatia* L.

The various ganglia within the central nervous system contain different numbers of nerve cells. The fewest neurons are in the buccal and the most numerous in the pedal ganglion. No appreciable difference was found in the number of cells between the left and right ganglion of the paired buccal, cerebral and pedal ganglia. In the case of the buccal and pedal ganglia this may be explained by the fact that the ganglia situated on the left and right side have a common area of innervation. The right and the left cerebral ganglia have different number of main nerve branches. The single nervus penis arises from the right cerebral ganglion. As according to our data, the two cerebral ganglia contain identic number of nerve cells, it seems probable that in the innervation of the nervus penis the left cerebral ganglion is likewise involved.

The right pleural ganglion contains by 60—100 neurons more than the left one. At present we cannot give a satisfactory explanation of this finding.

As regards the number of neurons the greatest difference can be found between the two parietal ganglia, the right containing about three times more cells than the left. It should be noted that KUNZE (1917) found but a slight difference in the number of neurons between the right and left parietal ganglia of *Helix pomatia* L. About 88—89% of the cells of the central nervous system of *Lymnaea stagnalis* are smaller than 50 μ in diameter, whereas the number of large cells (over 200 μ) is insignificant. In the basal part of the parietal ganglion and in the abdominal ganglion of the specimens examined (5—7 pieces) one to three giant cells were nearly always present, while in the cerebral ganglia a giant cell was seldom encountered *(Fig. 1)*. As regards ganglionic distribution and localization of cells over 100 μ in diameter a certain regularity was noted.

The large cells of the buccal ganglion were always found in the caudal part (zone I), around the sites of origin of the cerebro-buccal connective and bucco-buccal commissure. In the oral part of the ganglion (zone III), large cells were very rare. A similar distribution was noted in the large cells of the cerebral ganglia, as well. The left ganglion was always found to contain more large cells than the right one *(Fig. 1)*. The majority of the large cells are located around the site of origin of the cerebro-cerebral commissure, where

Nissl cells ranging from 70 to 140 μ in size are also present. According to BOER (1965) the Nissl cells are in relation with the small nerve cells of the medio-dorsal and medio-lateral bodies. Their number in the left ganglion is about 50—70 which is about the double of the Nissl cells found in the right ganglion.

In the pleural and pedal ganglia there are very few cells exceeding 100 μ in diameter. Their numerical distribution is about the same in all three zones of these ganglia. In the pleural ganglion of *Helix pomatia* the number of large cells is likewise low but in the pedal and parietal ganglia their number is much higher (KUNZE, 1917).

The abdominal ganglion is characterized by the presence of large cells. About 4—5% of all its cells are larger than 100 μ in size displaying an identic numerical distribution in all three zones, in particular in the area of the origin of the two parieto-abdominal commissures (PAC), as can be seen in *Fig. 1.*

As regards location of large cells, the left parietal ganglion differs from all the other ganglia, zones I and II containing but a few large cells, while zone III is relatively rich in them. Two large cells are always present in the basal part of the ganglion, one of them sending direct fibers to the nerve branch innervating the heart (GUBICZA and S.-RÓZSA, 1969), while the fibers emitted by other are directed towards the cerebral ganglion and left pallial nerve (JAZMIZINA, 1968).

There is a difference in location of the large cells between the right and left parietal ganglia. In the right ganglion their number is always high in zone I, relatively reduced in the median zone (II) and high again in zone III. The considerably higher number of cells over 100 μ in diameter and their different location in the right parietal ganglion raises the question whether these ganglia are an identic pair. The dissimilarity between them seems to be confirmed by the difference in their size, the right ganglion being much larger than the left, and by the presence of a well visible protuberance in its lateral part. Therefore, some authors (NABIAS, 1899; KARNAUKOV et al., 1968) suggested the right parietal ganglion to be termed as "large" and the left one as "small". Such a distinction between these ganglia does not seem to be entirely satisfactory as besides the difference in size, there are also other dissimilarities between them, e.g. the lateral protuberance differs in structure from the median part of the ganglion. A further difference is that two main nerves take origin from the right parietal ganglion, namely the right internal and the right external pallial nerves, while only one: the left pallial nerve arises from the left ganglion. Moreover, the innervation area of the right ganglion is larger than that of the left one (NABIAS, 1889; CARRIKER, 1946).

In a previous work (GUBICZA and S.-RÓZSA, 1970) we have reported that different results were obtained in the two parietal ganglia after trans-section of various nerve branches and connectives. The response of the right parietal ganglion to nerve trans-section was similar to that of the abdominal ganglion. As it has been demonstrated (SALÁNKI and KISS, 1969), some nerve cells of the abdominal and right parietal ganglion exhibit identic electro-physiological properties.

From our investigations it was concluded that the ganglion regarded as the right parietal one has come into being from the fusion of the right parietal and right abdominal ganglia, therefore; the term "parieto-abdominal ganglion" seems to be more appropriate for it. Correspondingly, for its connective to

the abdominal ganglion the term "parieto-abdomino-abdominal connective" (PAAC) and for its connective to the pleural ganglion the term "pleuro-parieto-abdominal connective (PPAC) are suggested.

Summary

From the results of cyto-topographic investigations performed on the central nervous system of *Lymnaea stagnalis* L. it was concluded that

1. The central nervous system of medium-sized specimens of *Lymnaea stagnalis* L. (weighing 14—16 g) contains about 13000—16000 neurons.

2. The pedal ganglion contains the highest and the buccal ganglion the lowest number of nerve cells.

3. The number of neurons in the right and left buccal, cerebral and pedal ganglia is nearly identic, whereas the right pleural ganglion in all the specimens examined contained more nerve cells than the left one.

In the right parietal ganglion the number of neurons was three times higher than in the left.

4. Most nerve cells exceeding 100 μ in diameter are comprised in the right parietal and the abdominal ganglia. No cells over 200 μ in diameter were found in the buccal, pleural and pedal paired ganglia.

5. In the distribution of large cells (over 100 μ in diameter) the following regularity was noted:

The majority of large cells are located in the caudal part (zone I) of the buccal and cerebral ganglia and in oral part (zone III) of the left parietal ganglion. In the other ganglia the number of large cells is nearly identic in all three zones.

6. On the basis of the anatomical and histological structure of the right parietal ganglion, cell number and distribution of large cells within it, and according to the data of earlier works, this ganglion should be regarded as one arisen from the fusion of the right parietal and right abdominal ganglia. Therefore, the term "parieto-abdominal ganglion" is suggested.

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A LYMNAEA STAGNALIS L. KÖZPONTI IDEGRENDSZERÉNEK CYTO-TOPOGRÁFIAI VIZSGÁLATA

Gubicza András

Összefoglalás

A *Lymnaea stagnalis* L. központi idegrendszerének cytotopográfiai vizsgálatainak adataiból az alábbi következtetések vonhatók le:

1. A közepes súlyú (14—16 gr-os) *Lymnaea stagnalis* L. központi idegrendszere 13—16 000 db idegsejtet tartalmaz.

2. A legtöbb idegsejt a ggl. pedalében, legkevesebb a ggl. buccalében van.

3. A ggl. buccale cerebrale és pedale jobb és bal dúcaiban közel azonos számú idegsejt van. A jobb ggl. pleurale minden vizsgált példánynál több idegsejtet tartalmazott, mint a bal. A ggl. parietale dexter háromszor annyi idegsejtet tartalmaz, mint a ggl. parietale sinister.

4. A központi idegrendszeren belül legtöbb 100 mikronnál nagyobb idegsejtet a ggl. parietale dexter és a ggl. abdominale tartalmaz. A ggl. buccale, ggl. pleurale és a ggl. pedale dúcpárban nincs 200 mikronnál nagyobb idegsejt.

5. A 100 mikronnál nagyobb idegsejtek dúcon belüli elhelyezkedésében az alábbi törvényszerűség tapasztalható:

A ggl. buccale és a ggl. cerebrale caudalis részében (I. zóna), a ggl. parietale sinister orális részében (III. zóna) találhatók a nagyméretű idegsejtek többsége, más dúcokban pedig mindhárom zónában közel azonos számban fordulnak elő *(1. ábra)*.

6. A jobb ggl. parietale-t az anatómiai szövettani szerkezete, sejtszáma s a nagyméretű idegsejtjeinek elhelyezkedése és a korábbi munkák eredményei alapján úgy kell tekinteni, mint két idegdúc — a ggl. parietale dexter és a ggl. abdominale dexter — összenövéséből létrejött ganglion. Ezért a ggl. parietale dexter helyett alkalmasabb a ganglion parieto-abdominale elnevezés.

ЦИТОТОПОГРАФИЧЕСКИЕ ИССЛЕДОВАНИЯ ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЫ

А. Губица

Из данных цитотопографических исследований центральной нервной системы большого прудовика были сделаны следующие выводы:

1. Центральная нервная система больного прудовика, со средним весом (14—16 гр), содержит 13 000—16 000 нейронов.

2. Больше всего содержится нейронов в педальных ганглиях меньше всего в буккальных ганглиях.

3. В правых и левых половинах буккального, церебрального и педального ганглиев содержатся нейроны приблизительно в одинаковом количесиве. У всех исследованных особей в правом плевральном ганглии было больше нейронов, чем в левом. Правый париетальный ганглий содержит в три раза больше нейронов, чем левый.

4. Нейронов, размером выше 100 мк, больше всего содержится в правом париеталном и в абдоминальном ганглиях. В буккальных, плевральных и педальных ганглиях не найдено нейронов выше 200 мк.

5. В расположении нейронов размером выше 100 мк, внутри ганглиев наблюдаются следующие закономерности:

Большинство гигантских нейронов найдены в буккальном ганглии, в каудальной части церебрального (I. зона) и в оральной части левого париетального ганглиев (III. зона); в других ганглиях во всех трёх зонах число нейронов одинаковое.

ANNAL. BIOL. TIHANY | 37 | 17—31 | HUNGARIA 1970

INVESTIGATIONS ON THE INTERGANGLIONIC AND PERIPHERAL NEURONAL PATHWAYS IN THE CENTRAL NERVOUS SYSTEM OF LYMNAEA STAGNALIS L.

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Received: January 22nd 1970

The simple structure of the central nervous system of *Lymnaea stagnalis* L., the large size and not too great number of its cells facilitate the investigations of morphologists and electrophysiologists on complex neural connections and central localization of physiological functions.

In a previous work (SALÁNKI and GUBICZA, 1969) we have determined the direct axonal connections between different ganglia and localization of neurons innervating the anterior and posterior adductors by the demonstration of retrograde regeneration following transection of various nerve branches in *Anodonta cygnea* L. The same method was employed to identify in *Lymnaea stagnalis* L. the central neurons involved in the direct innervation of the heart following transection of the branch of the intestinal nerve running to the heart (GUBICZA and S.-RÓZSA, 1969).

The purpose of the present work was to identify the neurons changed in consequence of transection of major nerve branches, and relying upon these findings, to describe the direct axonal connections, between different ganglia and organs.

Material and method

For the investigations medium-sized adult specimens of *Lymnaea stagnalis* L. were used. The snails were anaesthetized in a solution of 0.05—0.08% of nembuthal until complete relaxation was achieved. Then the central nervous system was exposed and the following commissures connectives and main nerve branches were cut:

1) cerebro-cerebral commissure
2) parieto-abdominal connective (left)
3) cerebro-buccal connective (left)
4) cerebro-pedal connective (left)
5) cerebro-pedal connective (right)
6) nervus labialis inferior (left)
7) nervus pallialis (left)
8) nervus pallialis (right)

Transection of each nerve branch was performed in 4—6 specimens. After the operative procedure the animals were placed in aquaria containing oxygenized circulating Balaton water for 24—48 hours. Only specimens that survived this post-operative period were used for histological preparations. After the

elapse of the period of 24—48 hours following operation the central nervous system was dissected out, fixed in Carnoy solution for 45 minutes, embedded in paraffin and serially sectioned (at 7—8 μ). Embedding was always preceded by careful orientation of the ganglia. Sectioning was made in a horizontal plane parallel with the horizontal posture of the animal. Staining of the sections was made with a mixture of malachite green (E. Gurr, No 315) and pyronine Y (GT Gurr, England) according to the method of BAKER and WILLIAMS (1965)

The sections were examined under the light microscope. In all the ganglia examined the neurons showing signs of regeneration were measured according to size and classified in three groups:

large cells (120 μ and larger)
medium-sized cells (from 50 to 120 μ)
small cells (under 50 μ)

Then the number of regenerating cells and their localization within the ganglion were determined. Localization of nerve cells was facilitated by dividing the sections of each ganglion into three equal zones:

dorsal third of central nervous system (zone I)
medial third ,, ,, ,, ,, (zone II)
ventral third ,, ,, ,, ,, (zone III)

On the basis of this division regenerating nerve cells exhibiting granular pyroninophilia could be localized in three zones of depth. The numbers of pyroninophilic cells given in the figures represent the mean values of 4—6 animals, with a limit of error of 10%. For control examinations 4 animals were used in which the central nervous system was only exposed without transecting any of the nerve branches. In addition 4 intact specimens were also processed. In the present work the results of experiments performed on 40 animals with nerve transection and 8 controls are reported.

Results

Transection of the cerebro-cerebral commissure (ccc)

After cutting the cerebro-cerebral commissure, in all the ganglia, except the buccal pair, cells showing signs of regeneration were present in varying numbers *(Fig. 1)*. About 40% of pyroninophilic cells appearing in the central nervous system after injury of the ccc were localized in the cerebral ganglia. In all, 153 neurons were found to send direct fibers to the ccc. Of these cells 59 were located in zone I, 55 in zone II and 39 in zone III. The approximate zonal localization of the cells is shown in *Fig. 1* and their numerical and size distribution in *Table 1*. The number of pyroninophilic cells appearing after injury of the ccc was nearly the same in the specimens examined.

No regularity was observed in the distribution of cells within the ganglion. There was, however, a difference in localization between the neurons of the left and right ganglia, except the parietal ganglia in which a giant cell was always present in the ventral third of each ganglion, localized symmetrically *(Fig. 1,* III). After transection of the ccc the left ganglia were found to contain

fewer pyroninophilic cells than the right ones. The difference was particularly great between the right and left parietal ganglion where the former contained 24 nerve cells, whereas the latter only 13 *(Fig. 1)*.

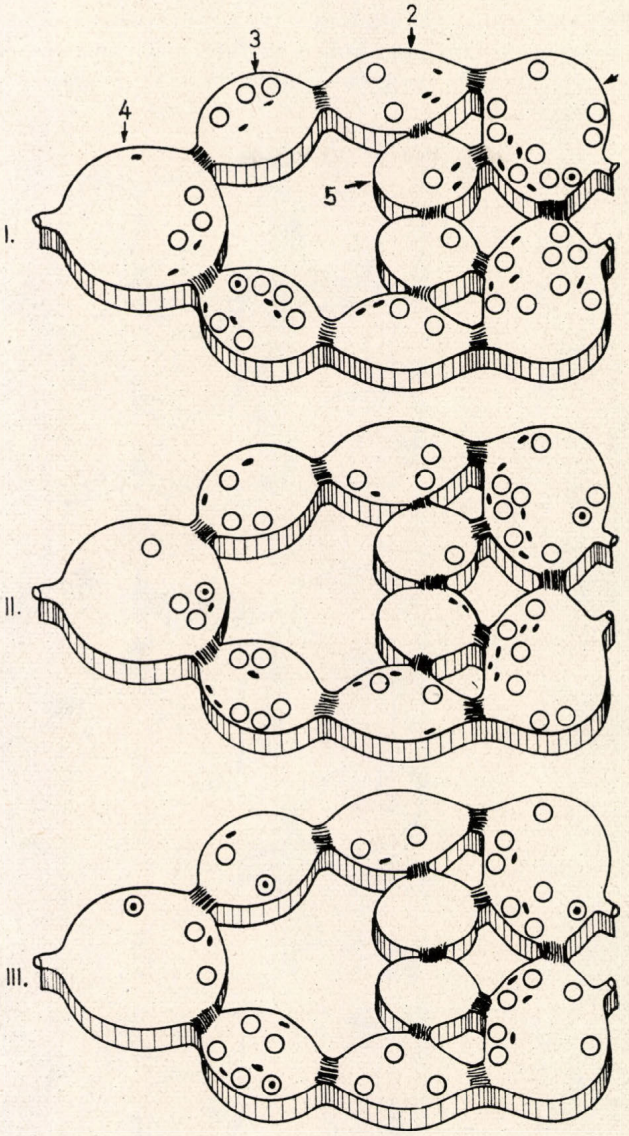

Fig. 1. Distribution of pyroninophilic nerve cells in the central nervous system of *Lymnaea stagnalis* L., after transsection of the cerebro-cerebral commissure
I = dorsal third, II = median third, III = ventral third of the central nervous system. Ganglia: 1. cerebral, 2. pleural, 3. parietal, 4. abdominal, 5. pedal. The left ganglia are marked on the *Figure*.
⊙ = large neurons (over 120 μ in diameter), ○ = medium-sized neurons (from 50 to 120μ)
– = small neurons (under 50 μ)
Legend to Figures refers to all the *Figures* (1—9)

By cutting the cerebro-cerebral commissure evidence was obtained that the cerebral ganglia receive nerve fibers from the contralateral pedal, pleural and parietal ganglia, as well as from the single abdominal ganglion. Thus, direct fibers are emitted from the left pedal, pleural and parietal ganglia and from the single abdominal ganglion to the right cerebral ganglion. On the other hand, the left cerebral ganglion is in direct axonal connection with the corresponding right ganglia and the single abdominal ganglion, at least in

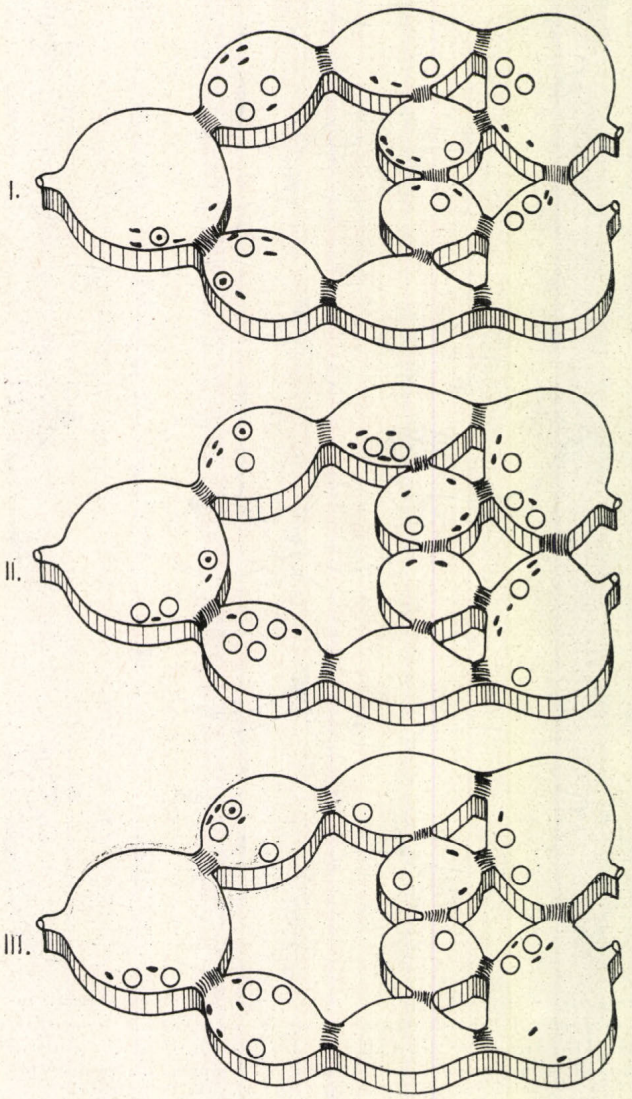

Fig. 2. Localization of pyroninophilic cells in the central nervous system following transection of the left cerebro-pedal connective

ascending direction. Existence of descending pathways from the cerebral ganglion to other ganglia was not demonstrable by cutting the ccc but only by transection of other connectives.

When cutting the ccc, in addition to the appearance of typical pyroninophilic cells, considerable changes were noted in the cells of the mediodorsal body attached to the cerebral ganglion. These cells are small, never exceeding 10 μ in diameter. Their nuclei are uncommonly large (7—10 μ) containing several small nucleoli hardly visible in the light microscope. In the control animals the diameter of the nucleoli was usually smaller than 1 μ,

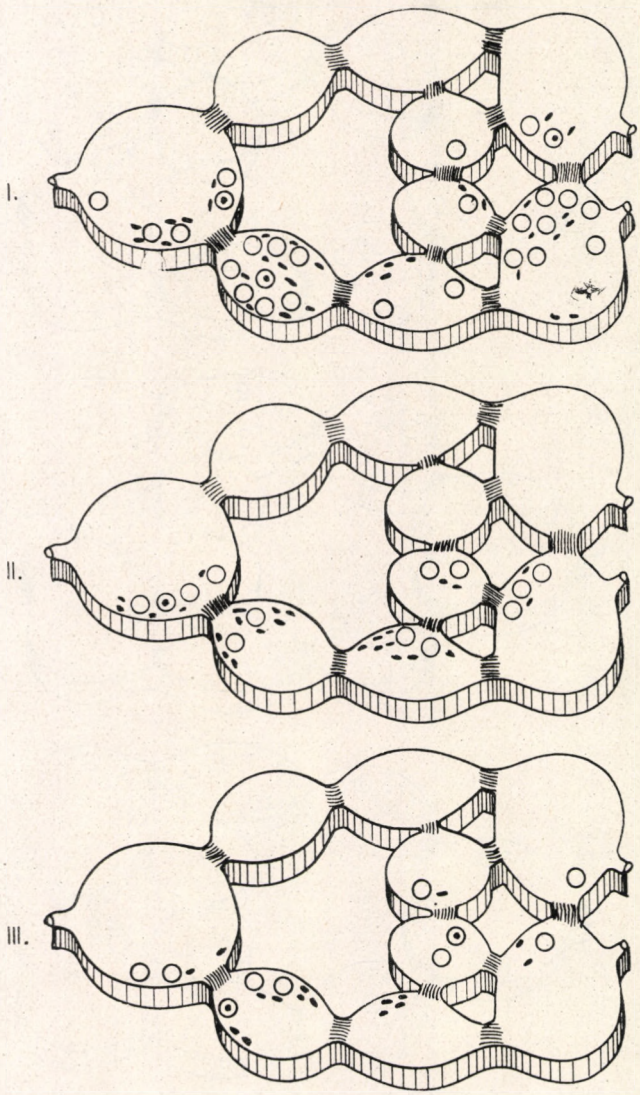

Fig. 3. Occurrence of neurons exhibiting granular pyroninophilia after cutting the right cerebro-pedal connective

whereas following transection of the ccc, the nucleoli in some cells of the mediodorsal body enlarged so much that they entirely filled the nuclei, ranging from 2 to 7 μ in diameter. Even nucleoli 8—9 μ in size were not rare. With pyronine these enlarged nucleoli stained a vivid red. The red colour could be removed by digestion with ribonuclease which is an evidence of the increased RNA content of the nucleoli.

These results have verified that the neurons of the mediodorsal body also send axons to the cerebro-cerebral commissure and injury of the latter brings about a characteristic reorganization of the nucleoli of the involved cells.

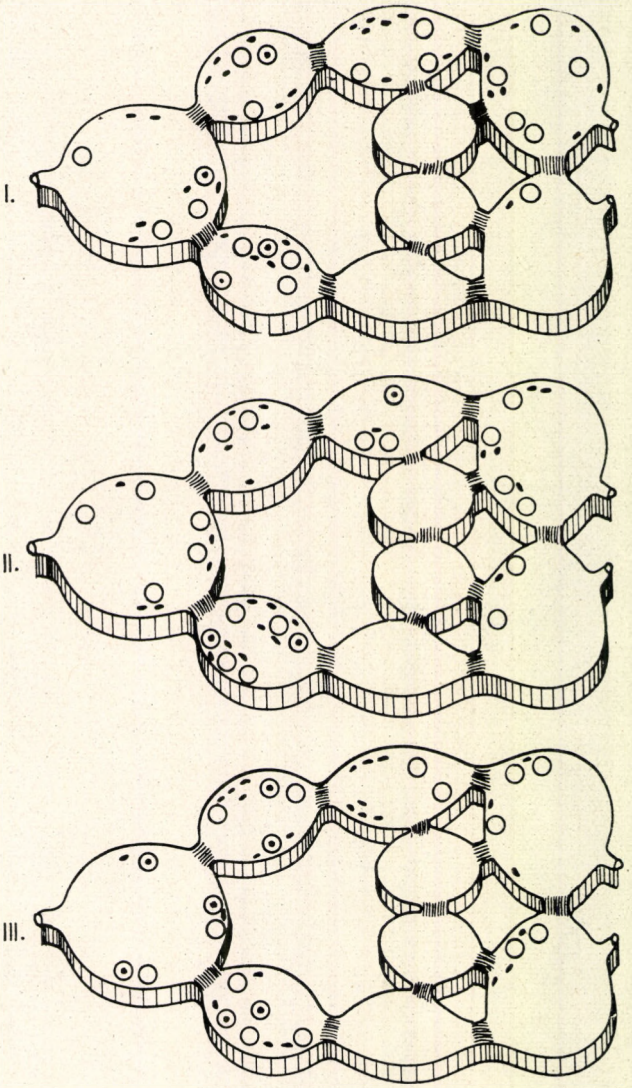

Fig. 4. Distribution of pyroninophilic cells in the central nervous system after cutting the left parieto-abdominal connective

Transection of the cerebro-pedal connectives (cpc)

By cutting the left cerebro-pedal connective cells exhibiting granular pyroninophilia were found in all the ganglia examined except the right pleural ganglion *(Fig. 2)*. In all, 112 pyroninophilic cells were present localized as follows: 38 in zone I, 42 in zone II, and 32 in zone III. When cutting the right cpc, no pyroninophilic cells were found in the left pleural and parietal ganglia in any of the specimens *(Fig. 3)*. Following transection of the right cpc 125 pyroninophilic nerve cells were counted in the central nervous system. Of these neurons 60 were found in the dorsal, 39 in the median and 26 in the ventral zone.

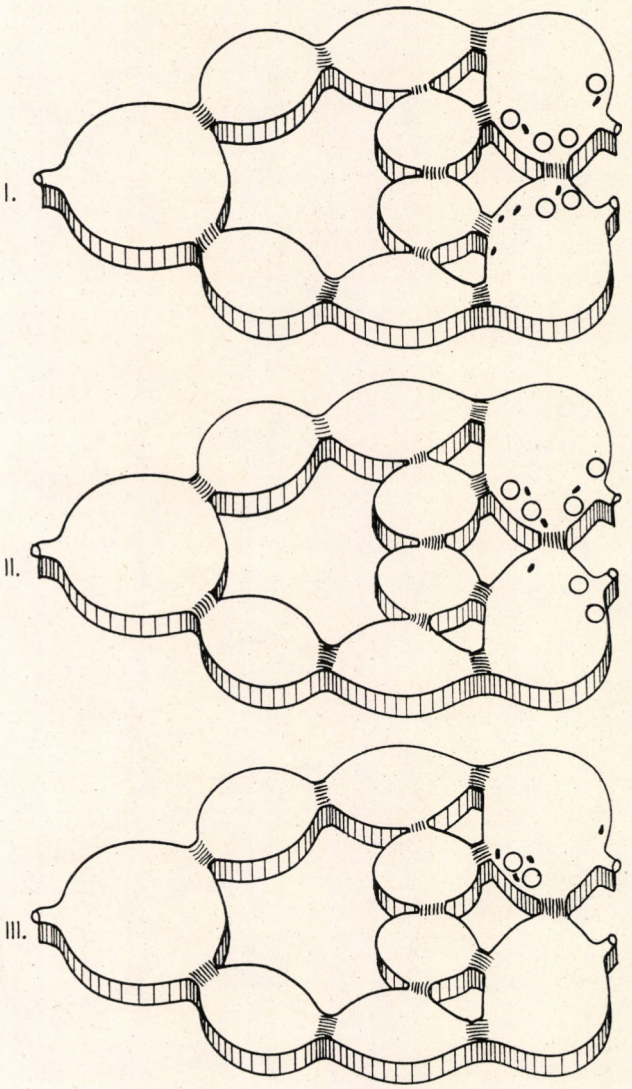

Fig. 5. Pyroninophilic cells after transection of the left cerebro-buccal connective

After cutting the cerebro-pedal connectives a total of 237 neurons exhibiting pyroninophilia were found in the central nervous system but only about 10—14% of these cells were localized in the pedal ganglia. Distribution of pyroninophilic cells according to size is shown in *Table 1.*

Localization of the involved cells within the ganglia was found to be identical in the various specimens. Injury of the right cpc was followed by the appearance of the greater number of pyroninophilic neurons in the right cerebral and right parietal ganglia *(Fig. 3).*

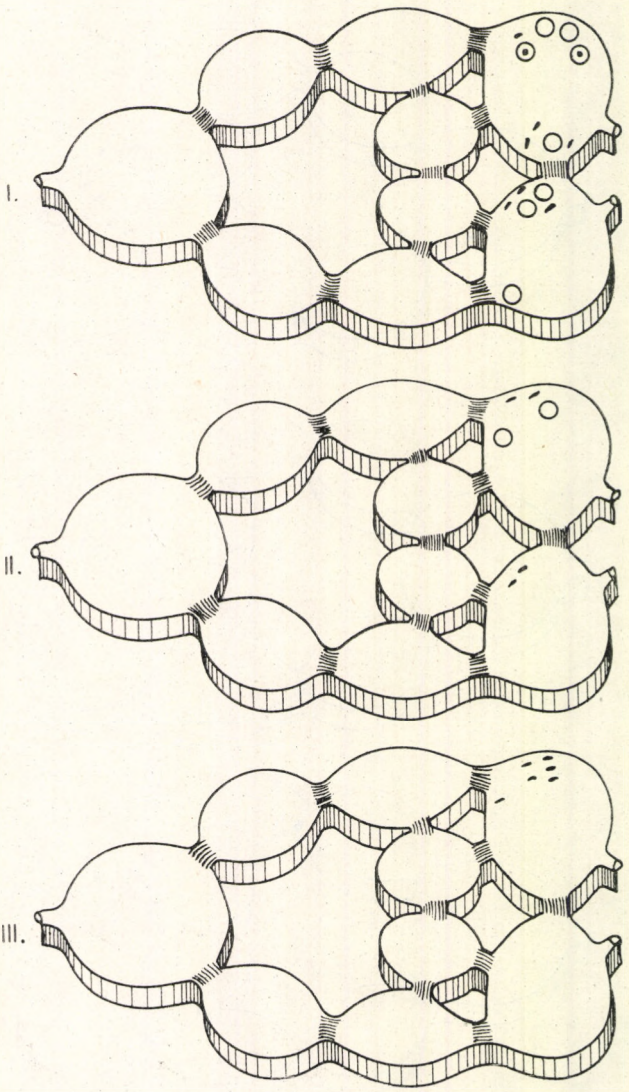

Fig. 6. Pyroninophilic cells after cutting the left nervus labialis inferior

Transection of the parieto-abdominal connective

Injury of the left parieto-abdominal connective resulted in the appearance of 148 pyroninophilic neurons in the central nervous system of the *Lymnaea stagnalis* L. In this case nearly the same number of neurons showing signs of regeneration were found in all three zones. The pedal pair of ganglia and the right pleural ganglion do not send direct fibers to the parieto-abdominal connective *(Fig. 4)*. In the right parietal ganglion the number of cells exhibiting pyroninophilia was strikingly high. Distribution of pyroninophilic cells in the right and left ganglia was assymetrical. By transection of the parieto-abdominal connective it was proved that direct axonal con-

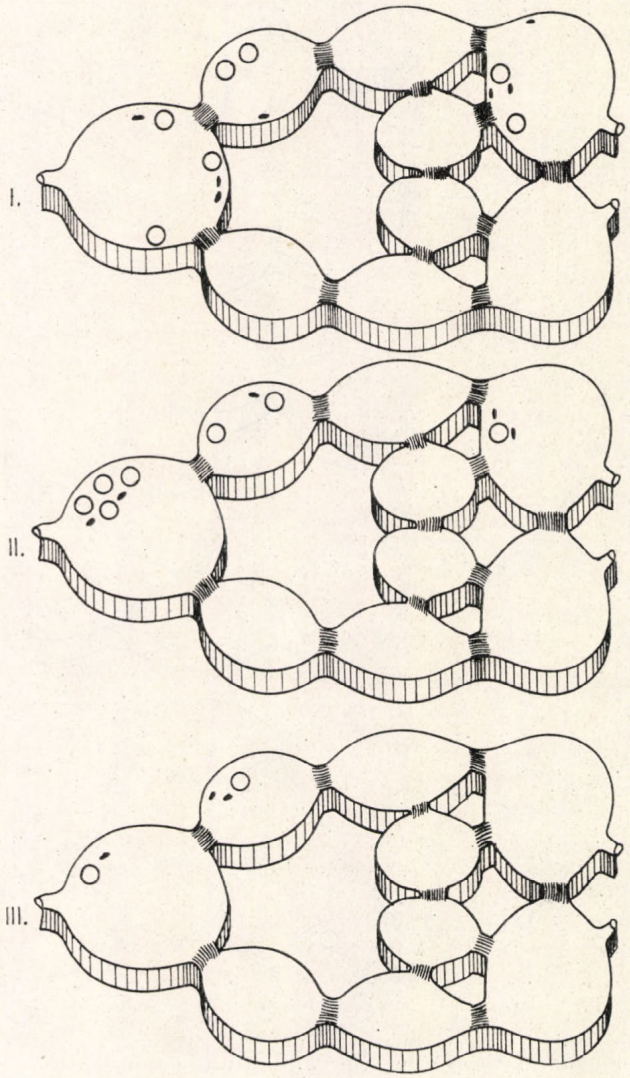

Fig. 7. **Pyroninophilic cells after cutting the left pallial nerve**

nections passing through the abdominal ganglion exist between the right and left parietal ganglia. Direct fibers are sent also from the abdominal ganglion through the left parieto-abdominal connective to the left pleural ganglion and both cerebral ganglia *(Fig. 4)*.

Transection of the left cerebro-buccal connective

After cutting this connective regenerating cells were present only in the cerebral ganglia *(Fig. 5)*. The number of these pyroninophilic cells was

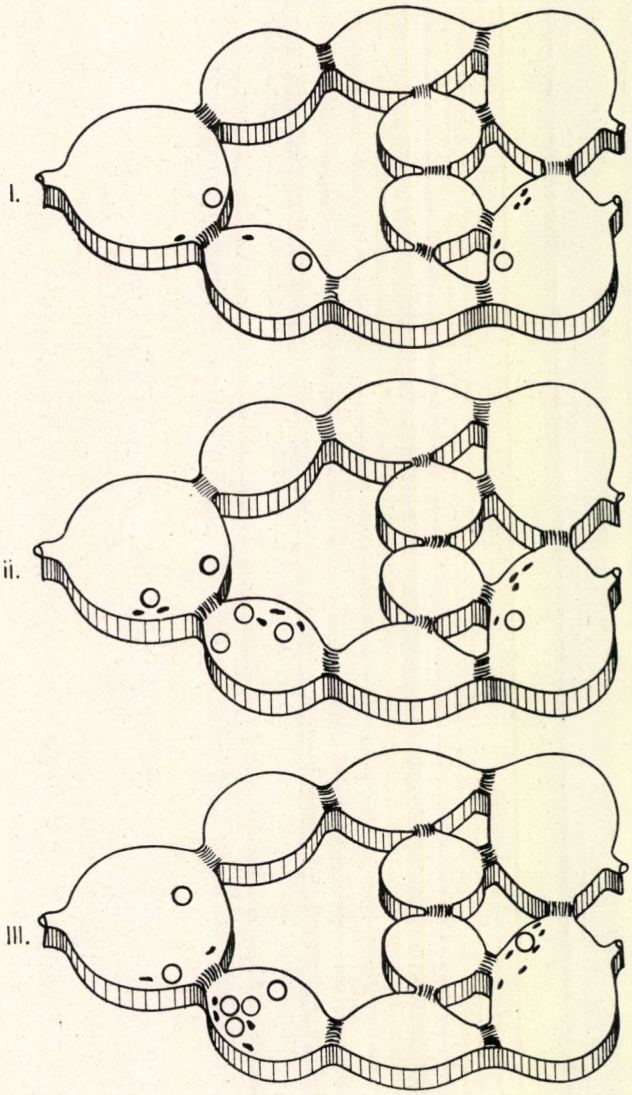

Fig. 8. Pyroninophilic neurons appearing after transsection of the right pallial nerve. Groups of pyroninophilic cells in the basal area (zone III) of the right cerebral and parietal ganglia

28: 12 in the dorsal, 10 in the median and 6 in the ventral area of the ganglion. No pyroninophilic cells were found in the buccal ganglion in any of the specimens examined following transection of any nerve branch.

Transection of the inferior labial nerve

By injury of the left inferior labial nerve similar results were obtained as by cutting the cerebro-buccal connective. Pyroninophilic neurons (in all, 27 cells) were found only in the cerebral ganglia. Distribution of these cells is shown in *Fig. 6*. Transsection of the nerves leaving the central nervous system was always marked by relatively few pyroninophilic cells.

Transection of the left and right pallial nerves

When cutting the left pallial nerve pyroninophilic cells appeared in the left cerebral and parietal ganglia and in the single abdominal ganglion *(Fig. 7)*. A total of 31 neurons became pyroninophilic, of which 14 were localized in

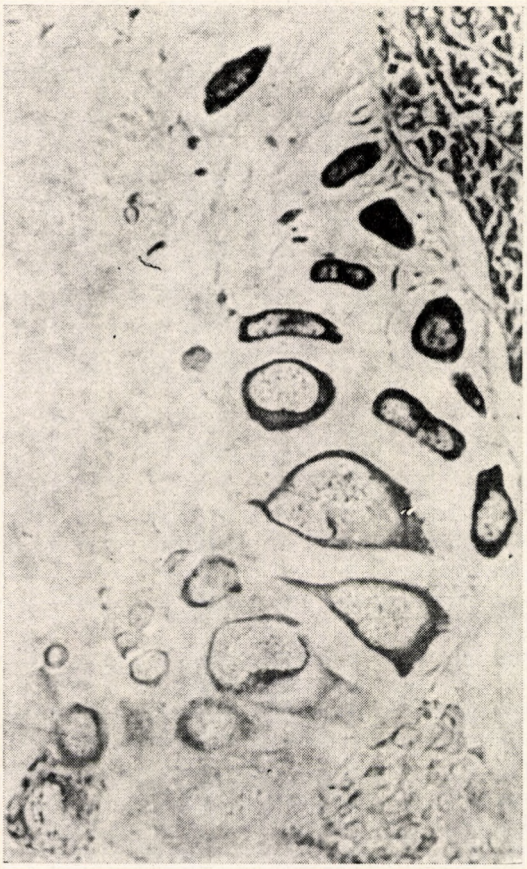

Fig. 9. Grouping of pyroninophilic cells in the third zone of the right cerebral ganglion after injury of the left pallial nerve

the dorsal, 12 in the median and 5 in the ventral zone. In the abdominal ganglion the pyroninophilic cells were grouped around the site of origin of the intestinal nerve.

Transection of the right pallial nerve resulted in the appearance of pyroninophilic cells in the right cerebral and parietal ganglia, as well as in the abdominal ganglion. In all, 43 neurons exhibited signs of regeneration. Their distribution within the ganglion is shown in *Fig. 8.*

Fig. 9 shows the grouping of small pyroninophilic cells in the third zone of the right cerebral ganglion after cutting the right pallial nerve. The basal part of the parietal ganglion also contains groups of pyroninophilic cells *(Fig. 8).*

Following transection of the inferior labial nerve and of the right and left pallial nerves, as well as of the cerebro-buccal connective the number of cells involved even all together, is smaller than that found after cutting the central commissures *(Table 1).*

TABLE 1

Distribution according to number and size of cells exhibiting pyroninophilia following transection of different commissures, connectives and nerve branches emerging from the central nervous system of Lymnaea stagnalis L.

	Over 120 μ	50—120 μ	Under 50 μ	Total
cerebro-cerebral commissure (ccc)	8	87	58	153
left cerebro-pedal connective (cpc)	5	41	66	112
right cerebro-pedal connective (cpc)	7	43	75	125
parieto-abdominal connective (left)	14	50	84	148
nervus labialis inferior (left)	2	8	17	27
nervus pallialis (left)	—	16	15	31
nervus pallialis (right)	—	16	27	43

Discussion

On the basis of our experimental results it was stated that following transection of different nerves pyroninophilic neurons in the central nervous system of *Lymnaea stagnalis* L. are rather scattered within the ganglia. Groups of cells containing a granular pyroninophilic substance were found only after cutting the right and left pallial nerves.

After cutting different interganglionic connections granular pyroninophilic neurons showing signs of regeneration were found in almost all the ganglia. Following transection of the cerebro-cerabral commissure great numbers of pyroninophilic cells were present in all the ganglia of the central nervous system. This finding supports the assumption that the pair of cerebral ganglia plays an integrative role within the central nervous system. In a previous work (Gubicza and S.-Rózsa, 1969) it has been reported that following injury of the intestinal nerve, namely, by cutting the branch innervating the heart, the greatest numbers of pyroninophilic cells were located in the cerebral ganglion. A further confirmation of the integrative role of the latter

ganglion seems to be provided by the fact that pyroninophilic cells were always present in this ganglion after transection of any nerve or connective. This finding can be taken as an evidence that the cerebral ganglion is the centre of the ascending and descending pathways.

By cutting the parieto-abdominal connective all the ganglia, except the right pleural ganglion and the pedal pair of ganglia, contained pyroninophilic nerve cells which proves that the parietal ganglion receives fibers from all the ganglia situated higher up and that the paths coming from the cerebral and pleural ganglia pass uninterruptedly through this connective in their way to the periphery.

After cutting the cerebro-pedal connectives it was surprising to find a relatively small number of pyroninophilic cells in the pedal ganglion, to which numerous direct fibers arrive from other ganglia. Presumably, the majority of the neurons of the pedal ganglia send direct fibres to the periphery, while the rest transmits information to the cerebral ganglion or receives information from it *(Figs 3 and 4)*.

No cells exhibiting pyroninophilia were found in the buccal ganglion after cutting any of the nerve branches which finding seems to support the assumption that the buccal ganglia send direct fibers only to the periphery (oral organs). According to our data the pair of buccal ganglia is not in close functional connection with the central nervous system.

Another question to be answered is why the right parietal ganglion contains more pyroninophilic cells than the left following transection of the cerebro-cerebral commissure and left parieto-abdominal connective. As shown in *Fig. 4*, when cutting the right cpc the right parietal ganglion numerous nerve cells exhibiting pyroninophilia, whereas in the left ganglion no such cells were present. On the other hand, when the left cpc was cut pyroninophilic cells were present not only in the left parietal ganglion but also in the right one, where their number was even somewhat higher. From the difference in the number of pyroninophilic cells between the right and left parietal ganglia following cutting of other nerve branches as well, it was concluded that the right parietal ganglion has a different functional role. As it is known from earlier anatomical descriptions of the central nervous system of the *Lymnaea stagnalis* L. there is a disparity between the nerve branches emitted by the two parietal ganglia and their areas of innervation are also different. Moreover, the right parietal ganglion contains about three times more neurons than the left (Gubicza, 1970).

In a previous work (Gubicza and S.-Rózsa, 1969) it has been reported that after cutting the intestinal nerve (originating from the abdominal ganglion) at its branch innervating the heart, pyroninophilic cells appeared in the pedal ganglia alike. After transsection of the parieto-abdominal connective, however, no such cells were present in these ganglia in any of the cases, which seems to prove that the nerve fibers running from the pedal ganglion to the heart pass through the right ganglia.

Enlargement of nucleoli exhibiting pyroninophilia in certain cells of the mediodorsal body attached to the cerebral ganglion is undoubtedly connected with the transection of the cerebral commissure as this was never encountered in control animals or when other nerve branches were cut. This observation seems to confirm the earlier assumption that the small cells of the mediodorsal and medio-lateral bodies are in direct connection with the neurons of the

cerebral ganglion (BOER, 1965; LEVER, 1958). Enlargement of nucleoli following neuronal injury has been described on various experimental animals (VOGT and VOGT, 1946; COHEN and JACKLET, 1965, SALÁNKI and GUBICZA, 1967).

No regularity was demonstrable in the distribution according to size of neurons forming the different nerve paths. The greatest number of pyroninophilic cells was found among the smallest cells and the fewest pyroninophilic cells were found among large cells (over 120 μ in diameter) but this may be in relation with the general cell number (GUBICZA, 1970).

Our results seem to indicate that the central representation of the different nerves is diffuse, several ganglia being involved in it.

Summary

By cutting the different commissures, connectives and nerve branches in the central nervous system of the *Lymnaea stagnalis* L. it was concluded that

1) Neurons containing a granular substance staining intensively with PMaG appearing 1—2 days following nerve transections were scattered in the various ganglia. Grouping of pyroninophilic cells was noted only after transection of the left and right pallial nerves.

2) After cutting different nerve branches, there was a disparity in the number and distribution of pyroninophilic cells between the right and left ganglia.

3) Granular pyroninophilia was found to appear in small, medium-sized and large cells alike. Most pyroninophilic cells were found among the small cells.

4) The number of pyroninophilic cells was higher after transection of the connectives and commissures of the central nervous system than after cutting peripheral nerves.

5) After transection of different nerve branches the right parietal ganglion differed from the left showing a similar pattern to that of the abdominal ganglion.

6) Following transection of the cerebro-cerebral commissure neurons with enlarged nucleoli containing RNA particles were found among the cells of the mediodorsal body. These cells stained intensively with pyronine.

7) The cerebral ganglia can be regarded as the integrative centre of the ascending and descending nerve paths in *Lymnaea stagnalis* L.

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GANGLIONKÖZTI ÉS PERIFÉRIÁS NEURONPÁLYÁK VIZSGÁLATA LYMNAEA STAGNALIS L. KÖZPONTI IDEGRENDSZERÉBEN

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Összefoglalás

A *Lymnaea stagnalis* L. központi idegrendszerének különböző comissuráit, connektivumait és idegágait átvágva, az alábbi megállapításokat tették:

1. Az idegátmetszések után 1-2 nappal jelentkező PMaG-vel jól festődött szemcsés anyagot tartalmazó idegsejtek a különböző dúcokban szórtan fordulnak elő. Csak a n. pallialis sinister és dexter átvágása után volt tapasztalható a pyroninofil sejtek csoportos előfordulása.

2. A különböző idegágak átmetszése után, a jobboldali és a baloldali dúcpárokban, eltérő számban és egymástól eltérő helyen fordultak elő pyroninofil sejtek.

3. Kis, közepes és nagyméretű idegsejt egyaránt található pyroninofil sejtek között. Legtöbb a kis méretű idegsejt.

4. A központi idegrendszer connektivumait és commissuráját átvágva több idegsejt vált pyroninofillá, mint a perifériás idegágak átvágása esetén.

5. A jobboldalon elhelyezkedő ggl. parietale a különböző idegágak átvágása esetén eltért a baloldali ggl. parietálétól és az abdominalis dúchoz hasonló képet mutatott.

6. A cerebro-cerebrális comissura átvágása esetén a medio-dorzális test sejtjei között pyroninnal jól festődött nagy nucleolusú RNS tartalmú sejtek fordultak elő.

7. A cerebralis ganglionok a *Lymnaea* integratív központjának tekinthetők, mivel mind a felszálló, mind a leszálló pályák végső, illetve kiindulási helyének felelnek meg.

ИССЛЕДОВАНИЕ МЕЖГАНГЛИОЗНЫХ И ПЕРИФЕРИЧЕСКИХ ПУТЕЙ В ЦЕНТРАЛЬНОЙ НЕРВНОЙ СИСТЕМЕ БОЛЬШОГО ПРУДОВИКА

А. Губица и *К. Ш.-Рожа*

На основе опытов с перерезкой различных комиссур, коннективов и нервов в центральной нервной системе большого прудовика авторы приходят к следующим выводам:

1. Через 1—2 дня после перерезки нервов нейроны, которые хорошо окрашиваются РМаГ обнаруживаются в различных ганглиях. Группировка пиронинофильных клеток появилась только после перерезки левого и правого паллиальных нервов.

2. После перерезки различных нервов, в левых и правых ганглиях пиронинофильные клетки появляются в различных местах и не в одинаковых числах.

3. Мелкие, средние и крупные нейроны в равной степени встречаются среди пиронинофильных клеток. Больше всего нейронов малого размера.

4. После перерезки комиссур и коннективов центральной нервной системы больше нейронов становится пиронинофильными, чем после перерезки периферических нервов.

5. После перерезки различных нервов, правый париетальный ганглий по разпределению пиронинофильных клеток отличался от левово париетального ганглия и проявлял сходство с абдоминальным ганглием.

6. В случае перерезки церебро-церебральной комиссуры среди нейронов медиодорзального тела обнаруживаются клетки, с большим ядрышком содержащим РНК, хорошо окрашиваемые пиронином.

7. Церебральные ганглии у большого прудовика являются интегративным центром, так как здесь оканчиваются восходящие пути и отсюда начинаются нисходящие пути.

ANNAL. BIOL. TIHANY | 37 | 33—41 | HUNGARIA 1970

EXAMINATION OF MONOAMINE SYNTHESIS AND BREAK DOWN IN THE NERVOUS SYSTEM AND OTHER TISSUES OF LYMNAEA STAGNALIS L.

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Received: 25th February, 1970

The distribution of serotonin (5 HT) and dopamine (DA) have been investigated in the nervous system of many molluscs. In Gastropods, serotonin and dopamine were demonstrated in *Helix aspersa* (KERKUT and COTTRELL, 1963), in *Helix pomatia* (DAHL et al. 1962, 1966), in *Lymnaea stagnalis* (SAKHAROV and ZS.-NAGY, 1968; HIRIPI, 1968). In many other molluscan species the distribution of serotonin was investigated by WELSH and MOORHEAD (1960) and that of dopamine by SWEENEY (1963).

Investigating the synthesis of these amines in *Busycon canaliculatum* (WELSH and MOORHEAD, 1959) and in *Helix aspersa* (KERKUT and COTTRELL, 1963) 5HTP-decarboxilase, in *Mercenaria mercenaria* (SWEENEY, 1969) DOPA-decarboxilase were demonstrated. 5HTP-DOPA decarboxilase was investigated in *Helix pomatia* (CARDOT, 1963a, b; 1966) and in *Anodonta cygnea* (HIRIPI and SALÁNKI, 1969).

The monoamine-oxidase (MAO), which is generally responsible for the inactivation of the monoamines was demonstrated in the digestive gland of *Buccinum undatum*, in different tissues of some Lamellibranches as well as in the nervous tissues of the Cephalopods (BLASCHKO and HAWKINS, 1952; BLASCHKO and HIMMS, 1954; BLASCHKO and HOPE, 1957). The monoamine oxidase was demonstrated also in the nervous tissues of *Helix pomatia* (CARDOT, 1963c; 1964) and in the kidney of *Helix aspersa* (KERKUT and COTTRELL, 1963).

However, in the different tissues of same species the whole metabolism has not investigated neither for serotonin nor for dopamine. Earlier the serotonin was demonstrated in the nervous tissues of *Lymnaea stagnalis* L. (HIRIPI, 1968) but no available data concerning the synthesis and break down of serotonin in the different tissues of this species.

The aim of the present study was the examination of the 5HTP-DOPA decarboxylase and that of the inactivation of serotonin in the nervous system of *Lymnaea stagnalis* L., as well as the break down of the serotonin was examined also in the tissues of the heart and kidney.

Methods

The pharyngeal ganglia of *Lymnaea stagnalis* L. was used for the examination of 5HTP-DOPA decarboxylase and that of the monoamine oxidase the tissues of the heart and kidney, too.

The tissues of the kidney can only be dissected together with the mantle and connective tissues so the measured weight of the kidney contains also

the weight of these tissues, and the given data concern this total weight. In some cases, after the careful separation of the kidney, the weight of the mantle and connective tissue were remeasured and the weight of the kidney was calculated. It was found that about 1/5 total weight of the kidney measured by us derive from the kidney.

During dissection the tissues were collected in ice-cold physiological saline, measured after drying on filter paper and homogenized in physiological saline with Potter—Elvehjein homogenizers.

Examination of 5HTP-DOPA decarboxylase: Enzymatic activity was assayed by fluorometric measuring the rate of amine formation. 5HTP decarboxylase was estimated by the method of KUNTZMAN et al. (1961) and that of the DOPA decarboxylase by the method of LOVENBERG et al. (1962).

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

The mixture was shaken throughout the incubation period. After a 15 minutes preincubation period, the incubations were carried out for 60 minutes in the case of 5HTP decarboxylase and for 30 minutes in the case of DOPA decarboxylase.

The composition of the incubation mixture was the follows: 20 mg/ml tissue homogenizate, piridoxal-5-phosphate 8.09×10^{-5} M, iproniazid 7.2×10^{-4} M, phosphate buffer 0.1 M. As substrates DL-5-hydroxytryptophan (DL-5HTP) in concentration 4.54×10^{-4} M and DL-3,4-dihydroxyphenylalanine (DL-DOPA) in concentration 7.6×10^{-3} M were used. Enzyme activity is reported as μg of amine formed/g of wet tissue per hour.

The excitation and fluorescent wavelenths (mμ, uncorrected instrument values, Aminco Bowman Spectrophotofluorometer) were as follows: serotonin 300 and 540; dopamine 282 and 330.

Examination of monoamine oxidase and the identification of the 5-hydroxyindoleacetic acid (5HIAA): Enzymatic activity was assayed by fluorometric measuring the rate of serotonin disappearance. At the beginning and the end of the incubation period 1 ml aliquot from the incubation mixture was assayed for serotonin by method of BOGDANSKI et al. (1956). The composition of the incubation mixture was the follows: kidney homogenate 25 mg/ml, heart and ganglia homogenates 50 mg/ml, phosphate-buffer 0.05 M pH 7.4, serotonin 1.13×10^{-4} M.

After a 15 minutes preincubation period the incubations were carried out for 60 minutes in the case of the kidney and for 120 minutes in the case of the heart and ganglia. During this period the enzyme activity was linear. The mixture was shaken throughout the incubation period at 25 $\pm$ 0.1 °C. As substrate serotonin creatinine sulphate was used. The enzyme activity are expressed in μg of disappeared serotonin/g wet weight per hour.

From the incubation mixture the 5HIAA was isolated and identified by the thin layer chromatographic method of ASCHROFT et al. (1968). In this case the incubation was carried out at 37 °C in order to increase the formation of the 5HIAA. ERLICH's reagent was used for the localization of the spots.

Results

5HTP-DOPA decarboxylase: The homogenate of *Lymnaea* ganglia is capable to synthetize both serotonin and dopamine. The enzyme activity is 220 μg serotonin synthetized/g wet weight per hour and 1600 μg dopamine synthetized/g wet weight per hour. The rate of the synthetized amines are (dopamine : serotonin) = 7.3: 1. The enzyme activity could be inhibited by α-methyl-DOPA. Among the two substrates we tested only the effect of DOPA,

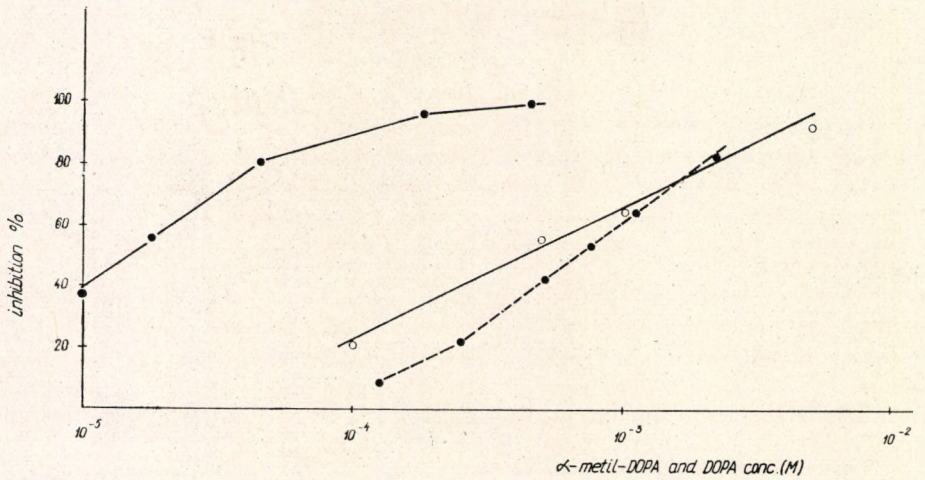

Fig. 1. Inhibition of the activity of 5HTP (• — • —) and DOPA (○ — ○ —) decarboxylases by α-methyl-DOPA (• – – •) and DOPA in the homogenate of *Lymnaea* ganglia

and it was found to inhibit the decarboxylation of 5HTP. The inhibition of serotonin and dopamine synthezis is shown in *Fig. 1* and the concentration of inhibitor necessary for 50% inhibition is given in *Tabe 1.*

TABLE 1

Concentration of inhibitors for 50% inhibition of 5HTP—DOPA decarboxylase

Substrate	Concentration of inhibitor for 50% inhibition	
	α-methyl-DOPA	DOPA
5HTP	1.6×10^{-5} M	6.2×10^{-4} M
DOPA	4.0×10^{-4} M	

An approximately K_M values were estimated with the Lineweaver-Burk plot, taking into consideration that we used DL-5HTP and DL-DOPA, however, the enzyme acts only on the L-form. As the D-form is no inhibitor and the L and D forms are present in equimolar concentrations in the DL form, we took into consideration during the estimation of K_M the half-value of the concentration of substrate for the DL form. This K_M corresponded 3×10^{-5} for 5HTP and 1.5×10^{-4} for DOPA.

Monoamine oxidase: The thin layer chromatogram of the extract from the incubation mixture shows that the serotonin was destroed to 5HIAA, because the R_f values are identity both to the destroing product and to the authentic 5HIAA *(Fig. 2)*. In the case of the kidney, where the enzyme activity is highest, three another spots *(Fig. 2.* I., II., III.) are seen which is failed to identity.

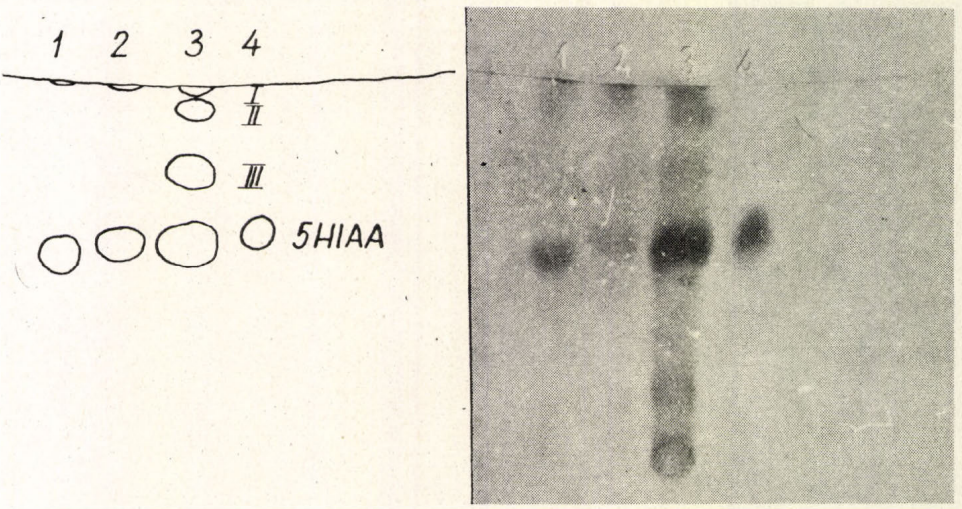

Fig. 2. Thin-layer chromatogram of the incubation mixtures derivated from *Lymnaea.* 1. ganglia, 2. heart, 3. kidney, 4. authentic 5HIAA

The spot I is present in the case of the ganglia and heart too, however its intensity is lower and it is possible that it derives from the carotine occurring in the tissues in a considerable amount.

This spot ran in each case with the front solvent. The spot II. is like a pigment, because its colour is yellow-brown before and after the localization of the spots.

The results show that the activity of the kidney is the most highest among the examined tissues, however, the nervous system have also a considerable activity *(Table 2)*. The homogenate of the kidney also contains the mantle and the connective tissue but the latter tissues have no activity.

TABLE 2

MAO activity in the ganglia, heart and kidney tissues of Lymnaea. The enzyme activity is reported as μg 5HT disappeared/g wet weight per hour

Tissue	μg 5HT disappeared/g wet wt/h
ganglia	77
heart	20
kidney	270

The rate of serotonin disappearance depending on the substrate concentration is illustrated in *Fig. 3.* The enzyme activity was inhibited by iproniazid and actomol. The inhibition was investigated in the case of the kid-

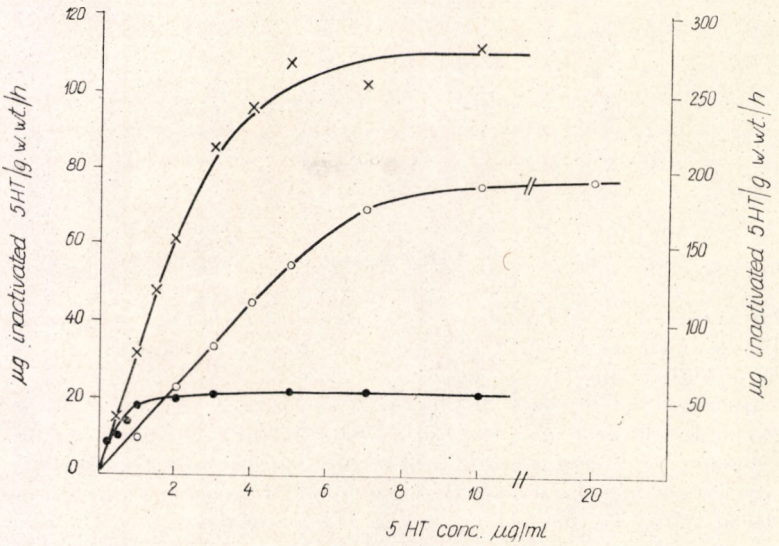

Fig. 3. Substrate curve of the inactivation of serotonin in case of ganglion (o o o) and heart (. . . . . .) homogenate of *Lymnaea* (left side) and that of the kidney homogenate (xxxxxx) (right side)

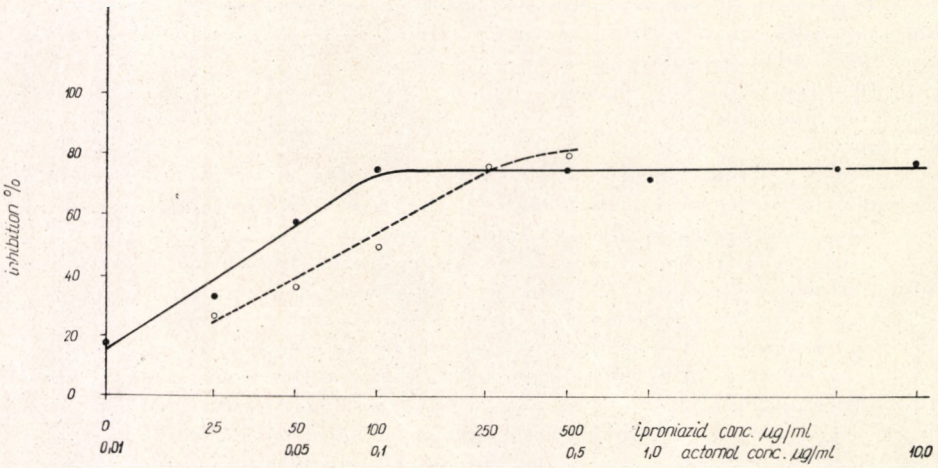

Fig. 4. Inhibition of the 5HT-disappearance in the kidney homogenate of *Lymnaea* by iproniazid (o o - -) and actomol (● — ● —)

ney at different concentration of iproniazid and actomol *(Fig. 4)* as well as in the case of each tissues at a concentration 500 µg/ml for iproniazid and 0.1 µg/ml for actomol *(Table 3).*

TABLE 3

The inhibition of enzyme activity in Lymnaea tissues by 500 μg/ml concentration of iproniazid and 0.1 μg/ml concentration of actomol

Tissue	Inhibition %	
	iproniazid 500 μg/ml	actomol 0.1 μg/ml
ganglia	90	80
heart	80	90
kidney	80	75

Discussion

The results show that the nervous tissues of *Lymnaea stagnalis* is capable to synthetize both serotonin and dopamine, however, its ability for dopamine synthesis is higher than for serotonin synthesis.

Comparing the present results with our earlier data gained with *Anodonta cygnea* L. (HIRIPI and SALÁNKI, 1969) it can be concluded that in the nervous tissues of *Lymnaea* about 4 times smaller serotonin concentration belongs to four times higher enzyme activity. Comparing the proportion of the synthesis (for *Anodonta* 6.0—6.4, for *Lymnaea* 7.3) it can be seen that in *Lymnaea* the direction of the synthesis even more moved toward dopamine.

This was supported by the comparison of the α-methyl-DOPA concentration necessary for 50% inhibition, because in the case of *Lymnaea* the α-methyl-DOPA concentration was one order lower than that of the *Anodonta*.

The synthesis of both amines by the same enzyme is proved by the inhibition of enzyme activity with the α-methyl-DOPA and DOPA the substrate of DOPA-decarboxylase.

It agrees with the data given in vertebrate animals (PLETSCHER et al. 1966) where the identity of two enzymes the 5HTP and DOPA-decarboxylase, is examined in detail.

The K_M values show that the affinity of the substrate to the enzyme is greater in the case of DOPA than in the case of 5HTP and correspond to that found in other cases (HAGEN and COHEN, 1966).

However in the molluscan nervous tissues the synthesis of serotonin is known, there are no identical opinion concerning its inactivation.

Our results suggest the present of the monoamine oxidase (MAO) in the nervous tissues as an inactivating system for serotonin.

This result is in agreement with the data of BLASCHKO and co-workers (BLASCHKO and HAWKINS, 1952; BLASCHKO and HIMMS, 1954; BLASCHKO and HOPE, 1957) who found MAO in the molluscan nervous tissues.

However, others found biochemical evidences that the MAO does not participate in the inactivation of 5HT by the nervous tissues. Monoamine oxidase has been found in the nervous system of *Helix pomatia* (CARDOT, 1963; 1964) but it seems to be inactive on a 5HT substrate.

MIROLLI (1968) found that MAO is not present in the nervous tissues of *Busycon canaliculatum*. According to his opinion, it is possible that in the nervous tissues the 5TH is inactivated by binding to other molecules and it may be in the kidney where serotonin is inactivated by MAO, because it is

known that the kidney homogenates are able to metabolize 5HT to 5HIAA (KERKUT and COTTRELL, 1963).

In the CILDA neurons of *Cryptomphallus* GERSCHENFELD and STEFANI (1968) have found evidence that the exogenous 5HT and probably the natural transmitter seem to disappear from their receptors by a diffusion mechanism.

In the nervous tissues of *Lymnaea* the diffusion is not regarded as an exclusive mechanism for 5HT inactivation because MAO is present and it is active on 5HT substrate. It was supported by the finding that the homogenate of the nervous tissues are able to metabolize 5HT to 5HIAA and the activity of the homogenate is inhibited by iproniazid and actomol inhibitors of MAO.

This result is in agreement with the data of SAKHAROV and ZS.-NAGY (1968) who on examinating the monoamine contents of the cerebral ganglia in *Lymnaea stagnalis* L. by histochemical method, found that the cell fluorescence was increased by nialamide.

However, the pigment formation may be also a metabolic pathway for the inactivation of serotonin because a yellow-brown spot, like a pigment spot, appeared on the chromatogram. Such a pigment formation was also demonstrated in other molluscan tissues (BLASCHKO and MILTON, 1960; AIELLO, 1964). Further investigations is needed in order to obtain evidence about the presence of pigment formation.

The fact, that the amount and activity of the enzyme is higher in the kidney than in the nervous system, suggest the idea that the majority of 5HT is destroyed in the kidney.

Summary

Examining serotonin metabolism in the different tissues of *Lymnaea stagnalis* L. the following may be said:

1. The nervous system is able to synthetize both serotonin and dopamine by 5HTP-DOPA-decarboxylaze enzyme.

2. The examined tissues of *Lymnaea* have MAO activity because the homogenates of the nervous system, heart and kidney are able to metabolize 5HT to 5HIAA in vitro and the enzyme activity is inhibited by iproniazid and actomol.

3. However, there may be another pathway for the inactivation of serotonin because such product is also formed in vitro which is not identical with 5HIAA.

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MONOAMIN SZINTÉZIS ÉS LEBONTÁS VIZSGÁLATA LYMNAEA STAGNALIS L. IDEGRENDSZERÉBEN ÉS EGYÉB SZÖVETEIBEN

Hiripi László

Összefoglalás

Vizsgálva a *Lymnaea stagnalis* különböző szöveteiben a szerotonin metabolizmust, azt találtuk, hogy:

1. Az idegrendszer jelentős mértékben képes szerotonin és dopamin szintézisre. A szintézist végző enzim az 5HTP-DOPA-dekarboxiláz.

2. A *Lymnaea* vizsgált szövetei tartalmaznak MAO-t, mivel az idegrendszer, a szív és vese szövetei 5HIAA-vá képesek bontani a szerotonint, és az enzimaktivitás iproniaziddal és actomollal gátolható.

3. A szerotonin lebomlás feltehetően nemcsak MAO révén következik be, minthogy az 5HIAA-val identikus termékek is képződnek.

ИССЛЕДОВАНИЕ СИНТЕЗА И РАЗЛОЖЕНИЯ МОНОАМИНОВ В НЕРВНОЙ СИСТЕМЕ И В ДРУГИХ ТКАНЯХ LYMNAEA STAGNALIS L.

Л. Хирипи

Исследуя метаболизм серотонина в различных тканях *Lymnaea stagnalis* нашли, что:

1. Нервная ситема в значительной мере способна синтетизировать серотонин и допамин. Синтетизирующий фермент 5HTP—ДОРА декарбоксилаза.

2. Исследованные ткани у большого прудовика содержат МАО, судя по тому что нервная система, ткани сердца и почек способны разлагать серотонин до 5HIAA, и активность фермента тормозится ипрониазидом и актомолом.

3. Предполагается, что разложение серотонина происходит не только с помощью МАО, посколько возникают и продукты, идентичные с 5HIAA.

ANNAL. BIOL. TIHANY | 37 | 43—53 | HUNGARIA 1970

PHOTODYNAMIC EFFECTS AND IRREVERSIBLE DAMAGE IN AUTOACTIVE NEURONS OF *HELIX POMATIA* EVOKED BY ROSE BENGAL AND METHYLENE BLUE EXPOSED TO VISIBLE LIGHT

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Received: 28th February, 1970

Photosensitization of different excitable tissues is a phenomenon being known since a long time and analyzed in details (LIPPAY, 1929; 1932; ROSENBLUM, 1960; LYUDKOVSKAYA and PEVSNER, 1964; LÁBOS, 1965; LÁBOS and TURCSÁNYI, 1966; LAKATOS and KOLLÁR-MORÓCZ, 1967; LAKATOS, 1969; SPIKES, 1967). Investigations at cellular level were started on squid giant axon (ARVANITAKI and CHALAZONITIS, 1953; CHALAZONITIS and CHAGNEUX, 1961). The natural photosensitivity of *Aplysia*-neurons (CHALAZONITIS, 1954; 1964; ARVANITAKI and CHALAZONITIS, 1960; 1961) due to endogenous neuronal pigments includes in a certain sense similar processes, but such phenomena in *Helix* neurons — because of their low pigment content — can be osberved only sporadically.

The cited authors generally emphasize that such a photoactivation is suitable tool when looking for electron processes be involved in the excitation. At the same time, it is also known that the photodynamic effect is noxious or even lethal (RAAB, 1902) and only the moderate effects are reversible.

Applying laser-pulses in the presence of methylene blue (ARVANITAKI et al. 1967) it succeeded to evoke discharges of *Helix* neurons, but long-lasting experiments were not carried out and effects of rose bengal on neurons — one of the most potent sensitizing dye — have not been reported yet. For these reasons in the present paper the photodynamic response evoked in the presence of methylene blue and rose bengal will be described up to its irreversible phase on molluscan neurons.

Methods

Spontaneous electrical activity of single or in some cases two neurons situated near the dorsal surface of the suboesophageal ganglion-complex was studied in the presence of 50—100 μg/ml rose bengal (Fluka, abbreviation: RB) and methylene blue (Merck, MC) in dark and in exposure to light. The composition of the physiological solution was the following: 3.0 g NaCl, 0.35 g KCl, 2.4 g $MgCl_2 \cdot 6\,H_2O$, 1.5 g $CaCl_2 \cdot 2\,H_2O$, 0.2 g $NaHCO_3$ pro lit. The recording was carried out with micropipettes of 4—10 MΩ filled with 2.5 M KCl, FET-input stages, and Tektronix 502 oscilloscope. The optical system had the following characteristics: tungsten-lamp of 500 watts; upper illumination from 50 cm; 15 mm heat-filter of BG 17 type; the surface of the focused spot was about 10 mm^2; effective illumination was near to 50 000 lx. The

exchange of solution was carried out at 100 lx. This latter illumination did not evoke any modification of neuronal activity even in the presence of sensitizing dyes.

The conditions of the leading off made possible experiments lasting for at least 5—6 hours without any essential decrease in the amplitudes of spike. In order to facilitate the input of stains to the cells, the thin layer of connective tissue covering the neurons was very carefully removed. To avoid mechanical excitations during the exchange of solution, it was carried out in a chamber of two compartments. The suction and exchange have taken place in the compartment where the ganglion was not present. The two parts of the chamber were communicating through a narrow gap.

50 neurons originating from about 40 ganglia were utilized in the experiments. In *Fig. 1* the localization of the neurons demonstrated in this paper is shown.

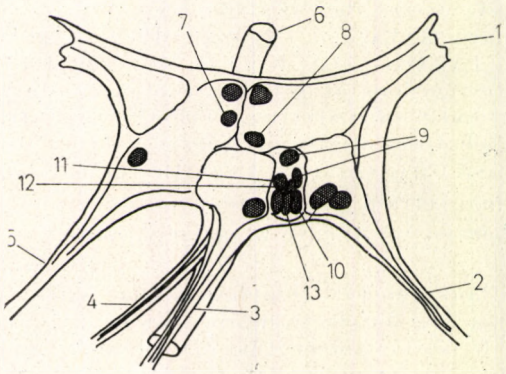

Fig. 1. A — Rough sketch of dorsal surface of suboesophageal ganglion. 1 — cerebropleural stub; 2 — n. intestinalis; 3 — n. analis; 4 — n. pallialis sin.; 5 — n. pallialis dext.; 6 — aorta; 7 — 170/8; 8 — 197/8 or 324/1 or 196/7; 9 — 190/2; 10 — 203/4; 11 — 195/1; 12 — 192/5; 13 — 196/6; 321/5; 198/1 (numbers of protocol).
Those neurons are demonstrated here whose reaction will be shown in the further figures (code-number see there)

Results

1. Effects of rose bengal in dark and light

In *Fig. 2* records obtained during an experiment carried out on a neuron discharging in constant intervals. During the control period of 25 min, the frequency was 22.6 $\pm$ 2 cpm. Exchanging the medium of incubation with rose bengal of 50 μg/ml, similarly for a period of 25 min, 21.4 $\pm$ 1.9 cpm frequency was observed. The resolution of these frequency-measurements was 20 sec. It is seen that these two values do not deviate significantly from each other. An illumination was applied after this period of 2×25 min. As an effect of the exposure to visible light, the frequency has doubled in 10—15 min and synchronously the peak to peak amplitudes have decreased. During a further 25—30 min the spikes get rarer and returning to the control a significant decrease in the amplitudes, the potential of resting membrane and

that of the overshoot was observed. In the given case, the decrease of frequency is fluctuating but that of the amplitude is monotonous.

In all experiments (25—30 neurons) we could notice a transient increase of frequency, a decrease of 25—30 mV in the membrane potential, an initially slow and after a rapid-decrease of amplitude and a diminuation of overshoot as well. In cases when the initial (control) amplitude of the action potential

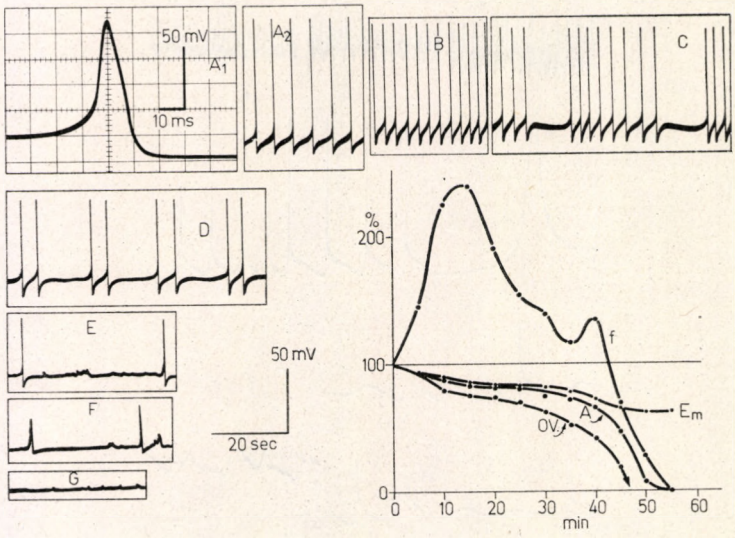

Fig. 2. Effect of 50 μg/ml RB in visible light. Neuron — 190/2. A_1 and A_2: control in RB and in dark. B—G: records in the 20, 30, 40, 42, 45 and 50th min of exposition. Time course of frequency (f), amplitude (A), membrane potential (EC) and overshoot (ov) changes in the per cent of control. The controls: 21 cpm, 107 mV, 54 mV or 33 mV (100 per cent)

was already smaller (peak to peak 60—80 mV) the above enumerated changes have taken place more quickly *(Fig. 3)*.

In *Fig. 3* the A_5—A_7 or B_7—B_8 squares demonstrate the terminal phases of the effect. These alterations are almost general at the end-period of the photo-dynamic effect. In such cases a polymorph oscillation of low amplitude appears. Some sufficently constant values of amplitude can be measured alternating bi- or multimodally. Synaptic noise, axon-spikes and abortive discharges of soma equally occur. In the final square of the *Fig. 3* a quasi-sinus oscillation is demonstrated yet. These diverse oscillations have amplitudes of about 1—20 mV.

The neurons modulated periodically (Br-like) are especially sensitive to this light-effect. In the case demonstrated in *Fig. 4* a short, transient increase of frequency, depolarization and finally a getting rare of frequency can be observed. The whole effect lasts for 10—15 min.

To verify that the observed destroying effects of rose bengal in light is not an accidental phenomenon caused by other influences, but it is in fact a photodynamic effect — we have recorded synchronously the alterations of

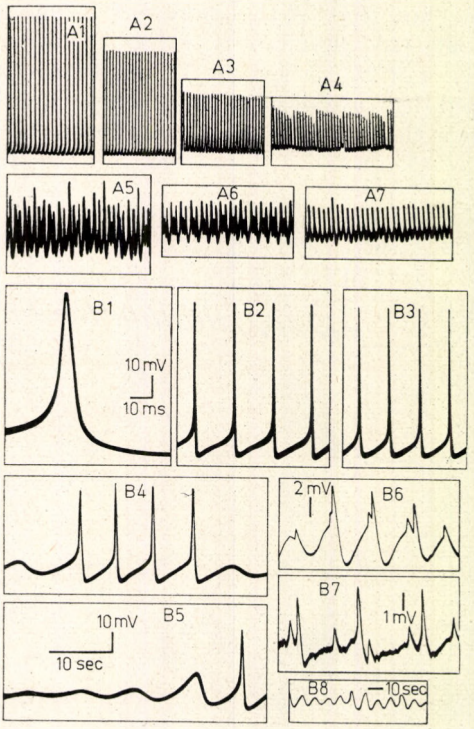

Fig. 3. Effect of 50 μg/ml rose bengal. A_1—A_7: neuron 192/5; B_1—B_8: neuron 170/8* A_1 — dark control in rose bengal; A_2—A_7 in the 10, 15, 17, 25, 30, 35 min of illumination. Calibration — A_1—A_4: 50 mV, 5 sec; A_5—A_7: 2 mV, 5 sec; B_1 — dark-control; B_2—B_5: 5, 10, 20, 32 min. of exposition; B_6—B_8: 40—50th min

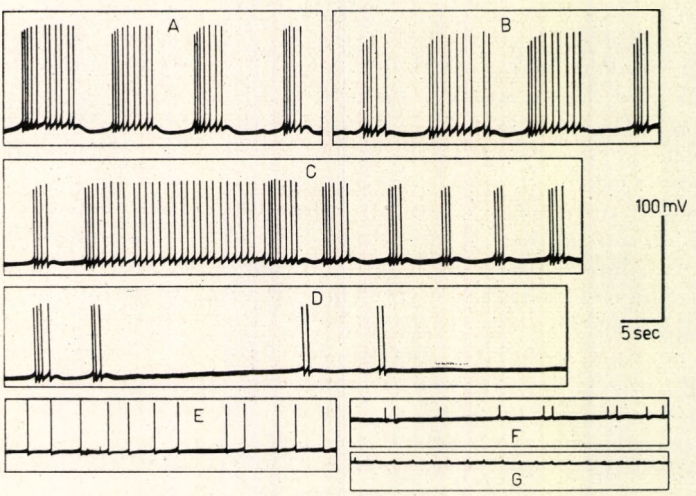

Fig. 4. Effect of 50 μg/ml RB on a cell of periodic activity (Br-like). Neuron 203/4. A — control; B — RB, in dark; C—G — RB in light: 1st, 2nd, 3rd, 10th and 12th min.

two different neurons *(Fig. 5)*. It is observable that in the cases of the two neurons (whose activities are, on the other hand, correlative) the decrease of amplitude, transient 2—5 fold increase of frequency and its terminal decrease are parallel events. In *Fig. 5D* and *5E* correlating periods are demonstrated. In *Fig. 5D* a seizure-like sequence of spike lasting for 2 sec is seen when in the other neuron a synaptic activation is notiecable. Such an activition of extremely high frequency in the given case between the 10th and 20th minutes of the exposition appeared 6 times and in all cases EPSP-s emerged in the record of the other neuron.

The destroying effect of rose bengal proved to be irreversible.

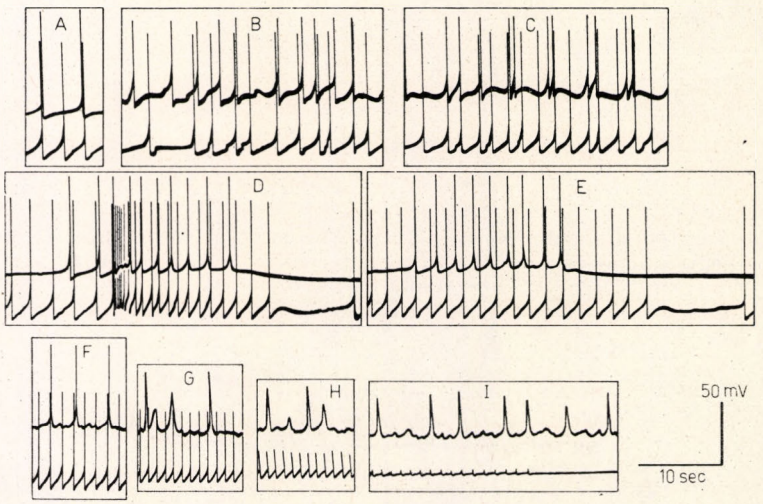

Fig. 5. 50 μg/ml RB. Two neurons of correlating activity. A — control in RB; B — I in light (5, 7, 10, 12, 20, 30, 40, 50th min of exposition). Neurons: 196/6 and 196/7

2. Effects of methylene blue in dark and light

Effects of 50 μg/ml methylene blue at the applied illumination are detectable already after an exposition of 5—10 min. It is observable furthermore that the dyestuff has effects even in dark, which effects become more explicit in light.

In the case illustrated in *Fig. 6* during the dye-free, control period and in the presence of the stain in dark or under the illumination, the amplitude of the action potential, its duration at the half-height or the frequency of discharge were measured. The values obtained are the following.

	98	92	80 mV (10—10 measurements)
18.6 ± 0.9	23.4 ± 1.4	28.6 ± 1.2	msec (11—11 measurements)
6.3 ± 0.45	6.48 ± 0.70	7.19 ± 2.27	spikes/20 sec (20—27—58 samples)

Changes can be observed in the shape of action potential already in dark. Both the decrease of amplitude and the prolongation are statistically significant (t-test, $P < 0.05$). It is noticeable, furthermore, that the relative dispersions

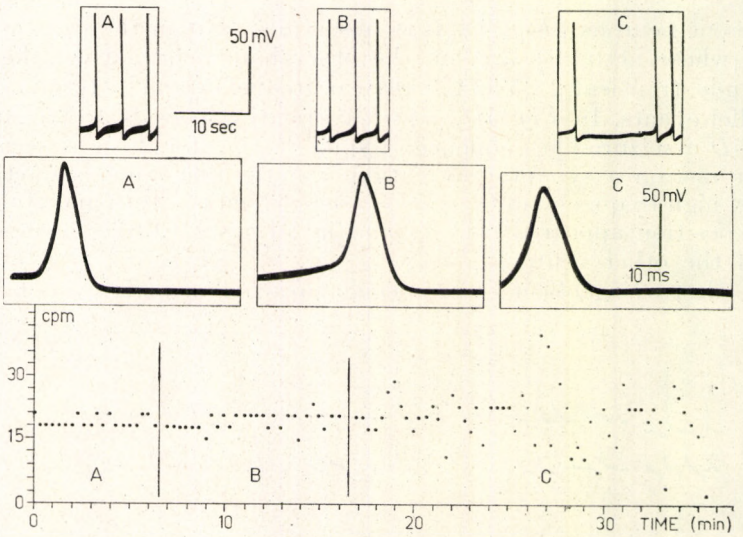

Fig. 6. Effect of 100 μg/ml MC. Neuron: 321/5. A: control; B: MC in dark; C: MC in light. The lower diagram demonstrates the frequency change in time for A, B and C periods. Time resolution is 20 sec

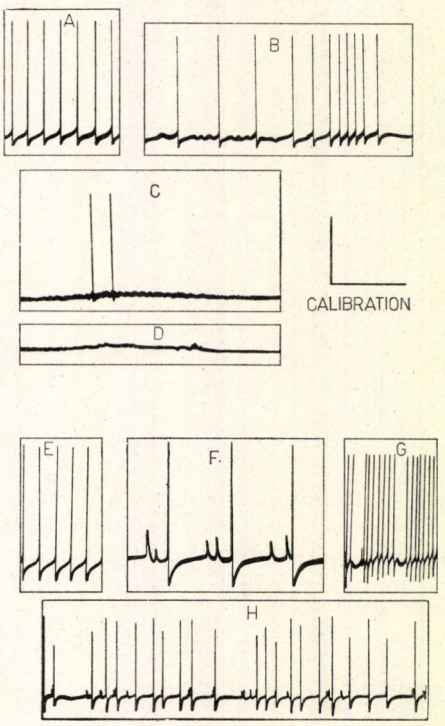

Fig. 7. Effect of 100 μg/ml MC in visible light. A—D — neuron 197/8; E—G: neuron 195/1. A — MC, dark, 5th min. B—D — MC, light 2nd, 5th and 8th min. E — control in MC; F—H: between the 40th and 50th min of exposition. Calibration — A—E: 50 mV, 10 sec; F: 25 mV, 4 sec; G: 25 mV, 40 sec; H: 60 mV, 10 sec

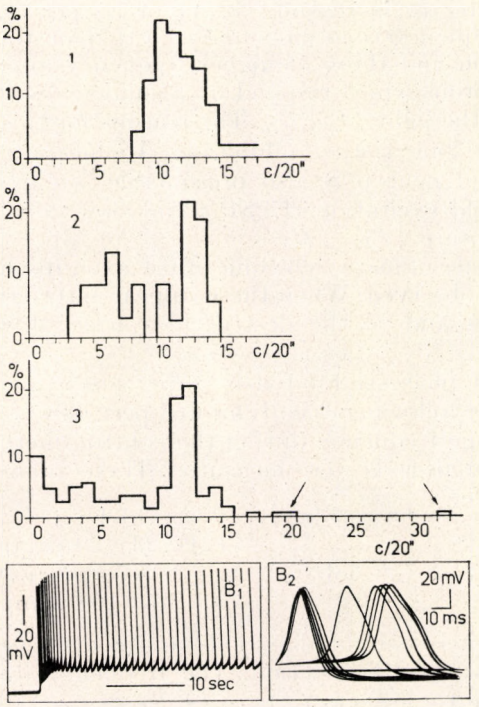

Fig. 8. Effect of 50 μg/ml MC. Neuron: 198/1. A — frequency-distribution; resolution is 20 sec; 1, 2, 3 — stain-free control, dark control in the presence of stain, in light. B_1 and B_2 — seizure-like burst in light; B_2 — shows the changes of shape, during the sequence

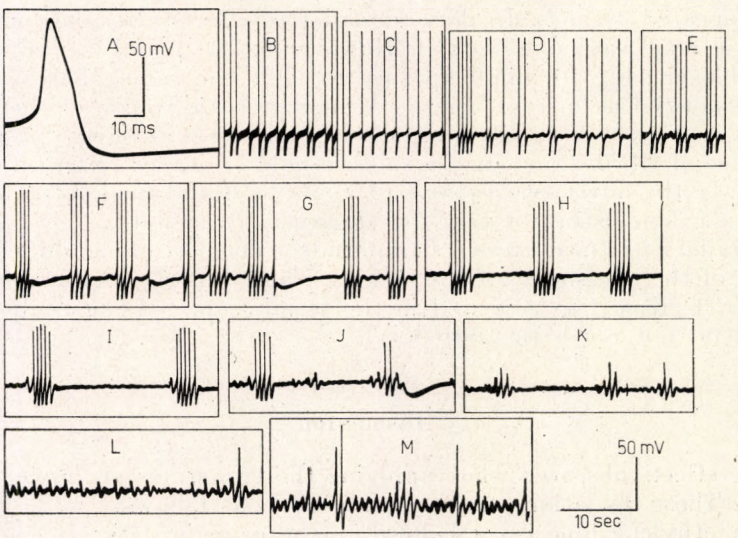

Fig. 9. Effect of 100 μg/ml MC in light. Neuron 324/1. A, B: dyestuff-free control; C — MC in dark 25th min; D—K: MC in light (1, 5, 12, 15, 25, 30, 35 and 40th min; L—M — records newly after some hours of total darkness

in the three periods are increasing: 7—11 or 32 per cent. It can be easily followed on the frequency-time plots in *Fig. 6* that on the effect of light both periods of inhibition and those of higher frequency appear.

There are neurons which respond by a sudden cessation of their activity after switching on the light *(Fig. 7)*. The reasons for this may be the appearance of EPSP-s or long lasting inhibition. An increase of threshold in the pacemaker region of neuron is also presumable, as in some cases only the signs of subthreshold excitation (EPSP-s and slow depolarizing waves) have survived light exposure *(Fig. 7D)*.

In the late-phase of methylene blue effect axon-discharge-like depolarizations can often be observed. When these appear interferring with spikes, the frequency decreases and in the destructive phase of effect the amplitude decreases as well.

The fact, that phases inhibited by other mechanisms as classic IPSP-s demonstrated by the following case. Inhibited periods whose duration exceeded 2 sec and the average frequency during the control, methylene blue/dark and methylene blue/light periods were measured. These values are:

4.2 ± 1.2	10.6 ± 3.9	19.6 ± 14.4 sec (124—23—29 samples)
11.4 ± 1.4	8.7 ± 3.3	8.7 ± 5.5 spikes/20 sec (50—37—108 samples)

A prolongation of inhibited periods and a decrease of frequency were observed already in the dark period. The frequency distributions of the 3 periods demonstrate these facts *(Fig. 8A)*. Cases marked by arrows refer to the presence of bursts with high frequency. Such a burst is demonstrated in *Fig. 8B*. These bursts are similar to those paroxismal discharges observed in rose bengal and in visible light *(Fig. 5D)*.

In the demonstrated case of sensitization with methylene blue such bursts appeared 4 times. In dark, destructive effects after the application of methylene blue was not observed. However in 100 μg/ml concentration and after a longer (30—50 min) exposure to light the same phases of damage could be noticed as in rose bengal. A characteristic course of the alteration is shown in *Fig. 9*. A significant change of rhythm, a decrease of amplitude, appearance of EPSP-s and finally subthreshold or graded, grouped oscillation are seen. In the latest two squares of *Fig. 9* a record made after 3 hours of dark period demonstrate a very low degree of reversibility.

Parallel with the decrease of amplitude, a decrease of membrane potential and that of the overshoot are also taking place. But the membrane potential has never decreased to zero and in the terminal period the peaks of action potential do not reach the zero level.

Discussion

The effects observed when applying the two stains are in certain sense different. These deviations can be summarized as follows:

1. methylene blue causes alterations already in dark;
2. in the effect of methylene blue perhaps the inhibitory phenomena are dominating;

3. in the case of methylene blue to evoke destroying effects a little higher concentration or longer exposition is needed

(50 μg/ml MC$\sim$156 μM MC; 50 μg/ml RB$\sim$50 μM RB)

Effects of the two stains are similar in the destructive phase of depolarization character and in the appearance of seizure-like bursts from time to time.

The higher sensitivity of certain neurons (for example Br-cells) perhaps is concerned with their higher endogenous pigment content. However, in stain-free medium no destructive reaction was observed. Because of such differences in the sensitivity of the individual cells, quantitative examinations, for example measurements of dose-effect curves can be carried out only when a great number of the same identified neuron is studied.

The destructive effect on the soma membrane is irreversible and taking place in discrete steps. Presumably in such cases different patches of the soma-membrane discharge without coordination which manifests in a more or less disordered fluctuation of amplitude. A probable mechanism of this that both inward and outward cation-transport are damaged as both the overshoot and membrane-potential decrease. According to this later fact in some cases a decrease in the resistance of membrane was observed (unpublished).

On the base of above outlined facts it can be stated that the photodynamic experiment in its early phase only, or after slight influences may be adequate to investigate the electron processes which are perhaps included in nerve excitation.

Summary

Spikes of 50 autactive giant neurons situated superficially on the dorsal surface of suboesophageal ganglion of *Helix pomatia* were studied in the presence of 50—100 μg/ml rose bengal and methylene blue in dark and visible light applying heat filter.

In the presence of rose bengal, the photodynamic effect is manifesting in a long-lasting increase of frequency, slow depolarization, decrease of amplitude, membrane potential and overshoot. In the terminal phase the frequency is newly decreasing and polymorph subthreshold oscillations originating from different sources having multimodal amplitude and frequency distribution, appear or remain. The effects are irreversible.

Methylene blue already in dark causes appreciable alterations of shape and frequency. In visible light the effect is more explicit. In the early phase of the influence the inhibitory phenomena may be more than before. In visible light a similar destruction takes place as in the case of rose bengal. Its effect is also irreversible.

Seizure-like burst were observed in the presence of both stains.

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AUTOAKTIV *HELIX POMATIA* NEURONOK FOTODINÁMIÁS BEFOLYÁSOLÁSA ÉS IRREVERZIBILIS KÁROSITÁSA BENGÁLVÖRÖSSEL ÉS METILÉNKÉKKEL

Lábos Elemér

Összefoglalás

Helix pomatia suboesophagealis ganglionjának 50, dorzális felszíni, autoaktív, óriás neuronjának kisüléseit vizsgáltuk 50—100 μg/ml bengálvörös, ill. metilénkék jelenlétében, sötétben, ill. látható fényben, hőszűrés mellett.

A fotodinámiás hatás bengálvörös jelenlétében a frekvencia tartós növekedésében, lassú depolarizációban, az amplitúdó, a membránpotenciál és az overshoot esésében nyilvánul meg. A hatás késői fázisában a frekvencia újra esik, és több forrásból eredő polimorf, multimodális amplitúdójú és frekvenciájú küszöb alatti oszcillációk jelennek meg. A hatás irreverzibilis.

Metilénkék már sötétben is észrevehető alak- és frekvenciaváltozást okozhat. Fényben a hatás kifejezettebb. A hatás kezdeti fázisában a gátlási jelenségek fokozódhatnak. Fényben a bengálvörös hatásához hasonló destrukció zajlik le. A hatás irreverzibilis.

Mindkét festék jelenlétében megfigyeltünk paroxizmális jellegű kisüléssorokat.

ФОТОДИНАМИЧЕСКОЕ ВЛИЯНИЕ НА НЕЙРОНЫ HELIX POMATIA СО СПОНТАННОЙ АКТИВНОСТЬЮ И ИХ НЕОБРАТИМОЕ ПОВРЕЖДЕНИЕ БЕНГАЛЬСКИМ КРАСНЫМ И МЕТИЛЕНОВЫМ СИНИМ

Э. Лабош

Была исследована спонтанная активность 50 поверхностных гигантских нейронов в подглоточном ганглии *Helix pomatia* в присутствии 50—100 μг/мл бенгальского красного или метиленового синего в темноте или при дневном свете при исключении тепловых волн.

Фотодинамическое действие в присутствии бенгальского красного проявляется в длительном увеличении частоты, в медленной деполяризации, и в падении мембранного потенциала, амплитуды потенциала действия и overshoot-та.

В последующих фазах действия красителья частота снова падает и проявляются полиморфные, с мултимодальной амплитудой и частотой подпороговые осцилляции, возникающие из разных источников. Дествие красителья необратимо.

Метиленовый синий способен вызвать заметное изменение формы и частоты потенциалов уже и в темноте. При дневном свете действие более выраженное. В начальной фазе тормозные процессы могут усиливаться. При дневном свете смеют места превращения, подобные эффекту бенгальского красного.

В присутствии обоих красителей наблюдались разряды, подобные к пароксимальным.

ANNAL. BIOL. TIHANY | 37 | 55—71 | HUNGARIA 1970

ACTIVATION OF THE ADDUCTOR IN *ANODONTA*-GLOCHIDIA BY N, N-DIALKYL-TRYPTAMINES, 5-METHOXY-TRYPTAMINE, β-ADRENERG-ANTAGONISTS, COCAINE, SCOPOLAMINE AND OTHER PHARMACONS

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Received: 7th Februay, 1969

Earlier it has been described by us that tryptamine, contrary to other indole compounds, is a relatively selective activator of the adductor in *Anodonta*-glochidia (LÁBOS et al. 1964; LÁBOS, 1966). It was found also that the most of the α- and β-adrenergic agonists and antagonists potentiate the tryptamine rhythm, moreover dichloroisoproterenol (DCI) is solely able to evoke rhythmic activity (LÁBOS, 1966). On the basis of these facts tryptamine was suspected as excitatory (LÁBOS et al. 1964) and some kind of adren ergic substance as inhibitory and/or excitatory mediators in the chemical control of adductor activity (LÁBOS, 1966). This supposition was supported by a chemical analysis according to which tryptamine and phenyl-alanine have been found in the extracts gained from the whole glochidial organism (S.-RÓZSA and LÁBOS, 1967). Electron microscopic observations of glochidia have shown nerve endings containing dense core vesicula (ZS.-NAGY and LÁBOS, 1969), the connection of which to catecholamines and tryptamines is presumed and disputed in mollusc (DAHL et al. 1963; COTTRELL and LAVERACK, 1968).

Tryptamine-sensitivity of the glochidia is variable, perhaps it is in connection with ontogenesis (LÁBOS et al. 1964). However, it has become clear, that certain non-specific factors able to modify the uptake and the effect of drugs also have to be taken into account (LÁBOS and LUKACSOVICS, 1968). Differences in the activiting effects of tryptamine, serotonin and different alkyl-tryptamines often are explained by their different permeation (VANE et al. 1069; MARLEY and VANE, 1963; OFFENMEIER and ARIENS, 1966). In other instances, the central excitatory effects of alkyl-tryptamines are considered as specific (LESSIN et al. 1965; SZARA, 1964; GERSCHON and BELL, 1963; OFFENMEIER and ARIENS, 1966).

By all means, therefore, it seemd to be reasonable to extend the experiments to substances with alkyl-indol-amine structure and of sympathetic type. Thus, the pronethalol and propanolol appeared to be important as being more selective-adrenolytic drugs as DCI (BLACK and STEPHENSON, 1962; KOCH-WESER, 1964) and also the N,N-diethyl-tryptamine (DET) was chosen, because it is one of the most complex central excitant indole-compound (SZARA, 1964). Among others, yohimbine (α-adrenolytic), cocaine (adrenaline-sensistor) and scopolamine (cholinolytic) were tested, to get complementary information to the pharmacology of glochidia.

Methods

By a method which has been described elsewhere (LÁBOS et al. 1964; LÁBOS, 1966), groups consisting of 10—25 larvae have been gained from the external gill of *Anodonta* were observed. We habe noted the number of rhythmic contractions in each minute and the ratio of glochidia being in closed state. The results were obtained from experiments (at least) on 100 animals or they refer to 100 animals. The total number of the glochidia used for the experiments was about 15.000. Only glochidia originating from the same population and tested in the same day were taken for comparisons.

Lake water of Balaton (BW) or distilled water (DW) were utilized as solvents. In general the concentrations of the applied materials refer to salts.

List of the applied substances: tryptamine HCL (TA; Schuchardt), N,N-diaethyltryptamine bioxalate (DET; Koch-Light), N,N-diaethyl (DET; Serva), serotonin-creatinine-sulphate (5HT; Sandoz), N,N-dimethyl-tryptamine-H-oxalate (DMT; EGA), 5-methoxytryptamine (5MeOTA; Mann Ltd), bufotenine H oxalate (5-OH-DMT; Fluka), melatonin purum (Fluka), cocaine HCl (Fluka), L-scopolamine (Fluka), atropin sulphate (Fluka), N-Br-methyl-atropine, Halidor (EGYT-201; 1-benzyl-1-3'-di-methylamino-propoxy cyclo-heptane), tetracaine HCl (EGYT), procaine HCL, ergometrine-H-tartarate (BDH), papaverine HCl (EGYT), γ-amino butiryc acid (Reanal; GABA), ergotoxine-methane-sulphonate (BDH), brom-lysergic acid-diethylamide (BOL-148, Sandoz), methysergide (UML-491, Sandoz), yohimbine HCl (Merck), chlorpromazine HCl (CPZ; EGYT), L-adrenaline-D-H-tartarate (EGA), L-noradrenaline-bitartarate (Serva), dopamine HCl (DA; Sigma), DL-isoproterenol (IPNA; Fluka), tyramine HCl (Fluka), ephedrine sulphate, dibenamine HCl, di-chloro-isoproterenole HCl (DCI, Eli et Co. Ltd.), alderlin HCl (nethalide, pronethalol; Wilmslow Ltd) propranolol HCl (Inderal; ICI), nicotinic acid, Vitamin B_6, pyrydoxale-5'-phosphate (Py-5'PO_4; Sigma), isonicotinicacid hydrazide (INH), iproniazide, actomol (ICI Ltd; Spinks and Whittle, 1966) carbamide, LiCl (BDH), NaCl, $CaCl_2$, $MgCl_2$, NaN_3, KCN, 2,4-DNP, ouabaine, α,α-dipirydyl (Chinoin), NaF, $CdCl_2$, cyclic-3',5'-adenosine monophosphate dibutyrate (cAMP); Boehringer Co), caffeine, theophylline, histamine HCl (Fluka), histidine, cystamine, acetylcholine Cl (Sandoz, ACh), d-tubocurare (d-TC), nicotine tartarate, reserpine (NCCo).

Results

1. Effects of pronethalol, DCI and propranolol

Already in 50—100 μM concentrations, pronethalol evokes a detectable rhythmic activity. Both in water of Balaton or distilled one, the activity often reaches 500 cmp maximal frequency *(Fig. 1)*. For higher concentrations (250 μM) it was typical that the activity stops suddenly after about 5 min. In DW the response is more prolonged. A tonic closure was not characteristic of a predominant majority of the populations even after applying 500 μM. However, sometimes such populations were found, which hardly responded by rhythmic actvity and after a certain pause a closure has taken place.

The pattern of individual rhythm follows the reaction observable in groups but there are glochidia whose maximal activity reaches a peak-frequency of 40—60 cpm/glochidium for 1/2—2 min. It is often noticeable that the maximal activity is followed by contractions organized in groups.

Approximately the same concentrations of DCI evoke a rhythm of similar degree and time-course as those of pronethalol. The attainable peak-frequency

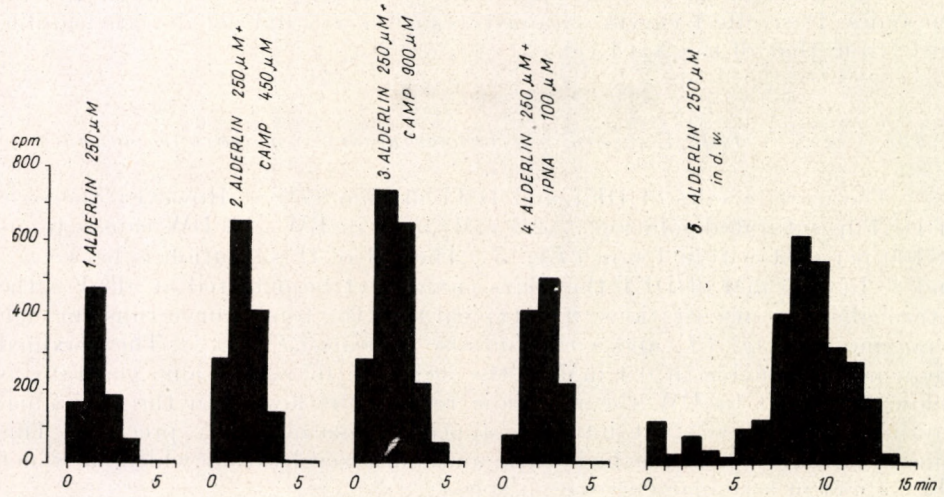

Fig. 1. Effect of pronethalol alone and in the presence of cAMP and IPNA. 100—100 glochidia. The solvents are distilled water (5th diagram) or Balaton-water (1st—4th diagrams)

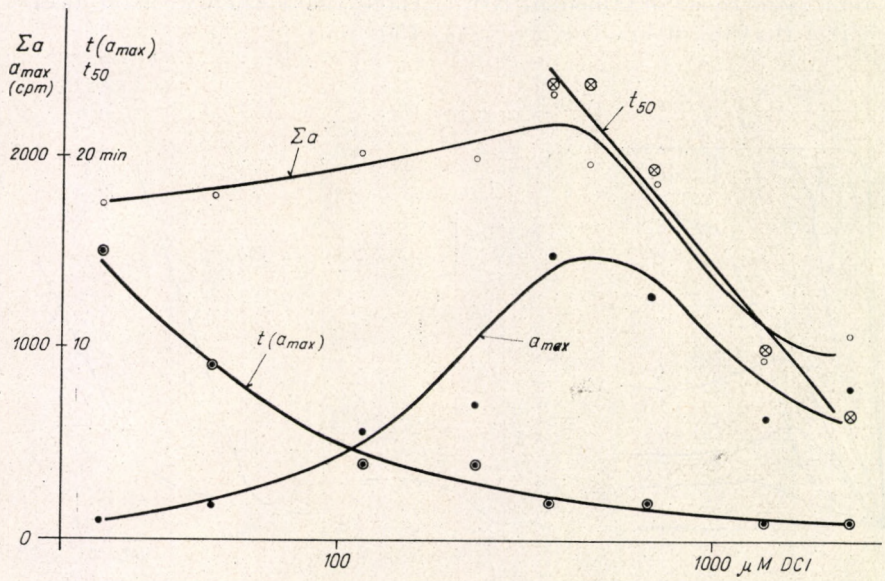

Fig. 2. DCI-effects. 100—100 animals. *Abscisse:* concentration; *ordinates:* total number of observed contractions (Σa), maximal frequency (a_{max}, cpm), time required for closure of 50 per cent (t_{50}) and time of maximum frequency ($t(a_{max})$)

is 400—1000 cpm. The sudden stop of activity is more typical. Independently from the rhythm following the stop by a considerable delay closure may appear *(Fig. 2)*. The time between the rhythm of maximal frequency and the closure can be even 10—20 min. In the individual cases it could not be observed that the activity of high frequency would damp gradually or by grouping activity (that is by an appearance of pauses).

The effect of propanolol was tested by diluting the content of Inderal ampoules. Dose-effect curves obtained in such a way did not deviate significantly from that of the NaCl vehicle.

2. *Effect of N,N-dialkyl-tryptamines, bufotenine and 5-methoxy-tryptamines*

The oxalate salts of DET and DMT are ineffective. However, the base of DET in saturated solution (~2.5 mM) both in BW and DW causes tonus of 100 per cent within 1 min *(Fig. 3)*. The pH of this solution is between 7 and 8. In 0.25 mM of DET the tonus cannot yet be detected at all. For the intermediated concentrations, it is typical that the tonus-curve runs through a maximum *(Fig. 3/1)* and a rhythm also appears *(Fig. 3/2)*. The maximal frequency attainable in 0.4 mM at the 3rd—5th min. Its value generally is below 200 cpm. In DW the rhythmic response is lower. In the individual rhythm patterns constant intervals appear, sometimes very precisely. This constant frequency is reached by monotonous acceleration which does not always end in a similar decrease in speed.

In DW, by lower concentrations of 5-methoxy-tryptamine a quick tonic closure can be elicited, almost without rhythmic activity. For example 250 μM leads to a closure of 50 per cent within 6—7 min. These results are different from those obtained in BW (LÁBOS, 1966) when we have been able to elicit a rhythm of low frequency (< 150 cpm).

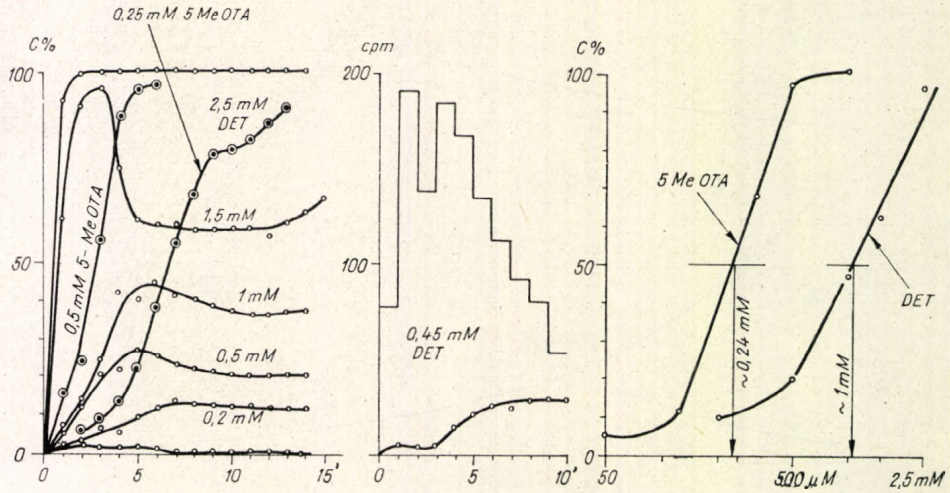

Fig. 3. DET (○) and 5-MeOTA (⊙) effects. 1. Tonus-time diagrams; 2. Frequency and tonus-time diagrams; 3. Dose-response (tonus-ratio)-curves. 100-100 animals. Solvents: lake-water (DET) and distilled water (5-MeOTA)

Earlier it has been published by us that 150—300 μM concentrations of bufotenine, examining for 20 min, were not effective (LÁBOS, 1966). Because of the effectiveness of DET a testing of higher concentrations for a longer time of incubation seemed to be reasonable. Indeed, it has become clear that already 160 μM can evoke a low-frequency rhythm, however only after about 1 hour of incubation. But when the concentration has been increased above 1 mM, the rhythmic activity was elicited in the 10th min, the frequency

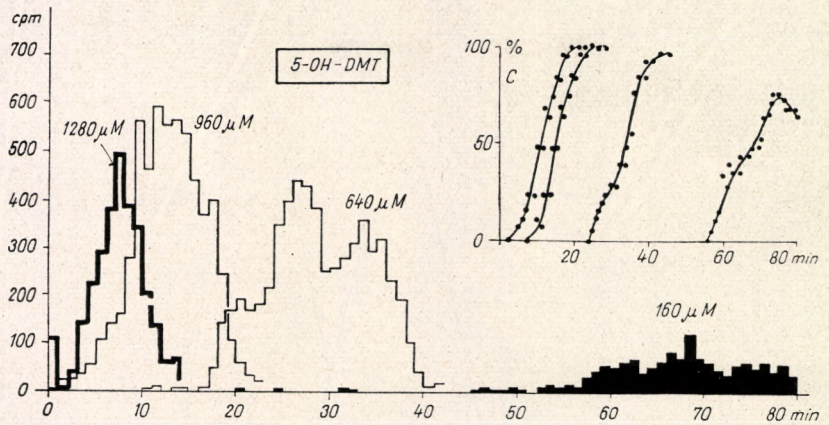

Fig. 4. Bufotenine-response. Frequency- and tonus-time diagrams. 100-100 animals. Balaton-water

of which is near to that of the tryptamine. The required concentrations of bufotenine are yet higher by 2—3 than those of the equipotent tryptamine (LÁBOS et al. 1964; LÁBOS, 1966). Also a further difference is, that bufotenine may lead to a closure of 100 per cent *(Fig. 41)*. In lower concentrations the closure-time curves have inflection or maximum.

3. *Effects of cocaine, scopolamine, atropine, novatropine, yohimbine, ergometrine, ergotoxine*

Both cocaine and scopolamine evoke a considerable and long-lasting rhythmic activity *(Fig. 5, 6,* and *7)*. Neither of them lead to tonus even in very high doses. The contractions are more complete in scopolamine than in cocaine, but in both compounds they become gradually of fibrillation-like. L-scopolamine causes an increase of activity already in 300 μM concentration. In higher concentrations the rhythm is of lower frequency and ceases earlier without any closing. A consequence of this that the dose-response curve shows a maximum. The threshold concentration of cocaine is about 150 μM. However its dose-response curve is similar to that of the scopolamine *(Fig. 5)*. The maximum of cocaine-curve is at 700 μM and that of the scopolamine s at 1600 μM. These values refer to BW. In DW a shift to right is observable. The dose-effect curve of atropine is comparable with those of the cocaine and scopolamine, except that its maximum is significantly lower than those of the

former ones. For the position or amplitude of dose-response curve-maxima the following is valid, respectively *(Fig. 5)*:

cocaine < atropine < scopolamine
or cocaine, scopolamine > atropine

The effect of atropine cannot be influenced by ACh.

In experiments for 50 min, novatropine is not effective even in concentrations above 1 mM.

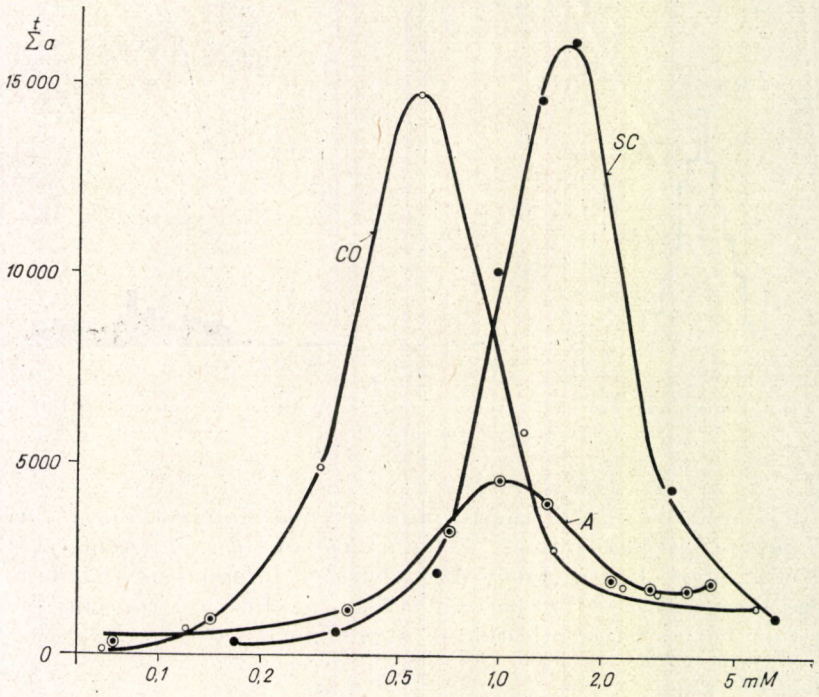

Fig. 5. Cocaine (CO), scopolamine (SC) and atropine (A) dose-effect curves. *Abscisse:* concentration (mM base); *ordinate:* number of produced rhythmic contractions in a given period. Summation has been carried out for 40, 80 and 20 min for the three drugs respectively, as it was necessary. Each points: 100 glochidia. Solvent: Balaton-water

Both in cocaine and scopolamine the time course of the individual patterns are rather variable. In lower concentrations, the rhythm accelerates gradually but slows down periodically. The periodicity of the slowing down is often very definite and even during observations of groups is detectable. Contractions organized in bursts or recurring in precise intervals have been observed only in cocaine. The scopolamine rhythm includes periods of less exact and its periodic modulation is less explicit.

250—500 μM yohimbine evokes a low-frequency rhythm, a tonus running through a maximum. Ergotoxine evokes a tonus while ergometrine does a rhythm of low frequency *(Fig. 8)*.

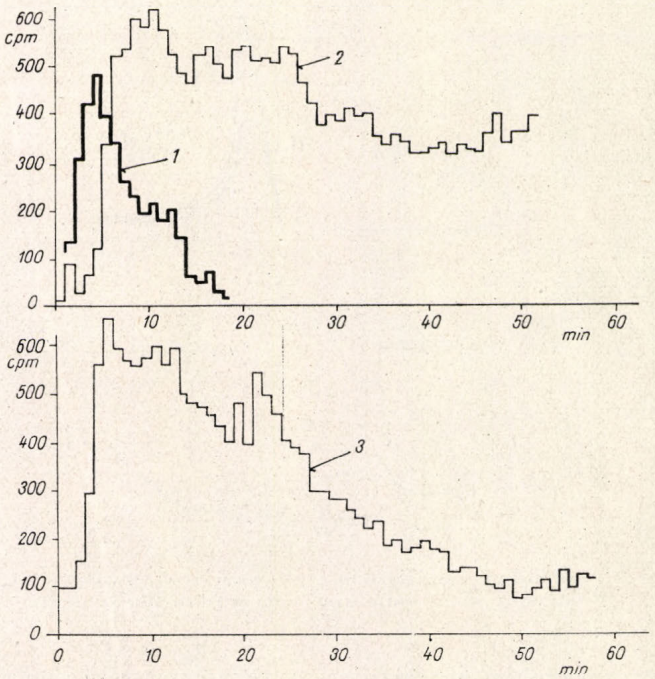

Fig. 6. Noradrenalin on scopolamine rhythm. 100-100 animals. 1. 1 mg/ml L-scopolamine; 2. 1 mg/ml L-scopolamine + 100 μg/ml noraderenaline; 3. 500 μg/ml scopolamine

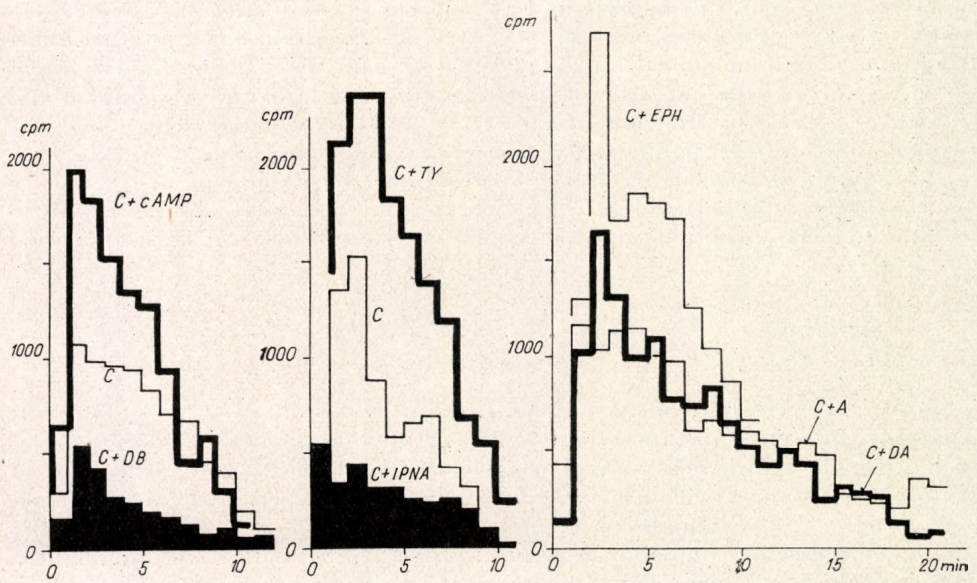

Fig. 7. Susceptibility of cocaine-response. Frequency-time curves. 100-100 animals. Balaton-water. C = cocaine; DB = dibenamine; EPH = ephedrine; DA = dopamine; A = adrenaline; IPNA = isoproterenol; cAMP = cyclic AMP; TY = tyramine

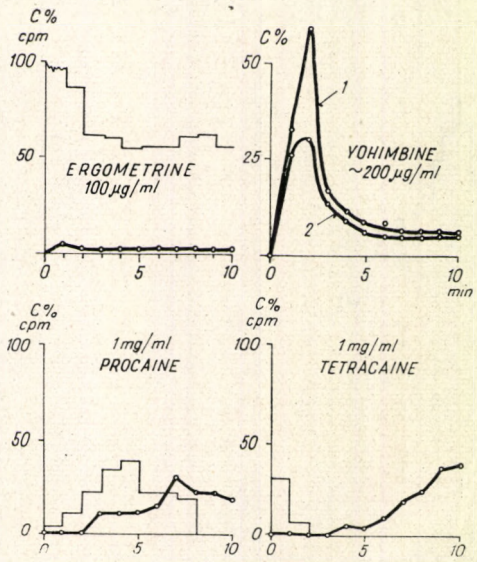

Fig. 8. Yohimbine, ergometrine, procaine and tatracaine effects
Frequency and tonus-time curves. 100-100 animals

4. Effects of scopolamine and cocaine in the presence of sympathomimetica, cAMP and dibenamine. Influence of reserpine preincubation

The effects of 200 µg/ml cocaine and 1 mg/ml scopolamine are inhibited by 100—200 µg/ml dibenamine and IPNA. The cocaine effect is more or less potentiated in the presence of 100—200 µg/ml adrenaline, noradrenaline, tyramine, dopamine, ephedrine and 200—400 µg/ml cAMP *(Fig. 7)*. The effect of 1 mg/ml scopolamine is not potentiated by adrenaline, dopamine and ephedrine. However, the potentiation by cAMP is of small degree and the scopolamine-effect is significantly prolonged by noradrenaline *(Fig. 6)*.

A preincubation in 10—20 µg/ml reserpine is not showing any influence on the DCI or pronethalol effect. A slight inhibition was observed only in cocaine at certain populations. This inhibition is present in the later period of activation *(Fig. 9)*.

5. Susceptibility of DCI-response. Pronethanol rhythm in cAMP and pyridoxale-5'-phosphate

We attempted to influence the rhythm evoked by 150—600 µM DCI, applying different substances in 10—200 µg/ml concentrations. This rhythm, as taking place in a relatively short period, is suitable to test on it a lot of different substances within a reasonable time. The experiments were carried out with 100—100 animals for 10 min.

The following compounds has been proved to be ineffective or a slightly potentiating (±20 per cent) on DCI: ACh, atropine, nicotine, dopamine, adrenaline, ephedrine, INH, tyramine, cystamine, GABA, ouabaine, $CaCl_2$, carbamide, creatinine.

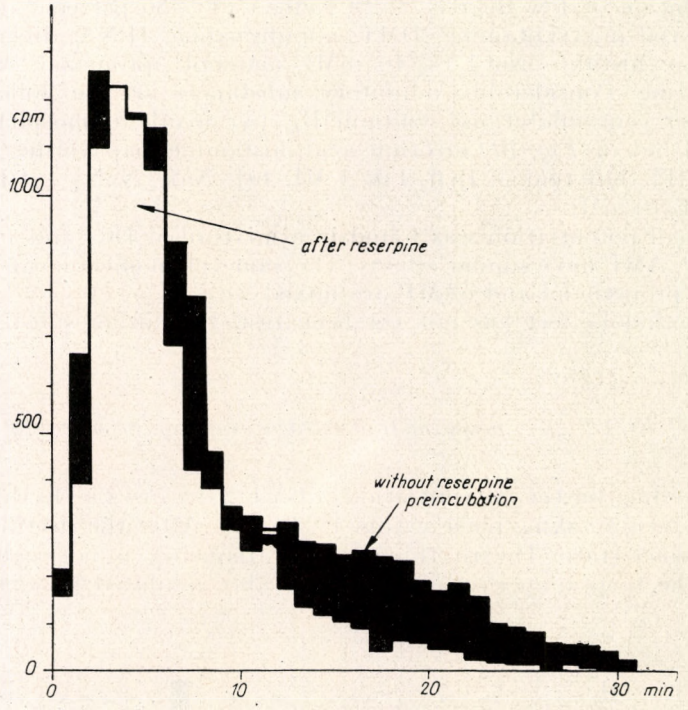

Fig. 9. Reserpine preincubation on the cocaine-rhythm. 100-100 animals. 200 μg/ml cocaine; 50 μg/ml reserpine preincubation for 3 hours. Solvent: Balaton-water

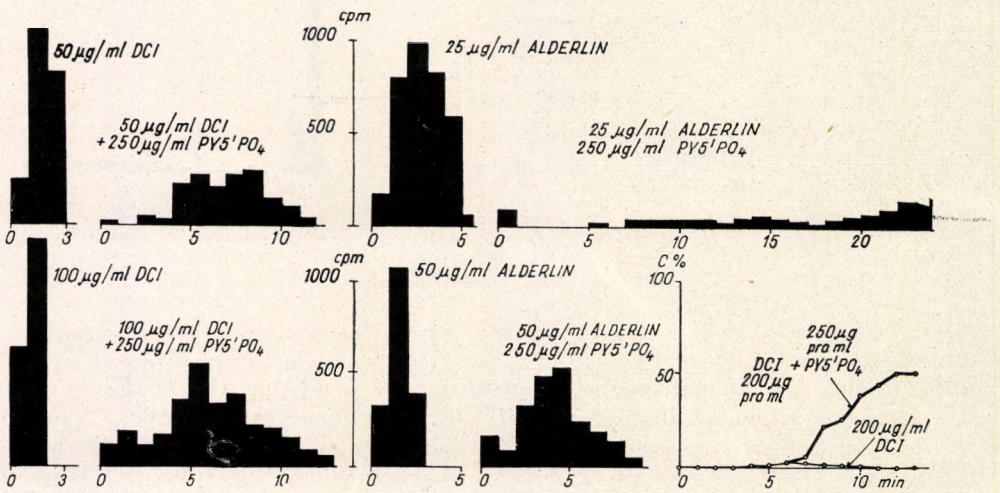

Fig. 10. DCI and pronethalol-rhythm in pyridoxale-5'-phosphate. Frequency-time curves. 100-100 animals. Balaton-water

Inhibition of low degree on DCI-effect (30—50 per cent decrease) has been observed in: tryptamine, DMT, noradrenaline, IPNA, dibenamie, CPZ, ergometrine, histidin, NaCl ($\leq$ 10 mM), actomol, iproniazid, $MgCl_2$ ($\leq$ 10 mM), creatine. Considerably inhibitory substances are the following (more than 80 per cent inhibition): vitamin B_6, pyridoxale-5'-phosphate (see also for pronethalol in *Fig. 10)* nicotinic acid, histamine, papaverine, theophyllin, caffeine, 5HT, bufotenine, BOL-148, UML-491, NaF, NaN_3, 2,4-DNP, $CdCl_2$, KCN, LiCl (10 mM).

A small potentiation was found in a mixture of DCI and 1 mM cAMP. ATP, ADP, AMP have similar effects. The same phenomenon can be observed also when pronethalol and cAMP are mixed.

Pronethalol-effect has not yet been tested by other substances.

6. *Effects of EGYT-201, procaine and tetracaine (Fig. 8 and 11)*

Depending on the concentration, EGYT-201 causes a rhythmic activity of high frequency taking place within 1—10 min. After the rhythm the larvae rest in opened state. The rhythm of peak frequency is noticeable at 100—200 μM. The tryptamin rhythm is considerably inhibited by this substance.

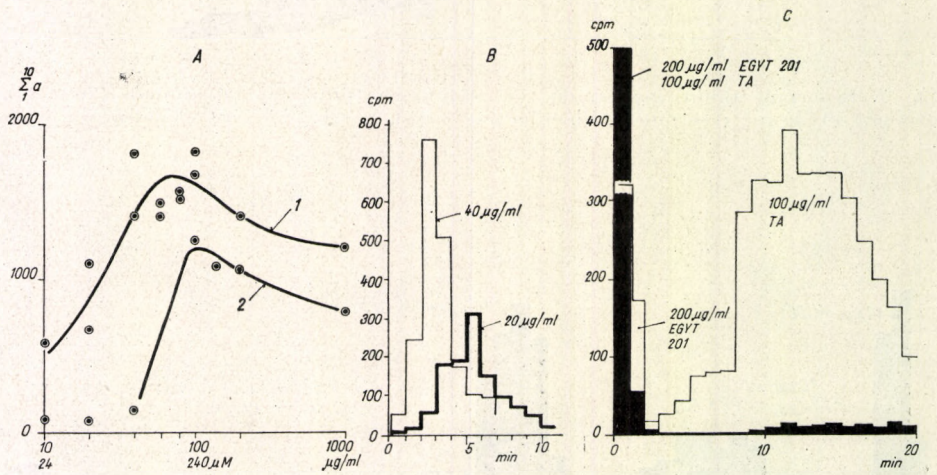

Fig. 11. Effects of EGYT—201. A — dose-response curves in Balaton (1) and in distilled water (2); B — frequency-time curves (Balaton-water); C — inhibition of tryptamine-rhythm (Balaton-water)

In 0.45 or even in 4.5 mM concentrations, the local anaesthetics do not evoke rhythmic activity of high degree. In similar concentrations, the β-blocking substances or the tropeine compounds can elicit an activity of 5—20 higher frequency. In *Fig. 8* the effect of high concentrations of procaine or tetracaine are demonstrated. A closure of low degree and a negligible rhythmic activity is typical (see *Table 1* and *Fig. 8).*

TABLE 1

Pharmacon	Threshold μM	Optimal μM	Tonus	Σa_{max}	a_{max}	Time course min	Rhythm	Pattern, modulation or timecourse of individual rhythm
DCI	10	500	late	2 000	1000	3—10	short, high	sudden stop
Pronethalol ...	50	350	late	3 000	500—800	5—15	short, high	clock-like accuracy of intervals, later periodic
Cocaine	150	600	Ø	15 000	500—1000	10—35	long, high	clock-like accuracy of intervals, later bursts
Scopolamine ...	350	1700	Ø	15 000	500—1000	10—80	long, high	slightly periodic
Atropine	150	1000	Ø	4 500	500—700	5—20	medium,high	irregular
Yohimbine			two phases	~100	<50	5—10	very low	Ø
Ergometrine ...			Ø	400	100	5—10	very low	Ø
DET	350	1000	two phases	2 000	200	10—15	low	clock-like accuracy
5MeOTA	150	240	sudden	Ø	Ø	3—10	Ø	Ø
Bufotenine	150	1000	synchron	6 000	600	15—80	high	regular
EGYT—201	20	100	late	1 700	800	3—10	short	short-lasting, regular
Procaine			low		Ø	1	Ø	Ø
Tetracaine			low		50	5	very small	Ø
Tryptamine ...	10	500	Ø	4 000	1000	5—50	high	regular

Explanations see in the text and legenda

Discussion

Before analyzing the possible specific effects of the tested substances we have to discuss the most important phenomena regarded to be non-specific.

As cocaine, yohimbine, atropine, ephedrine and EGYT-201 have an accessory effect of local anaesthetic (HAUSCHILD, 1961, p. 698), this possibility seems to be important. As that the true local anaesthetica are not activator *(Fig. 8)*, for this reason the activator effects cannot be considered as coming from such a property of the compounds even in the case of EGYT-201 which proved to be a potent local anaesthetic agent on Anodonta nerve (unpublished). Exclusively the cessation of the rhythm and perhaps the late and not consequent tonus evoked by pronethalol may originate from this. However, the descending parts of the dose-response and those of the frequency-time curves of cocaine, scopolamine or atropine may originate from a conduction block as well.

One may interpret the non specific origin for example as a "general membrane activation". But this term is too much undifferentiated and the fact that the effects are generally distinguishable from each other contradicts this possibility *(Table 1)*. Therefore, their points of attack may be different in the different groups of substance (cocaine-scopolamine-atropine or DCI-pronethalol). During the experiments showing the heterogenous susceptibility of DCI-effect, several sympathetic pharmacons have proved to be more or less effective as potentiating or inhibitory agents.

These facts require to discuss the question of specificity for DCI and pronethalol in detail and also for similar reasons the possible effects of cocaine, scopolamine and atropine have to be raised and discussed as well.

As two of the examined β-antagonists (DCI and pronethalol) cause rhythmic activity ending by a relatively sudden stop without closure we do not think that the possibility of an adrenergic control (LÁBOS, 1966) may be out of question when considering these factors playing a role in the adductor activity. The effect of propranolol in NaCl-milieu cannot be compared to these effects as simply attributed to the different conditions. Consequently further experiments are desirable.

It is known that DCI and pronethalol have properties of β-agonists (POWELL and SLATER, 1958; BLACK and STEPHENSON, 1962; KOCH-WESER, 1964; BLOOM and GOLDMAN, 1966; ARIENS, 1967). For this reason it is difficult to decide whether the observed activity — if it is specific — is a consequence of a β-agonist or β-antagonists property. The IPNA itself is not an activator but it inhibits the DCI rhythm (LÁBOS, 1966). Applying solely, cAMP is also ineffective, nevertheless, it potentiates slightly the effects of DCI and pronethalol. However this latter fact have to be considered as non-specific for cAMP, in accord with the observations of KIM et al. (1968), that AMP, ADP and ATP also can elicit similar effects. All these circumstances support an interpretation that the effects would originate — if they are specific — from "antagonist-activation". This would correspond with the findings of POWELL and SLATER (1958) concerning DCI and with the AHLQUIST's definition (1948) of the β-agonist which includes inhibitory effect.

The specificity concerning the influences evoked by different substances on DCI rhythm can be proved only by a quantitative method, testing the competitive mode of interrelations which may be a severe criterium (ARIENS,

1967) for each substances separately. Nevertheless, it is presumable, that IPNA inhibits the DCI response acting at the same point, where DCI evokes its effect. On the other hand, the non-competitive character (as an aspect of non-specificity) is evident for several substances (for example indole-compounds, metabolic inhibitors, cations, etc. . . .) influencing the DCI-response.

The activation evoked by the cholinolytic L-scopolamine which lasts for an exceptionally long time and is of high frequency is conspicious. It has been noticed before that atropine also causes a rhythmic response but this has considerably lower frequency (LÁBOS et al. 1964). A comparison of the dose-response curves in *Fig. 5* supports this observation. In higher animals scopolamine is mainly but not always (MÉHES, 1927), tranquillant and atropine is excitant (BRADLEY and ELKES, 1953; FORREZ, 1951; RINALDI and HIMWICH, 1955; RINALDI, 1965; ISBELL et al. 1964; WADA et al.1963).

As cocaine, scopolamine and atropine have similar structure and cocaine is adrenomimetic agent it may be raised that the effect of these drugs somehow is related to the presumed adrenergic control, because their local anaesthetic and cholinolytic activator effects are less probable or even can be excluded.

This is not surprizing for cocaine, but scopolamine or atropine are generally not considered as agents with direct sympathomimetic effect. However in our case the cholinergic control is not probable (LÁBOS et al. 1964; LÁBOS, 1966). This is supported also by the fact, that ACh does not inhibit the atropine-rhythm. On the other hand we must take into account that quaterner substances as ACh or novatropine may be ineffective because of their retarded permeation. Finally the sympathomimetic effect is just which cannot be excluded in interpreting the effect of the three tropeine-like pharmacons. Whether these effects are really sympathomimetic or other receptors being able to respond to these agents are responsible — it is an open question.

It is interesting to consider a recent paper of KALSNER and NICKERSON (1969). They explain the cocaine-potentiation of responses to amines by a direct hyperresponsive influence of cocaine on the effectors which is not related to uptake or storage of amines and at the same time the potentiation deviates from the procaine-like properties of cocaine. A possibility of a purely pharmacological interpretation without any endogenous sympathetic or adrenerg system is also possible.

The presence of an "adrenergic" control may be supported by the results showing that dibenamine, IPNA and perhaps reserpine inhibit the cocaine rhythm. But there are differences between scopolamine and cocaine concerning to the potentiation of their effects by catecholamines and ephedrine. These differences may be in connection also with the high concentrations of scopolamine required to attain an equipotent effect with cocaine *(Fig. 5)*.

It is more difficult to make these activating and potentiating effects consistent with the similar activating character of DCI and pronethalol. It is not impossible that an undifferentiated receptor system plays a role in the two groups of phenomenon. This is supported by the inhibition of cocaine-rhythm by dibenamine and IPNA and also by its potentiation by adrenaline, dopamine, noradrenaline, tyramine, ephedrine. In this respect numerous examples are known from the literature when α- and β-effects cannot be well distinguished (ARIENS, 1967; PATIL et al. 1968; GOVIER, 1968; LÁBOS, 1966). Furthermore the fact that ineffective sympathomimetics (for example ephedrine) are of MAO-inhibitor and are able to prolonge the effect of others

(MÉHES, 1927) also may be responsible for potentiations. On the other hand certain metabolites or metabolic inhibitors (nicotinic acid, vitamin B_6, INH, iproniazide, actomol, pyridoxale-5'-phosphate) are more or less effective on the DCI or pronethalol rhythm. An interpretation of such phenomena is rather complex, as for example, β-adrenolytic drugs also can inhibit MAO (GREEF and WAGNER, 1966).

Connection of the activations with an adenyl-cyclase system (BUEDING et al. 1966; BLOOM and GOLDMAN, 1966) does not seem to be close if it exists at all, as cAMP effect is of small degree.

The hallucinogen and central excitant DET and bufotenine (SZARA, 1964; LESSIN et al. 1965; DOWNING, 1964) are activators as it has been expected. It must be mentioned that besides the specific differences in the effects of indole compounds or their different permeation (VANE et al. 1960; WOOLEY and SHAW, 1962; MARLEY and VANE, 1967) — because of the ineffectivity of DMT and DET-oxalate — disturbing influence of counter-anion may also play a role. Numerous examples have been given here that even the solvent itself is not neutral. Thus, the distilled and Balaton-water have different influences on the effects of drug. The latter is rich in ions and it is possible that different cations are responsible for the observed differences.

Summary

Effects of activator substances were compared which elicit rhythmic or tonic adductor activity of *Anodonta* glochidia (see *Table 1).*

It can be stated that:

1. DCI and pronethalol evoke a rhythm in 100 μM concentration cessing suddenly. A late tonus was observed independent from the rhythm. Propanolol in NaCl does not evoke proper rhythmic activity.

2. DET leads to rhythm and a tonus of medium degree; 5-MeOTA evokes tonus almost without rhythm. Bufotenine in 1 mM concentration elicits a rhythm of high frequency.

3. The rhythm evoked by DCI can be influenced by indole compounds, secale-alkaloids, metabolic inhibitors (KCN, NaN_3, 2,4-DNP), cations (Na^+, Li^+, Ca^{2+}). Typical inhibitions are caused by pyridoxale-5'-phosphate. nicotinic acid, vitamin B_6, INH and actomol. The cAMP potentiates slightly.

4. Cocaine evokes in 100—2000 μM concentrations a high frequency rhythm which is sometimes periodic; maximal activity is at 600 μM.

5. Scopolamine can produces a long-lasting rhythm (80 min). It is effective in 500—5000 μM concentration; maximum is at 1700 μM.

6. IPNA and dibenamine inhibits, 0.5—1 mM cAMP and catecholamines ($\sim$0.5 mM) potentiates the cocaine- or scopolamine-effects. A reserpine-preincubation slightly inhibits the late phase of cocaine-effect.

7. Yohimbine and ergometrine causes a low-frequency rhythm; the latter evokes a transient tonic closure as well.

8. EGYT-201 (spasmolyticum and local anaestheticum) elicits a short-lasting and very frequent rhythmic activity. True local anaesthetica (procaine and tetracaine) do not activate.

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N, N-DIALKIL TRIPTAMINOK, 5-METOXI-TRIPTAMIN, β-ADRENERG-ANTAGONISTÁK, KOKAIN, SZKOPOLAMIN ÉS EGYÉB FARMAKONOK AKTIVÁLÓ HATÁSA *ANODONTA*-GLOCHIDIUMOK ZÁRÓIZOM-TEVÉKENYSÉGÉRE

Lábos Elemér

Összefoglalás

Anodonta glochidiumok ritmusos és tónusos záróizom-tevékenységét aktiváló anyagok hatását hasonlítottuk össze. Megállapítottuk, hogy

1. a DCI és nethalide 100 μM koncentrációban általában hirtelen leálló magas frekvenciájú ritmikus választ váltanak ki; tónust nem észleltünk;
2. a DET ritmikus választ és közepes fokú tónust hoz létre, míg az 5MeOTA tónust és igen kisfokú ritmust okoz. A bufotenin 1 mM koncentrációban igen nagy frekvenciájú ritmust vált ki;
3. a DCI és Alderlin-ritmus befolyásolható indolvázas vegyületekkel, anyarozs-alkaloidákkal, anyagcseregátlókkal (KCN, NaN_3, 2,4-DNP), kationokkal (Na^+, Li^+, Ca^{2+}). Jellegzetes gátlást piridoxál-5'foszfát, nikotinsav okoz a B_6-vitamin és INH. A cAMP kissé potencíroz;
4. a kokainnal 50—2000 μg/ml koncentrációban magas frekvenciájú — esetenként periodikus — ritmikus tevékenységet lehet kiváltani; maximális tevékenység 700 μM-nál észlelhető;
5. a szkopolamin igen hosszan (80 perc) elnyúló, magas frekvenciájú ritmust hoz létre 200—2000 μg/ml koncentrációban; maximális a ritmus 1,6 mM-nál;
6. IPNA és dibenamin gátolja, 200—400 μg/ml cAMP és katecholaminok (100 μg/ml) általában fokozzák (egyes esetekben hatástalanok) a kokain és szkopolamin hatását. Reszerpin-előinkubáció gátolja a kokain-hatást;
7. a yohimbin, ergometrin alacsony frekvenciájú ritmust okoz; előbbi átmeneti tónusos zárást is kivált;
8. az EGYT—201 (spazmolitikum és lokalanesztetikum) rövid lefolyású igen magas frekvenciájú ritmikus aktivitást vált ki.

ВОЗБУЖДАЮЩЕЕ ВОЗДЕЙСТВИЕ N—N ДИАЛКИЛ ТРИПТАМИНОВ, 5-МЕТОКСИТРИПТАМИНА, β-АДРЕНЕРГИЧЕСКИХ АНТАГОНИСТОВ, КОКАИНА, СКОПОЛАМИНА И ДРУГИХ ФАРМАКОЛОГИЧЕСКИХ ВЕЩЕСТВ НА АКТИВНОСТЬ ЗАПИРАТЕЛЬНОЙ МЫШЦЫ ГЛОХИДИЕВ БЕЗЗУБКИ

Э. Лабош

Возбуждающее действие веществ было сравнено в отношении тонической и ритмической деятельностей глохидиев беззубки. Было установлено, что:

1. ДЦИ и нефалид в концентрации 100 μм вызывают быстро развивающийся ритмический ответ высокой частоты. Наблюдается и поздний, независящий от ритма тонус.

2. ДЕТ вызывает ритмическую реакцию и тонус средней величны, а 5-метокситриптамин вызывает тонус и слабое увеличение ритма. Буфотенин в концентрации I mм вызывает ритм высокой частаты.

3. Ритм вызванный под влиянием ДЦИ и алдерлина видоизменяется при даче индольных соединений, алкалоидов, спорыньы, инхибиторов обмена веществ (KCN, NaN_3, 2,4-DNP) и катионов (Na^+, Li^+, Ca^{++}). Характерное торможение наступало под действием пиридоксал-5-фосфата, никотиновой кислоты, витамина $Б_6$, INH и актомола. Цикличный АТФ некоторое усиление ответа.

4. Кокаин в концентрации 50—2000 μг/мл вызывает ритмическую деятельность высокой частаты и иногда-периодическую реакцию; максимальный эффект был регистрирован при 700 μм.

5. Скополамин вызывает ритм высокой частоты и продолжительности (80 мин) в концентрации 200—2000 μг/мл; максимальное увеличение ритма наблюдалось в концентрации 1,6 мМ.

6. Изопропилнорадреналин и дибенамин тормозят, цикличный АТФ (200—400 μг/мл) и катеколамины (≈100 μг/мл) вообще увеличивают эффект кокаина и скополамина, или иногда неэффективны. Обработка резерпином предотвращает позднюю стадию эффекта кокаина.

7. Йохинбин и эргометрин вызывают ритм низкой частоты. Первое из них вызывает и временное тоническое закрывание.

8. EGYT—201 (спазмолитическое и локаланастетическое средство) вызывает кратковременную ритмическую активность очень высокой частоты.

ANNAL. BIOL. TIHANY | 37 | 73—83 | HUNGARIA 1970

INITIAL VALUE LAWS APPLIED TO SPONTANEOUS RHYTHM, EVOKED OSCILLATIONS AND APERIODIC RESPONSES OF ANODONTA ADDUCTOR MUSCLES

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Received: 1st January, 1970

The law of initial value (L.I.V.) was formulated firstly by WILDER (1931) and describes correlations between the initial value of a biological parameter and its change after stimulation. It shows also that on repeating a stimulus, its effect becomes less and less; and when the system is unbalanced, its behaviour is often oscillatory (WILDER, 1962; SOLLBERGER, 1965).

It had been observed earlier, that when the cerebrovisceral-connectives (CVc) of *Anodonta* are excited, the responses of the posterior adductor muscle (PAM) are variable, even when the stimulus parameters are constant. In such cases the parameters of the response seem to be influenced by the initial muscle-length, its eventual change in a given direction, previous stimulation and the phase of the periodic activity (SALÁNKI and LÁBOS, 1963).

After stimulating the CVc, the adductor muscle in many instances, performs rhythmic contractions and approaches the initial or a new tonus level. Such homeostatic or servomechanism properties are characteristic of a system which follows the L.I.V. (SOLLBERGER, 1965). Because of this close analogy, a systematic and more exact analysis of the related phenomena was considered desirable.

Methods

Both spontaneous and evoked contractions and relaxations of the freshwater mussel's *(Anodonta cygnea* L.) posterior adductor muscle (PAM) were recorded and analysed. The animal was taken out of the water and the anterior and posterior adductors were disconnected mechanically as described earlier (SALÁNKI and LÁBOS, 1963). One of the shells was fixed while the other was connected to a lever, recording the muscle displacement on a kymograph. The muscle was loaded only by the force of the ligament ($\sim$0.5 kg) connecting the two shells.

Evoked contractions were elicited by stimulation of the cerebro-visceral connective (CVc) with square pulses. The electrodes were placed in the middle of the uninjured nerve.

A given level on the kymograph records *(Fig. 1)* represents an actual muscle-length (l). The level representing the muscle-length when the shells are closed is designated with l_0. The value l_0 is the shortest under the experi-

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mental conditions, as further contraction is prevented by the apposition of the shell margins. The level l can be measured as the deviation from l_0 that is by $(l - l_0)$.

The size of contraction (c) was measured at its maximum, while that of the relaxation (r_T) at a given time (T) after the onset of stimulation. The values of "c" are given in arbitrary units which are the same for each experiment.

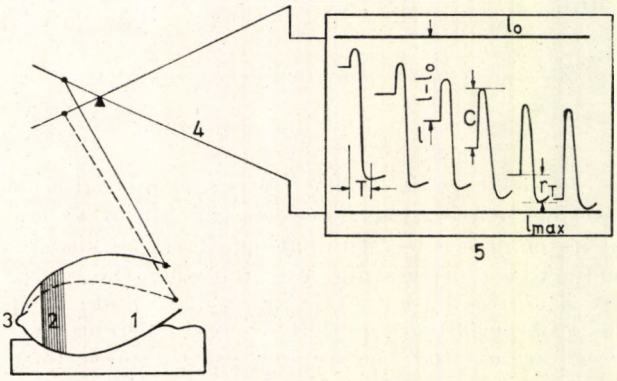

Fig. 1. Experimental arrangement and designations
1 — mussel; 2 — posterior adductor muscle (PAM); 3 — ligament; 4 — lever recording displacements; 5 — records; l_0 = muscle length at closure of the shells; l_{max} = maximal length of PAM; l = an actual muscle length; c = amplitude of contraction; T = time after stimulus-onset; r_T = effective relaxation below initial level at T moment

Results

1. The dependence of the amplitude of evoked contractions on the initial length of the muscle

As the PAM is a mixed tonic and phasic muscle, it can work at different lengths and after spontaneous rhythmic contractions is able to return to the actual working level. The contractions represent an increase in the muscle tension, as at shorter muscle length the load is represented by the increased tension of the ligament, which is higher. For this reason both steady state muscle length and tension vary from animal to animal, as do the temporal relations as well. Their levels influence the size and time course of the spontaneous or evoked phasic contractions and relaxations.

On stimulating the CVc by uniform trains of pulses, the evoked contraction is smaller if the initial level of tonus is higher i.e. when the initial muscle length is shorter. This correlation is always close. In *Fig. 2* the typical negative linear correlation is demonstrated by line A. Its linearity is equivalent to the following law: the steady state amplitude (c) of the evoked contraction is always a constant ratio of the maximal response that could be elicited at the given muscle length. In the demonstrated case (A on *Fig. 2)* the slope-parameter of the regression line is 0.53. In short experiments this ratio (k) is constant for a given animal, but it deviates with different animals and depends on the parameters of the stimulus. The average of k calculated from data of 20 animals was 0.50 ± 0.19 (mean $\pm$ s.d.) being 0.10 and 0.95 the extreme values. The stimulus-parameters were: 4 msec, 8 cps, 20 V, 60 sec.

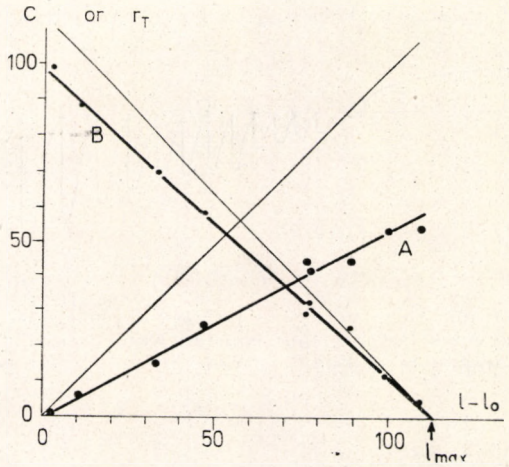

Fig. 2. Conctractions (c) and relaxations (r_T) are plotted against initial level ($l-l_0$)

$$A \; - \; c \sim 0.53 \cdot (l - l_0); \; B \; - \; r_T \sim -0.88 \cdot (l - l_0)$$

Coefficients of correlation are near to ± 1, resp. The origin means l_0 and the abscisse is intersected by r_T line at l_{max}. The two further lines follow slopes of ± 1 (45°) and represent the maximal possible contractions and relaxations in the actual case. Parameters of stimulus-train: 20 volts, 4 msec, 8 cps, 60 sec

2. *The dependence of the amount of relaxation following evoked contractions, on initial muscle-length*

In cases when the relaxation period was not interrupted by spontaneous contractions a clear dependence of the size of relaxation on the initial muscle length was found (B-line in *Fig. 2)*. The size of relaxation increases with increasing initial tonus level, that is with decreasing initial length of the muscle. This linearity represents a similar law as that was described for the contractions, however the linearity of this dependence is not always so strict as that of the contraction-response.

3. *The differences in the magnitude of contraction evoked at a constant length of the muscle*

When the CVc is repeatedly stimulated at a constant muscle length, the evoked contractions are still not uniform. *Fig. 3* shows that the amplitudes of the successive responses elicited by identical trains of pulse at a medium level of length (or tonus) decrease successively. The steady state magnitude of the n-th response (c_n) is proportional to that of the first one (c_0) and nearly exponentially decreases with the sequence number of repeated stimuli *(Fig. 4A)*.

The time-intervals between the successive contractions in *Fig. 3* are determined by the returning to the initial muscle length. If these intervals (τ_n) were measured and plotted against the amplitude of the following contractions (c_{n+1}) a correlation with a linear section was found *(Fig. 4B)*.

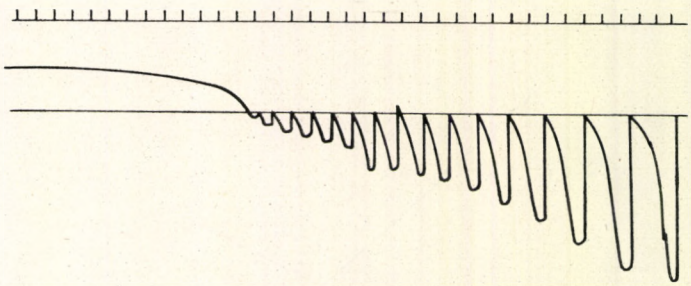

Fig. 3. Responses of PAM. The stimuli to CVc were applied at the moments when the length of relaxing muscle reached a certain, constant value (horizontal line on the figure). Time-scale 60 sec. Parameters of stimulus-train. 10 volts, 4 msec, 8 cps, 30 sec

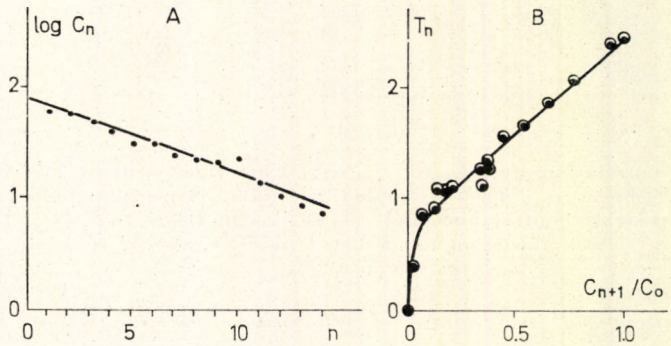

Fig. 4. A — Logarithms of amplitudes of evoked contractions (log c_n) are plotted against the sequence number (n) of successive stimulus trains. Data are taken from Fig. 3. The regression-line represents an equations $c_n \sim c_0 \cdot \exp(-0.16 \cdot n)$. Coefficient of correlation $r \sim 0.9$. — B Recurring times (τ_n) are plotted against the relative amplitude of n + 1-th response (c_{n+1}). Approximation of linear part:

$$\tau_n \sim 1.95 \cdot \frac{c_{n+1}}{c_0} + 0.68\,(\text{min})$$

4. Oscillatory after-effect

In general the prepared PAM may contract rhythmically with very different average frequencies (5—60 cph); and on stimulating the CVv, its evoked response consists of contraction and relaxation, often below the initial level. Depending on the stimulus strength and the initial muscle length, the PAM may become more or less relaxed, than it was before stimulation.

In a number of instances the evoked response is followed by a series of rhythmic contractions; whose frequency and amplitude is higher than during the control period. The time course of these oscillatory after-effects is variable. The tonus may return to the original or to a new steady level finally *(Fig. 5a—j)*.

The trend of the change in tonus depends on the ratio of the amplitudes of contractions and subsequent relaxations and on the frequency of after-oscillation as well. When relaxations are less than the contractions *(Fig.*

5c, h) or absent *(Fig. 5d)* the tonus increases. The high frequency of the rhythm is favourable to a higher tonus because the contractions following each other in short intervals interrupt the actual relaxation *(Fig. 5e, g)*. The

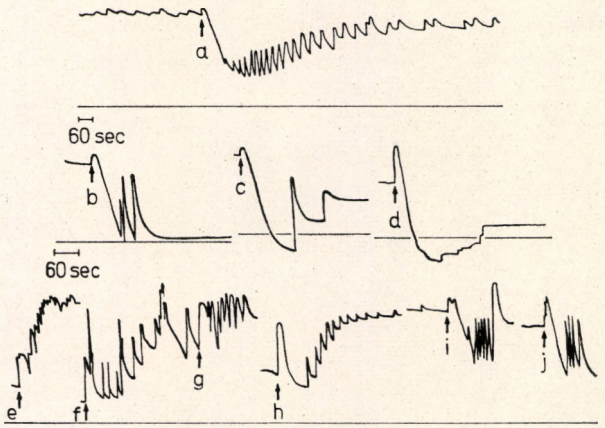

Fig. 5. Different cases of evoked after-oscillations taken from three different animals (a; b—d; 4—j). Parameters of stimuli to CVc: 10 V, 4 msec, 8 cps, 20 sec. The onset of stimulations is marked by arrows. Horizontal lines show the same length of muscle. Time-scale of b is valid for c—j as well

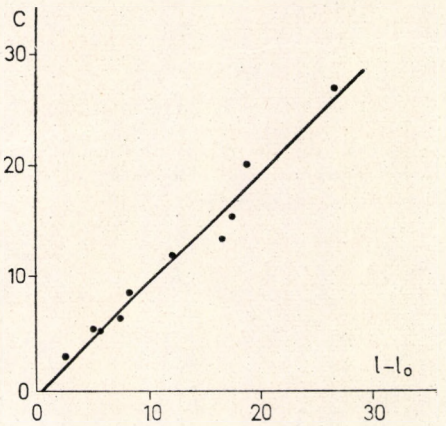

Fig. 6. Correlation between the size of phasic, evoked after-contractions (c) and the level of muscle length ($l - l_0$) from that they were starting. The regression line is $c \sim 0.94 \cdot (l - l_0)$. Coefficient of correlation $r > 0.9$

evoked oscillation starts usually after a latent period. Its frequency decreases, but sometimes in a short initial period a slight increase is observable *(Fig. 5a, h, i)*.

The amplitudes of spontaneous oscillatory contractions bear a relation to the tonus level from which they start. The higher the initial level, the smaller the amplitude. The function is fairly linear at most values of the initial tonus *(Fig. 6)*. In the cases analyzed in detail (8 animals) the constant of L.I.V. was $k = 0.68 \pm 0.11$ (mean $\pm$ s.d.; 0.28 — 1.00).

The first few contractions (and its k-value) sometimes do not decrease notwithstanding the increasing initial level *(Fig. 6a)*.

The oscillatory after-effect evoked by repeated stimulation becomes, step by step, less oscillatory *(Fig. 7)*. In this case the decreasing number of contractions after four successive stimulations were 17—7—3—0. In general

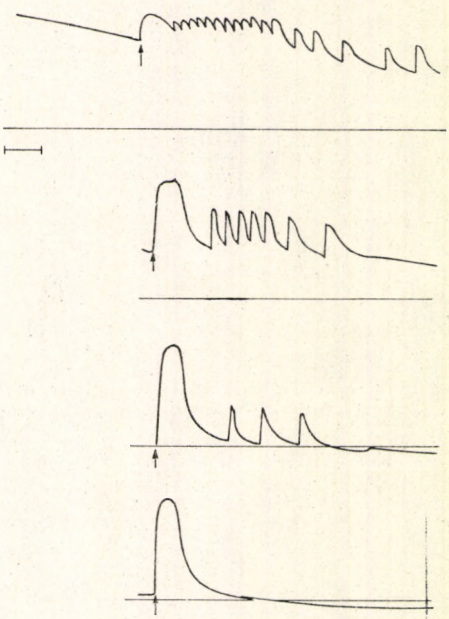

Fig. 7. Adaptivity of after-oscillation. The repeated stimulation abolish the after-effect. The horizontal lines show the reference-level. The onset of stimulation marked arrows. Parameters of stimulus-trains: 20 V, 4 msec, 8 cps, 60 sec. Time mark: 60 sec

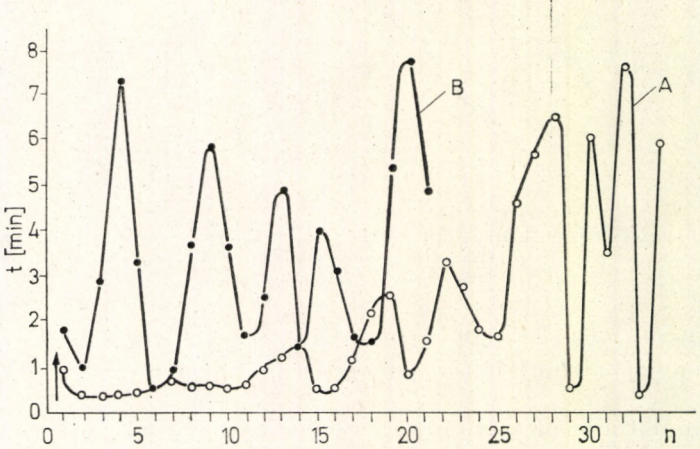

Fig. 8. The length of successive time intervals between the evoked after-oscillations are plotted against their sequence-number. A — CVc is intact; B — The same plot but after cutting the CVc After A an interval of about 30 min was left to pass. Parameters of stimulations: 10 V, 4 msec, 8 cps, 60 sec

after a few stimuli the oscillations are abolished. It is possible to subsequently elicit a steplike increase of tonus by mechanically stimulating the syphon. The previously abolished oscillation can be seen yet again.

The frequency of after-oscillations changes considerably when the connection of CVc with the cerebral ganglia is cut. In Fig. 8 the successive intervals between the rhythmic contractions of after-oscillation are plotted against their sequence number. *Fig. 8A* shows the control, where the decrease of frequency is well shown. Immediately after cutting the CVc, the cut end was stimulated by the same stimulus used in the control. The intervals between the evoked oscillatory after-contractions are demonstrated in *Fig. 8B*. It can be seen, that the frequency is decreased and its trend is less than in the control.

5. L.I.V. for spontaneous contractions at the end of the active period

The adductor muscles of Anodonta undergo alternating periods of activity and inactivity. During activity the muscles are partially relaxed and rhythmic contractions are observed. Towards the end of an active period characteristic contractions appear. These manifest themselves as adductions of decreasing amplitude. It was found that the amplitude decreases with the decreasing initial legth of muscle. This correlation proved to be linear except for contractions starting at very high tonus levels *(Fig. 9A)*. In the example shown the constant of L.I.V. is 0.58.

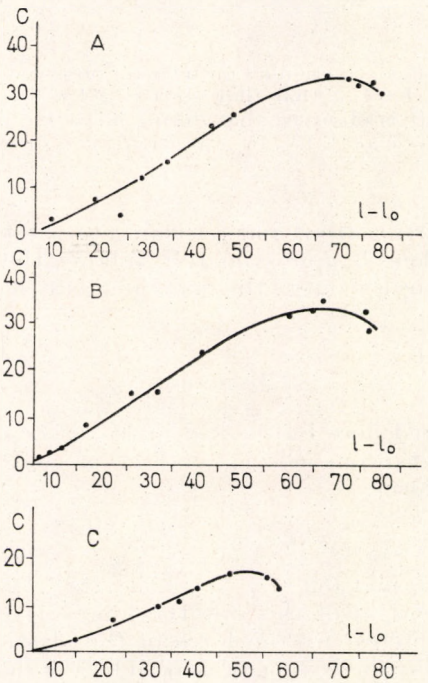

Fig. 9. The amplitude of contractions (c) are plotted against the initial muscle length ($l - l_0$). L.I.V. for A — spontaneous catch-contractions; B — evoked aperiodic response; C — after-oscillations

The described linearities in sections 1, 4 and 5 are not valid at extremely high or low initial levels, where a decrease of the constant k was found *(Fig. 9A)*. Similar non-linearity occurred also in case of evoked contractions *(Fig. 9B)* and oscillatory after-effect *(Fig. 9C)* at extreme initial values.

When contractions of a whole active period were analyzed, a more complex phenomenon was found. The plot of amplitudes of consecutive contractions (c) against the initial muscle length ($l - l_0$) does not follow a single line but describe a loop *(Fig. 10)*, which can not be characterized by a single k

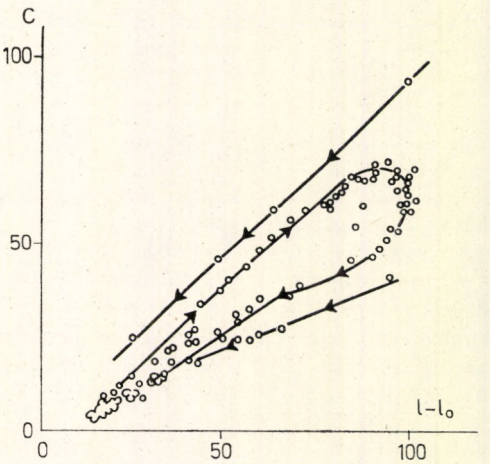

Fig. 10. Plot of all phasic contraction-amplitudes (c) observed in a whole active period of an animal against the level at that they start ($l - l_0$). The linear parts represent a L.I.V. with constant k. It is observed that during an active period an inhomogeneous cycle of L.I.V. — variation takes place

value. The arrows starting from and returning to the point corresponding to the shortest muscle length approximately follows the consecutive contractions. Some contractions are out of the main loop but they seem to form organized pattern.

Discussion

The L.I.V. applied here for evoked aperiodic or oscillating phasic contractions, for the spontaneous closing contractions and for relaxations can be written in a general form:

$$\Delta l \sim k \cdot (l - l_0)$$

when l is not extreme in value.

Concerning the origin of this law the following can be stated. In situ the adductors are stretched by the force of the elastic ligament connecting the two shells. When the shells are more closed by the shortening of the muscle, this force is greater. A given extension of the ligament can be evoked by a force being nearly proportional to its deformation (extension and/or compression; unpublished). Therefore a tension-increase in the muscle proportional

to the magnitude of contraction must be produced during the shortening. Thus the L.I.V. is equivalent to a length-active tension or to a load-active tension diagram. In such a way the muscle is not working isotonically but against a nearly linearly increasing load.

A source of the observed differences in the responses of the adductors when stimulating at different initial length with identical stimuli (SALÁNKI and LÁBOS, 1963) has been found mainly in the above described length-tension-correlation.

The L.I.V. for artifical stimulation is seen to be valid when applying the same stimulus. As the same law is valid for either spontaneous or evoked oscillatory, free running contractions, a conclusion is suggested that these actions must be generated by the excitation of same magnitude of the innervating centres. On the other hand, when the contractions of the same muscle working spontaneously cannot be described by a single k value *(Fig. 10)*, different level of nervous excitation must be supposed. In this manner the k of L.I.V. can give information about the excitation running out from ganglia towards the muscle.

The L.I.V. of relaxation can be interpreted also by taking into account the tension of ligament. At lower initial muscle-length, when the loading force of ligament is higher, the relaxation under the initial level is proportionally higher *(Fig. 2B)*. Nevertheless by this explanation the differences in the final relaxation level can not understood. For this reason during the relaxation-process intrinsic changes of the mechanical properties of muscle must be taken into consideration as well.

Phenomena demonstrated in *Fig. 3* and *4* clearly show that the explored L.I.V. is not valid when the stimulation is too frequent. In such a case a decrease of k value was observed. It could be considered as a result of the accumulative effect of previous stimuli and/or as a dependency on the initial speed of relaxation.

Systems studied by basimetry show all the phenomena observed in the behavior of *Anodonta* adductor:

1. law of initial value *(Fig. 2, 9)*
2. law of initial speed *(Fig. 3)*
3. oscillatory behavior after disturbing *(Fig. 5)*
4. adaptivity of oscillatory behaviour and of the evoked contractions *(Fig. 7* and *3)*.

It seems to be reasonable that such properties are concerned with an automatic, adaptive control (servomechanism) of adductor behavior. The sources of the phenomena or distribution of parameters in the components (muscles, ganglia, synapses, ligament, receptors) of the whole system are not known exactly. It is very probable that different functional feed-back loops are responsible for the whole behavior. A suitable and quantitative description or modelling of the behavior must be carried out in terms of automatic control. Approximations given by an application of basimetry are less general and purely phenomenological but it gives a unified explanation and may form a first step in a cybernetic evaluation of the regulation of adductors.

Summary

Initial value laws were demonstrated as being valid for spontaneous rhythmic contractions, and evoked responses (contraction and relaxation) of *Anodonta* adductor muscle.

The following group of phenomena has been observed and analyzed

1. quasi-linear relationship between responses and initial values,
2. homeostatic after-oscillations,
3. adaptive behavior of evoked contractions and that of after-oscillations.

The idea of basimetry seems to be useful in explaining some of the phenomena observed, however it does not provide a complete explanation. Conclusions may be drawn about the roles of innervation, the muscle itself and the ligament, in the bahavior of the adductors.

Acknowledgements

One of us (B.G.) wishes to thank Professor L. BRENT of the Department of Zoology, The University of Southampton, for his kind encouragement and assistance. He also wishes to thank the Medical Research Council for grants provided.

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KEZDETI ÉRTÉKTÖRVÉNYEK ALKALMAZÁSA ANODONTA ZÁRÓIZOM SPONTÁN ÉS KIVÁLTOTT OSZCILLÁCIÓIRA, VALAMINT APERIODIKUS VÁLASZAIRA

Lábos E., B. Glaisner és *J. Salánki*

Összefoglalás

Kezdeti értéktörvények érvényességét demonstrálták *Anodonta* záróizom spontán ritmikus válaszaira, valamint kiváltott oszcillációira (kontrakció és ernyedés).

Az alábbi jelenségcsoportokat vizsgálták és analizálták:

1. a válasz és a kezdeti érték közötti kvázi-lineáris viszony;
2. homeosztatikus utóoszcilláció;
3. a kiváltott kontrakció és utóoszcilláció adaptív jellege.

A bazimetria alapelvei használhatónak bizonyultak a vizsgált jelenségek magyarázatában, azonban elégségesnek nem tekinthetők.

Az eredmények alapján következtetések vonhatók le az innervációra, valamint az adduktorok működésében szerepet játszó izom és ligamentum sajátosságaira vonatkozóan.

ПРИМЕНЕНИЕ НАЧАЛЬНЫХ КОЛИЧЕСТВЕННЫХ ЗАКОНОВ ДЛЯ СПОНТАННОЙ И ВЫЗВАННОЙ ОСЦИЛЯЦИИ И АПЕРИОДИЧЕСКИХ ОТВЕТОВ АДДУКТОРА АНОДОНТЫ

Э. Лабош, Б. Глезнер и *Я. Шаланки*

Проверяли приложимость начальных количественных законов к спонтанным ритмическим ответам и вызванным осцилляциям аддуктора Анодонты (сокращение и расслабление).

Исследовались и анализировались следующее группы явлений:

1. Квази-линейное отношение между ответом и начальной величиной,
2. гомеостатическая пост-осцилляция,
3. адаптивный характер вызванного сокращения и пост-осцилляции.

Основные принципы базиметрии оказались пригодными для объяснения исследованных явлений, в то же время нельзя их рассматривать достаточным. На основе данных можно сделать выводы об иннервации, и о свойствах лигамента и мышцы, важных для работы аддукторов.

ANNAL. BIOL. TIHANY | 37 | 85—97 | HUNGARIA 1970

PHARMACOLOGICAL PROPERTIES OF THE HEART OF LUMBRICUS TERRESTRIS L.

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Received: January 22nd 1970

Phylogenetically, the Annelids are the first animals possessing a special organ to ensure the circulation of body fluids. There are but incomplete reports on this primitive type of heart, though it deserves attention being the first stage of development of the heart, an organ of vital importance.

It is known that on excitation of motor nerves acetylcholine is released in the nerve endings of Annelids (WU, 1939), at the same time the muscle of the body wall of these animals, sensitive to acetylcholine, is insensitive to γ-amino-butyric acid and glutamate (FLOREY, 1967). The digestive tract of *Lumbricus* is regulated by cholinergic and adrenergic innervations (WU, 1939; FLOREY, 1967), in which adrenaline is an inhibitory and acetylcholine a stimulatory factor.

Relatively little is known of the circulatory system of Annelids. According to GASKELL (1919) adrenaline increases and acetylcholine decreases the activity of contractile vessels in *Hirudo*, whereas the vessels of *Arenicola* and *Lumbricus* are stimulated by acetylcholine, and hardly affected by adrenaline (PROSSER and ZIMMERMAN, 1943).

It has been demonstrated that in the central nervous system of *Lumbricus terrestris*, adrenaline, noradrenaline and 5-hydroxytryptamine are present (OESTLUND, 1954; EULER, 1961; WELSH and MOORHEAD, 1960; KERKUT et al. 1967). In spite of the data mentioned above, there is no satisfactory explanation regarding the role of 5-hydroxytryptamine in Annelids and it is not clear whether adrenaline and noradrenaline can be transmitters in this group of animals. As regards the effect of other substances there are no data available at all.

The purpose of the present experiments was to investigate which of other animal branches are related to *Lumbricus* regarding the chemical sensitivity of its primitive heart and which agents can play the roles of inhibitory and stimulatory transmitters in the heart activity of this animal. In addition, the pharmacological characteristics of this type of heart has been also described.

Material and method

For the experiments 15—30 cm long specimens of earthworm, *Lumbricus terrestris* L., were used. Examinations were made on the ring-shaped heart (vasa dorsoventro-commissuralia) of which 6 pairs are found in segments 7—11 (IVANOV et al. 1958).

The animals were fixed dorsum-up to a wax-board and the hearts exposed. After a period of rest lasting about 25—30 minutes, the heart rate returned to normal. The number of contractions per minute of the pulsating heart was counted for 15 minutes. Then various pharmacological agents were applied to the hearts and the number of beats was counted as with the control hearts. Care was taken to use the same heart for the control and for the test measurements.

The effect of the drugs applied was studied over the range of 10^{-10} M to 10^{-3} M. Each concentration of all the drugs was tested at least on 6 hearts. The results obtained were then plotted on a graph, the abscisse showing the time in minutes and the ordinate the number of heart contractions per minute. For the investigations the physiological saline prepared according to PROSSER and ZIMMERMAN (1943) was used. The test compounds were diluted in the same type of saline.

The experiments were performed between September and May, at room temperature (20—22 °C). The animals had been collected earlier and stored in earth enriched with leaf-mould.

The following drugs were used for the experiments: adrenaline (EGA), noradrenaline (Sigma), isopropylnoradrenaline (Serva), dopamine (Sigma), 5-hydroxytryptamine creatinine sulphate (Sigma), histamine (Fluka), acetylcholine chloride (Fluka), γ-aminobutyric acid (Calbiochem), dichlorisoproterenole (Schuchardt), benzoquinone chloride (mytolon) (St, W. Res. Inst.), methysergide bimaleate (Sandoz), nicotine chloride (Sandoz), 2-brom-d-lysergic acid diethylamide (BOL-148) (Sandoz).

Results

1. Heart rate of control animals

The rhythmic activity of control hearts showed considerable fluctuations. The rhythm of the hearts is asynchronous, though the two hearts of a segment tend to contract together. The frequency of heart rate of control animals showed seasonal variations, namely, in autumn (October—November) the number of contractions of normal hearts ranged from 16 to 32 *(Fig. 1,* curves a and b), while in winter (December—January) the number of beats was much higher, reaching the values of 45 to 75 *(Fig. 1,* curves c and d). Therefore, great care was taken to measure the control heart activity. Each control value is the mean of 5 measurements.

The high or low pulse rates caused no difficulties in evaluating the results as in both cases the effects of inhibition or stimulation of the test compounds could be registered.

2. Effects of biologically active agents on the heart rate of the earthworm

For the experiments biologically active agents known to act as transmitters in other animal groups have been employed.

Acetylcholine (ACh) at concentrations ranging from 10^{-8} M to 10^{-4} M accelerated the heart rate of *Lumbricus.* The effect was not strictly dependent on concentration, as maximum stimulations was observed at 10^{-7} M and at 10^{-4} M *(Fig. 2).*

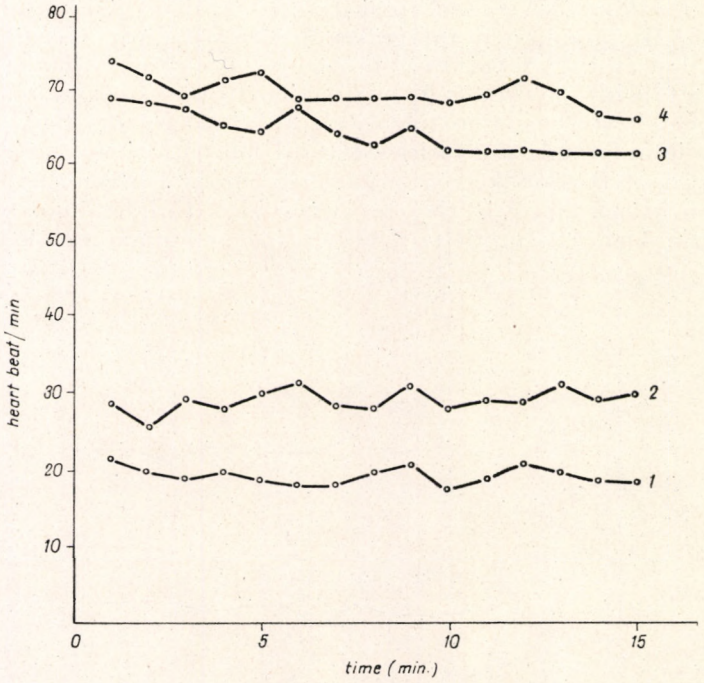

Fig. 1. Control values of the heart activity of *Lumbricus terrestris*
Curves 1 and 2 = autumnal period; Curves 3 and 4 = winter period

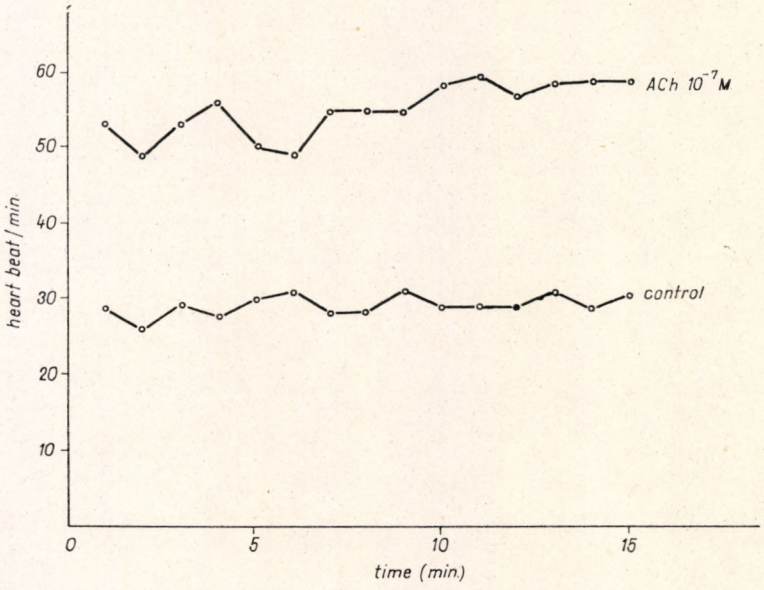

Fig. 2. Effect of acetylcholine on *Lumbricus* heart at 10^{-7} M concentration.
Abscisse: time in minutes; *Ordinate:* number of contractions per minute

Noradrenaline (NA) when applied at 10^{-10} M or higher concentrations (up to 10^{-4} M) produced a rather small increase (not exceeding 25%) in frequency, but at 10^{-3} M this drug proved to be a strong stimulant *(Fig. 3)*.

The effect of *adrenaline* (A) was similar to that of noradrenaline, their threshold concentrations were identical: 10^{-10} M. Nearly the same extent of increase in amplitude was observed at all concentrations applied *(Fig. 4)*.

Dopamine (DA) unlike the former two catecholamines, never produced acceleration in heart rate in the earthworm. The threshold concentration of DA is much higher than that of adrenaline and noradrenaline producing

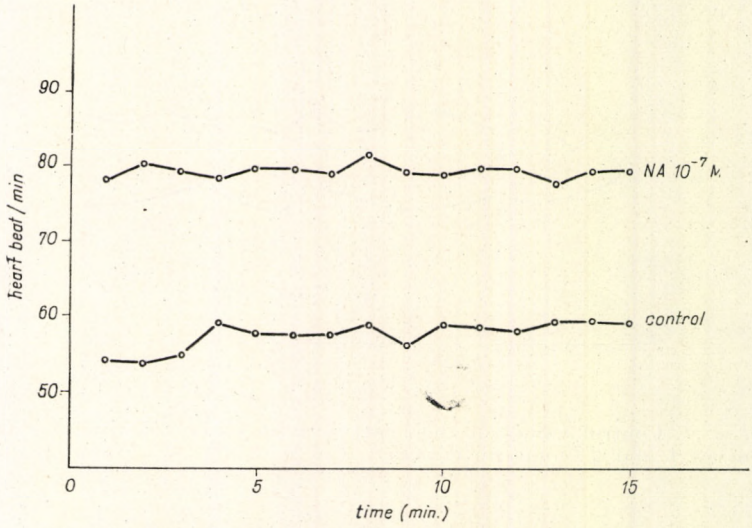

Fig. 3. Effect of noradrenaline on *Lumbricus* heart

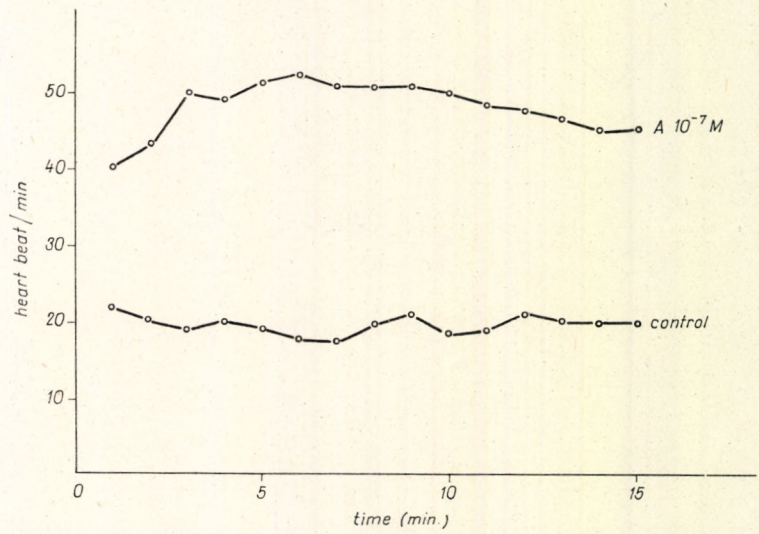

Fig. 4. Effect of adrenaline on *Lumbricus* heart

inhibition only from 10^{-5} M concentration. Considerable decrease in frequency of heart activity occurred, however, only at 10^{-3} M *(Fig. 5)* when the number of heartbeats per minute was reduced by about 50%.

Isopropylnoradrenaline (IPNA), like dopamine, produced but inhibition in heart rate at 10^{-6} M and in higher concentrations. The strongest inhibition

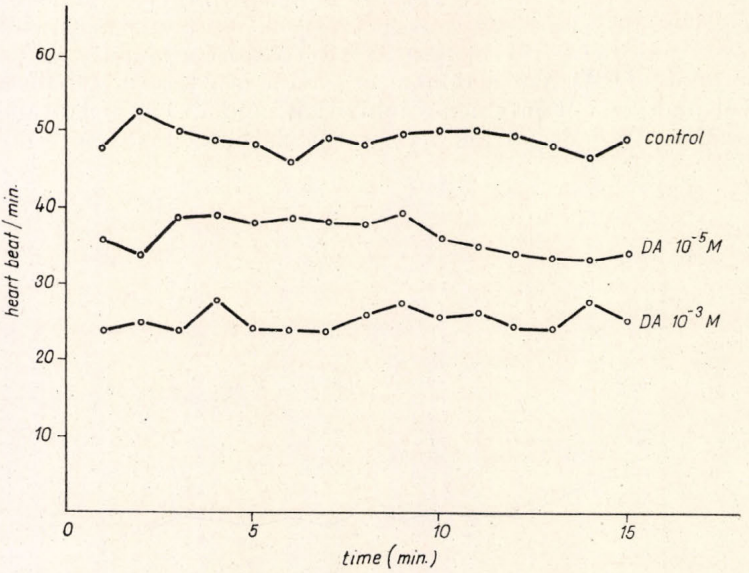

Fig. 5. Effect of dopamine on the heart rate of *Lumbricus*

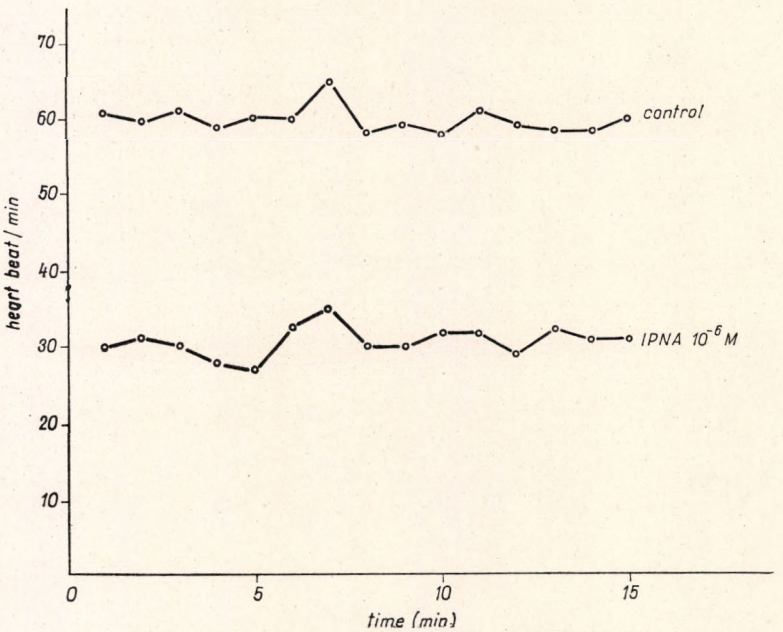

Fig. 6. Effect of isopropylnoradrenaline on *Lumbricus* heart

was noted at the threshold concentration and smaller degrees of inhibition were noted at 10^{-5} M and at 10^{-4} M *(Fig. 6)*.

5-hydroxytryptamine (5HT) was found to inhibit the heart rate of the earthworm in concentrations from 10^{-7} M *(Fig. 7)*. By raising the concentrations increased inhibition was observed.

Tryptamine (TA) at concentrations ranging from 10^{-9} M to 10^{-4} M had an accelerating effect on heart rate. The extent of stimulation was dependent on concentration, being the highest at 10^{-4} M *(Fig. 8)*.

Tyramine (TRA) had a double effect on heart rate, at concentrations of 10^{-10} M and 10^{-9} M producing inhibition, and in concentrations ranging from 10^{-8} M to 10^{-4} M evoking acceleration in heart rate. Both effects were

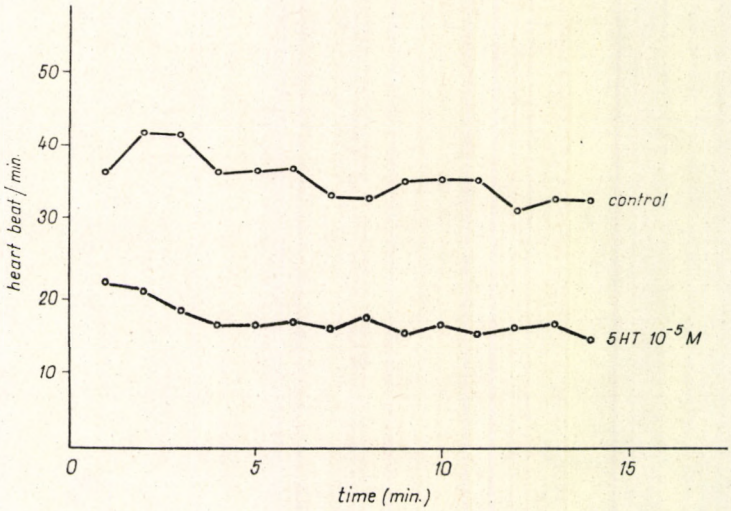

Fig. 7. Effect of 5-hydroxytryptamine on *Lumbricus* heart

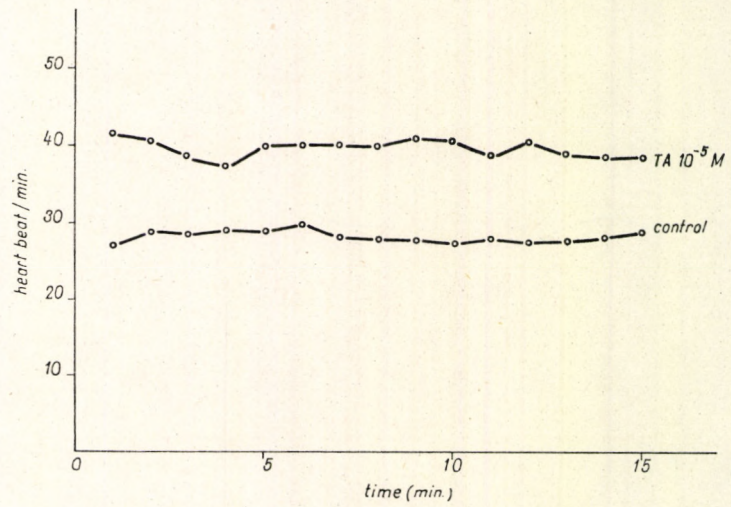

Fig. 8. Effect of tryptamine on *Lumbricus* heart

weak, neither of them causing an increase or decrease of frequency exceeding 20% *(Fig. 9)*.

γ-amino-butyric acid (GABA) inhibited heart activity at 10^{-7} M and higher concentrations *(Fig. 10)*. The extent of inhibition depended on concentration, the strongest inhibition was observed at 10^{-3} M.

Histamine (HA) produced both inhibition and stimulation, depending on the level of concentration, i.e. at 10^{-9} M and at 10^{-8} M the heart rate was accelerated, whereas at higher concentrations (from 10^{-7} up to 10^{-3} M) inhibition was observed *(Fig. 11)*. The degree of inhibition, at all concentrations, was approximately the same.

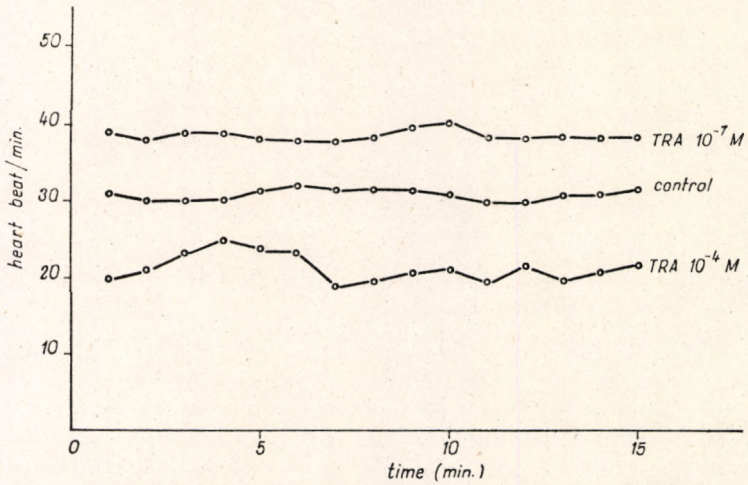

Fig. 9. Effect of tyramine on *Lumbricus* heart

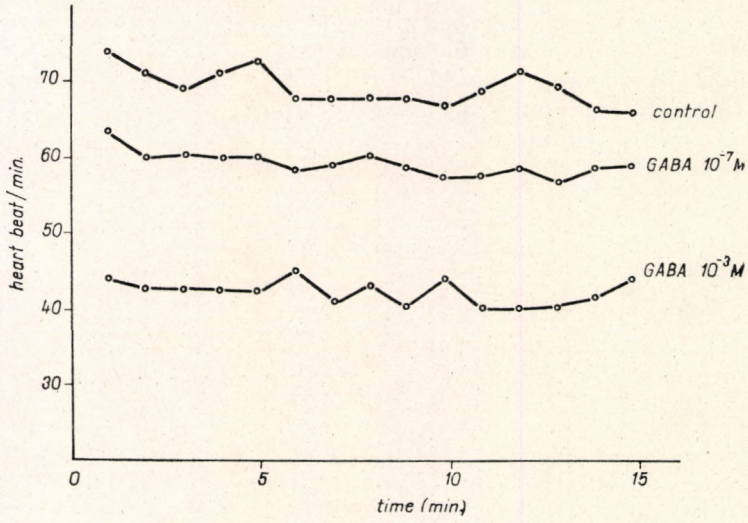

Fig. 10. Effect of GABA on *Lumbricus* heart

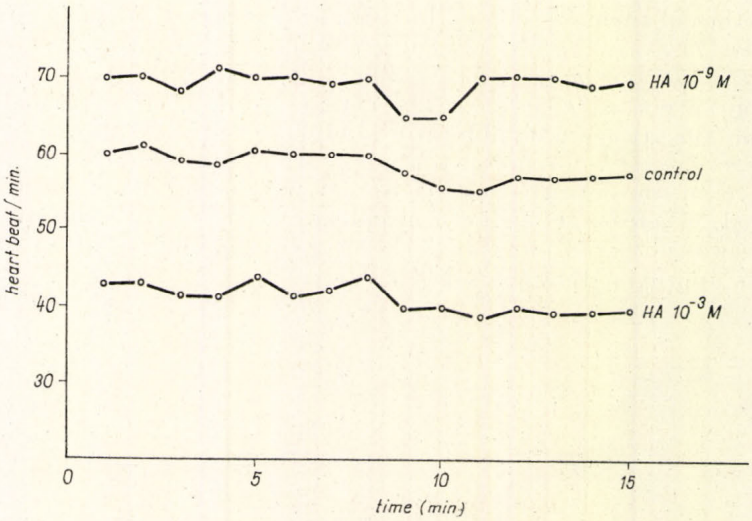

Fig. 11. Effect of histamine on *Lumbricus* heart

3. Effects of anticholinergic, 5HT antagonistic and adrenergic blocking agents on the Lumbricus heart

To settle the question which agents may be involved in the regulation of heart rate of *Lumbricus* we have investigated the effects of drugs influencing the level of endogenous substances or acting on their receptors.

Nicotine acted predominantly as a stimulant, as in concentrations ranging from 10^{-9} M to 10^{-4} M it accelerated the heart rate of the earthworm. The strongest stimulation was observed at 10^{-6} M and 10^{-7} M, when the frequency of heart rate was increased by about one third. Inhibition was produced only at 10^{-3} M.

Dichlorisoproterenol (DCI) at 10^{-10} M threshold concentration and up to 10^{-3} M had a marked stimulatory effect.

Mytolon accelerated the heart rate at concentrations ranging from 10^{-10} M to 10^{-3} M. Maximum stimulation was produced at 10^{-6} M concentration. The extent of stimulation was similar to that evoked by nicotine.

BOL-148 produced a decrease in frequency at concentrations from 10^{-6} to 10^{-4} M. No stimulatory effect of BOL-148 was observed in any of the cases.

4. Pharmacological investigation on the sites of action of some biologically active agents

In these experiments we have investigated how do drugs acting on known receptors influence the action of biologically active agents on the heart of *Lumbricus terrestris*. The substances were given in combination (1 : 1) at 10^{-6} M concentration. The sites of action of acetylcholine, adrenaline, 5-hydroxytryptamine and GABA were analyzed by simultaneous application of nicotine, DCI, mytolon, BOL-148 and methysergide. The results are summarized in *Table 1.*

TABLE 1

Modification of the effects of monoamines, acetylcholine and GABA by drugs

	Agent alone	Nicotine + agent	DCI + agent	Mytolon + agent	BOL-148 + agent	Methysergide + agent
Acetylcholine	+	—	0	—	+	0
Adrenaline	+	+	—	—	—	—
5-hydroxytryptamine	—	+	—	—	0	—
GABA	—	0	0	—	—	0

+ stimulation — inhibition 0 ineffective

From the experiments it appears that more drugs influence the stimulatory effects than the inhibitory ones. BOL-148 was the only drug having no effect on stimulation evoked by acetylcholine, DCI and methysergide protected the heart from the stimulatory effect of ACh, while nicotine and mytolon reversed the effect of ACh to the opposite direction.

The stimulatory effect produced by adrenaline remained unaltered during nicotine treatment but it was reversed by all the other drugs used *(Table 1)*.

DCI, mytolon and methysergide did not alter the inhibitory effect evoked by 5-hydroxytryptamine, but it was completely eliminated by BOL-148 and reversed to stimulation by nicotine.

The inhibitory effect of GABA was not reversed by any of the drugs used. Mytolon and BOL-148 had no effect on the action of GABA, while nicotine, DCI and methysergide abolished it.

Different concentrations of the above drugs evoked the same modifications in the action of the agents tested.

As can be seen from *Table 1*, there are overlappings in the effects of blocking and antagonistic agents, and except mytolon influencing only the action of two stimulating amines, all the drugs alter both the action of inhibitory and that of stimulatory factors. This seems to indicate the presence of mixed pharmacological receptors in the heart of *Lumbricus*.

Discussion

Our experiments have shown that the normal heart rate of *Lumbricus terrestris* is much more varying than it was described earlier. According to STUBE (1909), CLARK (1927), PROSSER and ZIMMERMAN (1943), the characteristic rate of contraction of the earthworm heart is 15—20 beats/min. We were not able to demonstrate such a slow rhythmic activity in any of the hearts examined *(Fig. 1)*. The disparity may be explained by the seasonal changes noted in these animals, in autumn their heart activity being slow, whereas in winter very fast. Perhaps the heart rate values measurable in spring and summer, which periods were not examined by us, are similar to those reported in literature.

Our experiments have verified that the *Lumbricus* heart responds selectively to biologically active amines and pharmacological agents. It is

accelerated by acetylcholine, noradrenaline and tryptamine, it is inhibited by dopamine, isopropylnoradrenaline, 5-hydroxytryptamine and γ-aminobutyric acid. Tyramine and histamine, on the other hand, have an inhibitory or stimulatory effect on it, depending on the level of concentration.

Our results are not entirely concordant with the pharmacological data obtained on other organs of *Lumbricus*. As has been reported by Wu (1939) and Florey (1967), adrenaline inhibits and acetylcholine stimulates the digestive tract of the earthworm. In our experiments both agents had a stimulatory effect on *Lumbricus* heart. In experiments on *Hirudo medicinalis* adrenaline was found to produce inhibition and acetylcholine stimulation on the contractile vessels of this Annelid (Gaskell, 1919). Prosser and Zimmerman (1943) found that acetylcholine accelerated and adrenaline was ineffective or had a slight inhibitory effect on the heart of *Lumbricus*. In our experiments it was the adrenaline that produced stimulation at lower threshold concentration (10^{-10} M) and not the acetylcholine (10^{-8} M) and no inhibition was ever observed with adrenaline in any concentration.

The heart of *Lumbricus*, like that of Arthropods, is accelerated by acetylcholine which proves that it is a neurogenic type of heart, as it is known that myogenic hearts are inhibited by acetylcholine (Clark, 1927; Prosser and Brown, 1962).

Of the different catecholamines the effect of adrenaline was investigated on the hearts of numerous invertebrates. On the hearts of Molluscs and Arthropods adrenaline was found to act as a stimulant (Prosser and Brown, 1962). In our experiments adrenaline and acetylcholine produced similar effects, both agents accelerating the heart rate of *Lumbricus*. Dopamine and isopropylnoradrenaline, on the other hand, showed an inhibitory effect and in this respect their effects differ from those observed on Molluscan heart (S.-Rózsa and Pécsi, 1967).

The inhibitory effect of 5-hydroxytryptamine indicates species specificity, as it is known that Molluscs and Annelids contain the highest amounts of 5HT (Welsh and Moorhead, 1960) and this agent is considered as the stimulatory mediator on the heart of Molluscs (Welsh, 1954; S.-Rózsa and Graul, 1964). Besides 5HT, dopamine is the dominant amine in the nervous system of Molluscs and Annelids (Welsh et al. 1965; Kerkut et al. 1967). Their action is, however, different in these animal groups as the heart rate of Molluscs is accelerated both by 5HT and dopamine (S.-Rózsa and Pécsi, 1967), whereas the *Lumbricus* heart is inhibited by both amines. The fact that dopamine acts at rather high threshold concentration (10^{-5} M), seems to indicate that it does not play the role of independent mediator in the regulation of heart rate of the earthworm but it may be the precursor of adrenaline and noradrenaline. This may explain its presence in large amounts, as well.

The effect of γ-amino-butyric acid on the heart of *Lumbricus* is similar to that observed on Crustacean hearts (Prosser and Brown, 1962). This similarity in pharmacological behaviour of Annelid and Crustacean hearts (Prosser and Brown, 1961). This similarity in phamacological behaviour of Annelid and Crustacean hearts refers again to the neurogenic nature of the *Lumbricus* heart. GABA is ineffective on myogenic vertebrate and Molluscan hearts (Florey, 1967).

According to our results, not only acetylcholine can be a stimulatory mediator on the *Lumbricus* heart as has been previously reported (Prosser

and ZIMMERMAN, 1943; FLOREY, 1967), but adrenaline and noradrenaline, as well. On the other hand, 5-hydroxytryptamine and GABA can be taken into consideration as inhibitory mediators on the Annelid heart.

The pharmacological analysis of the sites of action of the above agents has shown that there are no separated receptors in the heart of the *Lumbricus*. The effect of acetylcholine is eliminated not only by anticholinergic substances but also by agents blocking the β-receptors and antagonists of 5HT. The effect of adrenaline is likewise altered by agents acting on other types of receptors. The same applies to the sites of action of 5HT and GABA. All these data verify that there is a considerable disparity in the pharmacological receptors of the hearts of *Lumbricus* and those of other animal branches.

Summary

1. The frequency of contractions of the blood vessels of *Lumbricus terrestris* L. showed seasonal variations. In autumn the number of heart contractions per minute was found to be 16—32, while in winter this number was 45—75.

2. Of the biologically active agents tested acetylcholine (10^{-10} M), adrenaline (10^{-10} M), noradrenaline (10^{-10} M) and tryptamine (10^{-9} M) increase the heart rate of *Lumbricus*, while dopamine (10^{-5} M), isopropylnoradrenaline (10^{-6} M), 5-hydroxytryptamine (10^{-7} M) and GABA (10^{-7} M) have a decreasing effect on heart activity. Histamine and tyramine have a double effect.

3. Acetylcholine, adrenaline and noradrenaline may act as stimulatory mediators, while 5-hydroxytryptamine and GABA may play the role of inhibitory mediators on the heart of *Lumbricus*.

4. Nicotine, DCI and mytolon, similarly to acetylcholine and adrenaline, produce acceleration of heart rate in *Lumbricus*, while BOL-148 and methysergide have an inhibitory effect.

5. On the heart of *Lumbricus* the effects of monoamines, ACh and GABA take place on mixed receptor structures which differ from receptors of other animal groups.

6. Concerning pharmacological sensitivity, the *Lumbricus* heart seems to be related to the neurogenic hearts of Arthropods and not to myogenic hearts of Molluscs and vertebrates. As to the effect of acetylcholine, the *Lumbricus* heart seems to stand near the insect heart, whereas on the basis of its response to GABA it seems to be nearer to the Crustacean heart.

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LUMBRICUS TERRESTRIS L. SZIVMŰKÖDÉSÉNEK FARMAKOLÓGIAI VIZSGÁLATA

S.-Rózsa Katalin és *V.-Szőke Ida*

Összefoglalás

1. Föld giliszta véredényein az összehúzódások gyakorisága szezonális ingadozást mutat. Az őszi hónapokban az egy percre eső szívösszehúzódások száma 16—32, míg télen 45—75.

2. A biológiailag aktív anyagok közül az acetylcholin (10^{-10} M), adrenalin (10^{-10} M), noradrenalin (10^{-10} M) és tryptamin (10^{-9} M) növelik a *Lumbricus* szívműködésének frekvenciáját, míg dopamin (10^{-5} M), izopropylnoradrenalin (10^{-6} M), 5-hydroxytryptamin (10^{-7} M) és GABA (10^{-7} M) csökkentik azt. A hisztamin és a tyramin kettős hatású.

3. *Lumbricus* szívén az acetylcholin, adrenalin vagy noradrenalin lehetnek serkentő mediátorok, míg a gátló mediátor szerepét az 5-hydroxytryptamin és GABA tölthetik be.

4. Nikotin, DCI és mytolon az acetylcholinhoz és adrenalinhoz hasonlóan serkentik a szívműködést, a BOL—148 és methysergide pedig gátló hatásúak.

5. *Lumbricus* szívén a monoaminok acetylcholin és GABA hatása kevert receptor struktúrákon zajlik le, s az különbözik más állatcsoportok receptoraitól.

6. Farmakológiai érzékenység tekintetében a *Lumbricus* szív a neurogén ritmusú Arthropoda szívekkel mutat rokonságot, s nem a myogén ritmusú Mollusca vagy gerinces szívekkel. Ezen belül acetylcholin hatás vonatkozásában rovarszívekhez, míg GABA-ra adott válaszreakció alapján a rákszívekhez áll közelebb.

ФАРМАКОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ НА СЕРДЦЕ ДОЖДЕВОГО ЧЕРВЯ

Lumbricus terrestris L.

К. Ш.- Рожа и *И. В.- Сёке*

1. Частота сокращения сердца дождевого червя проявляет сезонные изменения. Осенью частота сокращения 16—32 в минуту, зимой 45—75.

2. Из биологически-активных веществ ацетилхолин (10^{-10} М), адреналин (10^{-10} М), норадреналин (10^{-10} М) и триптамин увеличивают частоту сокращения сердца дождевого червя, допамин (10^{-5} М), изопропилнорадреналин (10^{-6} М), 5-окситриптамин (10^{-7} М) уменьшают ёё. Гистамин и тирамин обладают двойным действием.

3. Возбуждающими медиаторами на сердце дождевого червя могут быть адреналин и норадреналин, в то же время тормозными медиаторами являются 5-окситриптамин и ГАМК.

4. Подобно действию ацетилхолина и адреналина, никотин, ДСІ и митолон возбуждают сердечную деятельность, БОЛ-148 и метисергид тормозят ёё.

5. На седце дождевого червя действие моноаминов, ацетилхолина и ГАМК осуществляется на общих рецепторных структурах, которые отличаются от рецепторов других животных.

6. С точки зрения фармакологической чувствительности сердце дождевого червя родственное нейрогенным сердцам членистоногих, и не проявлает сходства с миогенными сердцами Моллюсков или позвоночных животных. В отношении действия ацетилхолина это сердце ближе к сердцам насекомых, а в отношении ответа на ГАМК ближе к сердцу ракообразных.

ANNAL. BIOL. TIHANY | 37 | 99—109 | HUNGARIA 1970

INVESTIGATIONS ON THE CHEMICAL SENSITIVITY OF INSECT HEARTS

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Received: February 23rd 1970

The insect heart belongs to the neurogenic type of hearts, its rhythmic activity is under neuronic control. The chemical regulation of neurogenic hearts is characteristic as far as acetylcholine is the common stimulating factor in these types of hearts (PROSSER and BROWN, 1962).

Sensitivity of insect hearts was investigated mostly in two species: *Periplaneta americana* and *Blatta orientalis* (METCALF et al. 1964; RICHTER, 1967; MILLER and METCALF, 1968), first of all in connection with classical mediators (acetylcholine, adrenaline).

In the present work the results of investigations performed on the hearts of some insect species living in Hungary are reported. The purpose of the examinations was to describe the effect of transmitters known from experiments in other animals, as well as their sites of action from which conclusions may be drawn regarding the nature of chemical mediation involved in the regulation of insect hearts.

Material and method

For the experiments hearts of *Phaneroptera nana*, *Ephippigera ephippiger*, *Gryllotalpa vulgaris*, *Carabus coriaceus* and *Leptinotarsa decemlineata* were used.

After collecting the insects the specimens of *P. nana*, E. *ephippiger*, *C. coriaceus* and *L. decemlineata* were used immediately, whereas the *G. vulgaris* was kept for a time in earth enriched with leaf-mould.

The hearts of all species were exposed and prepared in the same manner: after cutting off the legs, the animals were fixed dorsum-down to a wax-board and the cuticle on the abdomen was completely removed. The dorsal tubular heart was cleaned of the adhering tissue remnants and perfused with physiological saline. Then the hearts were exposed to the effect of various agents by removing the normal saline solution and replacing it with saline containing the test compound. After observation of the effectiveness of the agent tested the solution was removed by suction and replaced by normal saline. The number of heart contractions in a minute was counted through a binocular microscope for a period of 10 minutes. The values obtained were illustrated graphically. Before application of the agents a control graph was always plotted. The effects of the different agents were studied over the range of the threshold concentration up to 10^{-4} M. Each concentration was tested

at least on 5 hearts. For the examinations physiological saline prepared specially for *Periplaneta* heart (LUDWIG et al. 1957) was used. The same solution was used also for dilution of test compounds.

The experiments were conducted in the period from June to January, at room temperature (20—24 °C). In winter only the hearts of *G. vulgaris* were investigated.

The effect of the following agents was investigated: acetylcholine chloride (Fluka), 1-adrenaline-d-hydrogentartarate (EGA), 1-noradrenaline bitartarate (Sigma), dopamine hydrochloride (Sigma), n-isopropyl-d 1-noradrenaline hydrochloride (Serva), 5-hydroxytryptamine creatinine-sulphate (Sigma), tryptamine hydrochloride (Schuchardt), tyramine hydrochloride (Fluka), γ-amino-butyric acid (Reanal), histamine di-hydrochloride (Fluka), nicotine chloride (Sandoz), dichloro-isoproterenol, DCI (Schuchardt), benzoquinonium chloride, mytolon (St. W. Res. Inst.), brom-d-lysergic acid diethylamide, BOL-148 (Sandoz), methysergide bimaleate (Sandoz).

Results

1. Normal heart activity of the insects examined

In the insect species examined the frequency of the heart beat was found to vary on a rather wide scale. *Table 1* shows the lowest and the highest values of the rate of heart activity.

TABLE 1

Characteristic activity of control hearts of the insects examined

Insect	Lowest frequency/min	Highest frequency/min
P. nana	75	145
E. ephippiger	55	141
G. vulgaris	65	110
C. coriaceus	30	65
L. decemlineata	130	150

As shown in *Table 1*, only the heartbeat rates of *P. nana* and *E. ephippiger* are approximately within the same limits. In the other insects examined varying values were obtained.

The extreme values do not, however, mean that the variation in heart rate shown above can be observed on the same heart. The frequency of heart activity is rather uniform within the same insect as shown in *Fig. 1* where control graphs for each species are presented. As can be seen in *Fig. 1*, there is no essential difference between the heartbeat rate of controls in the first and 10th minute. There are, however, considerable individual variations and each heart has an activity of its own. Therefore, the effect of the agents tested was always compared to the control values measured on the same heart. No appreciable differences were noted between the control values of various specimens measured on the same days. The cause of individual differences was not investigated.

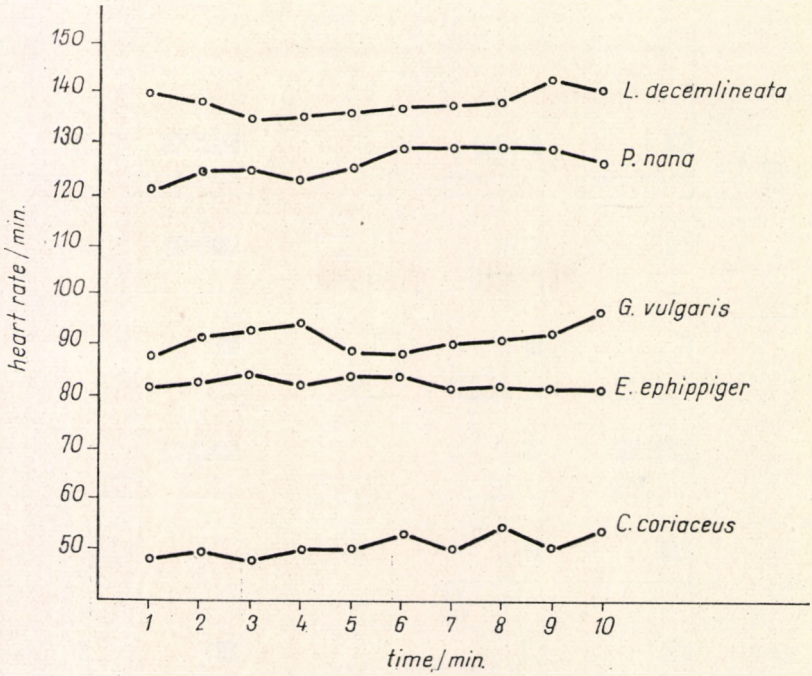

Fig. 1. Graphs showing control activity of normal hearts.
Abscisse: time in minutes; *Ordinate:* number of contractions per minute

2. Effects of some transmitter-like compounds and pharmacological agents

To facilitate the comparison of the effects of various agents tape diagrams were made which show the changes in per cent of inhibition and stimulation as compared to the control.

The effect of *acetylcholine* (ACh) on heart activity was examined in all five insect species. Acetylcholine in concentrations of 10^{-9} and 10^{-8} M had an accelerating effect on the heart of *G. vulgaris*. Increase in frequency never exceeded 20%. At 10^{-7} M and higher concentrations the heart of *G. vulgaris* was also inhibited *(Fig. 2)*, though to a lesser degree (15%). ACh was found to inhibit the hearts of the other four species in all the concentrations used beginning from the threshold concentration of 10^{-8} M. The extent of inhibition was always dependent on concentration. The maximum value was obtained at 10^{-4} M *(Fig. 2)*.

Noradrenaline (NA) when applied at 10^{-8}—10^{-9} M concentration produced an acceleration of about 50% in the rate of heartbeat in *G. vulgaris*. At higher concentrations (up to 10^{-4} M) the compound exerted an inhibitory action of about 15% *(Fig. 2)*. The hearts of *P. nana*, *E. ephippiger* and *C. coriaceus* were found to be inhibited by noradrenaline at concentrations ranging from the threshold value up to 10^{-4} M when the strongest inhibition was observed. The extent of inhibition was closely dependent on concentration *(Fig. 2)*.

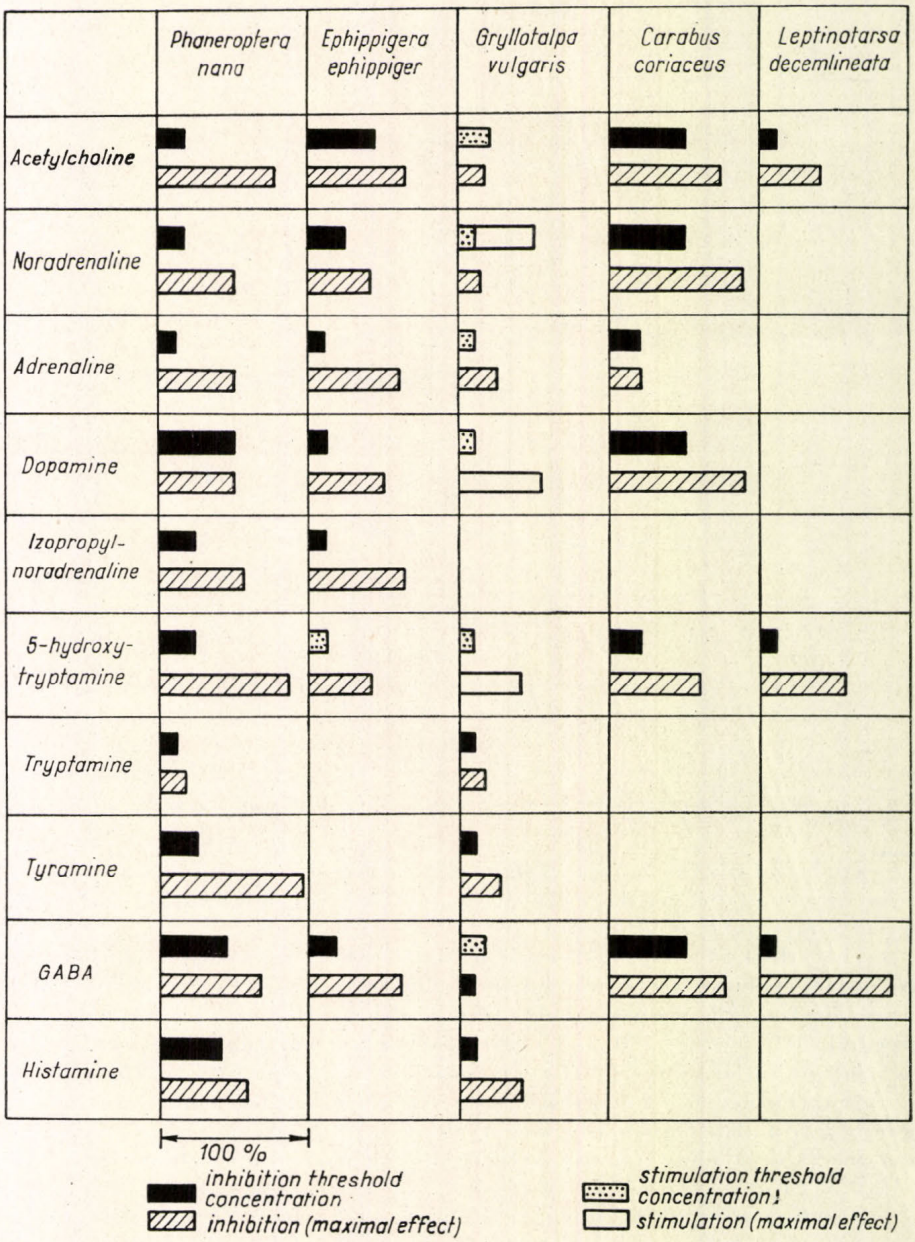

Fig. 2. Effect of biologically active agents on insect hearts. The extent of inhibition or stimulation is expressed in percentual value related to the control

Adrenaline (A) when applied at about the threshold concentration (10^{-8} M), produced an increase of about 10% in the rate of heartbeat of the *G. vulgaris*. Increasing the concentration to 10^{-7} M and higher, an inhibition of 25% was observed. The hearts of *P. nana* and *E. ephippiger* were inhibited by adrenaline at 10^{-8} M threshold concentration and beyond this value. Inhibition was found to grow with the increase in concentration. The threshold concentration for the heart of *C. coriaceus* was 10^{-7} M and the inhibition of about 20% was not dependent on concentration.

Dopamine (DA) showed an effect similar to that of the two catecholamines mentioned above: when applied at 10^{-8} M it accelerated the heart of *G. vulgaris* by about 10% and at higher concentrations by 25%. The hearts of *P. nana, E. ephippiger* and *C. coriaceus* were inhibited by dopamine. The inhibitory effect of the compound was the strongest in the heart of *C. coriaceus (Fig. 2)*.

Isopropylnoradrenaline (IPNA) at 10^{-7} M threshold concentration and at higher concentrations had a decreasing effect up to 65% on the heartbeat rate of *P. nana* and *E. ephippiger*. The effect was dependent on concentration *(Fig. 2)*.

A characteristic feature of all catecholamines examined is the appearance of the strongest inhibition in the 4th—5th minute of treatment and its gradual disappearance by about the 10th minute which seems to indicate the adaptation of the heart.

5-hydroxytryptamine (5HT) when applied at threshold concentration (10^{-8} M) produced an acceleration not higher than 10% on the hearts of *E. ephippiger* and *G. vulgaris*. Higher concentrations inhibited the rate of heartbeat by about 40%. The compound showed an inhibitory effect on the heart of *P. nana* (from 10^{-6} M), *C. coriaceus* (from 10^{-8} M) and *L. decemlineata* (from 10^{-7} M). In all insect hearts examined, the inhibitory effect of the compound depended on concentration. The strongest inhibition (77%) was produced in the heart of *P. nana (Fig. 2)*.

Tryptamine (TA) produced a decrease of 10—15% in the heart activity of *P. nana* and *G. vulgaris* at concentrations increasing from 10^{-8} M. The effect was independent of concentration.

Tyramine (TRA) was the only amine arresting the heart of *P. nana* at 10^{-4} M. The threshold concentration was 10^{-6} M causing an inhibition of 20% in the heartbeat rate of this insect. In *G. vulgaris* the threshold concentration was 10^{-7} M which was, however, less effective than in *P. nana*, as even at 10^{-4} the decrease of heartbeat rate was but 24% *(Fig. 2)*.

Gamma amino butyric acid (GABA) at threshold concentration (10^{-8} M) accelerated by 15% the heart activity of *G. vulgaris*, then at 10^{-7}—10^{-4} M concentrations an inhibition of 10—15% was observed in the heart of this insect. The action of the drug was definitely inhibitory when applied to hearts of *P. nana, E. ephippiger, C. coriaceus* and *L. decemlineata*. The threshold concentrations was 10^{-8} M. The effect depended on concentration.

Histamine (HA) inhibited the rate of heartbeat in *P. nana* and *G. vulgaris* at all concentrations beginning from 10^{-8} M. The extent of inhibition increased with higher concentrations but never exceeded 60 per cent.

The effect of pharmacological agents was studied in the hearts of *P. nana* and *G. vulgaris*.

Mytolon had a double effect on the hearts of *P. nana* and *G. vulgaris:* at about the threshold concentration (10^{-7}, 10^{-8} M), it produced an acceleration of 30% in heart activity, whereas beginning from the concentration of 10^{-6} it decreased the rate of heartbeat. Inhibition of the heart of *P. nana* was 90%, while that of the heart of *G. vulgaris* amounted only to 30%.

DCI when applied at threshold concentration (10^{-7} M) caused no acceleration but a decrease in heartbeat rate. The maximum inhibition was 40% *(Fig. 3).*

Nicotine at low concentrations (10^{-8}—10^{-7} M) accelerated the heart activity of *P. nana* and *G. vulgaris*, while at high concentrations (10^{-5}—10^{-4} M) it produced inhibition in all specimens examined *(Fig. 3).* The extent of inhibition, as well as that of stimulation remained below 30%.

BOL-148 inhibited the hearts of these insects and no acceleration of heartbeat rate was observed in any of the cases. The threshold concentration of the compound was 10^{-7} M. Decrease in frequency was 50% in *P. nana* and 25% in *G. vulgaris (Fig. 3).*

Methysergide was the only drug that had antagonistic effects on the hearts of *P. nana* and *G. vulgaris*, namely on the heart of *P. nana* at 10^{-5}—10^{-4} M concentration it produced a 30% inhibition and had no effect beyond this limit. In hearts of *G. vulgaris*, on the other hand, at all concentrations beginning from 10^{-8} M produced an acceleration of about 15% *(Fig. 3).*

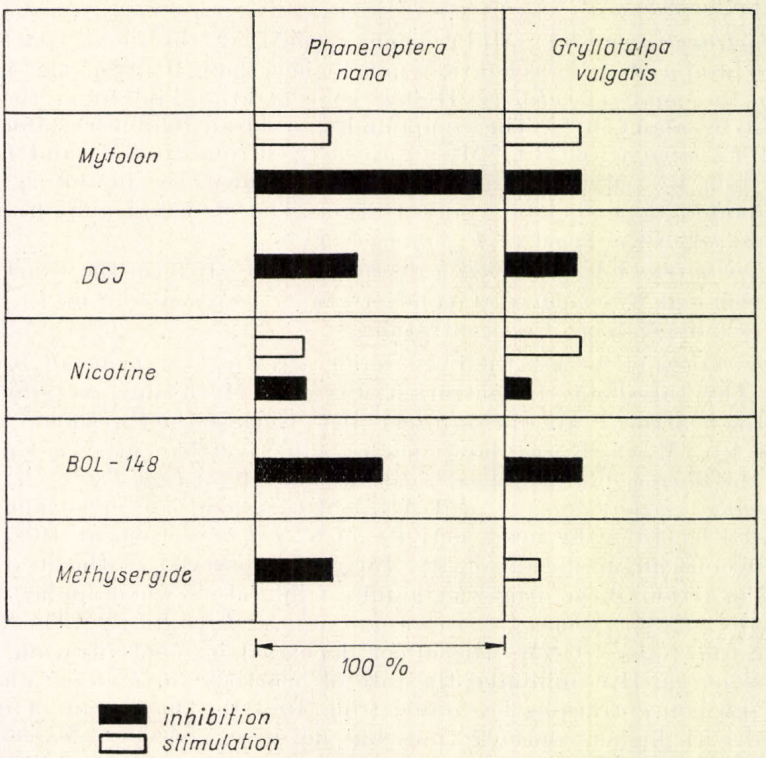

Fig. 3. Effect of some drugs on the activity of insect hearts. The extent of inhibition or stimulation is expressed in percentual value related to the control

3) Pharmacological analysis of the site of action of acetylcholine, adrenaline, 5HT and GABA

Investigations on the site of action of the agents tested were performed on the hearts of *G. vulgaris*, as this insect responded both with stimulation and inhibition to the action of biologically active agents applied. On the hearts of other insect species practically only inhibition was observed when various agents were applied to the heart preparations.

In the present experiments the biologically active agent and the drug were given in a mixture of equal volumes, at 10^{-6} M concentration. The results are summarized in *Table 2*.

As shown in the *Table 2* the original effect of acetylcholine is affected by all the drugs employed. DCI and methysergide eliminate the inhibitory effect of acetylcholine, whereas mytolon, BOL-148 and nicotine reverse the action of acetylcholine which becomes a stimulant of heart activity. All the drugs mentioned above, except nicotine and methysergide, when are given alone at the concentrations applied produced no stimulation but only an inhibition in heart activity.

Nicotine does not affect the action of adrenaline, where as DCI, mytolon, BOL-148 and methysergide reversed its inhibitory action to a stimulatory one.

With the drugs investigated the smallest degree of modification was observed in the effect of 5HT, DCI, mytolon, methysergide and nicotine had no effect on 5HT and only the BOL-148 was found to decrease or to stop the increase of heartbeat rate produced by 5HT. BOL-148 showed no effect on GABA action, while nicotine and methysergide reversed its inhibitory action to a stimulatory one.

TABLE 2

Effects of drugs on the action of acetylcholine, adrenaline, 5HT and GABA

Agent	Effect of agent alone 10^{-6} M	DCI + agent 10^{-6} M	Mytolon + agent 10^{-6} M	BOL—148 + agent 10^{-6} M	Methysergide + agent 10^{-6} M	Nicotine + agent 10^{-6} M
Acetylcholine	—	0	+	+	0	+
Adrenaline	—	+	+	+	+	—
5-hydroxytryptamine	+	+	+	0, +	+	+
GABA	—	+	+	—	+	0

+ stimulatory — inhibitory 0 ineffective

Reversal of inhibition to stimulation owing to drug effect is a characteristic feature of the heart of *G. vulgaris*. The stimulatory effect is, however, rather small causing usually an increase of about 15% in the amplitude and even the strongest stimulation never exceeded the level of 30%. Stimulation produced in this way could be easily eliminated by repeated washing and in some cases it was not observable after 9—10 minutes of treatment and the heart resumed its normal activity.

Discussion

Our results have verified that in spite of the considerable variation in the rate and amplitude of beating of insect hearts examined, measurements of activity performed on one specimen render possible the employment of insect hearts for bioassay. The heart activity of one specimen is constant enough to be usable for the evaluation of the effects of various agents *(Fig. 1)*.

The investigations have shown that there are no great differences as regards chemical sensitivity in the hearts of the insect species examined. The threshold concentrations of all biologically active agents were nearly identical (10^{-8}—10^{-7} M) in all insect hearts. The only difference in this respect was that the heart of *G. vulgaris* showed increased rate of amplitude in response to considerably more agents than the hearts of the other four species *(Fig. 2)*. Acetylcholine which was found to accelerate the hearts of *Blatta orientalis*, *Apis mellifera*, *Melanoplus differencialis*, *Periplaneta americana* and *Stenopelmatus* sp. (Hamilton, 1939; Roeder, 1953; Prosser and Brown, 1962), in our experiments produced but a rather small increase in the rate of heartbeat of *G. vulgaris* and in other insect hearts it had an inhibitory effect. Recently, some authors have questioned the view, generally accepted so far, that all insects should be considered to possess neurogenic hearts. So in the works of Metcalf et al. (1964) and Miller and Metcalf (1968) reference was made to myogenic insect hearts.

Considering that distinction between myogenic and neurogenic hearts is made on the basis of their response to acetylcholine, our data seem to point to the possibility of myogenic heart activity in some insects.

Of all catecholamines only the action of adrenaline was tested on insect hearts. The heartbeat of *Periplaneta americana* was found to be accelerated by adrenaline, whereas the heart activity of *Stenopelmatus* sp. was inhibited by this agent (Roeder, 1953; Prosser and Brown, 1962). According to our results the effects of the four catecholamines tested (NA, A, DA, IPNA) were similar on the insect hearts examined. The heart of *G. vulgaris* showed a different pharmacological response, namely, it was stimulated, whereas the other insect hearts were inhibited by these catecholamines. Tyramine proved to be an effective inhibitor producing a complete arrest in the heart of *P. nana*.

Indolalkylamine 5HT evoked stimulation of the heart of *G. vulgaris*, but the reverse effect was observed in other insect hearts. Of all biological agents tested tryptamine proved to be the least effective evoking but a small change (not exceeding 15%) in heart activity.

GABA was found to inhibit the Crustacean heart (Welsh, 1942; Crescitelli and Geissman, 1962) and — according to our results — the insect hearts as well, except the heart of *G. vulgaris* where at low concentrations it produced a slight stimulation *(Fig. 2)*. Histamine like-wise had an inhibitory effect when applied to the insect hearts under investigation.

There is no distinction between the numerous inhibitory effects acting on the hearts of the fours insects, either as regards threshold concentration or intensity of the effect *(Fig. 2)*. The data indicate pharmacological and not transmitter-like effects.

Up to now only the effect of nicotine has been investigated on the heart of *Periplaneta americana* and *Melanoplus* sp. where at low concentration it produced a stimulation of heartbeat rate (Jaeger and Gahan, 1937; Roeder

1953) and protected the heart from the action of low concentrations of acetylcholine (PROSSER and BROWN, 1962).

According to our observations, nicotine at low concentrations produced a stimulation, while at higher concentrations inhibited the heart of *G. vulgaris*. This finding is in agreement with the observation of HAMILTON (1939) on the heart of *Melanoplus* sp. Mytolon showed similar effects on the hearts of *P. nana* and *G. vulgaris*. DCI and BOL-148 produced inhibition on these hearts, while methysergide was found to inhibit the heart of *P. nana* and stimulate that of *G. vulgaris*.

Analyzing the sites of action of acetylcholine, adrenaline, 5HT and GABA, we concluded that in the heart of *G. vulgaris* the action of 5HT takes place on receptor structures that can be blocked by BOL-148. These receptors are highly specific as the effect of 5HT is not altered by mytolon, DCI, methysergide and nicotine. The acetylcholine receptors are less specific, as the effect of adrenaline is prevented both by DCI, an inhibitor of beta-adrenergic receptors, and by antiserotoninergic methysergide. This latter drug was found to have a similar effect on the rectum of *Tapes* (GREENBERG and JEGLA, 1963). Mytolon, BOL-148 and nicotine reverse the inhibitory effect of acetylcholine which is comparable to the pharmacological behaviour of Molluscan hearts (CHONG and PHILLIS, 1965).

Of the drugs used only nicotine proved to be ineffective on the action of adrenaline. Mytolon, DCI, methysergide and BOL-148 reverse the inhibitory action of adrenaline to stimulation. The effect of the above drugs on other insect hearts has not been described. In a previous work (S.-RÓZSA and PÉCSI, 1967), in which the effects of these drugs on the action of adrenaline in hearts of *Helix* and *Anodonta* were described, the same phenomenon was observed, namely a reversal in the inhibitory action of adrenaline to stimulation by BOL-148 and mytolon.

The effect of GABA was not influenced by BOL-148, but it was eliminated by nicotine and transformed to a stimulatory effect by DCI, mytolon and methysergide.

Our results obtained with blocking agents have verified that, except 5HT, the action of the drugs occurs on mixed receptor structures.

From our finding no conclusions can be drawn on the nature of the rhythm of the insect hearts but only on their chemical sensitivity. Why the great majority of these drugs produced inhibition on the insect hearts examined, can be answered only on the basis of further investigations.

Summary

Investigations were performed on the chemical sensitivity of the heart of *Phaneroptera nana*, *Ephippigera ephippiger*, *Gryllotalpa vulgaris*, *Carabus coriaceus* and *Leptinotarsa decemlineata*. From the results of the experiments it was stated that the heartbeat of *G. vulgaris* was accelerated by acetylcholine (10^{-9} M), noradrenaline (10^{-8} M), adrenaline (10^{-8} M), dopamine (10^{-8} M), 5-hydroxytryptamine (10^{-8} M) and GABA (10^{-8} M). Higher concentrations of these agents however, — except dopamine and 5-hydroxytryptamine — inhibited the heart of *G. vulgaris*. In addition, increase of cardiac activity was observed with *E. ephippiger*, when 5-hydroxytryptamine was applied at 10^{-8} M concentration to the heart of this insect.

The hearts of *P. nana*, *E. ephippiger*, *C. coriaceus* and *L. decemlineata* were inhibited by the following biologically active agents, in all concentrations used: acetylcholine (10^{-8} M), noradrenaline (10^{-8} M), adrenaline (10^{-7} M), dopamine (10^{-8} M), isopropylnoradrenaline (10^{-7} M), 5-hydroxytryptamine (10^{-8} M), tryptamine (10^{-8} M), tyramine (10^{-7} M), GABA (10^{-8} M), histamine (10^{-8} M). Of these drugs tested mytolon and nicotine had a double effect on the heart of *P. nana* and *G. vulgaris*. DCI and BOL-148 produced inhibition in both, whereas methysergide inhibited the heartbeat rate of *P. nana* and increased that of *G. vulgaris*.

Analyzing the sites of action of acetylcholine, adrenaline, 5-hydroxytryptamine and GABA, it was stated that only the effect of 5HT occurs on separated receptors, while the rest of biologically active agents act on mixed receptor structures.

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INSECTA SZÍVEK KÉMIAI ÉRZÉKENYSÉGÉNEK VIZSGÁLATA

S.-Rózsa Katalin és *V.-Szőke Ida*

Összefoglalás

Vizsgálták *Phaneroptera nana*, *Ephippigera ephippiger*, *Gryllotalpa vulgaris*, *Carabus coriaceus* és *Leptinotarsa decemlineata* szívének kémiai érzékenységét. Megállapították, hogy serkentést a *G. vulgaris* szívén az acetylcholine (10^{-9} M), noradrenaline (10^{-9} M), adrenaline (10^{-8} M), dopamine (10^{-8} M), 5-hydroxytryptamine (10^{-8} M) és

GABA (10^{-8} M) hoz létre. A dopamine és 5-hydroxytryptamine kivételével azonban a fenti anyagok magasabb koncentrációi gátolják a *G. vulgaris* szívműködését. A fentieken kívül serkentést még egy esetben regisztráltak; *E. ephippiger* szívén az 5-hydroxytryptamine 10^{-8} M koncentrációjú adásakor.

A *P. nana*, *E. ephippiger*, *C. coriaceus* és *L. decemlineata* szíve az alábbi biológiailag aktív anyagokra minden koncentrációban gátlással reagál: acetylcholine (10^{-8} M), noradrenaline (10^{-8} M), adrenaline (10^{-7} M), dopamine (10^{-8} M), isopropylnoradrenaline (10^{-7} M), 5-hydroxytryptamine (10^{-8} M), tryptamine (10^{-8} M), tyramine (10^{-7} M), GABA (10^{-8} M), histamine (10^{-8} M). Farmakonok közül a *P. nana* és *G. vulgaris* szívén a mytolon és a nicotine kettős hatású, a DCI és BOL = 148 csak gátlást, míg a methysergide az előbbi faj szívén gátlást, az utóbbin serkentést okoz.

Az acetylcholine, adrenaline, 5-hydroxytryptamine és GABA hatáshelyét vizsgálva megállapították, hogy a rovarok szívén csak az 5HT hatás valósul meg tiszta receptoron, a többi biológiailag aktív anyag kevert receptor-struktúrákon hat.

ИССЛЕДОВАНИЯ ХИМИЧЕСКОЙ ЧУВСТВИТЕЛЬНОСТИ СЕРДЦА НАСЕКОМЫХ

К. Ш.- Рожа и *И. В.- Секе*

Была исследована химическая чувствительность сердца *Phaneroptera nana, Ephippigera ephippiger, Gryllopalpa vulgaris, Carabus coriaceus, Leptinotarsa decemlineata.*

Установлено, что на сердце медведъки ацетилхолин (10^{-5} М) норадреналин (10^{-9} М), адреналин (10^{-8} М), допамин (10^{-8} М) 5-окситриптамин (10^{-8} М) и ГАМК (10^{-8} М) вызывают возбуждение. Однако за исключением допамина и 5-окситриптамина выше перечисленные вещества в болших концентрациях вызываыт торможение сердечной деятельности медведъки. Кроме выше перечисленных веществ, возбуждающее действите наблюдалось ещё в одном случае: во время добавления 5-окситриптамина с концентрацией 10^{-8} М к сердцу *E. ephippiger.*

Сердце *E. ephippiger P. nana*, *E. ephippiger*, *C. coriaceus* и *L. decemlineata* на следующие биологически-активные вещества отвечало торможением: ацетилхолин (10^{-8} М), норадреналин (10^{-8} М), адреналин (10^{-7} М), допамин (10^{-8} М), изопропилнорадреналин (10^{-7} М), 5-окситприптамин (10^{-8} М), триптамин (10^{-8} М), тирамин (10^{-7} М), ГАМК (10^{-8} М), гистамин (10^{-8} М). На сердцах *P. nana* и *G. vulgaris* митолон и никотин обладают двойным действием, ДСІ, и БОЛ = 148 вызывает только торможение, в то же время метисергид обладает тормозным действием на сердце первого и возбуждающим на сердце второго. При изучении места приложения ацетилхолина, адреналина, 5-окситриптамина и ГАМК, было установлено, что на сердце насекомых только действие 5-окситриптамина осуществляется на чистых рецепторных структурах, влияние остальных биологически-активных веществ осуществляется на смещанных рецепторах.

ANNAL. BIOL. TIHANY | 37 | 111—130 | HUNGARIA 1970

PHARMACOLOGICAL ANALYSIS OF THE SPONTANEOUS ACTIVITY AND TRANSMISSION OF IMPULSES ON THE VISCERAL GANGLION OF ANODONTA CYGNEA L.

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Received: 14th January 1970.

The results of pharmacological analyses of the regulation of excitatory processes in Pelecypoda are not so concordant as in other animal species. The role and action of bioactive agents have not been clarified as yet in spite of the fact that it was demonstrated by histochemical and biochemical methods that the central nervous systems of various species of molluscs contain serotonin (5HT) and catecholamines (WELSH and MOORHEAD, 1959; DAHL et al., 1962; PUPPI, 1964), as well as acetylcholine (WELSH, 1956; COTTRELL, 1966) and acetylcholinesterase (SALÁNKI et al. 1966), essental for cholinergic mediation. By electron microscopic examinations axo-somatic and axo-axonic synapses were demonstrated in the nerve cells of *Anodonta cygnea* (ZS.-NAGY, 1964) which — judging from the presence of numerous vesicles — seem to function by the release of some chemical substances.

In the course of numerous pharmacological investigations on the cerebral ganglion of *Mya arenaria* (HORRIDGE, 1961), cerebral and visceral ganglia of *Anodonta* (PUPPI, 1963a, b), visceral ganglion of *Anodonta* (SALÁNKI and LÁBOS, 1964) and on the cerebral and visceral ganglia of *Unio* (SOKOLOV, 1967a, b) spontaneous activity, as well as evoked potentials were recorded.

According to HORRIDGE, ACh has an inhibitory effect on the transmission of nerve impulses, PUPPI, on the other hand, observed an initial stimulation besides inhibition, and lasting stimulary effects in the cerebral ganglion when low concentrations of ACh were applied. Both authors found 5HT to stimulate spontaneous activity. Most of the drugs applied had, however, slight effects and the results were often contradictory.

As has been shown in a previous work (SALÁNKI and VARANKA, 1969) the spontaneous activity of the ganglia in *Anodonta cygnea* has varying patterns in different nerves. It has also been demonstrated that when the posterior margin of the mantle (region of the sypho) was mechanically stimulated, burst could be recorded from the nervus pallialis posterior maior (nppm) running toward the visceral ganglion. The burst could be conducted both from the contralateral nppm and from the cerebrovisceral connective (CVc). As has been pointed out by SALÁNKI and GUBICZA (1969) in the latter pathway there are no fibres running through the visceral ganglion and thus the impulse is conducted by synaptic transmission from the nppm to the CVc. One can, however, reckon with the presence of direct fibres between the two pallial nerves (nppm), as has been described by SALÁNKI and VARANKA (1969).

As the spontaneous activity of ganglionic origin can be conducted from the nppm and CVc, and transmission can occur in two directions, we have

applied direct chemical treatment on the visceral ganglion of *Anodonta* to see which possible chemical mechanisms are involved in the regulation of spontaneous electrical activity of the visceral ganglion and in the transmission of impulses.

Material and method

For the experiments, specimens of fresh-water mussel (*Anodonta cygnea* L.) 16—21 cm in length were used. One of the shells was removed and the ventral surface of the visceral ganglion (VG) and the initial portions of the nerves originating from it were exposed. Conduction of spontaneous and evoked activity was usually made from the left nppm and right CVc. The experimental arrangement is shown in *Fig. 1.*

When studying spontaneous electrical activity, the nerves were transected at about 10—15 mm from the VG and suction electrodes were applied for con-

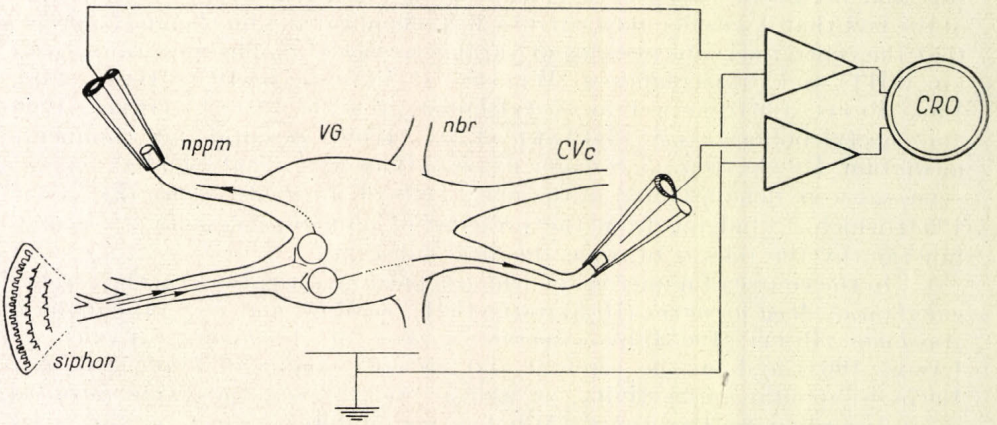

Fig. 1. Experimental arrangement. VG = visceral ganglion; nppm = nervus pallialis posterior maior; nbr = nervus branchialis; CVc = cerebro-visceral connective

duction of activity from the central stump. When evoked activity was studied, the sypho region with intact nerve was excited by touching it with a fine paintbrush. To protect the preparation from desiccation the free surface of the VG and the tissues in its vicinity were sprinkled from time to time, with Ringer solution. Under such conditions the animals could be kept alive even for 24 hours.

The agents tested were diluted in physiological solution (Marczynski, 1959) and applied to the VG with a swab of cotton wool. The pH of the solution was 7.0 $\pm$ 0.1. The effect of the agents was evaluated beginning from the 2nd minute after application, as manipulation with the cotton wool itself produces for about a minute a change in activity.

The effects of the following agents were investigated: acetylcholine chloride, tetraethylpyrophosphate, picrotoxin (Fluka), eserine sulphate, tetraethylammonium iodide, atropine sulphate, ergometrine maleate (BDH), neostigmine bromide, natrium glutamate (Merck), d-tubocurarine chloride, tetramethylammonium bromide, dichlorisoproterenol hydrochloride (Schu-

chardt) mytolon chloride (benzoquinonium), mytelase chloride (ambenonium) (St. W. Res. Inst.), L-adrenaline d-hydrogentartarate (EGA) dopamine hydrochloride, 5-hydroxytryptamine-creatininsulphate (Sigma), DL-5-hydroxytryptamine (Mann Research Lb.), L-noradrenaline bitartarate (Serva), dibenamine hydrochloride, decamethonium iodide (Koch-Ligth), BOL-148 (2-bromo-lysergic acid diethylamide), methysergide bimaleate (Sandoz), γ-aminobutyric acid (Reanal).

Results

Drugs acting on the cholinergic system

Acetylcholine evoked two kinds of response depending on concentration, namely, at 10^{-7} and 10^{-8} M the spontaneous basic and burst activity, as well as the amplitude of the transmitted potential were found to increase *(Fig. 2)*. When no spontaneous bursts were recorded in the control, they frequently appeared after ACh treatment.

In concentrations from 10^{-4} to 10^{-6} M ACh decreased the spontaneous activity of the visceral ganglion, abolished the bursts and inhibited the transmission of impulses evoked by mechanical stimulation of the sypho region *(Fig. 3)*. Within this range of concentrations sometimes a small stimulation could be observed after adaptation, showing that ACh has a biphasic effect. This applies also to the amplitude of spontaneous bursts and transmitted impulses. The effect of ACh can be eliminated by washing.

Atropine at high concentrations (10^{-3}—10^{-4} M) increased the spontaneous basic activity, elicited the initiation of spontaneous bursts and potentiated the transmission of evoked activity. At 10^{-5} M it still has an increasing effect on transmission but below this concentration level it is ineffective. Atropine prevented the inhibitory effect of ACh *(Fig. 4)*. Its stimulating effect on burst activity could not be washed out even after one hour.

d-Tubocurarin (d-TC) at 10^{-3} to 10^{-4} M potentiated the transmission of impulses and had a slight increasing effect on spontaneous basic activity, as well. Its effect can be washed out in a relatively short time.

Spontaneous burst activity was increased and transmission potentiated by *tetraethylammonium* (TEA). This drug antagonized the inhibitory effect of ACh. Its effect on spontaneous activity can be washed out rapidly but its potentiating effect on transmission persisted even after one hour *(Fig. 5)*.

The effect of *tetramethylammonium* (TMA) was similar to that of TEA, but it was effective only at 10^{-3} M. Its stimulating effect can be prevented by ACh.

Decamethonium at 10^{-4} and 10^{-5} M increased spontaneous basic activity and potentiated the amplitude of spontaneous bursts and transmission at 10^{-4} M. Below this level of concentration it is ineffective.

Eserine at 10^{-4} M inhibited transmission and to some extent also the spontaneous activity. Inhibition takes place rapidly, but incompletely as through several fibres transmission remains unaffected even after 30 minutes of treatment *(Fig. 6)*. The effect is not easily washed out.

Neostigmine even at 10^{-3} M had an inhibitory effect only on transmission and was easily washed out.

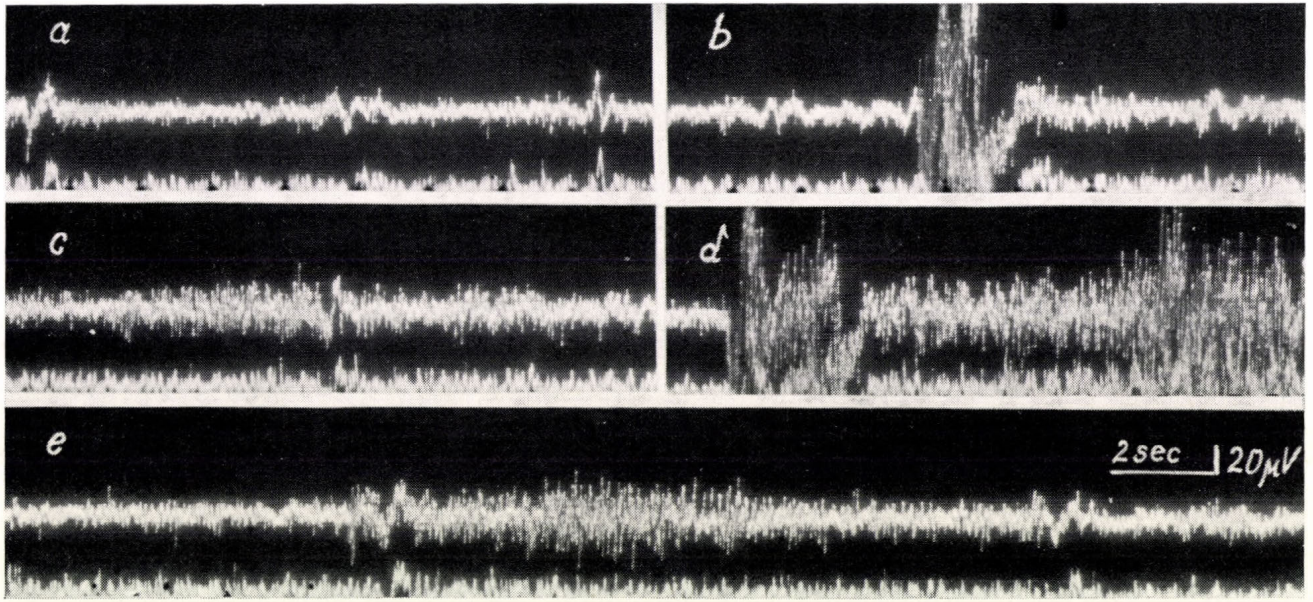

Fig. 2. Effect of 10^{-7} M ACh on spontaneous burst activity (a, c, e) and on transmission (b, d). a and b = before treatment; c and d = 20 minutes after treatment; e = after 30 minutes; Up : nppm = Down : CVc (on each Figure)

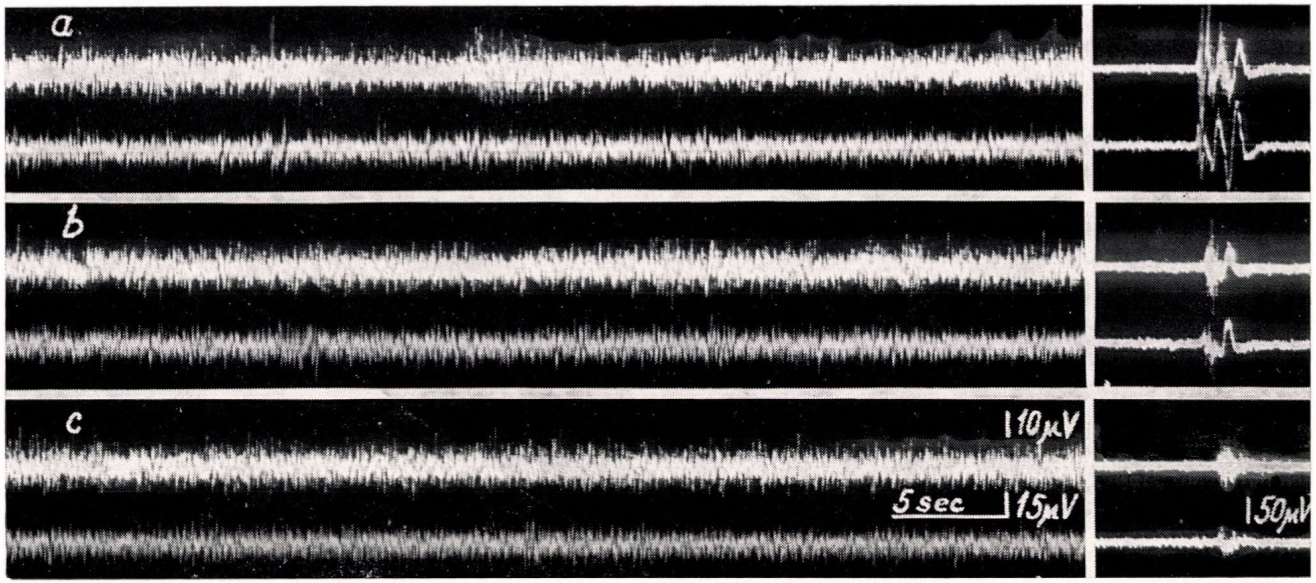

Fig. 3. Effect of 10^{-4} M ACh on VG spontaneous activity and transmission
a = before treatment; b = 10 minutes after treatment; c = after 30 minutes

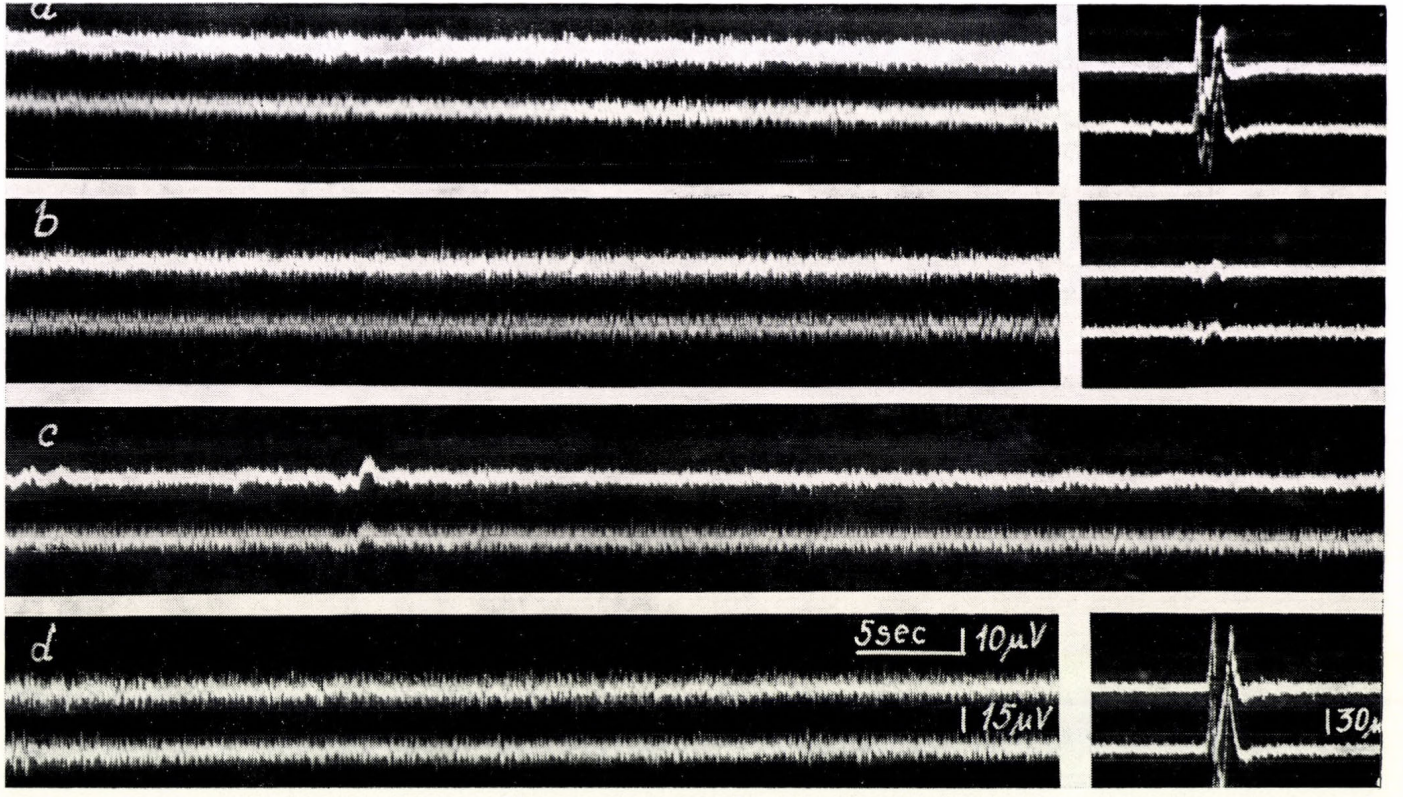

Fig. 4. Atropine antagonization of ACh inhibition
a = before treatment; b = after 5 min of treatment with 10^{-4} M ACh; c = after 20minutes of treatment; d = after 25 minutes following addition of 10^{-4} M atropine

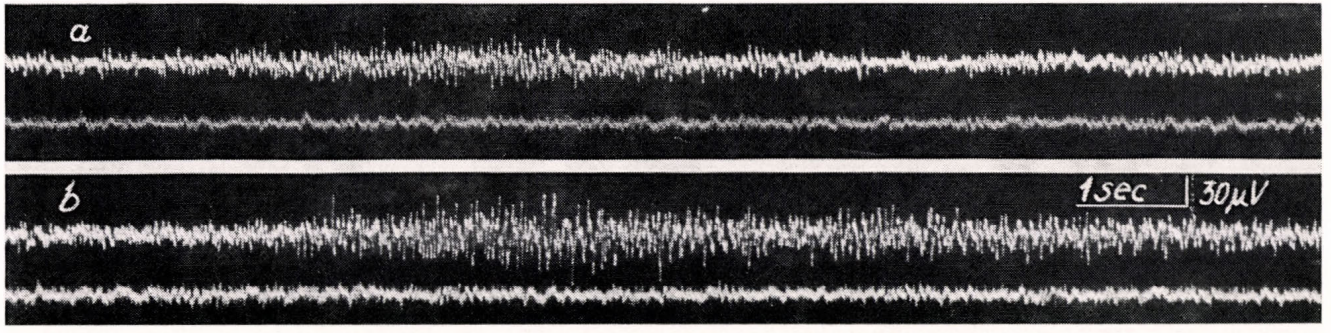

Fig. 5. Effect of 10^{-4} M TEA on spontaneous burst activity
a = before treatment; b = 20 minutes after treatment

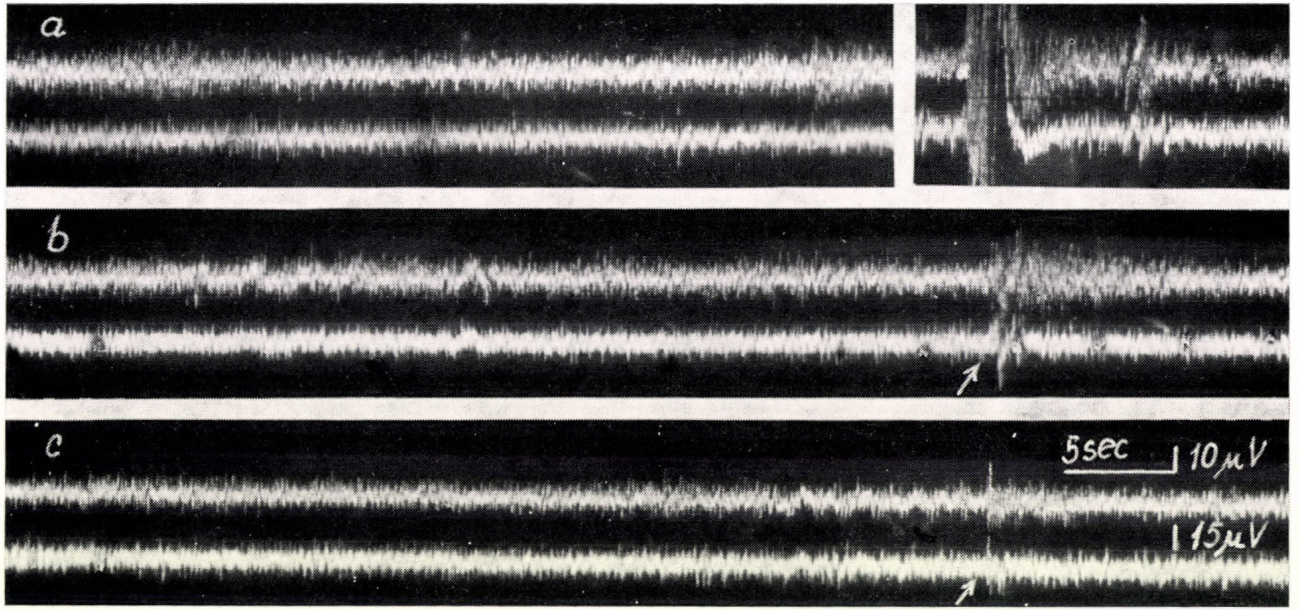

Fig. 6. Effect of 10^{-4} M eserine on spontaneous activity and on transmission.
a = before treatment; b = 5 minutes after treatment; c = 20 minutes after treatment

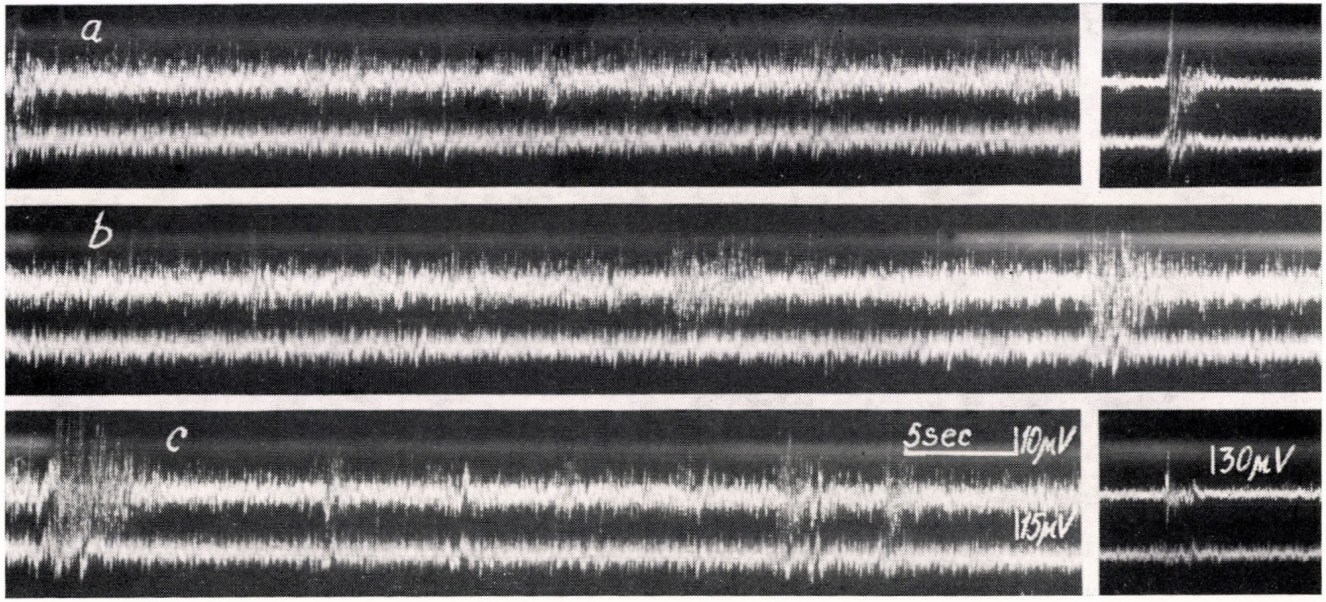

Fig. 7. Effect of 10^{-5} M Mytelase on spontaneous activity and on transmission.
a = before treatment; b = 10 minutes after treatment; c = 30 minutes after treatment

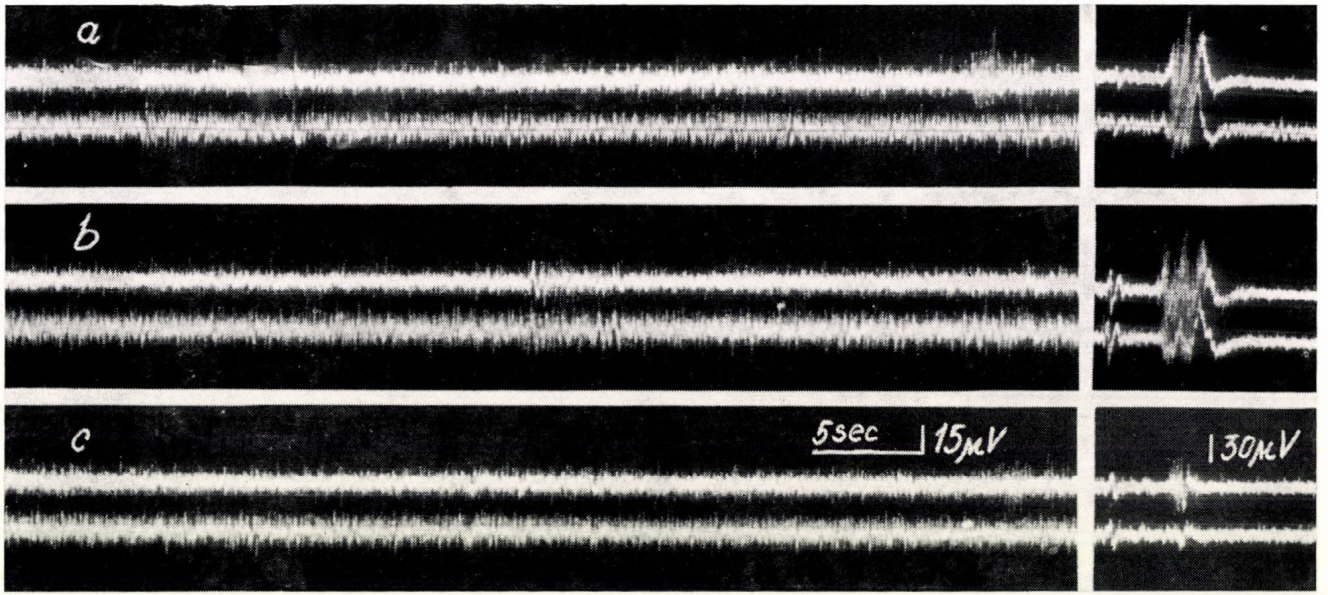

Fig. 8. Effect of 10^{-4} M noradrenaline on spontaneous activity and on transmission.
a = before treatment; b = 10 minutes after treatment; c = 20 minutes after treatment

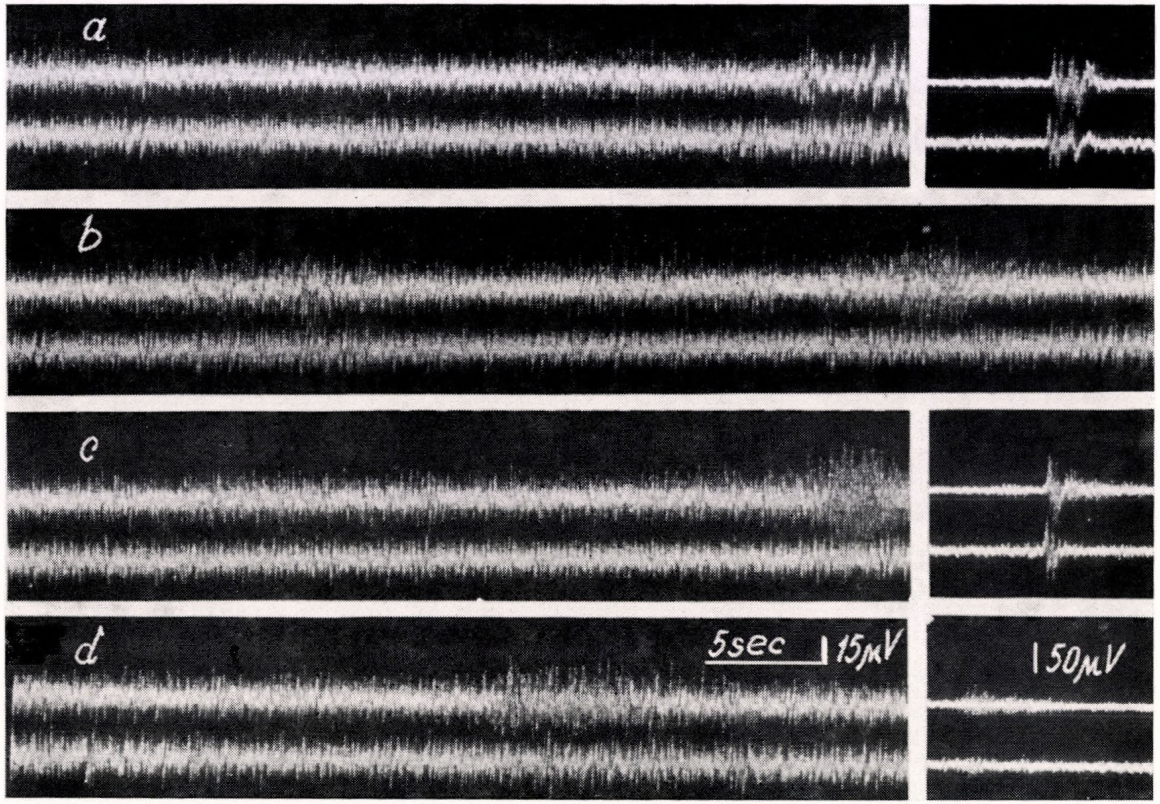

Fig. 9. Effect of 10^{-6} M DCI on spontaneous activity and transmission
a = before treatment; b = 5 minutes after treatment; c = 10 minutes after treatment; d = 30 minutes after treatment

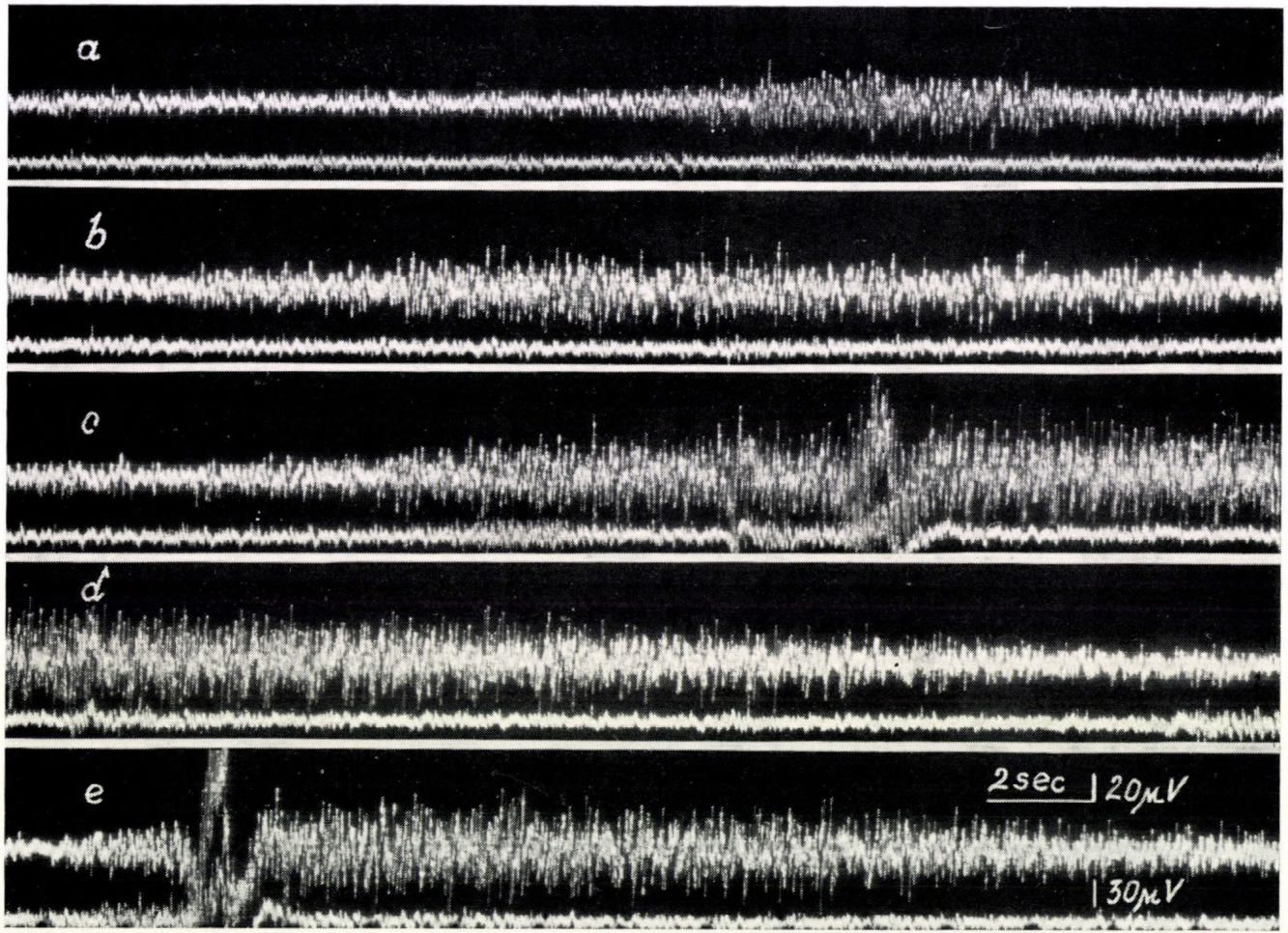

Fig. 10. Effect of 10^{-6} M 5HT on spontaneous activity
a = before treatment; b = one minute after treatment; c, d = continuous record after 10 minutes; e = after 20 minutes

Tetraethylpyrophosphate showed no effect even at 10^{-3} M.

Mytolon at 10^{-4} and 10^{-5} M enhanced spontaneous activity conducted from nppm, and increased the amplitude and frequency of bursts. The activity conducted from CVc showed no change as compared to the control. Transmission was inhibited at 10^{-4} M.

Mytelase at 10^{-4} and 10^{-5} M had no effect on spontaneous basic activity but increased the duration and amplitude of spontaneous bursts. Transmission was intensively inhibited under these circumstances *(Fig. 7)*, but inhibition, like with eserine, was incomplete. Application of 10^{-6} M mytelase potentiated burst activity, but was ineffective on transmission.

Picrotoxin at 10^{-4} and 10^{-5} M elicited spontaneous bursts or increased their frequency. It showed no effect on spontaneous basic activity and on transmission.

Drugs acting on the adrenergic system

Application of *adrenaline*, *noradrenaline* and *dopamine* at 10^{-4} M and in higher concentrations led to inhibition of transmission *(Fig. 8)*, whereas spontaneous activity remained unaffected.

Dibenamine exerted a slight increasing effect on spontaneous basic and burst activity, but was ineffective on transmission.

Application of 10^{-4} to 10^{-6} M *dichlorisoproterenol* (DCI) caused but a slight increase on spontaneous basic activity but had a strong activating effect on spontaneous bursts, particularly on their frequency. Its effect on the amplitude was less marked. When lower concentrations were used adaptation was observed after 20 minutes of treatment. Transmission was inhibited by DCI in the concentrations applied *(Fig. 9)*.

No appreciable alterations in electrical activity were observed with the application of *ergotamine* and *ergometrine* at 10^{-4} M or below this concentration level.

Drugs acting on the tryptaminergic system

Serotonin at 10^{-8} M and in higher concentrations enhanced spontaneous burst activity *(Fig. 10)* by increasing the amplitude and frequency of bursts or eliciting burst activity. The strongest effect was noted when the drug was applied at 10^{-6} M concentration. After 20—25 minutes of serotonin treatment (10^{-8} M) adaptation was observed. When higher concentrations were applied spontaneous basic activity was sometimes, also increased. Though the drug occasionally showed some potentiating effect on transmission, it did not affect it substantially.

5-hydroxytryptophan proved to be ineffective even at 10^{-3} M.

BOL-148 and *methysergide* at 10^{-4} to 10^{-6} M concentrations have usually no effect on spontaneous basic activity, but inhibited the appearance of spontaneous bursts or decreased their amplitude. Transmission was likewise inhibited *(Fig. 11)*.

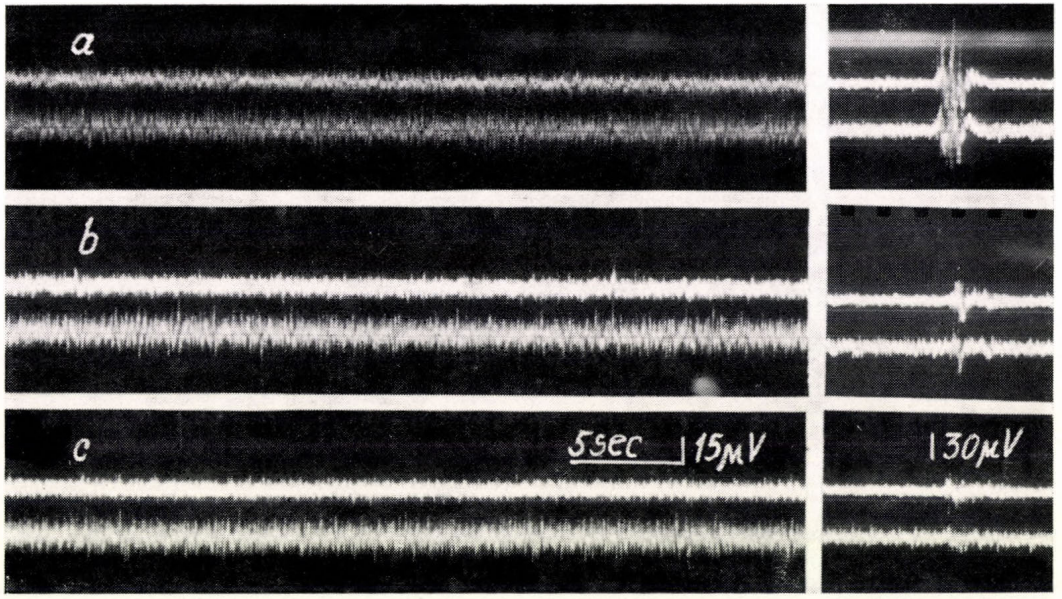

Fig. 11. Effect of 10^{-4} M BOL—148 on spontaneous activity and on transmission.
a = before treatment; b = 10 minutes after treatment; c = 20 minutes after treatment

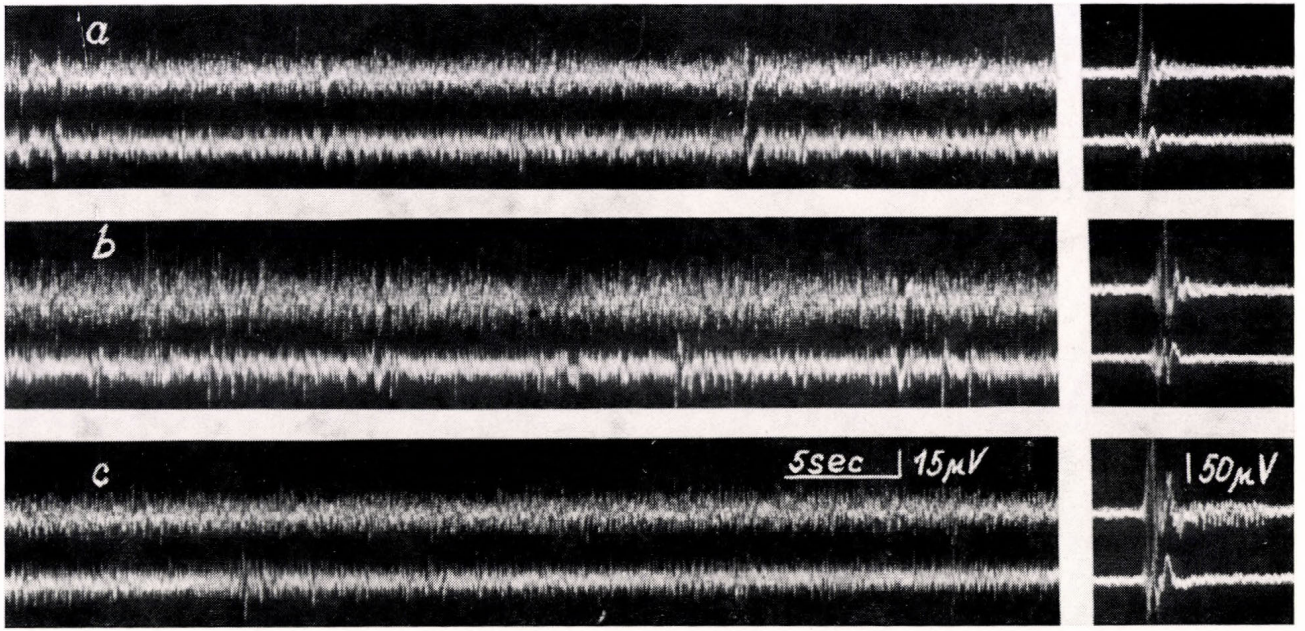

Fig. 12. Effect of 10^{-4} M Na glutamate on spontaneous activity and on transmission. a = before treatment; b = 5 minutes after treatment; c = 20 minutes after treatment

Other possible transmitters

γ-amino-butyric acid at 10^{-4} M and below this concentration level was ineffective, but at 10^{-3} M, inhibited both spontaneous activity and transmission of impulses.

Increase of spontaneous activity was observed when 10^{-4}—10^{-6} M concentration of *glutamate* were applied *(Fig. 12)*. The effect lasted about 15—20 minutes followed by adaptation or even by a slight inhibition, as was registered in some cases. Spontaneous burst activity and transmission were not altered by these concentrations of glutamate.

Discussion

Acetylcholine was found to evoke different responses, depending on concentration, on the visceral ganglion of *Anodonta cygnea*. On the whole, this finding is in agreement with the data of PUPPI (1963a) on cerebral ganglion of Lamellibranchiata. Cholinergic antagonists: atropine, TEA, TMA and decamethonium, as well as d-TC given alone stimulate spontaneous activity and transmission of potentials. Atropine and TEA eliminate the inhibitory effect of exogenous ACh. Though according to PUPPI (1963a) atropine produces inhibition on the cerebral ganglion, we have never observed inhibition by application of cholinergic antagonists. Considering moreover, that the ganglia of Pelecypoda were found to contain ACh (WELSH, 1956; COTTRELL, 1966) and cholinesterase (SALÁNKI et al. 1966) the assumption that cholinergic mechanisms may play a role in the regulation of spontaneous activity and transmission of evoked potentials on the visceral ganglion of *Anodonta*, may be accepted.

Dependence on concentration of ACh effect seems to be connected with different sites of action. Low concentrations of ACh may increase by depolarization the activity of certain nerve cells, as has been described in the "D" cells of Gastropoda (TAUC and GERSCHENFELD, 1962). A similar mechanism may result in the potentiation of the postsynaptic response. This may constitute the stimulatory cholinergic system.

Higher concentrations of ACh may also produce inhibition by prolonged depolarization of high degree, but the stimulating effect of cholinergic antagonists which themselves do not, as a rule, influence the permeability of non-cholinergic membranes, seems to indicate that besides a stimulatory cholinergic system, an inhibitory cholinergic system is also present in the visceral ganglion. Thus the effect of higher ACh concentrations, besides the depolarization, blocking the effect of the stimulatory system, may be explained by the activation of this inhibitory system. The inhibitory effects of eserine and neostigmine and the burst activating effect of picrotoxin also refer to the physiological presence of inhibitory cholinergic systems.

The fact that only stimulation and no inhibition was observed when cholinergic antagonists were applied seems to indicate that the cholinergic inhibitory system, as compared to the stimulatory one, is more sensitive to pharmacological treatment, or at least this is predominant after one minute of treatment. We may also suppose that the stimulatory effect of ACh is aspecific. This is, however, contradicted by the long duration of stimulation

and by the fact that not only the increase of spontaneous activity, but also the potentiation of transmission invariably occur with low concentrations of ACh. Mytolon and mytelase may be regarded rather as ACh synergists than antagonists in the visceral ganglion, increase of spontaneous activity may be interpreted as a stimulatory effect on the somatic membrane, while inhibition of transmission as blocking effect on the stimulatory synapses. The presence of a few fibres of evoked postganglionic potential, after application of eserine and mytelase, show that inhibition of transmission is not exclusively connected with cholinergic mechanisms.

The catecholamines employed were found to inhibit transmission only in relatively high concentrations, which seems to refer to their inhibitory role in the regulation of transmission. This is, however, contradicted by the fact that neither dibenamine, an α-adrenergic blocking drug, nor the β-blocking DCI had a potentiating effect on transmission, on the contrary, the latter drug inhibits transmission by itself. Both drugs increased spontaneous activity which was, however, unaffected by catecholamines. On the other hand, there are data indicating the ganglionic role of catecholamines. Thus, adrenaline and noradrenaline applied to the cerebral ganglion increase the rhythm of the posterior adductor muscle (SALÁNKI, 1963) and the ganglia are known to contain considerable amounts of catecholamines (DAHL et al. 1962; PUPPI, 1964; ZS.-NAGY, 1967). The presence of catecholamines in the synaptic vesicles (ZS.-NAGY, 1968) also indicate their involvement in transmission of nerve impulses. The question is raised that if these all are inhibitory synapses why they cannot be blocked by the best known adrenergic blocking agents? For the elucidation of the role of adrenergic system further investigations are required.

In the course of our investigations serotonin proved to be the most effective drug. The fact that it has a strong stimulatory effect on the burst activity of the visceral ganglion seems to point to its role played in the generation of spontaneous activity. This assumption is supported by the finding that serotonin-antagonistsڅ: BOL-148 and methysergide evoke inhibitory effects. This is in agreement with the data of HORRIDGE (1958), PUPPI (1963b) and SALÁNKI (1963) who likewise found that the application of serotonin produced stimulation in burst activity.

5HT did not alter the transmission of impulses. Therefore its function as an interneuronal transmitter along the path nppm-CVc and nppm-nppm, is unlikely. It should be noted that the role of 5HT as a transmitter within the ganglion has been questioned also on histochemical basis (ZS.-NAGY, 1967).

Of the other substances tested, glutamate seems to deserve attention. GABA does not appear to play a physiological part. The comparatively low level of the effective concentration of glutamate seems to indicate that similarly to the data obtained in other animals (KRNJEVIC et al. 1966; SALÁNKI, 1968), glutamate may be involved in the regulation of VG activity in *Anodonta*, as well.

So far morphological investigations of the cerebral and viseeral ganglia have not disclosed any microscopic and electron microscopic differences in structure between these ganglia. Physiologically, on the other hand, functional differences were observed as the cerebral ganglia are the sites of the primary relaxing centres of the regulation of the adductor muscle (PAWLOW, 1885;

Barnes, 1955; Salánki and Lábos, 1963). Pharmacological investigations also indicate the different nature of these ganglia. Thus, when ACh was applied, biphasic effect appeared only in the cerebral ganglion, whereas in the visceral ganglion only inhibition was observed (Puppi, 1963a). Similarly, a disparity in effect to atropine was also noted in these ganglia. Sokolov (1967b) described the different pharmacological conditions of the cerebral and visceral ganglia. It is therefore, not surprising that our data differ in part from those obtained on the cerebral ganglion. This pharmacological dissimilarity may be utilized in the investigations on spontaneous ganglionic activity and transmission of nerve impulses on different pathways for the demonstration of functional differentiation — insufficiently known so far — of the central nervous system of Pelecypoda.

Summary

From the investigations on the spontaneous activity of the visceral ganglion and on transmission of potentials evoked by mechanical stimulation of the sypho region in *Anodonta cygnea* it was concluded:

1) Acetylcholine in low concentrations (10^{-6}—10^{-8} M) increases while in higher concentrations (10^{-4}—10^{-5} M) inhibits both spontaneous activity and transmission of impulses.

Cholinergic antagonists exert a stimulating effect, cholinesterase inhibitors, on the other hand, block the transmission of evoked potentials, which indicates the presence of a stimulatory and an inhibitory cholinergic system.

2) Adrenergic drugs inhibited only the transmission of impulses. Their role is not clear as the effect of adrenergic antagonists does not confirm the inhibitory effect of catecholamines.

3) Serotonin in low concentrations (10^{-6}—10^{-8} M) had a stimulatory effect on burst activity but was ineffective on transmission. It may play a role in the generation of spontaneous activity.

4) Of the other substances tested, GABA does not seem to participate, while glutamte may be involved in the processes of transmission of the visceral ganglion.

5) Pharmacological difference is suggested between the cerebral and visceral ganglia. Detailed analysis of this difference may help to demonstrate the functional differentiation of the central nervous system in Anodonta cygnea.

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A SPONTÁN AKTIVITÁS ÉS INGERÜLETÁTTEVŐDÉS FARMAKOLÓGIAI VIZSGÁLATA ANODONTA CYGNEA VISCERÁLIS GANGLIONJÁN

Varanka István és *Salánki János*

Összefoglalás

A viscerális ganglion spontán aktivitásának, valamint a szifóingerléssel kiváltott potenciál áttevődésének vizsgálata során megállapítást nyert:

1. Az acetylcholin kis (10^{-6}—10^{-8} M) koncentrációban serkentő, nagyobb (10^{-4}—10^{-5} M) koncentrációban gátló hatású mind a spontán aktivitásra, mind az áttevődésre. Kolinerg antagonisták serkentő hatásúak, cholineszteráze gátlók az áttevődést blokkolják. Mindez serkentő és gátló kolinerg rendszer jelenlétére utal.

2. Adrenerg szerek viszonylag nagy dózisban (10^{-4} M) is csak az áttevődést befolyásolják, gátolják. Szerepük nem világos, minthogy az adrenerg antagonisták hatása a catecholaminok gátló szerepét nem támasztja alá.

3. Szerotonin a burst aktivitást fokozza már 10^{-6}—10^{-8} M koncentrációban is, az ingerületáttevődést azonban nem befolyásolja. A spontán aktivitás generálásában lehet szerepe.

4. Egyéb anyagok közül a GABA valószínűleg nem, a glutamát esetleg szerepet játszik a viscerális ganglion ingerületi folyamataiban.

5. A cerebrális és viscerális ganglion között farmakológiailag kimutatható különbség van, s ennek részletes vizsgálata alkalmas lehet a központi idegrendszer funkcionális differenciáltságának feltárására.

ФАРМАКОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ СПОНТАННОЙ АКТИВНОСТИ И ПЕРЕДАЧИ ВОЗБУЖДЕНИЯ НА ВИСЦЕРАЛЬНОМ ГАНГЛИИ ANODONTA CYGNEA

И. Варанка и *Я. Шаланки*

На основе исследования спонтанной активности и передачи возбуждения, вызванного раздражением сифонального нерва, можно сделать следующие выводы:

1. Влияние ацетилхолина в малой концентрации (10^{-6}—10^{-8} М) возбуждающее, в большей концентрации тормозное как на спонтанную активность так и на передачу. Холинолитики обладают возбуждающим действием, а вещества тормозящие холинэстеразу, блокируют передачу. Всё это показывает на присуствие возбуждающей и тормозящей холинергической системы.

2. Адренергические вещества в сравнительно большой дозе (10^{-4} М) влияют только на передачу тормозя ёё. Их роль не ясная, так как действие адреналитиков не подтверждает тормозящего действия катехоламинов.

3. Серотонин усиливает зальповую активность уже в концентрациях 10^{-6}—10^{-8} М, на передачу возбуждения он не влияет. Может быть, что серотонин играет роль в генерации спонтанной активности.

4. Предполагается, что из других веществ ГАМК не играет, а глутамат, может быть, играет роль в процессах возбуждения в висцеральном ганглии.

5. Между церебральным и висцеральным ганглиями существуют фармакологические различия, исследования этих различий могут содействовать обнаружению функциональной дифференцированности в центральной нервной системе.

ANNAL. BIOL. TIHANY | 37 | 131—143 | HUNGARIA 1970

THE FINE STRUCTURE OF NEUROMUSCULAR AND INTERMUSCULAR CONNECTIONS IN THE ADDUCTORS OF ANODONTA CYGNEA L. (MOLLUSCA, PELECYPODA)

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Received: 20th January, 1970

The innervation of adductors in bivalves has only been studied at light microscopic level by silver impregnations, methylene blue staining or cholinesterase histochemistry (BOWDEN and LOWY, 1955; BOWDEN, 1958; ÁBRAHÁM and MINKER, 1957, 1959). The published data are contradictory first of all because of the irreproducibility of the impregnation and staining methods. On the other hand, that time the cholinesterase activity was generally believed to indicate only nervous elements. Later, however, it became clear that in the adductors of mussels it is not the case (ZS.-NAGY, 1964a). Therefore, the histological investigations could only evidence that the adductors contain nerve fibres. According to BOWDEN (1958) neuromuscular end plates are present; others could not confirm his findings (ÁBRAHÁM and MINKER, 1957, 1959); BOWDEN (1958) described also nerve cells in the adductors whereas the latter authors denied this finding categorically.

Starting from the above mentioned contradictions we wanted to investigate electron microscopically the structure of axons and neuromuscular junctions as well as the presence or absence of nerve cells. Since, however, during the investigations light has been thrown to some intermuscular connections which can be significant in the muscle function, we treat them also here.

Material and method

The investigations were carried out on the 12—20 cm long specimens of *Anodonta cygnea* L. The adductors were fixed as follows:

a) 2% OsO_4 buffered with s-collidine at pH 7.2 (BENNETT and LUFT, 1959) for 2 hours at 0 °C.

b) 2.5% glutaraldehyde in tap water at pH 7.0 for 16—20 hours at room temperature. The glutaraldehyde was purified by ion-exchange according to VADÁSZ (1966). After the glutaraldehyde fixation we washed the material in tap water for 2 hours than it was postfixed in 2% OsO_4 for 1—2 hours.

After fixation the material was dehydrated by ethanol and embedded in Araldite (Durcupan ACM, Fluka) on the usual way. The sections were cut on an LKB Ultrotome III and contrasted with uranylacetate and lead citrate (REYNOLDS, 1963). The micrographs were taken with a TESLA BS 413A electron microscope.

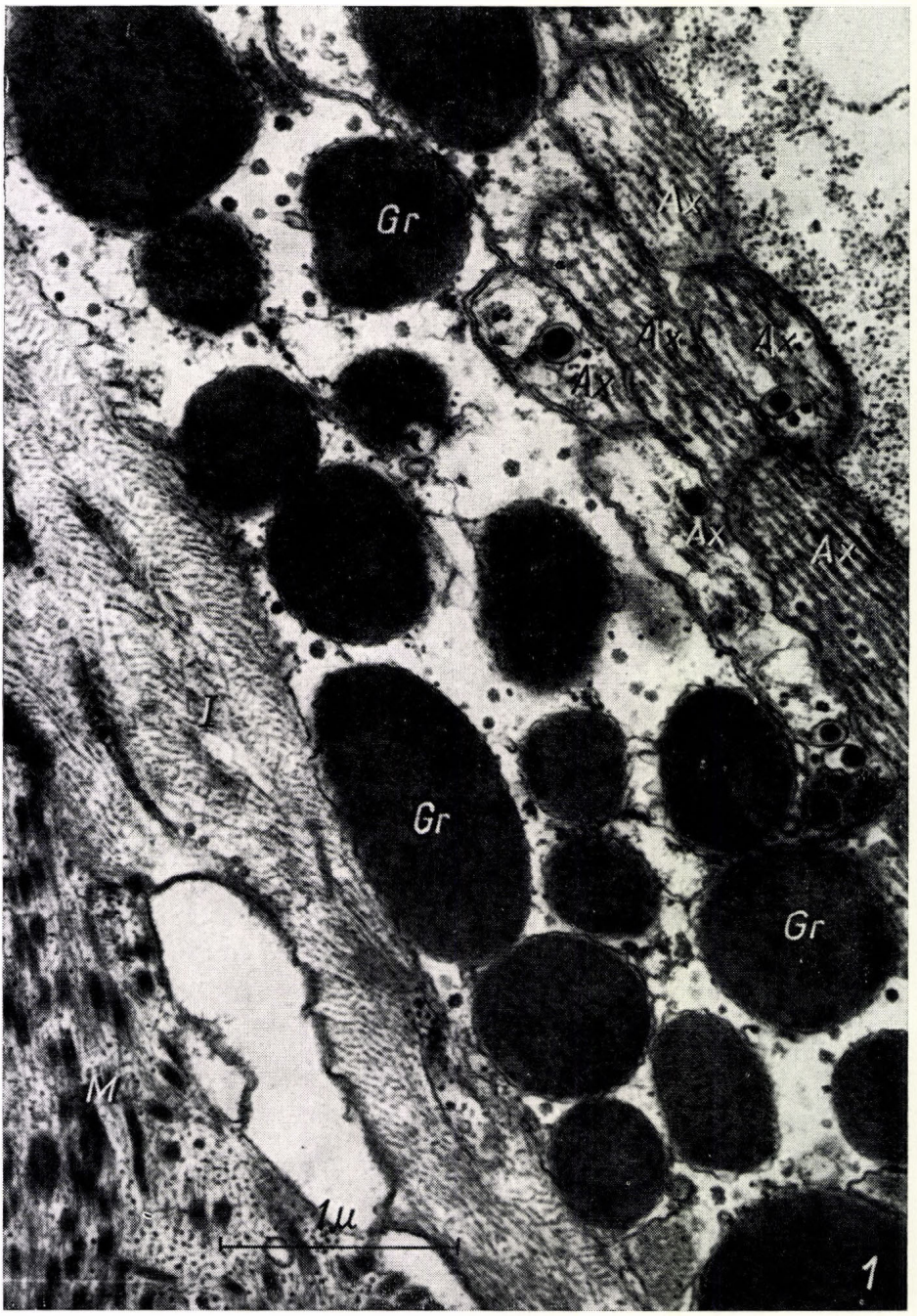

Fig. 1. Nerve fibres (Ax) in the white part of the posterior adductor. Gr — large granules in the cytoplasm of the special cell adjoining the axons. I — interstitial connective tissue; M — detail of a muscle cell. ×30,000

Results

1. The structure of innervating axons

The nerve branches consist of several axons having neither myelin nor Schwann sheaths. There frequently occur cells of special structure containing in their cytoplasm large ovale-shaped granules of high electron density *(Fig. 1)*. Neither these cells form a closed sheath around the axons, only adjoin them on certain places. The structure of axoplasm varies. In some places a lot of parallel arranged microtubuli are to be seen *(Fig. 2)*, however, numerous dense-cored vesicles (DCV) or empty vesicles also occur among them. The vesicles are also present in other parts of axons devoid of microtubuli. The thickness of axons is different; from several tenths of micron it can reach 1—2 micron. The axons of larger diameter have usually a very poor axoplasm.

2. The structure of neuromuscular junction

In some places between the muscle cells solitary axonal enlargements filled in with vesicles can be observed. They usually adjoin muscle cells and correspond to the nerve endings *(Fig. 3)*. On the place of contact both the axolemma and the sarcolemma are intact and of normal thickness. The intermembranic cleft is about 200 Å in width. In the nerve ending clusters of vesicles are frequently seen near the point of contact. The vesicles are always mixed, empty and DCV forms occur together, their diameter is about 400—1200 Å. Sometimes we could observe some morphological characteristics suggesting the fusion of vesicles with the presynaptic membrane *(Fig. 3)*. There are endings forming contacts with more than one muscle cells. The postsynaptic part never showed any specialization.

3. The presence of nerve cells

In either adductors both in white and yellow parts we observed cells *(Fig. 4)* the cytoplasm of which bears a close resemblance to that of the central neurones of mussels. These cells occur individually or in small groups usually together with axons. Sometimes the axons show a neuropile-like gathering near to these cells *(Fig. 4)*. The cells are rather small, we failed to observe any larger than 20 μ. The low number of cytosomes in these cells represents a difference as compared to the central neurones. The neurones of the adductors contain also dense-cored vesicles *(Fig. 5)*. The fibrocytes of the muscular interstitium can be strictly distinguished from the nerve cells on the basis of their characteristic rough endoplasmic reticulum being absent in the nerve cells.

4. The structure of intermuscular connections

Usually between two muscle cells there is a narrow intercellular space filled in by atypic collagen fibres. This space in some places has dilatations containing also fibrocytes. The sarcolemma here and there shows some specific structures resembling desmosomes *(Fig. 6)*. The cell membrane is trilaminated, on its internal side there is a dense material and the membrane itself

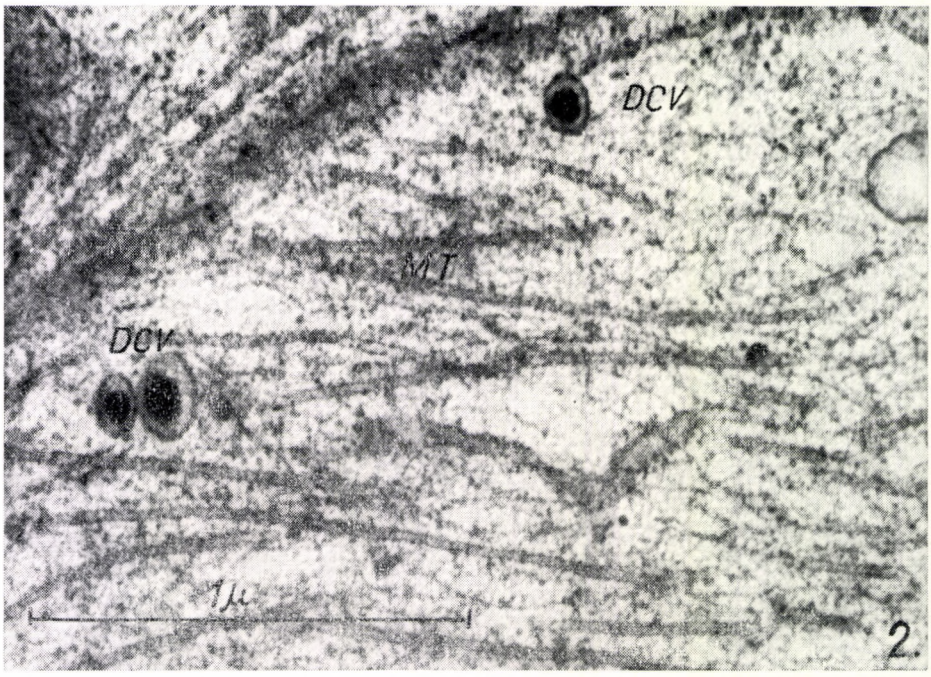

Fig. 2. Structure of the axoplasm in the white part of the posterior adductor. MT — microtubuli, DCV — dense-cored vesicles. ×58,500

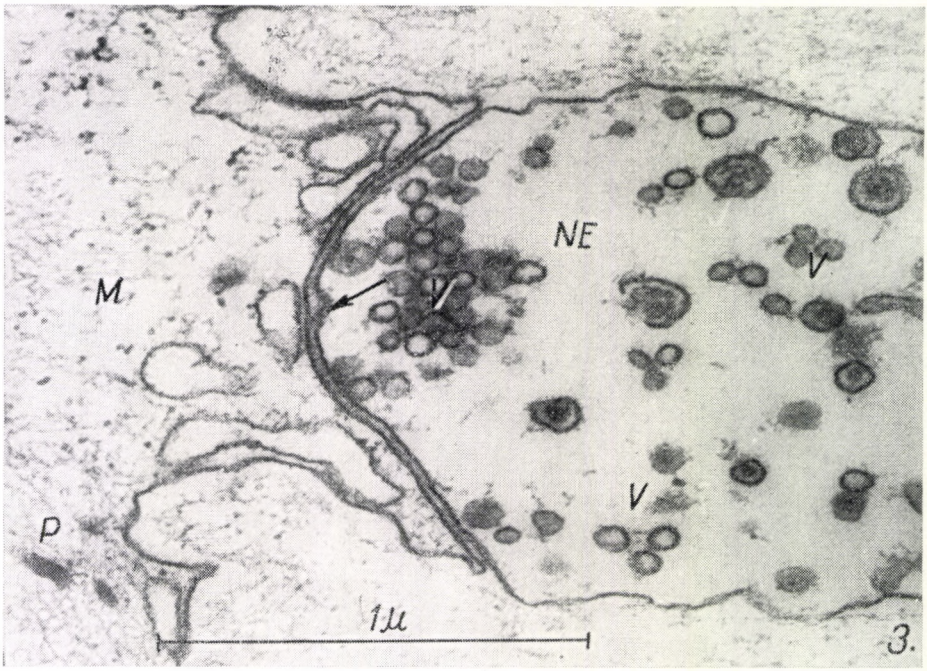

Fig. 3. Neuromuscular junction in the anterior adductor. NE — nerve ending; V — synaptic vesicles; M — muscle cell; P — paramyosin filament. The arrow indicates a possible place uf fusion of vesicles into the presynaptic membrane. ×58,500

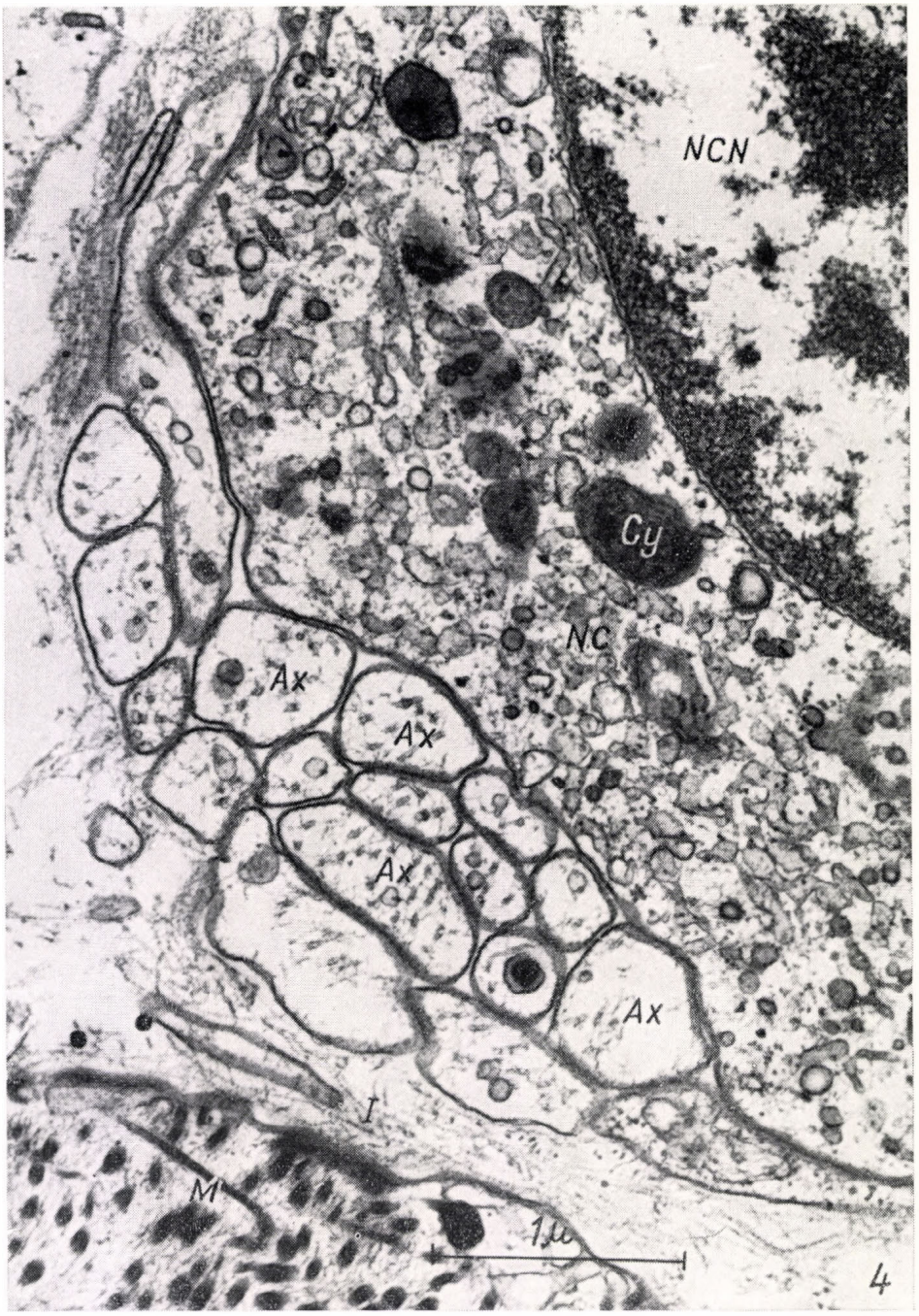

Fig. 4. Detail of a nerve cell in the posterior adductor (NC). NCN — nerve cell nucleus; Cy — small cytosome; AX — axons contacting with the nerve cell; M — muscle cell; I — interstitium. ×35,000

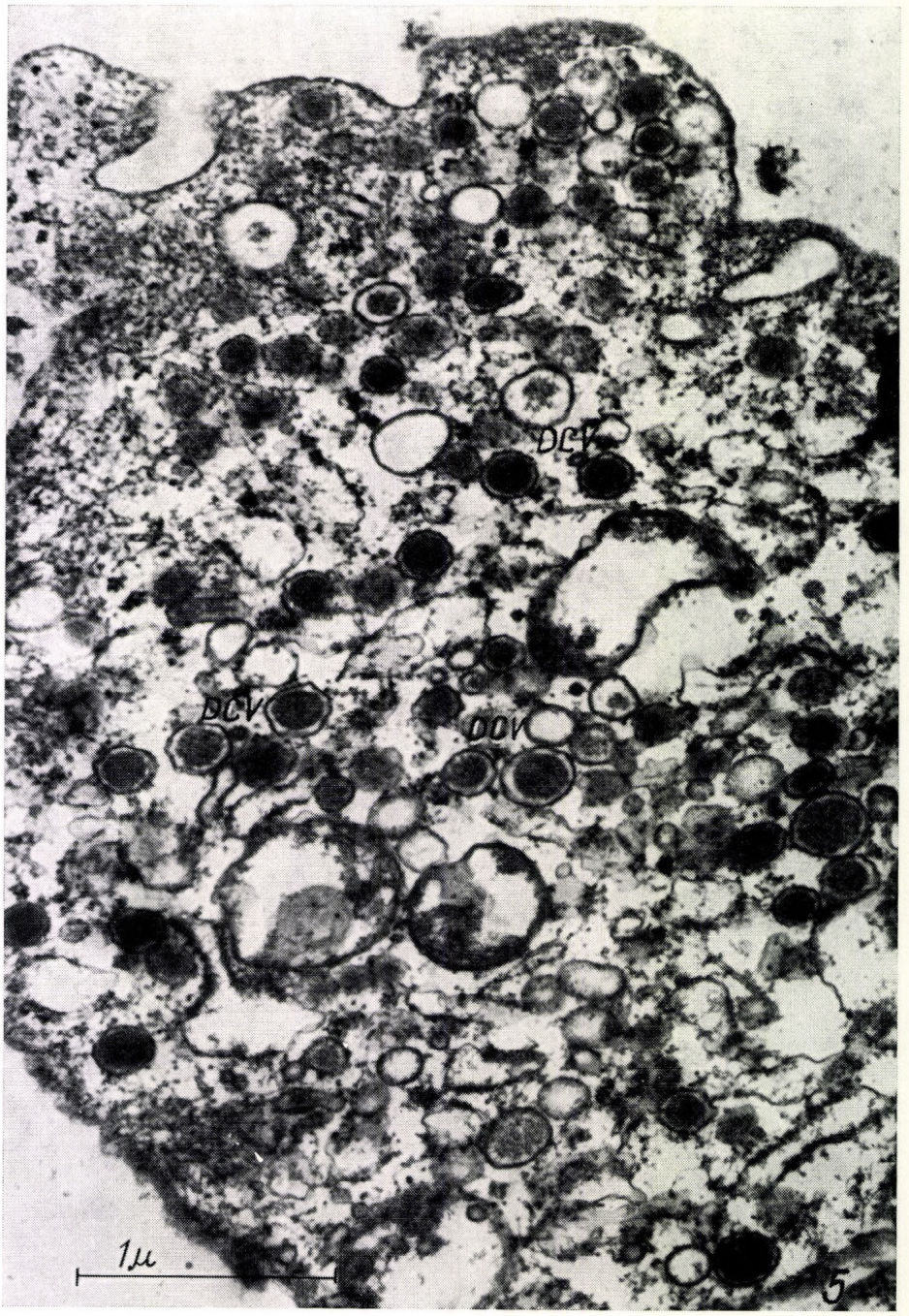

Fig. 5. Dense-cored vesicles (DCV) in the neurones of the posterior adductor. Their diameter is about 2000 Å. ×35,000

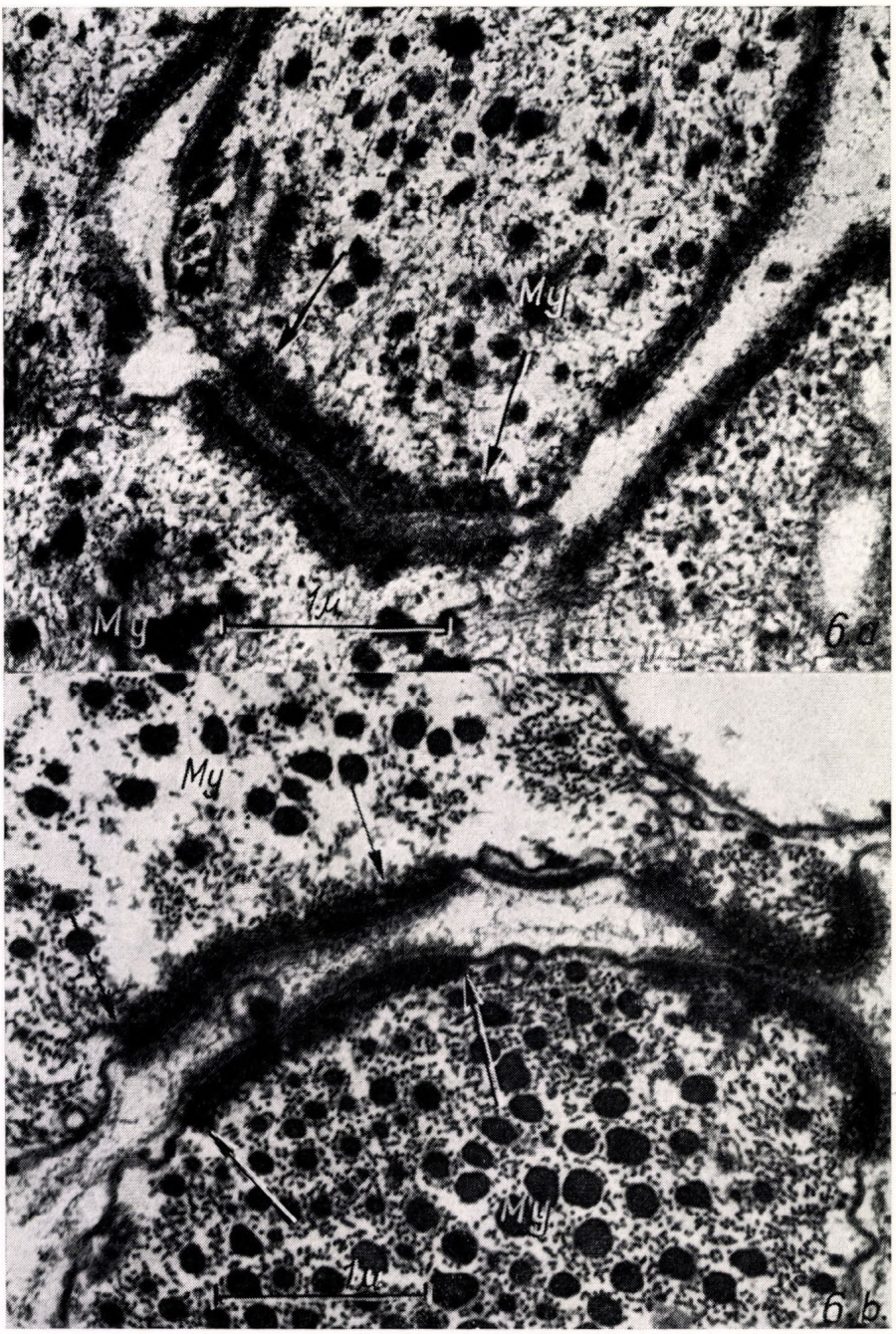

Fig 6. a) Desmosome between two muscle cells (arrows) in the white part of the posterior adductor. My — myofilaments, ×30,000. b) Half-desmosomes from the same muscle (arrows). On the right side of the picture partially connected half-desmosomes are to be seen. ×30,000

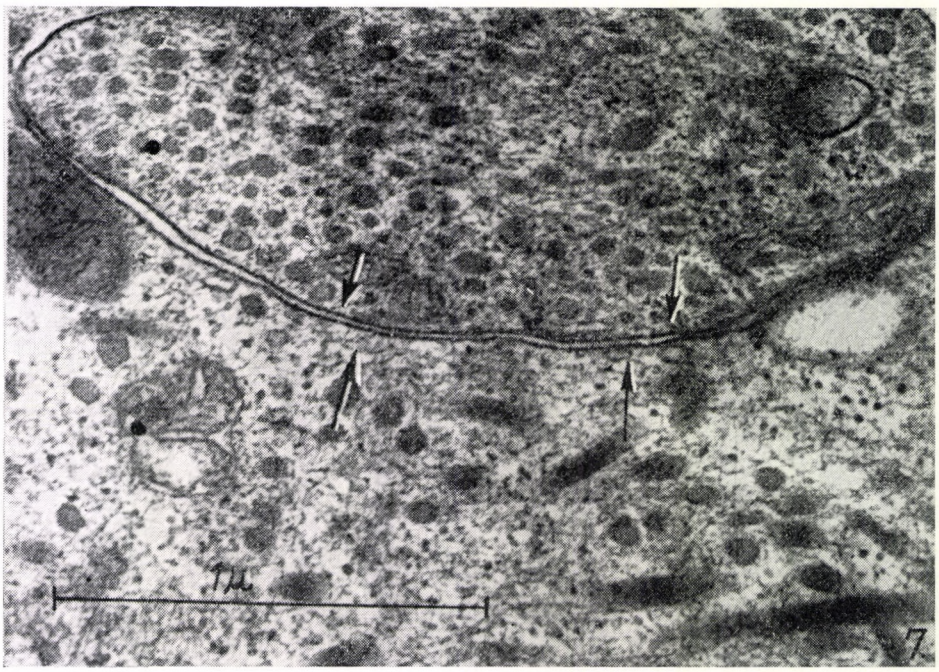

Fig. 7. Membrane appositions between two muscle cells (arrows). ×58,500

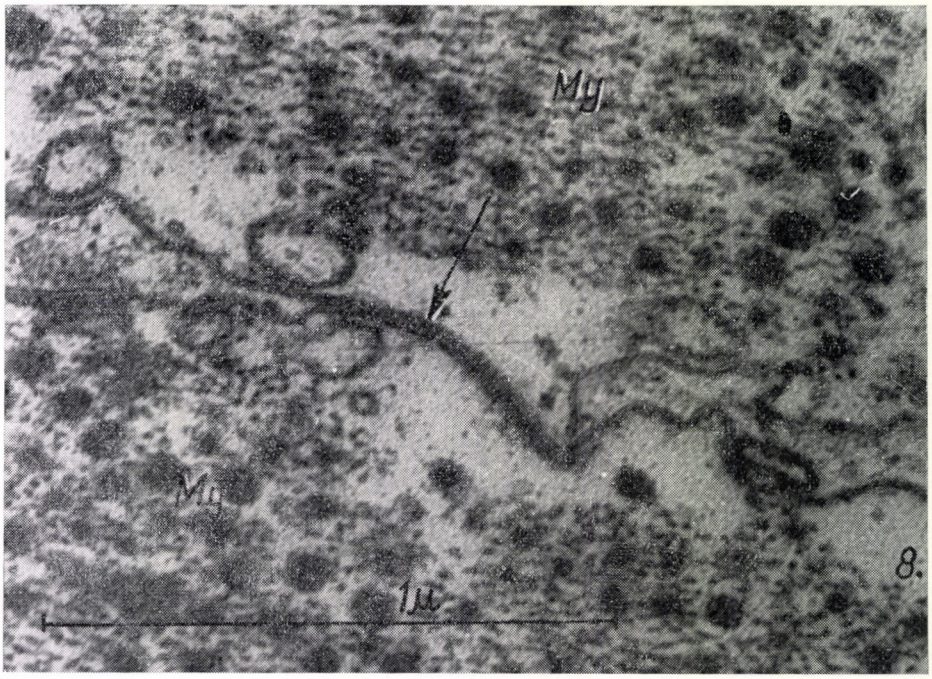

Fig. 8. Nexus between two muscle cells (arrow). My — myofilaments. ×78,000

is also thicker than at other places of the sarcolemma. At these desmosomes the muscle cells come nearer to each other than elsewhere. There occur also halfdesmosomes where the sarcolemma of the opposite muscle cell shows no specialization. At such places the intercellular space is also of normal width. The half-desmosomes can especially frequently be seen on the surface of muscle cells showing a small diameter in cross sections.

Apart from the desmosomes there are, however, intersarcolemmic contacts between the normal muscle membranes, too. The intercellular cleft is only 150—200 Å width on such places *(Fig. 7)*. We could only rarely observe nexuses or "gap junctions" between the muscle cells *(Fig. 8)*.

Discussion

There is no doubt that the adductors have no motor end plate. The innervating axons are similar to those found in the ganglia (Zs.-Nagy, 1964b, 1968a). The structure of neuromuscular contacts is generally identical with that of the neuromuscular (Schlote, 1962, 1963; Graziadei, 1966; Kerkut and cow. 1966; Rogers, 1968; Benjamin and Peat, 1968; Zs.-Nagy and Lábos, 1969) and neuro-interstitial (Nicaise, 1967; Nicaise and cow. 1968) contacts observed in other Molluscs. In the ganglia of *Anodonta cygnea* the DCV and empty vesicles represent the different physiological stages of the same elements (Zs.-Nagy, 1968b). Therefore, we are of the opinion that only on the basis of the morphological difference they cannot be assumed to contain different transmitter substances either in the adductors. The DCVs contain dopamine in the ganglia (Zs.-Nagy, 1968b), thus, also the innervation of the adductors is most probably monoaminergic. We failed to observe morphologically neuromuscular junctions of cholinergic character. This contrasts with the presence of cholinesterase activity in the adductors (Bowden, 1958; Zs.-Nagy, 1964a; Salánki and cow. 1967). It is extremely difficult to interpret the significance of this enzyme taking into consideration that its general sarcolemmar localization (Zs.-Nagy, 1964a) by no means agrees with the distribution of nervous elements.

On the basis of their physiological behaviour the adductors must be assumed to have a double innervation, namely the stimulation of the motor nerves causes either a tonic contraction or relaxation depending on the parameters of the stimulation. The former effect can experimentally be brought about by catecholamines whereas the latter by serotonin (Salánki and Lábos, 1969). As we mentioned above the nerve endings containing DCVs must function with catecholamines, i.e. they represent the stimulating factor. Nothing is known, however about the localization of serotonin. Since the stimulation of the cerebro-visceral connectives causes an increase of the serotonin level in the posterior adductor (Salánki and Hiripi, 1970) meanwhile the adductor relaxes, one cannot call in question the physiological significance of serotonin. This is why the innervating axon-endings are to be considered functionally of two different kinds even despite of the morphological identity. One cannot deny, however, that the catecholamines and the serotonin are localized together in the same nerve endings, only the character of the nerve impulse determines their liberation.

Our investigations show that the adductors contain also nerve cells which are not negligible in the interpretation of the muscle function. Their presence in the adductors elucidates the physiological fact, that even after a total denervation a rhythmic activity of the adductors remains, which is not a myogen rhythm (PAVLOV, 1885; SALÁNKI and ZS.-NAGY, 1970). It is curious that they were not seen in silver impregnated sections (ÁBRAHÁM and MINKER, 1957, 1959). We do not know wether the cholinesterase-positive structures described by BOWDEN (1958) as nerve cells are identical or not with the nerve cells found by us in the electron microscope. Anyway the occurrence of these nerve cells is not so frequent that it could explain — even if they contain cholinesterase — the high activity of this enzyme in the adductors. It should be noted that recently FOH and BOGUSCH (1969) described light microscopically nerve cells of the same size in the penis retractor muscle of *Helix pomatia*.

The presence of desmosomes and half-desmosomes show that the muscle cells are connected mechanically to each other and to the interstitial connective tissue; i.e. they do not extend from shell to shell. HANSON and LOWY (1961) failed to find desmosomes in the yellow adductor of an oyster. It is possible, however, that in the glycerinized muscle used mainly by them the desmosomes are not well preserved. Desmosomes have been found also in smooth muscle of vertebrates (FAWCETT and SELBY, 1958; SHOENBERG, 1958). The mechanical connecting function of desmosomes is also indicated by their frequent occurrence at the taper ends of the spindle-shaped muscle cell. It should be noted, however, that EBARA (1964) holds as possible that the desmosomes take part in the electric coupling in the heart of the oyster.

The other intersarcolemmic connection corresponds to a usual membrane apposition and as such it probably does not take part in the electric coupling. The very infrequent places where the membranes form a nexus (DEWEY and BARR, 1962) or "gap junction" (REVEL and KARNOVSKY, 1967) the electric coupling can take place since these structures represent a well-known low electric resistance. Since the neuromuscular connection itself is not more specialized than the usual membrane apposition and the chemical impulse-transmission takes place in it, it seems to be possible that also the intermuscular membrane appositions play some role in a chemical transmission process maintaining the synchronized function of the muscle cells in the adductors. One may assume that the cholinesterase activity localized diffusely in the sarcolemma has also some functional significance in such a process.

Summary

The anterior and posterior adductors of *Anodonta cygnea* have been studied by the usual electron microscopic techniques. It was found that the axons between the muscle cells are unmyelinated and no Schwann sheath is present. They are morphologically similar to the axons of the central nervous system. They contain empty or dense-cored vesicles of about 400—1200 Å diameter. The enlarged endings of axons are especially rich in vesicles. The endings are in connection with the sarcolemma on some places forming unspecialized membrane appositions representing the neuromuscular synapses. Near these contacts there often occur clusters of vesicles in the endings.

We failed to find any specialized neuromuscular end plate in the adductors. Nerve cells of small size are also present in the adductors.

There are membrane appositions between the muscle cells and rarely nexuses or "gap junctions" can also be seen. These intersarcolemmic contacts can have some functional significance in the maintenance of synchronity of muscle contraction by chemical or electrical transmission of impulses. Desmosomes and half-desmosomes occur in great number on the surface of the muscle cells.

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A NEURO-MUSZKULÁRIS ÉS INTERMUSZKULÁRIS KAPCSOLATOK FINOM SZERKEZETE AZ ANODONTA CYGNEA L. (MOLLUSCA, PELECYPODA) ZÁRÓIZMAIBAN

Zs.-Nagy Imre és *Salánki János*

Összefoglalás

Vizsgáltatott az *Anodonta cygnea* elülső és hátsó záróizmának beidegzése szokásos elektronmikroszkópos technika segítségével. Megállapítást nyert, hogy az izomsejtek között található axonok velőtlenek és Schwann-hüvellyel sem rendelkeznek, morfológiailag hasonlítanak a központi idegrendszer axonjaira. Bennük 400—1200 Å átmérőjű üres vagy dense-core vezikulák láthatók. Az axonok tágult végződései különsen sok vezikulát tartalmaznak. E végződések helyenként hozzáfekszenek az izomsejtekhez, azok membránjaival appozíciós kontaktusokat képeznek, amelyek specializációt nem mutatnak. Ezek felelnek meg a neuromuszkuláris szinapszisoknak. E helyeken gyakori a vezikuláris állomány tömörülése az idegvégződésekben. Neuromuszkuláris véglemezt nem találtunk a záróizomban. Sikerült megfigyelni az izomsejtek között kisméretű idegsejtek jelenlétét is.

Membránappozíciók az izomsejtek között is találhatók, sőt ritkán ugyan, de nexusok, illetve „gap junction"-ok is előfordulnak. Ezen interszarkolemmáris érintkezési formáknak jelentőségük lehet a záróizomműködés szinkronizásának biztosításában ingerületi folyamatok kémiai vagy elektromos átadása révén. Desmosomák és féldesmosomák nagy számban láthatók az izomsejteken.

ТОНКАЯ СТРУКТУРА НЕРВНО-МЫШЕЧНЫХ И МЕЖМЫШЕЧНЫХ СВЯЗЕЙ В АДДУКТОРАХ ANODONTA CYGNEA

И. Ж.- Надь и *Я. Шаланки*

Были исследованы передние и задние аддукторы Anodonta cygnea методом обычной электронноймикроскопии. Установлено, что аксоны среди мышечных клеток безмиэлиновые и не обладают даже Шванновской оболочкой, морфологически они сходны с аксонами центральной нервной системы. Внутри них видны пустые или dense-core везикулы диаметром 400—1200 Å. Особенно много везикул содержится в расширенных концах аксонов. Эти окончания в некоторых участках прилегают к мышечным клеткам, их мембраны образуют аппозиционные контакты, которые не показывают специализации. Эти контакты соответствуют нервно-мышечным синапсам. В этих местах часто наблюдается скопление везикул в нервных окончаниях. В аддукторе не нашли концевую пластинку. Удалось наблюдать наличие мелких нейронов среди мышечных клеток. Изредка наблюдались мембранные аппозиции между мышечными волокнами, nexus или «gap junction» тоже могут встречатся. Эти интерсерколеммарные формы связей, может быть, играют значительную роль в синхронизации аддукторов, помощью химической или электрической передачи возбуждения. Десмосомы и полу-десмосомы в большом количестве видны в мышечных клетках.

ANNAL. BIOL. TIHANY | 37 | 145—164 | HUNGARIA 1970

INVESTIGATION OF GROWTH OF PIKE-PERCH (LUCIOPERCA LUCIOPERCA L.) IN LAKE BALATON

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(Received: 28th January, 1970)

The age of fish together with the length of body and weight may provide information as to the composition of the stock, their growth, sexual maturation, life span production and mortality (TESCH, 1968). In the determination of age and investigation of growth various scalimetric methods are in use, by which the growth biology of fish can well be studied with sufficient accuracy. The summary and criticism of these methods have been published in a variety of papers (VOVK, 1956; BRYUZGIN, 1963; SHENTYAKOVA, 1966; TESCH, 1968; RICKER, 1969; and many others). BERTALANFFY's model (1938, 1957) has been successfully applied in the mathematical description of growth of a number of fishes; this model is the best known of its kind and extensively used in the description of fish production studies (FROST and KIPLING, 1968; SILLIMAN, 1969). The method of application of this model has been introduced into fish biological fields by BEVERTON and HOLT (1957) and by RICKER (1958). The diagrammatic representation of growth was first given by WALFORD (1946). The improved BERTALANFFY model was given quite recently by ALLEN (1969) in the form of a generalized equation of growth.

Early data bearing reference on the growth of pike-perch in Lake Balaton (UNGER, 1931; TÖLG, 1962) are partly contradictory, and partly have no reference whatsoever as to the growth of scales, the allometric relation of body weight and body length, and furthermore, these data lack the detailed description of the rate of the increasing body length. TÖLG's (1959, 1962) experimental results have drawn attention to the fact that the specimens of pike-perch in Lake Balaton up to the age of 4—5 years develop slowly and unevenly, whose cause was explained by the special characteristics of the lake and more especially in the lack of food-stuff touching mainly the younger age groups.

The aim of our scale investigations was to survey the characteristic parameters of growth of the pike-perch population in the North-eastern basin of Lake Balaton. Furthermore we endeavoured to gain data as to the relation existing between the increasing length of scales and body dimensions.

Material and methods

A part of our experimental material was collected between 7th—11th of December, 1968. We have altogether examined 432 pike-perches, out of them 340 were 3—9 years old (all these specimens were supplied by the Balaton Fishing Company), 32 were 1—2 years old, while 60 individuals hatched

that summer. Specimens younger than 3 years old were collected by us with a trawlnet of 5 mm mesh during the years 1968 and 1969.

For age estimation and growth investigation we detached 15—20 scales from the area below the lateral line behind the posterior margin of the left pectoral fin of the fish *(Fig. 1)*, the scales were stored between the pages of a note-book. We measured the standard (L_c) and total body length (L_t) of each specimen in mm, and also its weight (W) in grams. The length and weight

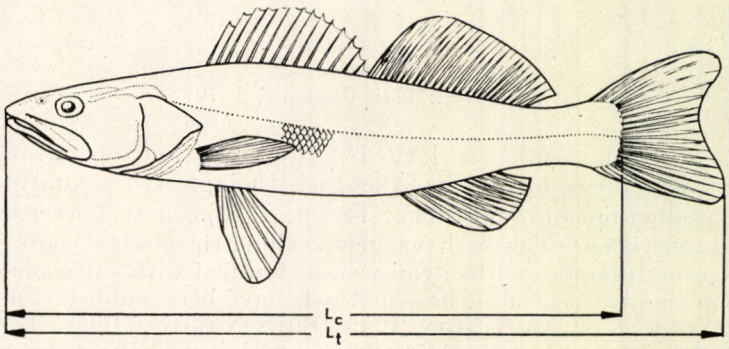

Fig. 1. Measured lengths and the place of scale sampling on the left side of pike-perch ("key-scales")

measurements of one year old or of younger specimens were taken on material preserved in 4% formalin. From among the scales we selected those which were entire — not regenerated — under a binocular microscope, each scale was cleaned and subsequently stained by the WALLIN (1957) and KEETON (1965) method, then they were washed in distilled water and dried between slides. The preparations were projected by a profile projector at a 50 times magnification (sometimes at a magnification of 20), by the help of a millimetre divided scale we measured from the focus of the scale the winter annual ring distances and the total oral radius *(Fig. 2)*. Age determination was done by counting the number of completely developed annual rings in differentiating age groups we used the currently accepted denotations (1+, 2+, etc.).

The relation of the standard length (L_c) and the total oral radius (S), and the body dimensions of first-summer fries concerning scale formation were all expressed by the equation of a straight line. With respect to the relation of radial distances of the winter year rings and body length a similar method of reasoning was followed. By knowing these data from the annulus-radii the body length (L_c and L_t) of individual age groups was determined by the FRASER (1916) method. The relation existing between body weight and body length was calculated by the allometric formula of HUXLEY (1924) (cit. BEVERTON and HOLT, 1957).

For the description of growth of pike-perch in Lake Balaton on the basis of back calculated lengths we employed BERTALANFFY's (1957) growth-model, according to which body length at every t period of time can be given as follows:

$$l_t = L_\infty \left[1 - e^{-K(t-t_0)}\right]$$

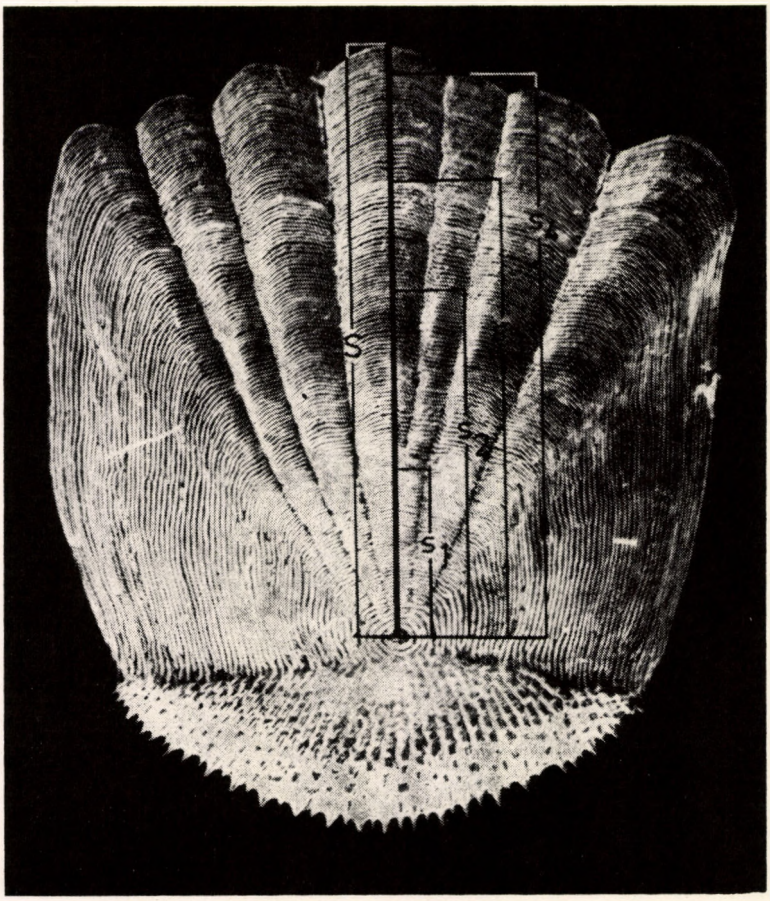

Fig. 2. Points of measurement on pike-perch scales: S = total oral radius, s_1, s_2, s_3, etc. = distances measured from the focus and winter rings

where L_∞ = the upper asymptotic limit of body length (maximal length)
K = the measure of rate at which body length approaches L_∞
t_0 = the parameter of that hypotetic period of time at which the length of fish would have been zero ($l_{t_0} = 0$)
e = the base of natural logarithm

Results

The extreme values for the standard length in the examined specimens of pike-perch, including the younger generation, too, were 22—630, while for the total body length these values varied between 27 and 710 mm, body weights were between 0.21—4000 g. The present paper deals with those specimens caught in nets with a mesh of 4 cm, whose age is not less than 3 years *(Table 1)*.

TABLE 1

Measured values of body length and body weight in pike-perch of Lake Balaton (samples were collected by fishermen)

Age		L_c	L_t	W
3+	A	31.0	36.0	349
	B	37.0	42.5	645
	C	34.0	39.1	510
4+	A	32.5	37.0	460
	B	42.5	48.5	975
	C	37.0	42.5	671
5+	A	37.5	43.0	805
	B	44.5	51.0	1593
	C	41.8	48.1	1012
6+	A	47.0	53.0	1341
	B	51.0	59.0	2160
	C	49.1	56.4	1698
7+	A	50.5	56.0	1900
	B	58.0	66.0	3000
	C	51.7	58.0	2715
8+	A	52.7	57.5	3725
	B	57.5	68.0	4000
	C	54.0	62.0	3862
9+	C	63.0	71.0	3812

L_c = standard length (cm); L_t = total body length (cm)
W = body weight (g)
A = minimum, B = maximum, C = average value

On the scales of pike-perch in Lake Balaton the winter rings appear generally as wide bands. In most cases the borderline of the first winter ring caused some difficulties in measurements. Similar problems also occurred when examining the scales of older specimens. The growth zones may be examined with greater precision by applying stains, on the other hand, in the case of 7—8 years old specimens age estimation becomes increasingly difficult because the annual rings are more dense and at times they even merge into one another, so not even staining brings out any significant features for differentiation. About 20—30 per cent of the collected scales were regenerated.

Taking into consideration all the fish samples in our investigation the relation of standard length and the total oral radius (S) was calculated as follows *(Fig. 3, A)*:

$$S = -0.0729 + 0.1108 \cdot L_c$$

where S was given in mm, L_c in cm. The equation gives a low value for the respective body length of key-scale formation, and according to our experiences this value indicates that body length which is measurable at the time of development of the first caudal scales. The values of S at back calculated

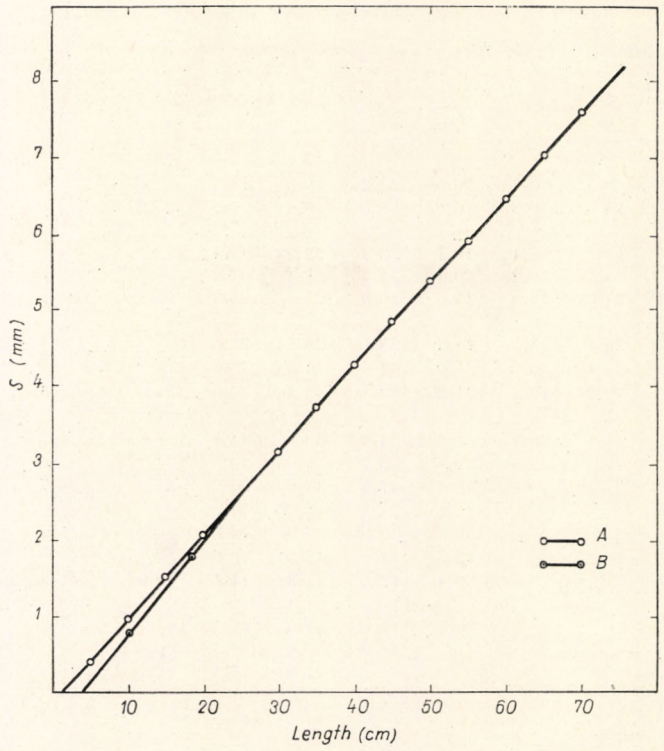

Fig. 3. Relation between standard length of pike-perch (L_c) and total oral radius of scale. A-straight line: calculated for all examined specimens (432 individuals) ($S = 0.0729 + 0.1108 \cdot L_c$), B-straight line: relation expressing measurable body length at the time of scale formation in first-summer fries evaluated by direct analysis (60 individuals):

$$L_c = 4.064 + 8.054 \cdot S\,,$$

its point of intersection on the abscissa corresponds to the measurable standard length at the time of key-scale formation (S given in mm, L_c in cm)

body dimensions *(Table 2*, average values) between 1+ and 8+ age groups gave the following values in mm: 1.86, 2.71, 3.39, 4.05, 4.75, 5.32, 5.78, 6.14. On first-summer specimens we established by direct investigations that standard length (L_c) of pike-perch fries is 40 mm at the formation of the lateral key-scales (in case of L_t it is about 4.5 cm) *(Fig. 1)*. At this stage of development for the standard length the following equation is valid *(Fig. 3,B)*:

$$L_c = 4.064 + 8.054 \cdot S$$

In Lake Balaton on the caudal peduncle of pike-perch fries at 2 cm body length developing scales may be perceived. When correcting back calculated body lengths we considered 40 and 45 mm measurements. The equation expressing the relation existing between the average radial distances of the winter rings and standard length is given below *(Fig. 4)*:

$$s_n = 0.3300 + 0.1153 \cdot L_c$$

TABLE 2

Back calculated body lengths of pike-perch in Lake Balaton (L_c given in cm)

Age group:		3+	4+	5+	6+	7+	8+	(9+)	Average	Increase	W (g)
1_1	A	12.5	11.9	11.3	16.7	17.7	17.6	—	—		
	B	20.7	23.2	20.5	22.2	19.3	18.2	—	—		
	C	16.9	16.8	16.9	18.6	18.3	17.9	17.3	17.5	7.6	62.5
1_2	A	18.3	18.4	20.7	22.3	22.5	23.4	—	—		
	B	28.7	30.2	30.4	31.3	28.4	27.2	—	—		
	C	24.9	23.9	25.0	26.0	25.0	25.4	25.7	25.1	6.3	196
1_3	A	25.0	23.5	28.2	28.1	28.2	29.8	—	—		
	B	35.1	35.6	35.6	36.0	34.5	35.6	—	—		
	C	30.2	29.4	31.2	32.7	32.4	33.0	31.3	31.4	5.2	397
1_4	A		29.4	33.2	34.3	33.5	34.2	—	—		
	B		39.3	39.8	39.7	39.0	39.5	—	—		
	C		33.8	35.7	37.9	37.6	37.7	37.3	36.7	5.7	645
1_5	A			36.3	39.9	37.9	40.0	—	—		
	B			43.5	44.9	46.5	46.6	—	—		
	C			39.4	42.6	43.3	44.1	42.0	42.3	4.6	1020
1_6	A				43.6	42.6	44.5	—	—		
	B				48.9	51.0	51.5	—	—		
	C				46.3	47.0	47.3	47.1	46.9	3.7	1414
1_7	A					47.5	48.9	—	—		
	B					55.0	53.1	—	—		
	C					50.4	50.0	51.3	50.6	2.9	1798
1_8	A						51.0	—	—		
	B						53.6	—	—		
	C						52.4	54.6	53.5		2145
1_9	C							57.4			

A = minimum, B = maximum, C = average value
Calculated weight: $\log W = -5.2996 + 3.1634 \cdot \log L_c$ according to allometric equation

where s_n = distance between the focus and the "n"th winter ring measured in mm, L_c is given in cm. The plotted points show that the distances between the annual rings (growth zones) indicate the rate of growth, and that this rate slackens year by year. Up to the fourth year the distance of winter rings decreases, after this period an increase in rate may be observed, then from the sixth year on a gradual deceleration may again be seen.

For both sexes the allometric equations received for the relation of body length and body weight were the following *(Figs 5* and *6)*,
between 22—200 mm:

$$\log W = -4.6088 + 2.8379 \cdot \log L_c$$

$$\log W = -4.8339 + 2.8880 \cdot \log L_t$$

above 200 mm:

$$\log W = -5.2996 + 3.1634 \cdot \log L_c$$

$$\log W = -5.6057 + 3.2077 \cdot \log L_t$$

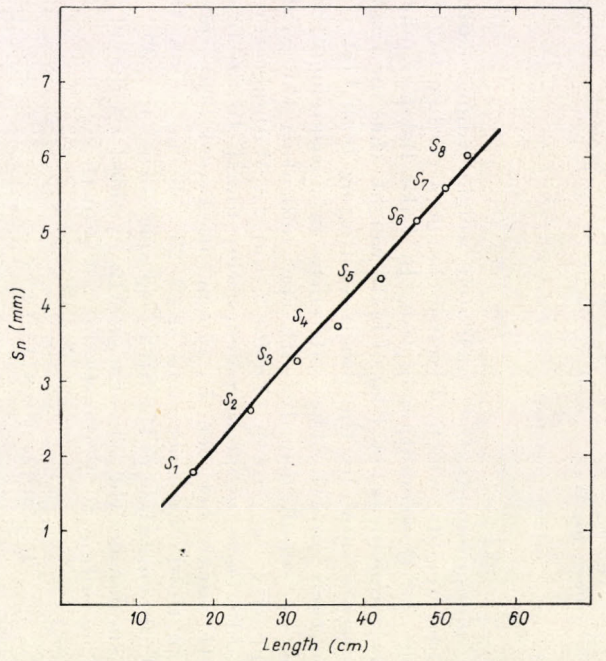

Fig. 4. Relation between back calculated standard lengths and the average radial distances of the winter rings: $s_n = -0.3300 + 0.1153 \cdot L_c$ (s_n given in mm, L_c in cm)

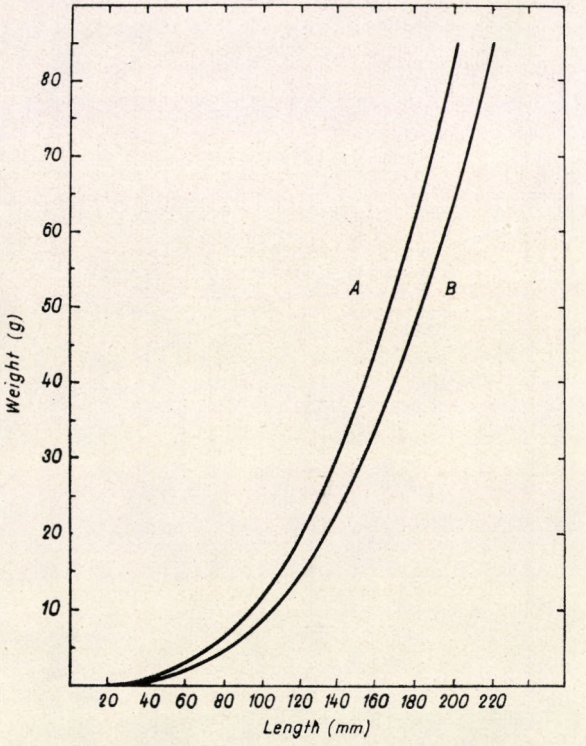

Fig. 5. Allometric growth curve of body length and body weight between 22 and 200 mm:

A-curve: $\log W = -4.6088 + 2.8379 \cdot \log L_c$
B-curve: $\log W = -4.8339 + 2.8880 \cdot \log L_t$

(W = body weight in g, L_c and L_t = standard and total body length in mm)

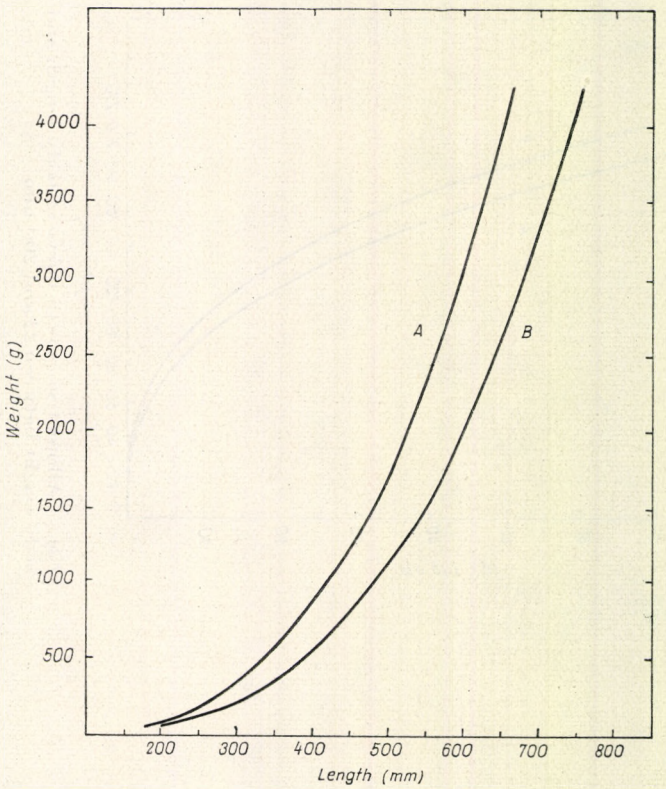

Fig. 6. Allometric growth curve of body length and body weight above 200 mm:

A-curve: $\log W = -5.2996 + 3.1634 \cdot \log L_c$
B-curve: $\log W = -5.6057 + 3.2077 \cdot \log L_t$

(abbreviations as in *Fig. 5*)

In fish of similar measurements the calculated weight values hardly fall short of the measured weights, which of course change according to the mass of stomach content and the condition of fish. In Lake Balaton pike-perch for the first five years remain below 1 kg of body weight, thus its weight and body length increase are slow, by the progress of aging both body characteristics slacken in their growth. The differences in measurement of each age group become less simultaneously with age, indicating the weakening of the potential capacity for growth *(Tables 2* and *3)*. Within certain age-groups significant differences in measurements were found. In the first year the standard length varied between 7—17 cm, in second year specimens (1+) 18—31 cm, in third year specimens (2+) 23—36 cm, in fourth year specimens (3+) 29—40 cm, while in the fifth year specimens (4+) 36—47 cm. In pike-perches of similar age the "diverse growth" yielded a value of 10 cm.

Body length according to Walford's line of growth:

$$l_t = 9.74 + 0.8713 \cdot l_{t-1},$$

TABLE 3

Back calculated body lengths of pike-perch in Lake Balaton (L_t given in cm)

Age group;		3+	4+	5+	6+	7+	8+	(9+)	Average	Increase	W (g)
	A	12.5	13.7	12.7	18.8	19.7	19.6	—	—		
l_1	B	23.8	26.8	23.6	25.7	21.6	22.4	—	—		
	C	19.5	19.4	19.6	21.4	20.8	21.5	20.9	20.4	8.5	63.5
	A	20.5	20.3	23.7	25.2	26,4	25.7	—	—		
l_2	B	33.2	34.3	34.5	36.2	33.0	31.0	—	—		
	C	28.7	27.4	28.7	29.9	29.4	29.3	29.3	28.9	6.9	194
	A	28.9	26.6	32.8	31.7	32.4	33.0	—	—		
l_3	B	40.0	39.9	40.5	41.7	39.3	39.4	—	—		
	C	34.7	33.7	35.8	37.5	36.8	36.8	35.2	35.8	6.4	386
	A		33,3	38,1	38,7	38,5	39,0	—	—		
l_4	B		44.0	45.2	45.6	44.4	47.2	—	—		
	C		38.8	41.0	43.6	42.4	45.6	42.1	42.3	6.0	654
	A			41.7	45.0	43.5	44.8	—	—		
l_5	B			49.9	51.9	52.9	53.0	—	—		
	C			45.4	49.0	48.9	50.6	47.3	48.2	5.3	1012
	A				49.1	48.9	51.0	—	—		
l_6	B				56.6	58.0	58.2	—	—		
	C				53.2	53.7	54.3	53.1	53.5	4.1	1400
	A					54.6	55.2	—	—		
l_7	B					62.6	59.8	—	—		
	C					57.5	57.4	57.9	57.6	3.2	1736
	A						59.0	—	—		
l_8	B						61.0	—	—		
	C						60.2	61.5	60.9		2110
l_9	C							67.8			

A = minimum, B = maximum, C = average value
Calculated weight: $\log W = -5.6057 + 3.2077 \cdot \log L_t$ according to allometric equation

(Fig. 7,B). When representing diagrammatically the back calculated body lengths (WALFORD-plot), the points showing the average values for body lengths closely follow the course of the line, whose slope ($a = e^{-K} = 0.4190$) determines the final body length. In our case, the diagonal line passing through the origin at an angle of 45 degrees intersecting the above line gives the following numerical relationship:

$$L_\infty = \frac{9.74}{1 - 0.8713} = 75.7 \text{ cm.}$$

According to this the body length of first-summer (0+) fish is 9.7 cm, while that of the second year specimens (1+) is 18.4 cm. The straight line representing the body length differences of the various age groups *(Fig. 7,A)* intersects the abscissa exactly at the calculated value of L_∞. The measurements of $\log (L_\infty - l_t)$ in the function of time displays linearity *(Fig. 8)*. In projecting

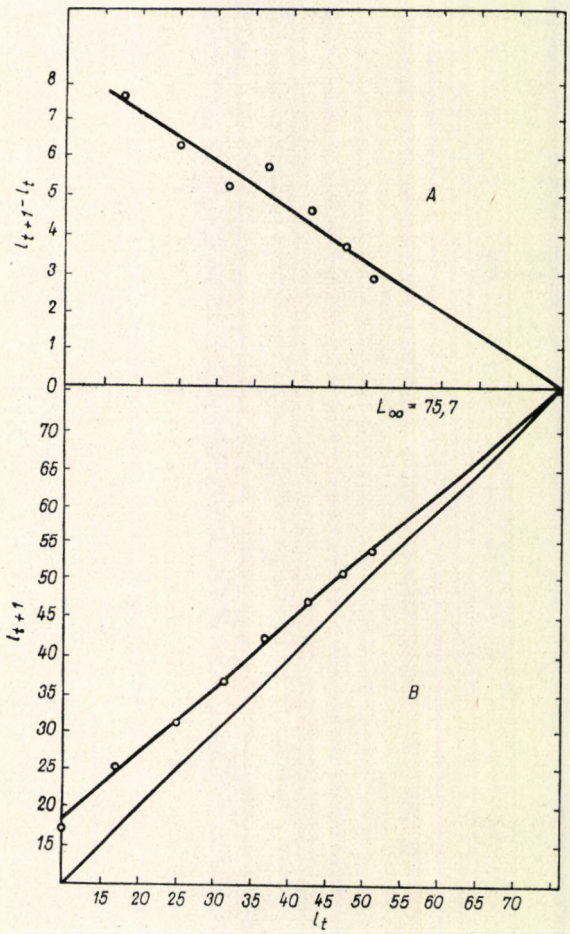

Fig. 7. Alternative representation of body length increase (L_c) of pike-perch and body length differences of individual age groups. A: body length difference in subsequent years, B: representation of $X = l_t$ values (body length in t time) in the function of $Y = l_{t+1}$ values (body length in t + 1 time) (WALFORD-plot). The equation of the straight line

$$l_t = 9.74 + 0.8713 \cdot l_{t-1}$$

its slope $a = e^{-K} = 0.4190$, maximum of standard length $L_\infty = 75.7$ cm

the point of intersection of log L_∞ value and the straight line determined by the points onto the horizontal axis we obtain $t_0 = -0.91$ year. The slope of the straight line is $K = 0.1376$, its point of intersection on the abscissa means the final age limit (roughly between 13+, 14+ years) of pike-perch in Lake Balaton. In substituting the above parameters into Bertalanffy's growth equation we receive that the body length of pike-perch at any t time *(Table 4, Fig. 9)* is as follows:

$$l_t = 75.7\ (1 - e^{-0.1376(t+0.91)})$$

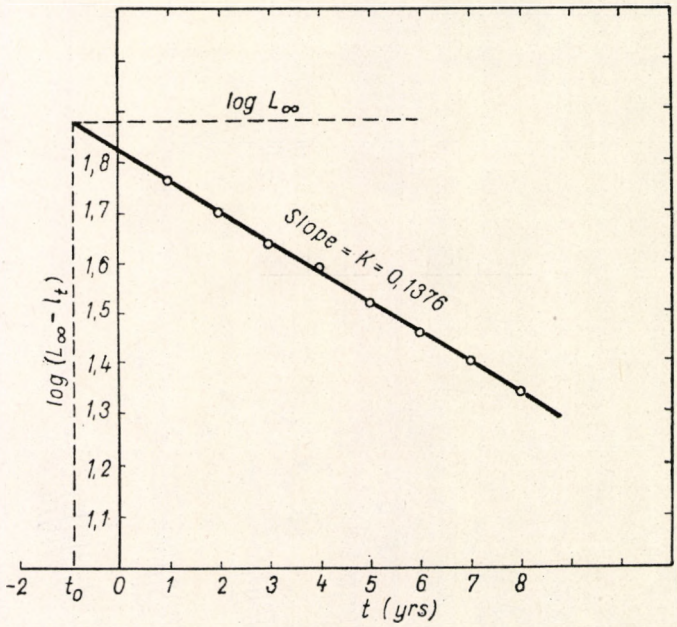

Fig. 8. Plotting of asymptotic body length (L_∞) and standard length differences at t time in the function of consecutive years given in log values. $t_0 = -0.91$ year, slope = K = 0.1376, maximum age (i.e. point of intersection of straight line on abscissa) is roughly between 13+ and 14+ years

TABLE 4

Back- calculated standard lengths (L_c) in cm of pike-perch in Lake Balaton

Age-group	Actual age in years	Calculated body lengths		
		From scales	Walford-plot	Bertalanffy's equation
0+	0.9	—	9.7	8.9
1+	1.9	17.5	18.2	17.3
2+	2.9	25.1	25.0	25.0
3+	3.9	31.4	31.6	31.1
4+	4.9	36.7	37.1	37.0
5+	5.9	42.3	41.7	42.0
6+	6.9	46.9	46.6	46.4
7+	7.9	50.6	50.6	50.2
8+	8.9	53.5	53.8	53.3

Discussion

The obliteration of annual rings of pike-perch in Lake Balaton and the inaccurate determination of age by the scalimetric method in the case of older specimens have already been mentioned by WOYNÁROVICH (1960). According to our experiences the confluency of the annual rings, the formation of subsidary, the so-called "pseudorings" are frequent phenomena. The forma-

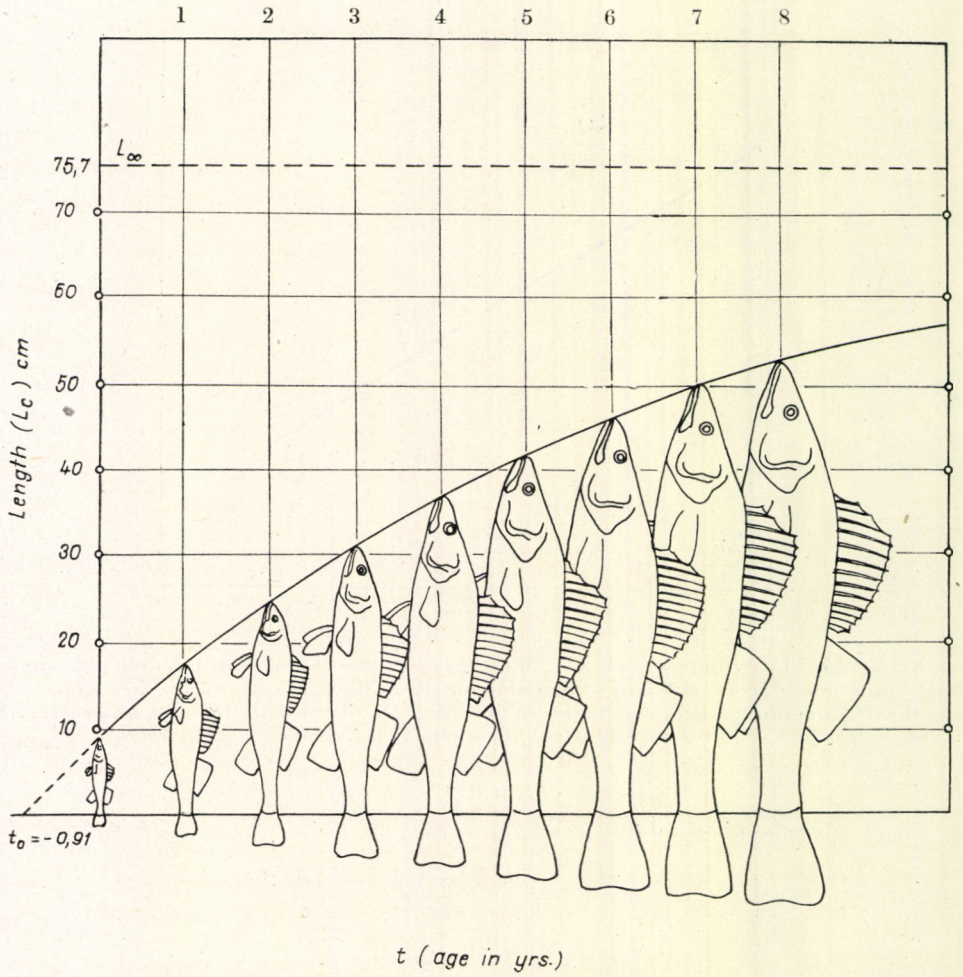

Fig. 9. Representing the annual average body length increase (L_c) of pike-perch population in Lake Balaton by the BERTALANFFY's growth model

tion of these may come about by the changes occuring in growth during spawning-season, the seasonal variation of the quantity of food, extreme climatic conditions and the changes in water level. From this point of view temperature is one of the most important factor in Lake Balaton. SVÄRDSON and MOLIN (1968) basing their opinion on the establishment of FREIDENFELT say that in pike-perch in Sweden the complete development of annual ring falls on the beginning of a new growth period, i.e. for May or June. CHUGUNOVA (1931) on examining the pike-perch in Azov Sea found that in sexually immature specimens the annual rings develop in spring, while in mature specimens the development of annual rings starts at the end of the year. The process likewise occurs in Lake Balaton.

Body measurements vary at the time scale formation, and the increase in length of the scales also during the development of the fish according to water area.

The length increase of scales — as it has been revealed in many species of fish — rarely follows in direct proportion the increase of body length (Lee's phenomenon). The relation between body length (L_c) and the complete oral radius (S) proved to be linear in the case of the examined pike-perch specimens caught in Lake Balaton, at the same time, minor divergencies may be observed in the relation existing between the radii of annual rings (s_n) and body length (age-groups 4+, 5+). The measureable standard length at complete scalation gives 40 mm which is in accordance with the data obtained by PRIEGEL (1969) *(Figs. 3—4)*.

The allometric relation between body length and body weight expresses a relative growth rate within each natural period of life (FÁBIÁN, 1959; SZÉKY, 1966). In the numerical values of allometric coefficients (or the initial growth index) show no great variation *(Figs. 5—6)*.

In the North-eastern basin of Lake Balaton the average growth of the pike-perch population may be given with satisfactory accuracy by Bertalanffy's model *(Table 4, Figs. 7—9)*. The differences occuring within the season and population cause changes in the numerical values of parameters, too. The final body length (L_∞), is influenced by the quantity of food and the density of population, while the slope of the growth curve (K) is governed by genetic and physiologic factors (BEVERTON and HOLT, 1957). In the northern part of the Caspian Sea, LUKASOV (1961) obtained the following values for the growth of pike-perch:

$$L_\infty = 80 \text{ cm}, \quad K = 0.235, \quad t = -1.00 \text{ year}.$$

Besides pike-perch, he calculated the constants for other species of fish, too *(Rutilus r. caspicus Jak., Abramis brama* L.) and he found that the method is applicable for the description of growth of all three species.

FROST and KIPLING (1967) applied the method in their analysis when examining pike *(Esox lucius* L.) inhabiting Lake Windermere in great numbers. Both for back calculated and for measured body lengths separately for males and females they calculated the parameters of the equation, the values received were close to one another. According to their opinion those specimens of pike which grew slowly in the first year will continue to develop slowly, while those whose body length increased considerably in the first year will continue to grow at a faster rate in their later years of life, too. Close relation has been established between temperature and growth rate. Another factor, at times having decisive role, is the intensity of light, which exerts effect through the visual faculty of the fish on the mode of obtaining food. Similar environmental factors (great fluctuation in temperature, muddy water, etc.) have far reaching consequences in the life conditions of pike-perch inhabiting the water of Lake Balaton.

SVÄRDSON and MOLIN (1968) suggest that the cause of slow growth is due to the low water temperature of the Swedish lakes. On the other hand, in Poland in the environs of Konin Lakes (Slesiń, Gosławice, Pątnów) pike-perch living under rather more temperature conditions grow very fast (B. ZAMOJSKA and H. WILKOŃSKA, personal communication).

DAVIS and WARREN (1968) called attention to the fact that in the food consumption and growth of fish a whole string of factors play important roles (e.g. concentration of dissolved oxygen). They especially emphasized that food consumption, digestion, metabolic rate, nitrogen balance and growth

TABLE 5

The growth of pike-perch

Item	Basin	l_1	l_2	l_3	l_4	l_5	l_6
1	Average of Northern Lakes (Sweden, Finland, Soviet Union)	10.8	19.5	26.2	32.6	38.1	42.2
2	Average of 24 Northern German Lakes	13.0	24.0	34.0	43.0	49.0	55.0
3	Average of 8 Central German Lakes ...	13.2	26.7	40.1	47.0	54.3	56.7
4	Average of 12 Northern Poland Lakes .	14.6	25.5	35.8	43.8	49.7	54.2
5	Szczeciń-Bay	14.9	30.8	47.2	55.8	64.2	68.2
6	Szczeciń-Bay	16.9	30.0	39.0	51.0	61.0	64.5
7	Stettin-Bay	—	44.4	56.2	64.7	66.5	68.7
8	Kurisches-Haff	12.9	25.8	39.2	—	53.3	60.9
9	Vistula-Bay	12.2	23.7	36.7	46.8	57.5	—
10	Don-delta	16.9	32.0	37.4	42.3	47.9	54.4
11	Kuban-River	16.8	37.2	43.8	48.8	56.0	61.3
12	Volga-River	20.6	28.0	34.3	41.2	46.8	53.0
13	Lake Razelm	—	25.6	41.1	51.7	55.6	63.2
14	Lake Balaton	20.0	28.6	39.4	47.1	57.0	55.0
15	Lake Balaton	17.5	25.1	31.4	36.7	42.3	46.9
16	Lake Balaton	20.4	28.9	35.8	42.3	48.2	53.5

itself change together with the alterations taking place in the behaviour and state of metabolism of the fish. In identical seasons temperature requires an optimum amount of energy and the ration of food utilizable for growth, which decreases at low temperatures. A decrease may also be observed in the rate of growth of larger and older fish specimens, because compared to their body lengths they consume a relatively smaller quantity of food. Other worker observed the daily food consumption of pike-perch with regard to the number of consumed animals and found that the quantity of the latter simultaneously decreases with the increase of body lengths (STEFFENS, 1960). The development of pike-perch fries comes to a halt if they had not started predatory life in the first summer. In such cases, specimens of similar age but of diverse mode of food consumption by the end of the first summer the difference in body length may be as much as 10 cm (NEUHAUS, 1934). In the Bay of Vistula, pike-perch developed very well in those years when the principal food-item the smelt *(Osmerus eperlanus* L.) was available in great numbers (FILUK, 1962).

TÖLG (1959, 1962) supposes that only those pike-perch fries may reach high age which started predatory life not later than by end of May or June. Thus, the growth of the fry may be faster than the average when appropriate food was consumed, while at the same time, those fries which feed on planktons fall behind in growth, and continue to grow slowly, or they may even die in great numbers during the first winter. WOYNÁROVICH (1959, 1968) states that the rate of growth is the function of available quantity of food, and he gives the following mean values for fish ponds: first-summer fish 15—25 cm and 30—100 g, two-years old specimens 30—40 cm and 200—500 g, while for three-years old pike-perch reach 37—55 cm in length and 500—1500 g in weight. He says that pike-perch does not develop well in muddy, shallow water (as is the case in Lake Balaton), because its natural habitat is deep water,

in various European waters

l_7	l_8	l_9	l_{10}	l_{11}	l_{12}	Author, year of publ.	Note
						JÄRNEFELT, KLIMOVA, SCHNEIDER,	
46.0	48.6	50.7	54.9	59.2	61.8	FREIDENFELT (after NAGIĘĆ, 1961)	L_t
						KLIMOVA:	L_c
56.0	57.0	—	70.0	—	—	BAUCH, 1955	L_t
59.0	—	—	—	—	—	HELFER, 1944	L_t
59.1	59.3	—	—	—	—	NAGIĘĆ, 1961	L_c
70.0	72.2	88.0	79.0	84.0	—	NEUHAUS, 1934	L_t
66.5	—	—	—	—	—	WIKTOR, 1954	L_t
72.6	77.6	—	81.5	—	—	NEUHAUS, 1934	L_t
—	65.0	—	73.0	—	—	MARRE, 1933	L_t
59.1	61.3	67.7	74.9	78.1	—	FILUK, 1955	L_t
60.9	68.1	—	—	—	—	CHUGUNOVA, 1931	L_c
69.6	72.6	—	—	—	—	CHUGUNOVA, 1931	L_c
59.1	65.2	68.0	—	—	—	LOGASEV, 1931 (after NAGIĘĆ, 1961)	L_c
64.8	—	—	—	—	—	GRIMALSCHI, 1938	L_t
62.0	—	73.0	68.0	73.0	—	UNGER, 1931	L_t
50.6	53.5	(57.4)	—	—	—	present investigation	L_c
57.6	60.9	(67.8)	—	—	—	present investigation	L_t

above a gravelly and sandy ground. Our previous works reveal that both quantitatively and qualitatively poor food consumption, the frequently occuring cannibalism and the high percentage of specimens with empty stomachs clearly reflect the unfavourable living conditions of the pike-perch population in Lake Balaton for the past few years. The three-four years old pike-perch in Lake Balaton consume but a small amount of food (0.46—14.6 g/day, yielding an average of 3.4 g/day) (BIRÓ and ELEK, 1969; BIRÓ, 1969).

Taking an average of many years fish catch around 1300—2000 q pike-perch from Lake Balaton (1.2—1.7 kg/cadastral yoke). The unfavourable composition of the stock form fisheries point of view is due to the comparatively small catch per cadastral yoke (TÖLG, 1962). This is due to the fact, that the majority of the catch comprises small, young generation. Because of insufficient intake of food the majority of 300—500 g pike-perch comprises slowly growing specimens. In this age-group specimens reaching a weight-group remain in it even when they pass their fourth year. During the fourth year there are hardly any specimens which reach the weight of 1 kg, on an average they reach this weight only in their sixth (5+) year. Of course, there are other causes for the slow growth of pike-perch in Lake Balaton, besides insufficient feeding. From the point of view of lacustrian life it is undoubtable that the shore arrangement of the lake has greatly influenced the stock of fish, so did the more modern method of fishing, the introduction of eel and last but not least the pesticide-accumulation which took place in the food-chain (BARON et al. 1968). Internal organic lesions can preceived almost in every group of generation, which closely resemble to pesticide poisoning. The effect of pesticide materials accumulated in the body tissues on growth is as yet not elucidated.

Fast growth and the differences in individual body lengths can be further influenced, besides the above mentioned factors, by social interrelationships

(population density, size of habitat, structure of environment), which finally result in size hierarchy (NAGOSHI, 1967). Considering local conditions the probability for an inclination to dwarf-size growth come to the foreground, like it happened with bream *(Abramis brama* L.) in Lake Balaton, whose observation clearly revealed a relationship between this phenomenon and stock-density (WUNDER, 1930). For the relationship between individual growth and population-density in the case of carp was found that the rate of growth at high population number very abruptly decreases (cit. HEPHER, 1967). Pike-perch in fish-ponds where the population generally in very high by the end of the first summer hardly reach 6—10 cm, on the other hand, at low density they reach a maximum length of 22.6 cm. At a 20,000 pike-perch /hectare density the average body length is 7.7 cm, at 1800 pike-perch/ hectare 12.7 cm (STEFFENS, 1960).

Comparing foreign data with our results obtained for the growth of pike-perch in Lake Balaton it is readily given that in here both the increase of body weight and body length remains below the growth rate measured in other waters. In northern waters (Sweden, Finland, Soviet Union), on the other hand, the rate of growth is somewhat slower than in Lake Balaton *(Table 5).*

Summary

1. The body length of the pike-perch fry at the time of scale formation is 40 mm:

$$L_c = 4.064 + 8.054 \cdot S;$$

where L_c = standard length in cm, S = total oral radius of scale in mm.

2. The following equation has been received for showing relation existing between the radii of winter rings (s_n) and the standard length:

$$s_n = -0.3300 + 0.1153 \cdot L_c;$$

where s_n is given in mm while L_c was measured in cm. The radii of the 4th—5th winter rings somewhat differ.

3. The following relations have been calculated for the allometric relation of body length and weight:
below 200 mm body length:

$$\log W = -4.6088 + 2.8379 \cdot \log L_c$$

$$\log W = -4.8339 + 2.8880 \cdot \log L_t$$

above 200 mm body length:

$$\log W = -5.2996 + 3.1634 \cdot \log L_c$$

$$\log W = -5.6057 + 3.2077 \cdot \log L_t$$

where W is the body weight in g, L_c and L_t are the standard and total length of the body respectively, given in mm.

4. We observed a slower rate of growth as compared to earlier data for Lake Balaton and also for foreign data. The following values were yielded for the annual increase of standard length:

(1+) 17.5, (2+) 25.1, (3+) 31.4, (4+) 36.7,
(5+) 42.3, (6+) 46.9, (7+) 50.6, (8+) 53.5 cm.

Age estimation for pike-perch older than 8 years is inaccurate. There is only a slight difference between the body lengths which has been back- calculated and originally measured.

5. Our investigations gave the following parameters for BERTALANFFY's growth-model: calculated for

$$L_\infty = 75.7 \text{ cm}, \quad K = 0.1376, \quad t_0 = -0.91 \text{ year}.$$

On the basis of these data the following equation is given to show the growth of pike-perch population inhabiting the North-eastern basin of Lake Balaton:

$$l_t = 75.7 \{1 - e^{-0.1376(t+0.91)}\}$$

where l_t is the standard length in every t period of time.

Acknowledgement

Author expresses his sincere thanks to Prof. Dr. TADEUSZ BACKIEL (Zakład, Gospodarki Rzecznej Institutu Rybactwa Śródlądowego, Żabieniec, Poland) for his invaluable guide in applying mathematical growth-models; thanks are also due to Dr. J. PONYI (Tihany, Hungary) for his useful criticism and help in preparing this paper.

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A FOGASSÜLLŐ (LUCIOPERCA LUCIOPERCA L.) NÖVEKEDÉSÉNEK VIZSGÁLATA A BALATONBAN

Biró Péter

Összefoglalás

1. A pikkelyképződés időpontjában a süllőivadék testhossza 40 mm: $L_c = 4{,}064 + 8{,}054 \cdot S$; ahol L_c = törzshossz cm-ben, S = pikkelyhossz mm-ben.

2. A téli évgyűrűk rádiuszai (s_n) és a törzshossz összefüggésére a következőt kaptuk: $s_n = -0{,}3300 + 0{,}1153 \cdot L_c$; ahol s_n-t mm-ben, L_c-t cm-ben mértük. A 4—5. téli évgyűrűk rádiuszai kis mértékben eltérnek.

3. A testhossz—testsúly allometrikus viszonyára a következő összefüggéseket számítottuk:

200 mm testhossz alatt $\log W = -4{,}6088 + 2{,}8379 \cdot \log L_c$
$\log W = -4{,}8339 + 2{,}8880 \cdot \log L_t$

200 mm testhossz fölött $\log W = -5{,}2996 + 3{,}1634 \cdot \log L_c$
$\log W = -5{,}6057 + 3{,}2077 \cdot \log L_t$

ahol W = testsúly g-ban, L_c és L_t = törzs és teljes testhossz mm-ben.

4. Korábbi Balatonra vonatkozó és külföldi adatokhoz képest a fogassüllő lassúbb növekedését figyeltük meg. Évenkénti törzshossz-növekedésre a következő értékeket kaptuk: (1+) 17,5; (2+) 25,1; (3+) 31,4; (4+) 36,7; (5+) 42,3; (6+) 46,9; (7+) 50,6; (8+) 53,5 cm. A 8 évnél idősebb süllők korbecslése bizonytalan. A pikkelyek alapján visszaszámított és eredetileg mért testhosszak között kis különbségeket találtunk.

5. Bertalanffy növekedés-modelljének paraméterei vizsgálatainkban a következők voltak: $L_\infty = 75{,}7$ cm törzshosszra számítva, $K = 0{,}1376$, $t_0 = -0{,}91$ év. Ezek alapján a tó ÉK-i medencéjében élő fogassüllőpopuláció növekedését leíró számszerű egyenlet:

$$l_t = 75{,}7\ \{1 - e^{-0{,}1376(t+0{,}91)}\},$$

melyben l_t = törzshossz minden t időpontban.

ИССЛЕДОВАНИЕ РОСТА СУДАКА (LUCIOPERCA LUCIOPERCA L.) В БАЛАТОНЕ

П. Биро

1. Образование чешуи у мальков судака завершается при длине тела 40 мм: $L_c = 4{,}064 + 8{,}054.S$; где L_c = длина корпуса в см, S = длина чешуи в мм.

2. Зависимость между радиусами зимних годовых колец (s_n) и длиной тела следующая: $s_n = -0{,}3300 + 0{,}1153.\ L_c$; где s_n в мм, L_c в см. Радиусы 4—5 зимних колец немного различны.

3. Для аллометрического отношения между длиной тела и весом рассчитаны следующие зависимости:

длина тела до 200 мм	$\log W$	$= -4{,}6088 + 2{,}8379 \cdot \log L_c$
	$\log W$	$= -4{,}8339 + 2{,}8880 \cdot \log L_t$
длина тела свыше 200 мм	$\log W$	$= -5{,}2996 + 3{,}1634 \cdot \log L_c$
	$\log W$	$= -5{,}6057 + 3{,}2077 \cdot \log L_t$

где W = вес тела в гр, L_c и L_t = длина корпуса и длина тела в мм.

4. В сравнении с прежними данными по Балатону и других водоемам нами наблюдался более медленный рост судаков. Рост корпуса по годам происходит следующим образом: (1+) 17,5; (2+) 25,1; (3+) 31,4; (4+) 36,7; (5+) 42,3; (6+) 46,9; (7+) 50,6; (8+) 53,5 см. У судаков старше 8 лет возраст нельзя определить с уверенностью. Между длинами тел вычисленных на основе чешуи, и результатами прямых измерений различия невелики.

5. В наших опытах параметри модели роста (Берталанффи) следующие: $L_\infty = 75{,}7$ см перечисленное на длину тела $K = 0{,}1376$, $t_0 = -0{,}91$ год. На основе этих уравнений описываемый рост популяции судаков, живущих в северо-восточном бассейне озера следующий:

$$l_t = 75{,}7\ \{1 - e^{-0{,}1376(t+0{,}91)}\}$$

где l_t = длина корпуса во время.

ANNAL. BIOL. TIHANY | 37 | 165—176 | HUNGARIA 1970

THE DYNAMICS OF FATTY ACIDS IN THE AQUATIC FOOD CHAIN, PHYTOPLANKTON, ZOOPLANKTON, FISH

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Received: January 28th, 1970

It has long been known that the fat of aquatic animals differs from that of land animals in the presence of long chain polyunsaturated fatty acids (HILDITCH, 1956). The fats of planktonic algae are in general poor in these fatty acids and resemble the fats of higher terrestrial plants (ACKMAN and TOCHER, 1968; CHUECAS and RILEY, 1969). It is clear that each constituent of the lower part of the food cycle must contribute in some way to the formation of the characteristic fatty acid pattern of the animals at the higher trophic levels (e.g. fish), but the way in which this conversion takes place is not clear as yet.

Detailed investigations of the composition of the fatty acids of organisms at different trophic levels of the aquatic food chain have been made at Tihany, and many accounts of the fat metabolism of planktonic crustacea and fish are available in the literature. The following account sets out to correlate the results obtained by the author with the findings of other workers, and an attempt has been made to determine the processes which take place at all trophic levels leading to, and including, the formation of the characteristic fatty acid composition of fishes.

Material and methods

The alga *Chlorella vulgare* was obtained from a large-scale culture and samples of *Diatomea vulgare* collected from the inshore waters of Lake Balaton.

Live specimens of the planktonic crustaceans *Daphnia cucullata* G. O. SARS (Cladocera), *Diaphanosoma brachiurum* LIEVIN (Cladocera) *Eudiaptomus gracilis* G. O. SARS (Copepoda), *Mesocyclops leuckarti* CLAUS (Copepoda) and the bleak, *Alburnus alburnus* L. were collected in Lake Balaton in the summers of 1968 and 1969. The knife, *Pelecus cultratus* L. were obtained from the Balaton Fisheries Association and the carp, *Cyprinus carpio* L. from a nearby fish hatchery. The fat for fatty acid analysis was obtained from 5—10 g of planktonic crustaceans, from a sample of 50 bleak, 5 knife and 5 carp. The body, intestinal and liver fats were investigated separately.

Fat was extracted according to the method of BLIGH and DYER (1959). The algae and planktonic crustaceans were homogenised in a Potter-Eljevhem homogeniser and the fishes in a Bio-Mix homogeniser.

One part of the fat obtained was transmethylated immediately, and the other fraction subjected to thin layer chromatography in order to separate

triglycerides and phospholipids. Silicagel G on 20.20 cm plates was used for this purpose and a mixture of petroleum ether : diethyl ether: acetic acid = 10 : 30 : 1 was utilized as a developing solvent. 0.01 per cent hydrochinone was added to protect long chain polyunsaturated fatty acids. The spots obtained were removed and transferred directly into ampoulles containing 5 per cent hydrochloric acid in 100% methanol and were then transmethylated in the presence of silicagel at 80 °C.

Details of gas chromatographic analysis, identification and quantification have been published elsewhere (FARKAS, 1970). In the present study, 15 per cent EGSS-Y on Gas Chrom P was used instead of EGS. This enabled the separation of 16 : 3 and 20 : 1 fatty acids from stearic and linolenic acid respectively.

Results

Few data are available on the fatty acid composition of species of fresh water algae living under natural conditions. *Table 1.* shows the fatty acid composition of a blue-green alga as described by PARKER et al. (1967), and of *Chlorella vulgare* and *Diatomea vulgare*. The blue-green alga collected by PARKER et al. is a marine species, although two related species *(Lyngbia limnetica* and *L. circumcreta)* are to be found in L. Balaton. The fatty acid composition of a number of species of alga has been extensively described by a number of workers (PASCHKE and WHEELER, 1954; SCHLENK et al. 1960; HOLTON et al. 1964; IWATA, 1964; WERESCHIAGIN and KLASHKO-GURBICH, 1965; PARKER et al. 1967; ACKMAN and TOCHER, 1969; KATES and VOLCANI, 1966; CHUECAS and RILEY, 1969). It is generally supposed that the fat of any algal species is characteristic for its own group, although the relative concentrations of the fatty acids present may change with variations in the salinity of culture medium, the environmental temperature, the degree of illumination and the age of the culture (HOLTON et al. 1964; REITZ et al. 1967; ACKMAN and TOCHER, 1968).

Table 1. also gives the fatty acid composition of four planktonic crustaceans under investigation and of the body fat of the bleak.

The blue-green algae have the simplest fatty acid composition of all the species in the food chain. Linolenic acid was absent in the species of blue-green algae listed in *Table 1*, although present in some other species investigated by PARKER et al. (1967) and palmitic, palmitoleic, and oleic acids were predominant in blue greens. The fat of the green algae is more complex. The data presented in this paper and in the publications of other workers clearly show that the fat of green algal species is rich in both linoleic and linolenic acids. The eicosa- and docosapolyenoic fatty acids which are characteristic of the fats of the higher aquatic animals are present, if at all, in proportions less than 1 per cent. A number of authors (IWATA, 1964; WERESCHIAGIN and KLASHKO-GURBICH, 1965) failed to detect the last mentioned acids at all, and while the present investigation demonstrated the presence of eicosapolyenoic fatty acids in the fat of *Chlorella vulgare,* docosapolyenoic acids were also absent from the samples investigated. According to SCHLENK et al. (1960) less than 0.1 per cent of these acids are present in the fat of *C. pyrenoidosa* and the fats of marine species investigated are also poor in eicosapolyenoic and docosapolyenoic fatty acids. In most cases oleic and stearic acid levels

TABLE 1

Fatty acid compositions in different members of food chain

Fatty acid	Blue-gren alga (*Lyngbia lageraimi*)	Green alga (*Chlorella vulgare*)	Diatom (*Diatomea vulgare*)	*Daphnia cucullata*	*Diaphanosoma brachiurum*	*Eudiaptomus gracilis*	*Mesocyclops leukarti*	*Alburcus lucidus*
SATURATED FATTY ACIDS								
12 : 0	—	0.68	0.36	1.13	2.29	0.26	0.47	0.35
13 : 0	—	0.71	0.44	0.95	1.99	0.09	0.45	0.14
14 : 0	2.0	2.19	7.73	4.50	5.63	4.46	5.57	3.20
15 : 0	1.7	2.83	1.40	1.87	2.49	0.70	1.04	0.87
16 : 0	36.0	6.17	7.61	12.40	12.23	14.01	13.77	10.32
17 : 0	—	8.29	5.76	3.16	2.56	2.50	2.82	4.07
18 : 0	1.9	1.64	4.14	5.24	5.07	7.84	6.52	3.89
total	41.6	22.50	27.44	29.25	33.26	29.86	30.64	22.84
MONOENOIC FATTY ACIDS								
12 : 1	—	—	—	0,44	0.90	—	0,38	0.15
13 : 1	—	—	1.06	0.49	1.44	0.17	0.35	0.17
14 : 1	0.8	—	1.57	1.64	2.54	1.67	2.07	2.31
15 : 1	—	—	0.50	1.75	2.33	0.28	0.54	1.05
16 : 1	15.0	7.41	9.71	11.34	12.11	5.44	9.24	10.55
17 : 1	—	—	5.13	3.00	3.52	1.41	1.64	2.21
18 : 1	31.0	17.17	8.60	13.52	11.24	9.19	10.09	12.83
20 : 1	—	—	2.42	—	0.66	1.55	1.40	1.12
total	46.8	24.58	28.99	32.18	34.74	19.71	25.71	30.39
POLYENOIC FATTY ACIDS								
16 : 2	—	—	—	—	—	0.60	—	—
16 : 3	—	—	—	—	—	—	0.67	—
16 : 4	—	9.68	—	—	—	—	—	—
18 : 2 ω 6	7.4	12.10	6.54	7.09	6.24	10.47	10.40	8.67
18 : 3 ω 6	—	1.54	—	0.37	0.64	0.96	1.59	1.93
20 : 2 ω 6	—	1.14	1.25	0.35	0.42	0.89	0.31	0.39
20 : 3 ω 6	—	3.41	—	0.44	0.52	0.16	0.23	0.39
20 : 4 ω 6	—	0.93	1.83	4.85	3.77	4.37	4.47	5.78
22 : 4 ω 6	—	—	0.45	0.32	0.30	0.34	0.25	+
22 : 5 ω 6	—	—	+	1.23	1.14	3.10	1.58	1.26
total	7.4	19.12	10.07	14.65	13.03	20.29	18.83	18.42
18 : 3 ω 3	—	21.01	3.45	6.49	4.75	6.71	5.47	4.45
18 : 4 ω 3	—	1.25	1.76	3.60	2.87	4.90	3.48	2.30
20 : 4 ω 3	—	+	+	0.72	0.27	0.22	0.28	1.72
20 : 5 ω 3	—	1.84	25.80	12.08	9.40	7.98	7.15	7.38
22 : 5 ω 3	—	—	—	1.23	—	0.37	0.77	1.21
22 : 6 ω 3	—	—	1.65	1.01	1.90	9.32	5.63	11.25
total	—	24.16	32.60	25.13	19.19	29.50	22.78	28.31

are low and the proportion of palmitolieic acid present does not exceed that of palmitic acid.

Results of the present work on the fatty acids of *Diatomea vulgare* agree with previous investigations on the fatty acids of other diatom species. The level of myristic acid is comparatively high and palmitoleic acid is always present at a higher level than that of palmitic acid. Linoleic and linolenic acids form only very small proportion of the total fatty acids. All diatoms are rich in eicosa pentaenoic and poor in docosahexaenoic acids. The fatty acid composition of *Nitella pelliculosa* and *N. closterium* analysed by KATES and VOLCANI (1965) seems to be comparable to the fats of marine species. It is probable that linoleic and linolenic acid levels and the ratio of palmitic to palmitoleic acid are useful criteria in differentiating between the fats of blue-green and green algae those of diatoms.

The fatty acid composition of the total fats of planktonic crustaceans and plankton-eating fishes qualitatively resembled that of the fats of diatoms. Crustaceans and fish fat contained significant amounts of eicosa- and docosapolyenoic fatty acids and differed in a number of ways from the fats of the species of blue-green and green algae listed in *Table 1.*

Palmitic and stearic acid levels were higher in the fats of planktonic crustaceans than in those of the aglal species investigated, and as a consequence the total amount of saturated fatty acids in the crustaceans was higher also. The amount of palmitic and palmitoleic acids in the fats of the cladocerans *Diaphanosoma brachiurum* and *Daphnia cucullata* was approximately the same, while in the copepods *Eudiaptomus gracilis* and *Mesocyclops leukarti* a higher proportion of palmitic than palmitoleic acid was detected. The fats of all the crustaceans in the investigation were equally rich in linoleic and linolenic acids. While more eicosapentaenoic acids were detected in the cladocerans than in the copepods, the latter proved to be richer in docosahexaenoic acids than the former.

The total fat fatty acid composition of the bleak agreed in general with that of the planktonic crustaceans, with the exception of the level of docosahexeanoic acid which was even higher than in crustaceans.

The distribution of the fatty acids in the triglycerides of the crustacean speciaes investigated was very similar *(Table 2.)*. The triglicerides prepared from the bleak and from the knife differred from those of planktonic crustaceans. The palmitic acid level in the triglicerides of planktonic crustaceans was higher than that of palmitoleic acid and as a result their ratio was greater than unity. In the fishes the situation was reversed, but the sum of the two acids was equal in fishes and crustaceans *(Table 3.)*. This was also the case with oleic and stearic acid. The levels of linoleic and linolenic acids in the triglycerides of fishes and crustaceans was approximately the same, while higher levels of docosa hexaenoic acid were detected in the fishes than in the crustaceans.

There are two striking differences between the fatty acid composition of triglycerides and phospholipids *(Tables 2 and 4)*. The level of linoleic acid (and that of linoleic acid also in *Eudiaptomus garcilis)* was considerably lower in the phospholipids than in the triglycerides. This was particularly evident in the copepods examined. The phospholipids and triglycerides of cladocerans were equally poor in docosahexaenoic acid, while the level of this fatty acid in the phospholipids of copepods was considerably higher than in their triglycerides. The level of docosahexaenoic acid in bleak body phospholipids was

TABLE 2

Fatty acid compositions of the triglicerides in planctonic crustaceans and fishes

Fatty acid	*D. cucullata*	*D. brachiurum*	*M. leukarti*	*E. gracilis*	*A. lucidus*, body	*A. lucidus*, adipose tissue	*P. caltratus* adipose tissue	*C. Carpio*, body
				SATURATED FATTY ACIDS				
12 : 0	0.12	1.45	0.33	0.34	0.44	0.23	0.26	0.10
13 : 0	0.09	2.16	0.16	0.17	0.17	0.08	0.17	0.04
14 : 0	8.17	11.16	7.37	6.89	4.49	4.60	3.41	1.11
15 : 0	1.02	3.95	1.30	1.07	0.93	0.89	1.00	0.40
16 : 0	17.06	19.84	15.56	18.14	10.76	11.49	12.17	12.09
17 : 0	3.15	4.25	3.48	2.68	2.09	2.64	3.39	—
18 : 0	7.18	4.36	6.28	8.13	2.14	2.10	3.23	3.98
otal	37.33	47.17	34.48	37.41	21.02	22.03	20.63	17.72
				MONOENOIC FATTY ACIDS				
12 : 1	0.11	1.11	0.16	0.19	0.19	0.40	0.20	0.07
13 : 1	0.17	1.42	0.16	0.23	0.17	0.08	0.17	0.07
14 : 1	2.52	2.21	3.17	2.22	2.48	1.94	1.65	0.39
15 : 1	0.28	0.97	0.50	0.24	0.22	0.33	0.31	0.19
16 : 1	8.36	9.89	8.47	7.74	12.07	11.09	12.11	11.98
17 : 1	1.40	—	2.25	0.87	2.09	2.17	2.23	0.87
18 : 1	10.81	11.61	11.52	3.19	15.78	16.57	19.64	38.15
20 : 1	2.06	—	1.25	1.30	1.66	1.12	1.43	6.50
22 : 1	—	—	0.22	—	—	—	—	—
total	25.71	27.21	27.70	15.98	34.66	33.70	34.74	58.22
				POLYENOIC FATTY ACIDS				
16 : 2	—	—	2.33	1.06	—	1.54	1.62	1.20
16 : 3	—	—	—	—	—	2.17	2.23	0.87
16 : 4	0.45	—	0.60	—	—	—	—	—
18 : 2 ω 6	12,60	9.68	11.38	13.96	10.48	11.75	9.20	13.96
18 : 3 ω 6	1.48	1.62	1.58	0.97	2.26	1.15	1.09	1.80
20 : 2 ω 6	0.13	—	tr.	0.82	0.86	0.80	0.42	2.82
20 : 3 ω 6	0.24	—	tr.	0.32	0.37	0.59	0.18	1.17
20 : 4 ω 6	1.87	2.80	2.97	4.02	4.74	7.02	0.13	—
22 : 4 ω 6	tr.	—	0.08	0.25	tr.	0.08	0.11	—
22 : 5 ω 6	0.64	—	1.00	1.94	0.55	0.47	0.33	—
total	16.96	14.10	17.01	22.28	19.26	21.86	11.46	19.75
18 : 3 ω 3	8.42	4.30	7.22	9.29	6.26	5.09	4.94	1.79
18 : 4 ω 3	6.81	2.18	5.18	6.94	3.29	2.01	1.22	0.48
20 : 4 ω 3	tr.	0.66	0.29	0.28	0.98	1.21	0.13	—
20 : 5 ω 3	2.29	2.45	4.31	4.73	6.87	8.56	7.63	—
22 : 5 ω 3	tr.	0.74	0.07	tr.	0.61	0.72	0.83	—
22 : 6 ω 3	0.65	—	1.66	2.01	5.24	3.77	4.53	—
total	18.17	10.33	18.73	23.25	23.25	21.36	19.28	2.27

TABLE 3

The ratio of saturated to monounisaturated fatty acids in the triglicerids of planctonic crustacens and fishes

Species	$\frac{\text{sturated}}{\text{monoenoic}}$	$\frac{16:0}{16:1}$	$\frac{18:0}{18:1}$	16 : 0 + 16 : 1	18 : 0 + 18 : 1
D. cucullata	1.48	2.10	0.70	25.42	17.99
D. brachiurum	1.64	2.00	0.37	19.73	15.97
E. gracilis	1.22	2.34	2.55	25.92	11.37
M. leukarti	1.23	1.84	0.55	24.03	17.80
A. lucidus	0.60	0.84	0.16	22.83	17.92
P. cultratus	0.52	1.00	0.16	24.28	22.87
C. carpio	0.55	1.00	0.10	24.07	42.13

similar to that of the copepods examined. The liver and body phospholipids of the carp also contained an appreciable amount of docosahexaenoic acid, which was completely absent from the triglycerides.

Discussion

Nutritional factors. The planktonic crustaceans investigated obtain the bulk of their food by filtration and the fishes feed mainly on zooplankton (with the exception of the carp which were artificially cultured and received a special diet).

The species composition of the phytoplankton in the northern part of L. Balaton (according to TAMÁS, 1969) is listed in *Table 5*. In terms of the number of individuals per litre and number of species present, members of the Chrysophyceae are dominant throughout the summer, followed by species of Cyanophyta, Chlorophyta and Pyrrophyta in that order.

The intestinal contents of planktonic crustaceans were not investigated and it is therefore not possible to demonstrate any species selectivity in relation to the algal species consumed. It is highly unlikely that very large, filamentous or gelatinous algae, or those having a complex sceletal structure play a significant role in the food of planktonic crustaceans.

Metabolic aspects. The following processes may modify the fatty acid composition of any member of the food chain:

1. De novo synthesis of fatty acids.
2. The fatty acid composition of the food ingested.
3. Interconversion of fatty acids taken up with the food or produced by the organisms.

It is evident that the situation is most simple in the planktonic algae, where all fatty acids are produced by de novo synthesis. Significant differences in the ability to synthetise different fatty acids may occur between the different taxonomic groups. The blue-green algae are the most primitive in this respect: even linolenic acid is absent from the fats of some species. Diatoms may be distinguished from species of Chlorophyta by their different abilities in synthetising long-chain polyunsaturated fatty acids. The fats of green algae are rather poor in these fatty acids, but are rich in linoleic and linolenic acids

TABLE 4

Fatty acid composition of the phospholipids in fishes and planktonic curstaceans

Fatty acid	*D. cucullata*	*D. brachiurum*	*E. gracilis*	*M. leukarti*	*A. dilucus* body	*C. carpio* body
			SATURATED FATTY ACIDS			
12 : 0	—	0.26	0.35	0.16	0.87	0.72
13 : 0	—	0.41	—	—	0.61	0.59
14 : 0	1.93	2.80	3.33	2.60	2.32	1.21
15 : 0	1.20	1.22	0.70	0.77	1.62	1.62
16 : 0	13.16	14.23	11.56	12.41	14.77	8.80
17 : 0	3.87	1.16	2.00	2.45	1.35	—
18 : 0	5.38	4.94	6.27	5.63	7.34	5.29
total	25.54	30.02	24.21	24.02	27.26	18.23
			MONOENOIC FATTY ACIDS			
12 : 1	—	0.16	—	—	—	0.46
13 : 1	—	0.09	—	—	0.69	0.59
14 : 1	1.10	0.28	0.92	0.97	1.22	0.79
15 : 1	1.80	0.31	0.25	0.32	0.94	0.90
16 : 1	9.76	10.32	4.10	7.52	6.41	6.08
17 : 1	3.37	1.79	0.47	15.00	0.87	0.62
18 : 1	12.43	14.68	6.14	9.54	10.90	14.47
20 : 1	1.97	0.81	—	0.71	—	4.47
22 : 1	—	—	—	—	—	—
total	30.93	28.44	11.88	34.06	21.03	28.38
			POLYENOIC FATTY ACIDS			
16 : 2	—	—	—	—	—	0.87
16 : 3	2.40	—	0.47	—	—	0.62
16 : 4	—	0.48	—	0.36	—	0.76
18 : 2 ω 6	10.28	10.70	6.81	12.08	5.13	6.66
18 : 3 ω 6	2.09	0.35	0.72	1.00	—	1.07
20 : 2 ω 6	tr.	—	0.20	0.20	0.18	5.98
20 : 3 ω 6	tr.	—	0.23	0.20	tr.	3.86
20 : 4 ω 6	8.53	11.77	7.82	7.90	9.15	14.77
22 : 4 ω 6	0.17	—	0.46	tr.	0.58	0.48
22 : 5 ω 6	0.24	1.00	4.83	2.39	2.08	1.92
total	21.31	23.82	21.07	23.77	17.12	34.74
18 : 3 ω 3	0.19	2.64	3.51	4.23	1.09	0.92
18 : 4 ω 3	3.21	—	2.37	1.66	—	0.44
20 : 4 ω 3	0.40	0.30	tr.	0.37	—	—
20 : 5 ω 3	10.39	11.52	15.97	12.01	6.38	5.66
22 : 5 ω 3	0.26	—	0.71	0.41	1.86	1.69
22 : 6 ω 3	1.06	1.39	19.40	11.55	21.65	8.28
total	15.51	15.85	41.96	28.23	30.98	16.99

which are their natural precursors. Accumulation of linoleic and linolenic acids may be the result of an inability of green algae to dehydrogenate and elongate these compounds to long-chain polyunsaturated fatty acids. One of the major components of the fat of diatoms is eicosapentaenoic acid (accompanied by lower level of docosahexaenoic acid), while linoleic and linolenic acids are present in very low quantities. Dinoflagellates have no signifcance

TABLE 5

Species composition of the phytoplankton in the Lake Balaton

Taxonomic group	Time of sampling	Number of species	Number of individuals	Per cent of total
Cynophyta	VII	9	13 700	10.2
	VIII	10	23 100	22.0
	IX	10	27 270	40.0
Pyrophyta	VII	3	8 650	6.4
	VIII	4	10 840	10.2
	IX	3	5 600	8.0
Chrysophyta	VII	23	95 190	71.0
	VIII	33	58 450	55.0
	IX	20	23 210	34.0
Chlorophyta	VII	25	12 120	9.0
	VIII	19	12 830	12.1
	IX	22	11 040	16.0

in the diet of planktonic crustaceans in L. Balaton, as the only representative in the Lake, *Caratium hyrundinella* has a complicated sceletal structure, but they may be of greater consequence in the nutrition of marine species. Since the members of the *Dinoflagellata* are able to take up particulate food as do the higher members of the trophic chain, it is to be expected that their fatty acid composition should resemble that of the higher animals (HARRINGTON and HOLZ, 1968).

The higher animals can synthetise de novo only saturated (i.e. palmitic, stearic, myristic) fatty acids, which are readily desaturated to the corresponding monoenoic fatty acids. The rate of these processes depends on the fat content and on the fatty acid composition of the food, and in the case of fat rich diet it may be supressed. There is no direct evidence indicating whether or not planktonic crustaceans are capable of converting the starch of algae into saturated fatty acids. The low fat and high starch content of blue-green and green algae (BARASKOW, 1962) and the high level of palmitic, stearic, and in some cases of the palmitoleic acids in the fat of planktonic crustaceans indicate that this may be the case however. It is probable that the fishes dehydrogenae palmitic and stearic acids taken up from planktonic crustaceans, to palmitoleic and oleic acids. This is also suggested by the data presented in *Table 4*. It seems likely that this is the case with carp, since carp on a carbohydrate-rich diet have fat containing substantial amounts of palmitoleic and oleic acids. Phospholipids have a more stable fatty acid composition than triglycerides, and this may assure the functioning of subcellular particles into which they are incorporated.

The level of linoleic and linolenic acids in the triglycerides of the fresh water crustaceans from L. Balaton was considerably higher than in diatoms. The values of 16 : 0/16 : 1 were higher than 1., suggesting that their diet consisted mainly of blue-green and green algae, rather than of diatoms. Since linoleic and linolenic acids are present in large amounts in the fat of plankton-

feeding and predatory fresh water fishes, it may be concluded that they are members of a food chain based on blue-green and green algae. From this it follows that differences in the fatty acid composition of fresh water and marine fishes may be explained by differences in the species composition of phytoplankton at the base of the food chain.

The mechanism of the biosynthesis of long chain polyunsaturated fatty acids has already been described in vertebrates including fishes, by MEAD (1960) and KLENK and KREMMER (1960). The factors influencing the intracellular conversion of linoleic and linolenic acids have also been outlined (BRENNER and PELUFFO, 1966, 1969; MORHUSER et al. 1967). It has been shown that linoleic und linolenic acid in the mitochondria of vertebrates are readily transformed into the corresponding long chain polyunsaturated fatty acids (KLENK and DETTE, 1960), and that the products are incorporated preferentially into the phospholipids (NERVI et al. 1968). The intensity of elongation and desaturation reaction is strongly influenced by the presence of linoleic, linolenic and docosahexaenoic acids (BRENNER and PELUFFO, 1966, 1969; MORHUSER et al. 1967). REISER et al. (1963) have noted that high intensity of conversion of linoleic and linolenic acids occurs only when the level of these acids is adequate in the diet ($>1\%$) while BRENNER et al. (1963) have also shown that docosahexaenoic acid inhibits this process. The presence of eicosa and docosa polyenoic acids in the phospholipids of the carp provides further evidence that fishes are capable of producing long chain polyunsaturated fatty acids, although the differences between the trigliceride fatty acid composition of the carp and plankton feeding fishes and the literature (BRENNER et al. 1963; TOYOMIZU et al. 1963; KELLY et al. 1958, 1963; KANEKO et al. 1967) suggest that the bulk of these fatty acids in the triglicerides of fishes is derived from the diet.

Comparison of the fatty acid composition of fresh water algae and planktonic crustaceans suggests that the long chain polyunsaturated fatty acids characteristic for the aquatic organisms accumulate in phospholipids of certain planktonic crustacean species. There are however few direct evidences on the origin of these fatty acids in crustaceans. The observation that the fats of species raised on green algae contained these fatty acids in greater quantities than are present in algae (KAYAMA et al. 1963; KELLY et al. 1963; FARKAS and HERODEK, 1964) suggests that the crustaceans are able to convert linoleic and linolenic acids in the diet to long chain polyunsaturated fatty acids. It is also likely that these fatty acids are preferentially incorporated into the phospholipids.

The present work suggests that the phospholipids of planktonic crustaceans are the source of the long chain polyunsaturated acids in the triglicerides of fishes. The accumulation of these fatty acids in fish triglycerides may be explained in terms of their specific metabolic properties. Long chain polyunsaturated fatty acids are esterified mainly into the β-position of phospholipids (BROCKERHOF et al. 1963, 1964) and are resistant to pancreatic lipolysis, even when transacilated into the α-position (BOTTINO et al., 1967). Because of this, the monoglycerides resorbed in the intestines of fishes contain long chain polyunsaturated fatty acids mainly in the carbonic atom 2 position. These monoglycerides serve as a starting point for the synthesis of both triglycerides and phospholipids in the intestinal wall and liver, while the triglicerides formed in this way are deposited in the adipose tissue.

Summary

Correlation of the present work on the fatty acid composition of algae *(Chlorella vulgare, Diatomea vulgare)*, planktonic crustaceans *(Daphnia cucullata, Diaphanosoma brachiurum, Eudiaptomus gracilis, Mesocyclops leukarti)* and plankton feeding fishes *(Alburnus alburnus, Pelecus cultratus)* and of the carp *(Cyprinus carpio)* with results obtained by other workers, suggests that the metabolism concerned in the formation of the characteristic fatty acid composition of aquatic animals may be as follows:

1. Biosynthesis of linoleic and linolenic acid in members of the phytoplankton.

2. Formation of eicosa and docosapolyenoic acids in some planktonic crustaceans. The bulk of these fatty acids accumulate inthe phospholipids.

3. Redistribution of long chain polyunsaturated fatty acids in the triglicerides and phospholipids of fishes.

4. De novo synthesis of saturated fatty acids from non lipid precursors and their desaturation to monoenoic fatty acids may take place in each member of the food chain.

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ZSÍRSAVAK DINAMIKÁJA A PHYTOPLANKTON – CRUSTACEA PLANKTON – HAL TÁPLÁLKOZÁSI LÁNCOLATBAN

Farkas Tibor

Összefoglalás

Vizsgálva a planktonalgák, planktonrákok és planktonevő halak zsírsavösszetételét és figyelembe véve ezen szervezetek zsíranyagcseréjére vonatkozó irodalmi adatokat, a vízi szervezetek jellegzetes zsírsavösszetételének kialakulását, a következő lépéseken keresztül képzeljük el:

1. linol és linolénsav szintézise a phytoplanktonban;
2. eicosa és docosa polyen zsírsavak képződése linol és linolénsavból és felhalmozódása bizonyos planktonrákok phospholipidjeiben;
3. a planktonrákokban felhalmozódott eicosa és docosa polyen zsírsavak „redistributio"-ja a halak trigliceridjeiben és phospholipidjeiben.

ДИНАМИКА ЖИРНЫХ КИСЛОТ В ПИТАТЕЛЬНОЙ ЦЕПИ: ФИТОПЛАНКТОН CRUSTACEA ПЛАНКТОН-РЫБА

Т. Фаркаш

Исследуя состав жирных кислот у планктонных водорослей, раков и у рыб, питающихся планктоном, и принимая во внимание литературные данные, касающиеся обмена жиров у этих органихмов, формирование характерного состава жирных кислот у водных организмов представляется таким образом:

1. синтез линолевой и линоленовой кислоты в фитопланктоне
2. образование eicosa и docosa жирных кислот из линолевой и линоленовой кислот и накопление в определенных фосфолипидах планктонных раков.
3. Усвоение планктоными раками eicosa и docosa полиен жирных кислот в триглицеридах и фосфолипидах рыб.

ANNAL. BIOL. TIHANY | 36 | 177—182 | HUNGARIA 1970

DESATURATION OF PALMITIC ACID-1- C^{14} AND STEARIC ACID-1- C^{14} IN GAMMARUS (RIVULOGAMMARUS) ROESELII GERVAIS (CRUSTACEA, AMPHIPODA)

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Received: February 18th 1970

Among Arthropods the synthesis of fatty acids has been studied mainly in Insects. The ability to synthetise saturated and monoenoic fatty acids and to transform stearic and palmitic acids to monoenoic acids was demonstrated in many species belonging to different Orders (ZEBE and MCSHAN, 1959; BADE, 1964; LAMBREMONT et al., 1965, 1966; SRIDHARA et al. 1966; KEITH, 1967; STEPHEN et al. 1969). The isotopic experiments however — contrary to earlier opinions — showed no signs of polyenoic fatty acid synthesis in Insects.

In Crustacea the fatty acid composition of many species, the effect of food and temperature on the fatty acid composition and the distribution of fatty acids in different lipid classes are known (HILDITCH, 1956; FARKAS and HERODEK, 1964; WOLFE et al. 1965; HERODEK and FARKAS, 1967; HERODEK, 1969; FARKAS, 1970 a, b; COLLATZ, 1969 a, b). Till now however the synthesis of fatty acids has been studied only in two Decapods, in *Astacus astacus* L. and *Homarus gammarus* L. (ZANDEE, 1966, 1967). After the injection of acetate-1-^{14}C the saturated and monoenoic fatty acids were labelled, but not the polyenoic ones.

The present work investigates to what extent can Amphipod crustaceans desaturate the palmitic acid-1-^{14}C and the stearic acid-1-^{14}C and how are these fatty acids distributed into the different lipid classes.

Material and methods

The stearic acid-1-^{14}C (REANAL, Budapest) had the specific activity of 1.379 mCi/mmole, the palmitic acid-1-^{14}C (REANAL, Budapest) 1.110 mCi/mmole.

From the labeled fatty acid 1 μCi was dissolved in 1 ml benzene and this solution was distributed on a Macherey-Nagel 640 m filter-paper disk as evenly as possible. The benzene was then carefully evaporated. The animals were collected from the Aszófő-Séd brook on the 20th October 1969. They were kept in the laboratorium in two glass aquaria. The water level was 5 cm. The water was permanently transaerated. Both aquaria contained some hundreds of animals. The plamitic acid-1-^{14}C impregnated filter-paper was placed into the one, the stearic acid-1-^{14}C impregnated filter-paper into the other aquarium. The crustaceans eat up the filter-papers in two days. For further two days they were fed on green and decaying plants originating from the sampling place. On the fourth day the animals were rinsed with pure water, blotted on filter-

paper and weighed. The fresh weights of the palmitic and stearic acid feeding groups were 26.0 g and 14.7 g respectively. Both groups were homogenized in chloroform : metanol 2 : 1 in a Biomix blendor.

The lipids were extracted according to FOLCH (1957). The distribution of the label in the lipid classes was determined from a part of the total lipid by thin layer chromatography as described earlier (HERODEK, 1968). Another portion of the total lipid was chromatographed in the same way, then from the silica gel the triglycerides were eluted three times with diethyl ether the phospholipids three times with chloroform : metanol 2 : 1 and once by methanol : ammoniumhydroxide 10 : 1. The triglycerides and phospholipids so obtained, and a further part of the total lipid were transmethylated with cc. HCl : abs. methanol 5 : 95. The methyl esters were purified by rechromatographing on silica gel G and eluted with diethyl ether. They were then subjected to thin layer chromatography on silica gel G containing 12.5 per cent silver nitrate to separate the methyl esters of the monoenoic, dienoic, trienoic and polyenoic fatty acids. The developing solvent was benzene, the bands were detected with Rhodamine B. The fatty acid composition of the bands was checked gas-chromatographically in control runnings. The methyl esters were eluted from the silver nitrate impregnated silica gel G with diethyl ether in a micro-Soxhlet apparatus for two hours, then their radioactivity was determined.

Radioactivity measurements were carried out with a USB—2 liquid scintillation detector (Office for Nuclear Engineering Equipment Pilot Plant, Warsaw). The samples were dissolved in 8 ml scintillation liquid consisting of toluene, containing 0.4 per cent 2.5-diphenyloxazole and 0.01 per cent 1,4-di[2-(5-phenyloxazolyl)]-benzene.

The gas-chromatographical analysis was carried out as described earlier (HERODEK, 1969).

Results and discussion

In the lipids of crustaceans 18.9 and 29.1 per cent of the activity of palmitic acid-1-^{14}C and stearic acid-1-^{14}C respectively, introduced by the food, was refound. The fatty acids were practically completely esterified, about two third of them incorporated into the triglycerides and one third into the phospholipids *(Fig. 1.)*.

Neither in palmitic acid-1-^{14}C nor in stearic acid-1-^{14}C feeding animals did the di-, tri-, and polyenoic fatty acids show any measurable radioactivity. The monoenoic fatty acids on the other hand had activities amounting in the palmitic acid-1-^{14}C fed group to 19 per cent, in the stearic acid-1-^{14}C fed group to 66 per cent of the total radioactivity. That only the smaller part of the palmitic acid but the greater part of the stearic acid was transformed to monoenoic acids corresponds to the fact that in the fat of Gammarus Roeselii there is more palmitic than palmitoleic but much less stearic than oleic acid *(Table I.)*.

In rats the ratios of the labeled palmitic /palmitioleic and stearic/ oleic acid incorporations are much higher in phospholipids than in triglycerides (GÖRANSSON and OLIVECRONA, 1964, 1965; GÖRANSSON, 1965 a, b). In *Gammarus Roeselii* there is no such difference between phospholipids and triglyce-

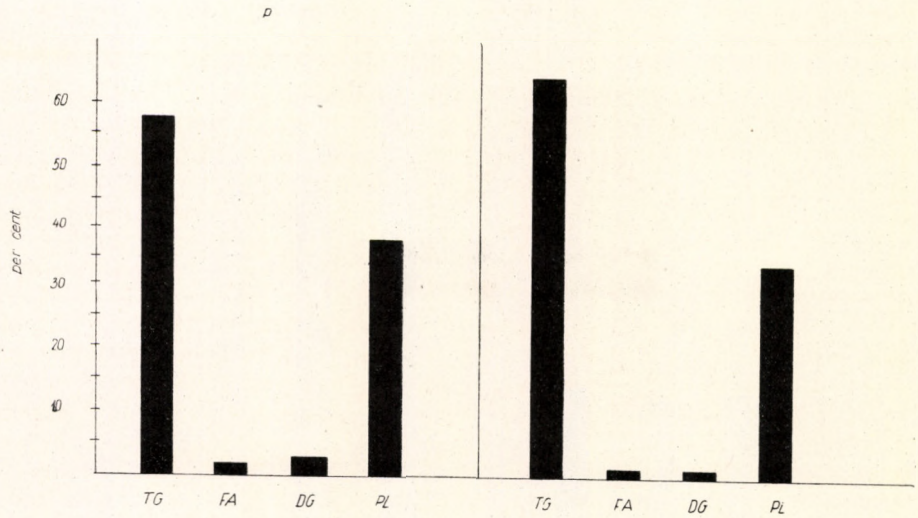

Figure 1. The distribution of radioactivity in the lipid fractions of palmitic acid-1-^{14}C and of stearic acid-1-^{14}C fed crustacenas.
Left = palmitic acid-1-^{14}C fed animals; Right = stearic acid-1-^{14}C fed animals; TG = triglycerides; FA = free fatty acids; DG = diglycerides; PL = phospholipids

TABLE 1

Fatty acid composition of the total lipid, triglycerides and phospholipids

	14 : 0	14 : 1	15 : 0	15 : 1	16 : 0	16 : 1	16 : 2	17 : 0	18 : 0
Total lipids	2.2	1.3	1.4	2.0	11.9	5.5	4.2	2.2	2.9
Triglycerides	1.4	1.2	1.0	2.0	12.2	8.5	4.6	2.1	2.8
Phospholipids	2.8	1.6	2.0	2.2	10.6	5.1	4.6	2.0	3.1

	18 : 1	18 : 2	18 : 3	18 : 4	20 : 1	20 : 4	20 : 5	22 : 5	22 : 6
Total lipids	25.8	15.3	11.0	2.5	1.4	2.6	4.0	0.4	3.4
Triglycerides	27.6	16.9	13.5	2.5	1.6	0.7	1.0	0.1	0.3
Phospholipids	19.8	13.3	8.0	1.9	1.3	5.8	8.7	1.2	6.0

The number before the colon indicates the number of carbonic atoms, that after the colon the number of double bonds.

rides. In the palmitic acid-1-^{14}C fed crustaceans 18 per cent of the radioactivity of triglycerides and 20 per cent of that of phospholipids, in the stearic acid-1-^{14}C fed crustaceans 63 per cent of the radioactivity of triglycerides and 69 per cent of that of phospholipids were in the monoenoic fatty acids *(Fig. 2.)* As compared to mammals there is more palmitoleic and much less stearic acid in the phospholipids of *Gammarus Roeselii*, therefore the saturated to monoenoic acid ratio of triglycerides and phospholipids does not differ so much in this animal.

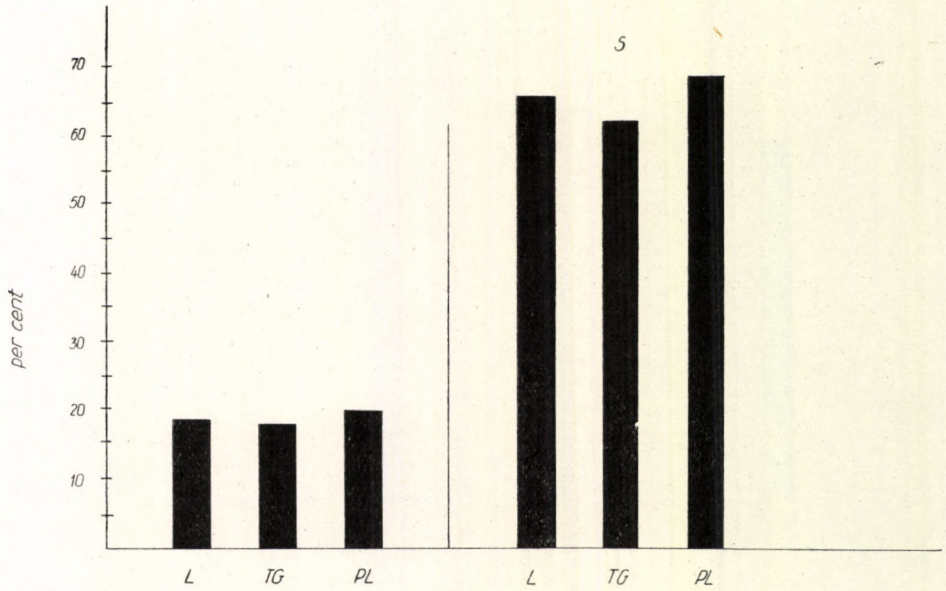

Figure 2. The radioactivity of monoenoic acids in per cent of the radioactivity of total fatty acids. L = total lipids, other abbreviations as in *Fig. 1*

The nutrition of *Gammarus Roeselii* was in detail studied by PONYI (1956, 1959). This crustacean chews with its strong mandibuls the green and decaying plants like caterpillars. Decayed plants contain a lot of cellulose. According to PONYI (1959) these animals, presumably due to their symbionts can break down cellulose. Kept only on filter-paper these crustaceans chewed it like the decaying leaves and survived longer than the control group fasting without filter-paper. This experiment gave the idea to feed crustaceans with labeled fatty acid impregnated filter-paper. Accordingly saturated and monoenoic fatty acids can be formed also from the glucose originating in cellulose. On the other hand *Gammarus Roeselii* similar to Vertebrates and Insects is not able to synthetise linoleic and linolenic acids, these compounds are taken up from green plants. The origin of highly unsaturated fatty acids of 20, 22 carbonic atoms is more problematic. These fatty acids are to be found mainly in the phospholipids, as demonstrated for several Crustacea species by FARKAS (1970 a, b). They are absent in higher plants, but may originate in epibiotic algae. It is also possible that crustaceans desaturate and elongate linoleic and linolenic acids in the way described for mammals (MEAD, 1960; KLENK and MOHRHAUER, 1960). Further feeding experiments with labeled linoleic and linolenic acids could decide whether this process exists also in crustaceans or not.

Summary

Crustaceans were fed on labeled fatty acid impregnated filter-paper for two days and on their natural food for further two days.

In crustaceans fed on palmitic acid-1-^{14}C 58 per cent of the radioactivity was incorporated into the triglycerides and 38 per cent into the phospholipids.

In the stearic acid-1-^{14}C fed group 64 per cent radioactivity was in the triglycerides and 34 per cent in the phospholipids.

In *Gammarus Roeselii* 19 per cent of the palmitic acid-1-^{14}C and 66 per cent of the stearic acid-1-^{14}C was transformed to monoenoic fatty acids.The ratio of radioactivity of saturated to monoenoic acids was similar in triglycerides and phospholipids

In the polyenoic fatty acids there was no measurable radioactivity.

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A PALMITINSAV-1-^{14}C ÉS SZTEARINSAV-1-^{14}C ÁTALAKULÁSA A GAMMARUS (RIVULOGAMMARUS) ROESELII Gervais AMPHIPODA RÁKBAN

Herodek Sándor

Összefoglalás

A rákokat két napig jelzett zsírsavval átitatott szűrőpapírral, majd még két napig a természetes táplálékukkal etettük.

A palmitinsav-1-^{14}C-vel etetett rákokban az aktivitás 58%-a a trigliceridekben, 38%-a a foszfolipidekben volt. A sztearinsav-1-^{14}C-vel etetett rákokban az aktivitás 64%-a volt trigliceridekben és 34%-a foszfolipidekben.

A palmitinsav-1-^{14}C-nek 19, a sztearinsav-1-^{14}C-nek 66%-a alakult át egyszer telítetlen zsírsavvá. A telített és monoen zsírsavak radioatkivitásának aránya a trigliceridekben és foszfolipidekben hasonló volt.

ПРЕОБРАЗОВАНИЕ I—C^{14}-ПАЛЬМИТИНОВОЙ И I—C^{14}-СТЕАРИНОВОЙ КИСЛОТЫ У РАКА *Gammarus (Rivulogammarus) Roselii Gervais* Amphipoda

Ш. Херодек

Раков кормили в течение двух суток бумагой, пропытанной меченной жирной кислотой, потом ещё в течение двух суток натуральным кормом.

У раков кормленных I-C^{14} -пальмитиновой кислотой, 58% активности было в триглицеридах, 38% в фосфолипидах. У раков, кормленных I-C^{14}-стеариновой кислотой 64% активности было в триглицеридах и 34% в фосфолипидах.

19% I-C^{14}-пальмитиновой кислоты, 66% I-C^{14}-стеариновой кислоты превращались в жирные кислоты с одной ненасыщеной связью. Соотношение радиоактивности в триглицеридах и фосфолипидах, насыщенных и ненасыщенных кислот было подобное.

ANNAL. BIOL. TIHANY | 37 | 183—197 | HUNGARIA 1970

DATA TO THE KNOWLEDGE OF COMPOSITION OF THE MOST FREQUENTLY OCCURRING REED-GRASS SPECIES IN LAKE BALATON

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Received: 17th March, 1970

During the synecological analyses of the reed-grass vegetation in Lake Balaton we wish to gain detailed information on the quantitative and qualitative conditions of the phytomass production. The present paper is to add data to the composition of the most frequently occurring reed-grass species in Lake Balaton. A carefully planned survey will subsequently follow our present preliminary collaction of data. We wish to gain a comprehensive picture about the organic material production of the reed-grass vegetation (Potametea) in the constant water surfaces of Lake Balaton, together with the shore-zonal reedery (Phragmition) and high sedge (Magnocarition) with respect to individual annual periods.

Material and method

The reed-grass samples used in the investigation were collected at the same time (1. X. 1968) from Lake Balaton *(Table* 1*)*. After washing in distilled water, the samples were dried at 60 °C, then the desiccated matter was groun to powder.

The compositional characteristics were determined by the following methods:

1. Determination of water and dry matter content was carried out in two phases:
   (a) Organic matter (after drying) = dry matter — raw ash
   (b) Extractable matter free of nitrogen = 100 — (water content + raw protein) + raw fat + raw fibre + raw ash
2. Determination of raw ash was carried out according to the pattern number MSZ6, B30.
3. Determination of raw fat by the Soxhlet method (extrahaling).
4. Determination of materials containing nitrogen:
   (a) Raw protein (total matter containing nitrogen), the determination was carried out according to the WAGNER-PARNASS microkjeldahl method
   (b) Digestible raw protein percentage was obtained by calculation.
5. Raw fibre (lignin) content was determined by the HENNEBERG—STOKMANN method.
6. Starch values were obtained by calculation.

Our investigations, in all cases, were carried out in accordance with the foraging standards in order to enable anyone to compare our data to those already published in Hungarian technical literature directly.

TABLE 1

Collecting data of read-grass samples

Serial N°	Note book N°	Species	Association	Collecting sites	Date of collecting
1	23	*Ceratophyllum submersum*	*Ceratophylletum submersi*	+ Fadd	1968. X. 1.
2	25	,, ,,	,, ,,	Balatonfüred	1968. X. 1.
3	35	,, ,,	,, ,,	Tihany	1968. X. 1.
4	38	,, ,,	,, ,,	Badacsonytomaj	1968. X. 1.
5	39	,, ,,	,, ,,	Vonyarcvashegy	1968. VIII. 30.
6	40	,, ,,	,, ,,	Badacsonytomaj	1968. X. 1.
7	63	,, ,,	,, ,,	Balatonfüred	1968. X. 1.
8	6	*Chara phoetida*	*Lemno-Utricularietum charetosum*	Tihany	1968. X. 1.
9	10	,, ,,	,, ,,	Balatonfüred	1968. X. 1.
10	14	,, ,,	,, ,,	Vonyarcvashegy	1968. X. 1.
11	19	,, ,,	,, ,,	Badacsonytomaj	1968. X. 1.
12	60	,, ,,	,, ,,	Vonyarcvashegy	1968. X. 1.
13	3	*Hydrocharis morsus-ranae*	*Hydrochari-Stratiotetum typicum*	Balatonfüred	1968. X. 1.
14	21	,, ,,	,, ,,	Balatonfüred	1968. X. 1.
15	58	,, ,,	,, ,,	Tihany	1968. X. 1.
16	17	*Lemna minor*	*Lemno-Utricularietum typicum*	Vonyarcvashegy	1968. X. 1.
17	2	*Lemna trisulca*	*Lemno-Utricularietum*	Balatonfüred	1968. X. 1.
18	37	,, ,,	lemnetosum trisulcae	Balatonfüred	1968. X. 1.
19	4	*Myriophyllum spicatum*	*Myriophyllo-Potametum*	Balatonfüred	1968. X. 1.
20	15	,, ,,	myriophylletosum spicati	Vonyarcvashegy, Kápolna	1968. X. 1.
21	16	,, ,,	,, ,,	Tihany	1968. X. 1.
22	26	,, ,,	,, ,,	Badacsonytomaj	1968. X. 1.

23	28	„ „	„ „	Vonyarcvashegy, Kápolna	1968. X. 1.
24	31	„ „	„ „	Vonyarcvashegy, Kápolna	1968. X. 1.
25	34	„ „	„ „	+Fadd	1968.
26	49	„ „	„ „	+Hanság	1968. XI. 13.
27	57	„ „	„ „	Tihany	1968. X. 1.
28	62	„ „	„ „	Balatonfüred	1968. X. 1.
29	29	*Najas marina*	*Myriophyllo-Potametum*	Tihany	1968. X. 1.
30	32	„ „	„	Badacsonytomaj	1968. X. 1.
31	30	*Nuphar luteum*	*Nymphaeetum albo-luteae*	Vonyarcvashegy	1968. X. 1.
32	55	*Potamogeton crispus*	*Myriophyllo-Potametum*	Tihany	1968. X. 1.
33	20	*Potamogeton pectinatus* ssp. *balatonicus*	*Myriophyllo-Potametum potametosum balatonici*	Badacsontomaj	1968. X. 1.
34	27	„ „ „ „	„ „	Tihany	1968. X. 1.
35	33	„ „ „ „	*Myriophyllo-Potametum potametosum balatonici*	Vonyarcvashegy, Kápolna	1968. X. 1.
36	36	*Potamogeton pectinatus* ssp. *balatonicus*	„ „	Tihany	1968. X. 1.
37	52	„ „ „ „	*Myriophyllo-Potametum potametosum perfoliati*	Badacsonytomaj	1968. X. 1.
38	9	*Potamogeton perfoliatus*	„ „	Badacsonytomaj	1968. X. 1.
39	53	„ „	*Hydrochari-Stratiotetum stratiotetosum*	Vonyarcvashegy	1968. VIII. 30.
40	54	„ „	„ „	Tihany	1968. X. 1.
41	5	*Stratiotes alloides*	„ „	Balatonfüred	1968. X. 1.
42	7	„ „	„ „	Balatonfüred	1968. X. 1.
43	11	„ „	„ „	Balatonfüred	1968. X. 1.
44	24	„ „	*Trapetum natantis*	Vonyarcvashegy, Kápolna	1968. X. 1.
45	56	*Trapa natans*	*Lemno-Utricularietum*	Tihany	1968. X. 1.
46	1	*Utricularia vulgaris*			

Coenotaxonomic survey of the reed-grass stands at the collecting sites

The coenotaxonomic conditions of the reed-grass vegetation in Lake Balaton are quite well known from the detailed works of BORBÁS, V (1900), Soó R. (1928, 1934, 1936, 1947, 1948), also Soó's Manual I, II and III (1964, 1966, 1968), and from the result of our investigations (KÁRPÁTI, I. and Mrs. KÁRPÁTI, I. 1967a, 1967b, 1967c, 1968). We have taken our material for investigation from these well defined associations. The significant associations with respect to sampling are presented with a view to coenotaxonomy:

A. Lemno-Potamea Soó, 1968

a. Hydrochari-Lemnetea
I. Hydrocharietalia Rübel 33
Lemnion minoris KOCH et Tx. 54
1. *Salvinio-Spirodeletum* SAVNIC 56
2. *Lemnetum minoris* RÜBEL 12
a) typicum
b) lemnetosum trisulcae
c) charetosum
Hyrocharition (VIERHAPPER) RÜBEL 33
3. *Lemno-utricularietum* Soó 28
a) lemnetosum minoris
b) charetosum
4. *Hydrochari-Stratitetum* (LANGENDONCK 35) WESTHOFF 42

b. Potametea Ty. et Prsg. 42
II. Potametalia W. Koch 26
Potamion eurosibiricum W. KOCH 26. (p.p.) Vlieger 37
5. *Anacharietum canadensis* (PIGN.) Soó
6. *Myriophyllo-Potametum* Soó 34
a) potametosum perfoliati
b) potametosum balatonici
c) potametosum crispi
d) myriophylletosum spicati
e) myriophylletosum verticillatii
Nymphaeion (Oberd. 56) Soó 64
7. *Polygono-Potametum natantis* Soó 64
a) polygonetosum amphibii
b) potametosum natantis
8. *Nymphaeetum albo-luteae* NOWINSKY 28
a) namphaeetosum
b) nupharetosum
9. *Trapetum natantis* MÜLLER-GÖRS 60
trapetosum natantis
potametosum perfoliati
10. *Ceratophylletum submersi* KÁRPÁTI I. et V. 68
ceratophylletosum demersi
potametosum balatonici

B. Cypero-Phragmitea Soó 68
c. Phragmitetea Tx. et Persg. 42

III. Phragmitetalia W. Koch 26

Phragmition communis W. Koch 26
11. *Scirpo-Phragmitetum* W. Koch 26
a) phragmitetosum
b) schoenoplectetosum
c) typhetosum
d) glycerietosum
e) phalaridetosum

Discussion of results

a) *Nutritive value of reed-grass elements*

Tables 2 and *3 give* the composition and nutritive values of reed-grasses. The nutritive material content has been expressed by the starch value calculated by Kellner, accepted and currently in use in Hungary. The utilization coefficients of individual nutritive materials were obtained on the basis of Sudan grass *(Sorgum vulgare sudanense)* from the standard No. MSZ 6890, for no coefficient numbers have as yet been calculated for reed-grass species, and because the chemical composition of reed-grass species is very similar to this arable land foraging plant.

The dry matter content of the reed-grass species fluctuated between 7.94 and 12.33%. The lowest dry matter content was measured (7.94—8.22%) in *Ceratphyllum submersum* samples, while the highest values were obtained for *Lemna trisulca* (11.98—12.33%) and *Lemna minor* (12.16%).

With respect to organic matter content the most favourable result was obtained with *Nuphar luteum* (9.17%), while the lowest value was shown in *Chara phoetida* (4.38%). The highest and lowest amount of ash residue (5.05% and 1.14%) were gained with *Myriophyllum spicatum*. Concerning raw protein content the lowest and the peak values were between 0.53 and 2.38%. The highest protein content was shown by *Myriophyllum spicatum*, while the lowest values were obtained with *Potamogeton pectinatus* ssp. *balatonicus*.

Among the nutritive materials the lowest values were displayed by raw fats (0.03—0.15%). The raw fibre content of reed-grass species fluctuated between 0.66—3.96%. The quantity of the extractable matter free of nitrogen varied between 0.58 and 5.24%.

The nutritive material quantity expressed in starch values fluctuated between 3.18 and 7.90 kg/g. The lowest nutritive values were displayed by *Chara phoetida*, while the highest values were shown by *Myriophyllum spicatum*. With respect to digestible raw protein the smallest value was yielded by *Potamogeton perfoliatus* (0.47%), while the highest value was given by *Myriophyllum spicatum* (1.83%) *(Table 1)*.

In summarizing the results we established that both in chemical composition and nutritive values great variations are perceiveable between species and even within the individuals of the same species. The most favourable nutritive material content (expressed in starch values) is shown in *Lemna minor*

TABLE 2

1000 g analised material contains

Serial N	Note-book	Species	Dry matter %	Organic matter %
1	23	*Ceratophyllum submersum*	8.19	5.06
2	25	,, ,,	8.07	6.49
3	35	,, ,,	8.16	5.29
4	38	,, ,,	8.22	5.90
5	39	,, ,,	8.00	6.26
6	40	,, ,,	8.15	5.52
7	63	,, ,,	7.94	6.03
		average	8.10	5.79
8	6	*Chara phoetida*	8.50	4.43
9	10	,, ,,	8.54	4.39
10	14	,, ,,	8.52	4.59
11	19	,, ,,	8.49	4.38
12	60	,, ,,	8.50	4.61
		average	8.51	4.48
13	3	*Hydroharis morsus-ranae*	8.17	5.34
14	21	,, ,,	8.03	5.42
		average	8.10	5.38
15	17	*Lemna minor*	12.16	8.94
16	2	*Lemna trisulca*	11.98	8.40
17	37	,, ,,	12.33	7.94
		average	12.15	8.17
18	15	*Myriophyllum spicatum*	10.77	6.50
19	16	,, ,,	10.65	5.85
20	26	,, ,,	10.64	6.58
21	31	,, ,,	10.86	6.48
22	34	,, ,,	9.91	8.77
23	49	,, ,,	10.53	8.25
24	57	,, ,,	10.73	5.68
		average	10.58	6.87
25	29	*Najas marina*	10.56	7.64
26	32	,, ,, average	10.55	8.12
		average	10.55	7.88
27	30	*Nuphar luteum*	10.71	9.17
28	55	*Potamogeton crispus*	10.65	6.94
29	20	*Potamogeton pectinatus* ssp. *balatonicus*	10.41	7.14
30	27	,, ,, ,, ,,	10.39	7.65
31	33	,, ,, ,, ,,	10.49	7.56
32	36	,, ,, ,, ,,	10.19	7.56
33	52	,, ,, ,, ,,	10.30	7.48
		average	10.35	7.47
34	9	*Potamogeton perfoliatus*	10.28	6.84
35	53	,, ,,	10.09	8.02
36	54	,, ,,	10.49	6.46
		average	10.28	7.10
37	5	*Stratiotes aloides*	10.62	6.96
38	17	,, ,,	10.24	7.93
39	11	,, ,,	10.63	6.34
40	24	,, ,,	10.55	6.89
		average	10.51	7.03
41	56	*Trapa natans*	10.21	7.70

calculated for original dry matter

Ash %	Raw protein %	Raw fat %	Raw firre %	NMK %	Starch value %	Digestible raw protein, %
3.13	2.09	0.06	2.33	0.58	3.70	1.61
1.58	1.97	0.08	1.52	2.92	4.72	1.51
2.93	1.02	0.06	1.88	2.33	3.84	0.79
2.32	0.59	0.06	1.88	3.37	4.27	0.45
1.74	1.12	0.07	1.49	3.58	3.63	0.86
2.63	0.74	0.05	1.47	3.26	3.98	0.57
1.91	1.52	0.06	2.34	2.11	4.39	1.17
2.32	1.29	0.06	1.84	2.59	4.07	0.99
4.07	0.90	0.06	1.89	1.58	3.23	0.69
4.15	0.74	0.03	0.66	2.96	3.18	0.50
3.93	0.80	0.03	1.72	2.04	3.32	0.62
4.10	0.75	0.04	2.61	0.98	3.20	0.58
3.89	0.78	0.03	2.96	0.84	2.82	0.60
4.02	0.79	0.03	1.96	1.68	3.15	0.59
2.83	1.07	0.05	1.54	2.68	3.86	0.82
2.61	0.99	0.08	1.17	3.18	3.94	0.76
2.72	1.03	0.06	1.35	2.93	3.90	0.79
3.22	1.55	0.09	2.90	4.40	7.55	1.19
3.58	1.65	0.15	2.75	3.85	7.90	1.27
4.39	1.78	0.08	3.32	2.76	5.78	1.37
3.98	1.71	0.11	3.03	3.30	6.84	1.32
4.27	1.10	0.05	2.87	2.48	4.68	0.85
4.80	2.38	0.07	1.53	1.87	5.13	1.83
4.06	0.84	0.08	2.41	3.25	4.78	0.65
4.38	1.14	0.05	3.65	1.64	4.72	0.88
1.14	1.91	0.13	1.55	5.18	7.90	1.47
2.28	1.44	0.08	1.49	5.24	5.95	1.10
5.05	1.07	0.06	2.67	1.88	4.13	0.82
3.71	1.27	0.07	2.31	3.07	5.32	1.08
2.92	1.17	0.09	3.15	3.24	5.56	0.90
2.43	1.20	0.10	2.77	4.05	5.90	0.92
3.67	1.18	0.09	2.96	3.64	5.73	0.91
1.54	1.14	0.10	2.68	5.25	6.65	0.88
3.71	1.13	0.08	2.76	2.97	3.25	0.87
3.27	0.84	0.08	2.80	3.42	5.19	0.65
2.74	1.27	0.11	3.04	3.23	5.56	0.98
2.93	0.82	0.07	2.77	3.90	5.47	0.63
2.63	1.02	0.08	2.54	3.92	5.62	0.92
2.82	1.36	0.08	3.00	3.04	5.44	1.04
2.87	1.06	0.08	2.83	3.50	5.45	0.84
3.44	0.53	0.07	3.96	2.27	4.97	0.41
2.07	1.01	0.11	3.60	3.30	5.84	0.78
4.03	1.34	0.08	1.45	3.59	4.69	1.03
3.18	0.96	0.08	3.00	3.05	5.16	0.74
3.66	2.23	0.07	2.49	2.17	5.10	1.71
2.31	1.39	0.08	2.75	3.71	5.76	1.07
4.29	1.00	0.07	2.68	2.59	4.61	0.77
3.66	1.20	0.07	2.95	2.68	5.00	0.92
3.48	1.45	0.07	2.71	2.78	5.11	1.11
2.51	1.15	0.09	1.70	4.76	5.56	0.89

TABLE 3

1000 g analysed material contains

Serial No	Note-book	Species	Dry matter %	Organic matter %
1	23	*Ceratophyllum submersum*	100.00	61.78
2	25	,, ,,	100.00	40.43
3	35	,, ,,	100.00	64.10
4	38	,, ,,	100.00	71.78
5	39	,, ,,	100.00	78.25
6	40	,, ,,	100.00	67.74
7	63	,, ,,	100.00	75.95
		average	100.00	71.43
8	6	*Chara phoetida*	100.00	52.11
9	10	,, ,,	100.00	51.40
10	14	,, ,,	100.00	53.87
11	19	,, ,,	100.00	51.59
12	60	,, ,,	100.00	54.24
		average	100.00	52.64
13	3	*Hydrocharis morsus-ranae*	100.00	65.36
14	21	,, ,,	100.00	67.49
		,, ,, average	100.00	66.42
15	17	*Lemna minor*	100.00	73.51
16	2	*Lemna trisulca*	100.00	70.12
17	37	,, ,,	100.00	57.51
		average	100.00	63.81
18	15	*Myriophyllum spicatum*	100.00	60.35
19	16	,, ,,	100.00	54.92
20	26	,, ,,	100.00	61.85
21	31	,, ,,	100.00	59.67
22	34	,, ,,	100.00	88.38
23	49	,, ,,	100.00	78.35
24	57	,, ,,	100.00	52.94
		average	100.00	65.20
25	29	*Najas marina*	100.00	72.35
26	32	,, ,,	100.00	76.97
		average	100.00	74.66
27	30	*Nuphar luteum*	100.00	85.63
28	55	*Potamogeton crispus*	100.00	65.17
29	20	*Potamogeton pectinatus* ssp. *balatonicus*	100.00	68.58
30	27	,, ,, ,, ,,	100.00	73.63
31	33	,, ,, ,, ,,	100.00	72.07
32	36	,, ,, ,, ,,	100.00	74.20
33	52	,, ,, ,, ,,	100.00	72.63
		average	100.00	72.22
34	9	*Potamogeton perfoliatus*	100.00	66.53
35	53	,, ,,	100.00	79.49
36	54	,, ,,	100.00	61.59
		average	100.00	69.20
37	5	*Stratiotes aloides*	100.00	65.53
38	7	,, ,,	100.00	77.44
39	11	,, ,,	100.00	59.64
40	24	,, ,,	100.00	66.97
		average	100.00	65.30
41	56	*Trapa natans*	100.00	75.42

calculated for absolute dry matter

Ash %	Raw protein %	Raw fat %	Raw fibre %	NMK	Strach value kg/g	Digestible protein, %
38.22	25.51	0.73	28.45	7.08	45.17	19.65
19.57	24.41	0.99	18.87	36.10	58.48	18.71
35.90	12.50	0.73	23.03	28.55	47.05	9.68
28.22	7.17	0.73	22.87	40.99	51.94	5.47
21.75	14.00	0.87	18.62	44.75	45.37	10.75
32.26	9.07	0.61	18.03	40.00	48.83	6.99
24.05	19.14	0.76	29.47	26.57	55.28	14.73
28.56	15.97	0.77	22.76	32.00	50.30	12.28
47.89	10.58	0.71	22.23	18.58	38.00	8.11
48.60	8.66	0.39	7.73	34.66	37.23	6.55
46.13	9.38	0.37	20.18	23.94	38.96	7.27
48.41	8.83	0.47	30.74	11.54	37.69	6.83
45.76	9.17	0.35	34.82	9.88	33.17	7.05
47.35	9.32	0.45	23.14	19.72	37.01	7.16
34.64	13.09	0.67	18.84	32.80	47.24	10.03
32.51	12.32	0.99	14.57	39.60	49.06	9.46
33.57	12.70	0.83	16.70	36.20	48.15	9.74
26.49	12.14	0.74	23.84	36.18	62.08	9.78
29.88	13.77	1.25	22.95	32.14	65.94	10.60
42.49	14.44	0.65	26.93	22.38	46.88	11.11
36.18	14.10	0.95	24.94	27.26	56.41	10.85
39.65	10.21	0.46	26.64	23.02	43.45	7.89
45.08	22.35	0.66	14.36	17.55	48.16	17.18
38.15	7.89	0.75	22.65	30.54	44.92	6.11
40.33	10.49	0.46	33.61	15.10	43.46	8.10
11.62	19.27	1.32	15.80	52.80	79.71	14.83
21.65	13.67	0.76	14.14	49.76	56.50	10.44
47.06	9.97	0.56	24.88	17.52	38.48	7.64
34.79	13.40	0.71	21.72	29.47	50.66	10.31
27.65	11.07	0.84	29.83	30.68	52.65	8.52
23.03	11.37	0.95	26.26	38.39	55.92	8.72
25.34	11.22	0.89	28.04	34.53	54.28	8.62
14.37	10.64	0.93	25.02	49.01	62.09	8.22
34.83	10.61	0.75	25.91	27.88	30.51	8.17
31.42	8.06	0.76	26.89	32.85	49.85	6.24
26.37	12.22	1.06	29.26	31.09	53.51	9.43
27.93	7.81	0.67	26.41	37.18	52.14	6.00
25.80	10.00	0.78	24.93	38.47	55.15	9.03
27.37	13.20	0.78	29.12	29.51	52.81	10.09
27.77	10.25	0.88	27.32	33.82	52.69	8.15
33.67	5.15	0.68	38.52	22.08	48.35	3.98
20.51	10.01	1.09	35.67	32.70	57.87	7.73
38.41	12.77	7.62	13.82	34.22	44.71	9.82
30.86	9.31	3.13	29.33	29.66	50.38	7.17
34.47	20.99	0.65	23.44	20.43	48.02	16.10
22.56	13.57	0.78	26.85	36.23	56.25	10.44
40.36	9.40	0.66	25.21	24.36	43.37	7.24
34.70	11.37	0.69	27.96	25.40	47.39	8.72
33.02	13.83	0.69	25.86	26.60	48.75	10.62
24.58	11.26	0.88	16.65	46.03	54.45	8.72

(7.55 kg/g), followed in order of sequence by *Lemna trisulca* (6.84 kg/g), *Nuphar luteum* (6.65 kg/g). The nutritive values of the rest of the reed-grass species was below 6.0 kg/g calculated for starch values. The highest value for the digestible raw protein content was found in *Lemna trisulca* (1.32%),while therest followed in order of sequence *Lemna minor* (1.19%), *Stratoides aloides)* (1.11%), *Myriophyllum spicatum* (1.08%). The digestible raw protein content of the rest of the species never reached 1.0% *(Table 2).*

The chemical composition and nutritive material values of reed-grass species were calculated for absolute (100%) dry matter in order to eliminate the differences caused by dry matter content and to obtain more favourable basis for comparison. The absolute dry matter included 52.64—85.63% organic matter. The lowest values were displayed by *Chara phoetida*, while the highest ones by *Nuphar luteum*. Concerning ash content, the lowest yield was given by *Nuphar luteum* (14.37%), while highest yield was obtained with *Chara phoetida* (47—37%). The most favourable result with respect to raw protein was obtained with *Ceratophyllum submersum* (15.97%), while *Pomatogeton perfoliatus* showed a mere 9.31% of raw protein. The highest raw fibre content was yielded in *Naja marina* (28.04%), while the lowest value was shown by *Trapa natans* (16.65%). The extractable matter free of nitrogen fluctuated between 19—72 and 49.01%. With respect to nutritive material content we found the following two limiting values 30.51 and 62.09 kg/g; at the same time the digestible raw protein content fluctuated between 6.00 and 19.65%. Considering the mean values of nutritive material content in eight species we found a value more than 50.0 kg/g for starch value *(Lemna minor, Trapa natans, Potamogeton perfoliatus, Nuphar luteum, Najas marina, Ceratophyllum submersum, Lemna trisulca, Potamogeton pectinatus).*

Only in four species have we found a value above 10.0% for digestible raw protein content *(Myriophyllum spicatus, Stratiotes aloides, Ceratophyllm submersum, Lemna trisulca).*

On the basis of our investigations we came to the conclusion that the nutritive value of reed-grass species is favourable, and under appropriate and economic exploitation suitable for foraging of domestic animals. However, we must point out that our data as yet are informatory in nature, for the exact determination of nutritive material content as now expressed in starch value will need extensive investigations in the future. That will render possible that the nutritive value of reed-g species be comparable to arable land fodder plants, in order to decide the bes method of utilization.

b) *Mineral material content of reed-grass elements*

During the evaluation of phytomass production of reed-grass species we endeavoured to obtain data throwing light on the mineral material conten too. We wrote a comprehensive study on the manganese content of *Potametea* elements (KÁRPÁTI I., KÁRPÁTI V. and TÖLGYESI, 1967), but we also have useful data at our disposal concerning the characteristics of macro- and microelement conditions of individual plant families (MODOR and TÖLGYESI, 1964; MÓCSY and TÖLGYESI, 1960; TÖLGYESI, 1962, 1963, 1965a, b, c, 1968). As it has been indicated in our paper, the well-nigh 5,000 data of the 44 profoundly investigated plant families unanimously support that the taxa included in Potamion associations have a very high Mn content *(Table 5).* At

TABLE 4

Mineral material content of some reed-grass species compared with that of fodder plants
(After: TÖLGYESI GY. 1965)

Species	CaO	Na	P_2O_5	Fe	Mn	Zn	Cu
	g/kg				mg/kg		
Potamogeton natans	37	11	7	690	1400	81	5
Potamogeton pectinatus	26	10	6	880	1800	47	6
Ceratophyllum demersum	36	7	9	1400	5000	140	8
Myriophyllum spicatum	62	12	4	1300	2400	65	5
Trapa natans	35	5	4	450	700	68	6
Hydrocharis morsus-ranae	12	9	8	1300	4000	100	7
Lemna minor	58	7	12	440	3100	110	16
Lemna trisulca	30	7	6	3000	7000	50	9
Hordeum vulgare (grain)	1	0.2	6	80	20	16	5
Zea mays (grain)	1	0.1	5	60	15	16	3
Medicago sativa (hay)	20	0.4	6	240	50	30	10
Meadow-hay	10	0.5	5	160	80	25	8

TABLE 5

Mineral material content of some plant families
After: TÖLGYESI GY. — *Acta Agr. Hung.* (1965) 287—301.

Pant family	No. of species	Sample No.	CaO	P_2O_5	Fe	Mn	Zn	Su
			g/kg		ppm			
Gramineae	98	466	3.7	4.2	179	71	28	5.6
Compositae	74	243	14.2	5.9	329	57	31	11.4
Papilionaceae	49	194	15.6	4.7	196	50	25	9.1
Salicaceae	18	117	17.0	4.8	149	103	85	7.7
Cyperaceae	41	109	4.8	4.9	273	199	24	2.0
Rosaceae	24	105	15.8	4.3	201	69	28	7.7
Labiatae	27	72	15.3	5.8	439	50	33	11.2
Cruciferae	27	71	14.0	6.4	270	46	27	5.3
Fagaceae	7	65	13.6	3.7	162	454	25	7.7
Ranunculaceae	19	64	15.5	6.2	176	51	35	9.8
Betulaceae	4	58	19.5	3.1	218	432	32	8.1
Scrophulariaceae	28	53	11.8	5.0	323	60	28	7.7
Caryophilaceae	18	49	11.7	5.7	281	128	32	6.7
Polygonaceae	14	48	10.2	5.4	277	81	28	6.6
Boraginaceae	18	46	20.6	6.1	372	66	27	11.7
Oleaceae	7	46	14.5	3.2	140	46	24	6.5
Rubiaceae	12	44	15.4	5.5	293	52	27	7.5
Aceraceae	6	44	15.0	5.1	181	94	27	7.0
Liliaceae	16	40	10.2	5.8	143	46	32	7.2
Juncaceae	13	32	3.0	3.6	210	141	24	6.6
Euphorbiaceae	8	25	16.9	6.1	183	109	32	8.4
Umbelliferae	14	24	17.4	5.9	196	47	29	8.9
Solanaceae	6	23	15.7	7.7	299	57	25	14.0
Plantaginaceae	5	22	15.2	5.4	261	38	31	9.4
Chenopodiaceae	9	20	12.7	5.5	270	55	46	7.1
Hydrocharidaceae	3	20	15.7	7.2	1300	496	108	5.3
Zosteraceae	7	20	35.6	6.0	875	1160	80	5.6

TABLE 6

Manganese content of some reed-grass species

(After KÁRPÁTI I.—KÁRPÁTI V.—TÖLGYESI GY. 1967)

Species	Collecting site	Depth of water	Association	Mn mg/kg
CHAROPHYTA				
Chara phoetida	Tatai-tó, Réti	60	Homogen-cous stand	1 320
NYMPHACEAE				
Nuphar luteum	Ludányhalászi, Ipoly	80—100	Nymphaeetum albo-luteae	2 760
N. luteum	Drégelypalánk, Ipoly	80—100	Nymphaeetum albo-luteae	1 960
N. luteum	Sérfenyősziget, Duna-holtág	100—150	Nymphaeetum albo-luteae	300
N. luteum	Sérfenyősziget, Kis-Duna	50—80	Nymphaeetum albo-luteae	420
CERATOPHYLLACEAE				
Ceratophyllum demersum .	Mosonmagyaróvár, Fekete-erdő, Duna-holtág	50—150	Myriophyllo-Potametum	10 600
C. demersum	Dunaharaszti	100—120	Myriophyllo-Pot2metum	3 000
C. demersum	Tatai-tó, Réti	60—80	Myriophyllo-Potametum	2 160
C. demersum	Velencei-tó	200—300	Myriophyllo-Potametum	450
HALORAGACEAE				
Myriophyllum spicatum .	Velencei-tó	200—300	Myriophyllo-Potametum	480
M. spicatum	Dunaharaszti	100—120	Myriophyllo-Potametum	2 470
M. spicatum	Tatai-tó, Cseke	100	Myriophyllo-Potametum	980
M. spicatum	Drégelypalánk, Ipoly	40—60	Myriophyllo-Potametum	8 400
M. spicatum	Öskü-Séd		Myriophyllo-Potametum	405
TRAPACEAE				
Trapa natans	Dunaharaszti	200—250	Trapetum natantis	1 100
T. natans	Szarvas, Bikazug	200—300	Trapetum natantis	620
T. natans	Taksony	180—190	Trapetum natantis	360
T. natans	Hercegszántó	200—300	Trapetum natantis	305
LENTIBULARIACEAE				
Utricularia vulgaris	Karapancsa, Ferenc-csatorna	200—300	Lemno-Utricularietum	1 090
U. vulgaris	Veresegyház	70	Lemno-Utricularietum	2 040
U. vulgaris	Karapancsa, Duna-holtág	100	Lemno-Utricularietum	4 580
U. vulgaris	Velencei-tó	50—150	Lemno-Utricularietum	303
HYDROCHARITACEAE				
Hydrocharis morsus-ranae	Kiskunlacháza	60—70	Hydrochari-Stratiotetum	1 100
H. morsus-ranae	Szarvas, Bikazug	200—300	Hydrochari-Stratiotetum	895
H. morsus-ranae	Ipolyszög	150—180	Hydrochari-Stratiotetum	5 200
H. morsus-ranae	Velencei-tó	50—100	Lemno-Utricularietum	625

Species	Collecting site	Depth of water	Association	Mn mg/kg
ZOSTERACEAE incl. POTAMOGETONACEAE				
Potamogeton pectinatus ..	Tata, Fényesfürdő	60—80	Myriophyllo-Potametum	840
P. pectinatus	Drégelypalánk	60	Myriophyllo-Potametum	2 350
P. pectinatus	Tatai-tó, Réti	60—80	Myriophyllo-Potametum	276
P. pectinatus	Soroksár, Duna-holtág	50	Myriophyllo-Potametum	1 320
P. pectinatus	Velencei-tó	100—200	Myriophyllo-Potametum	240
Potamogeton perfoliatus ..	Keszthely		Myriophyllo-Potametum	600
P. perfoliatus	Magyaróvár, Fekete-erdő	50—100	Myriophyllo-Potametum	336
P. perfoliatus	Bankháza	100—150	Myriophyllo-Potametum	385
Potamogeton crispus	Veresegyház	200	Myriophyllo-Potametum	1 510
P. crispus	Dunaharaszti	100—120	Myriophyllo-Potametum	1 150
P. crispus	Ludányhalászi, Ipoly	80—100	Myriophyllo-Potametum	1 200
NAJADACEAE				
Najas minor	Szarvas, Káka	30—50	Myriophyllo-Potametum	1 100
Najas marina	Velencei-tó	200—300	Myriophyllo-Potametum	285
N. marina	Hercegszántó, Ferenc-csatorna	200—300	Myriophyllo-Potametum	620
LEMNACEAE				
Lemna triculca	Veresegyházá hz	70	Lemno-Utricularietum	16 100
L. trisulca	Drégelyplánk	40—50	Lemno-Utricularietum	9 680
L. trisulca	Ipolyszög, Ipoly	80—100	Lemno-Utricularietum	34 600
Lemna minor	Tatai-tó, Réti	30	Lemno-Utricularietum	1 190
L. minor	Karapancsa, Duna-holtág	200—280	Lemno-Utricularietum	9 000

the same time, in the specific elements of Rosaceaea, Fabaceae, Umbelliferae, Runiaceae, Labiatae, Solanaceae, etc. reealy acclimatized to dry land habitats manganese occur as an average between 50 and 70 mg/kg. The problem is discussed in more detail in our previously mentioned paper (KÁRPÁTI, I., KÁRPÁTI, V. and TÖLGYESI, 1967) providing ample space both for ecological and phylogenetical references.

The principle just indicated does not only appear in the relation of Mn content but we can also see from the comparison of systematical taxa (TÖLGYESI, 1965 b, c) that both the macro- and microelements occur in greater quantities in the reed-grass elements in plant species living under dry land conditions.

Especially their manganese, sodium, iron, and zinc contents are greater than many of the plant species living on the dry land. The calcium oxide content is equivalent to the highest limit of known dry land fodder plants In phosphorus and copper content no significant difference may be observed between them and that of the plants living on dry land *(Tables 4* and *5)*.

The data on hand reveal that this is not the outcome of macro- and microelement quantity of habitats. It can rather be explained by the better

absorbing and concentrating capacity of aqueous plants although, we must bear in mind that given habitats with prevailing conditions play a sgnifican role in the accumulation of mineral material of the individual plant species.

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ADATOK A BALATONBAN ELTERJEDTEBB HÍNÁRFAJOK BELTARTALMÁNAK ISMERETÉHEZ

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A balatoni hínárvegetáció fitobiomassza-produkciójának vizsgálata során szükségessé vált a termelt zöld- és száraz növénytömeg kémiai jellemzőinek megismerése. Elemzéseinket a takarmányozástani gyakorlatban elterjedt módszerekkel végeztük el, a tóban legnagyobb tömeget adó fajok vonatkozásában.

Elemeztük a következő jellemzőket:

a) *hínárelemek táplálóértéke:* nyersfehérje, nyerszsír, keményítőérték.

b) *hínárelemek ásványanyagtartalma:* különösképpen a mangán, nátrium, vas, cinktartalom vonatkozásában állapítható meg, hogy az lényegesen nagyobb mennyiségben fordul elő, mint a szárazföldi növényekben.

A vizsgálati eredmények *elméleti szempontból* hasznos támpontot nyújtanak a balatoni táplálékláncban lényeges jelentőségükre vonatkozóan.

Gyakorlati vetületében pedig a hazai nagy tömegű hínárprodukció esetleges takarmányozási hasznosításához nyújtanak vizsgálataink támpontot, adatokat.

ДАННЫЕ О ХИМИЧЕСКОМ СОСТАВЕ НАИБОЛЕЕ РАСПРОСТРАНЕННЫХ ВИДОВ ВОДОРОСЛЕЙ БАЛАТОНА

Карпати Иштванне и *Бедѐ Шандорне*

Знание химических характеристик зеленой и сухой массы водорослей необходимо для исследований фитобиопродукции Балатона. В данной работе наиболее рапространенные водоросли Балатона характеризировались методами, принятыми в кормоводстве. Определялись следующие показатели:

а) питательность водорослей (сырые белки, сырые жиры, крахмал),

б) содержание минералов. Установлено, что содержание в первую очередь, марганца, натрия, железа и цинка гораздо выше в водорослях, чем в наземных растениях. С теоретической точки зрения, данные полезны для понимания места растений в пищевой цепи Балатона. С точки зрения практики, полученные результаты дают информацию о возможностях испльзования богатых запасов водорослей в качестве кормов.

ANNAL. BIOL. TIHANY | 37 | 199—207 | HUNGARIA 1970

SHORT PERIODIC CHANGES IN THE MICROBAL PLANCTON QUANTITY OF LAKE BALATON

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Received: 18th February, 1970

The monthly investigations carried out in the years of 1966—1968 in five standard sections of Lake Balaton throw light on the complexity of seasonal changes occurring in the quantity of bactrioplankton. In the very same year in various sections, or in different years for the same section the seasonal dynamics rather varied. These data called the attention to the fact that in order to be acquainted with the seasonal dynamics of the quantity of bacterioplankton and with the factors exerting influence on it in the case of shallow waters of vast extension, as is the case in Lake Balaton, we must carry out short periodic investigations (OLÁH, 1969a, b).

On the basis of these conclusions we carried out short periodic investigations in the years of 1968—1969 at some points of the open water of Lake Balaton. During these seasonal investigations we carried out a series of measurements which noted the daily changes in the quantity of saprophytic and total microbial plankton, in wind condition, in Secchi-transparency in temperature and in COD. Besides surveying the quantity of bacterioplankton we paid due attention to the quantity of phyto- and zooplankton as well during our winter and early spring investigations, the former were taken under the ice cover.

Material and method

Our short periodic investigations were carried out in the open water, some 500 metres from the shoreline beside a buoy in front of our Research Institute. On the 8th—27th September, 1968, 5th—13th February, 1969, 8th—12th April, 28th April to 7th May and between 13th and 16th August generally on every day, in some cases with a few days interval. Between 5th and 13th February, 1969 we carried out parallel investigations with the open water examination in the reedery in front of Research Institute. Besides the sites enumerated above, in front of our Institute, we made two surveis in Keszthely-Bay, between the 24th Juny and 4th July, and between the 8th—27th September, on every day, on the standard G section (TAMÁS, 1967) at some 500 metres from the shoreline.

To determine the quantity of the total microbial plankton we made good use of RAMUZOV's (1932) direct method, while the determination of phytoplankton quantity was made by DE NOYELLES's (1968) method. The vertical distribution of the quantity of zooplankton was determined from the

filtrates secured by a 90—100 μ bronze sieve of 5—5 litre of water. To render easy counting of the filtrate we used a mixture of dyes according to DE NOYELLES made of analine blue and eosine Y. By this differential staining the rotifers, and both young and mature crustaceans and their females with eggs stained with the various hues of blue and red, and by the different colour tones they could be easily distinguished. In counting saprophytic microorganisms we used the sodium-caseinate agar; and sealing-was made in the following hour of sample taking. The saprophytes were counted in the section M by using OPPENHEIMER's (OPPENHEIMER an ZOBELL, 1952) medium. The chemical oxygen demand (COD) was determined by $KMnO_4$.

Results and discussion

In section G, on the second day of our investigation of short period changes, on the 19th September, 1968 a high wind disturbed the sediment so much that Secchi transparency decreased to 12 cm from 108 cm *(Fig. 1)*.

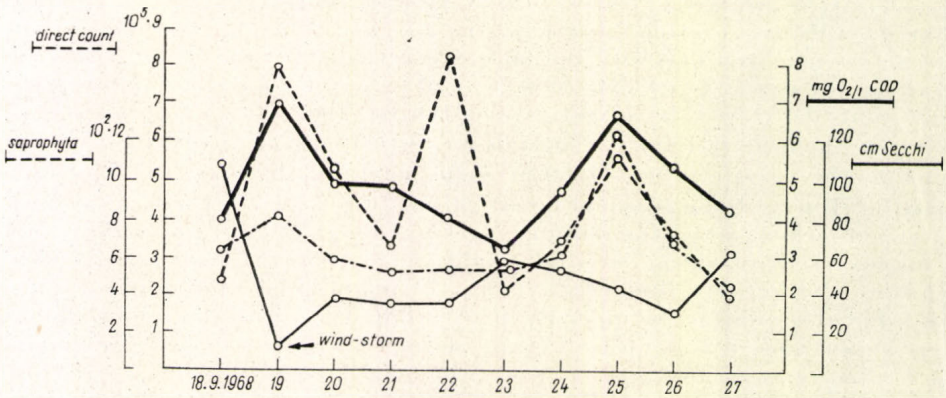

Fig. 1. Short periodic investigations in section "G" between 18th and 27th September, 1968

Subsequent to this the Secchi gradually increased until the 25th September, when again on the effect of gusts of wind it began to decrease. The COD of of the water in the 10-days period of investigation displayed an inverted value when compared to Secchi transparency values. On the 19th September, at the same time when the sediment was disturbed the COD values reached their peak, then as Secchi values increased they proportionately decreased, and on the 24th, when Secchi transparency decreased COD values again increased. The close relationship between the two factors seem to prove that due to the disturbance of water from the sediment a large amount of organic material passes into the water, especially at the site of investigation where the lake is very shallow. The quantity of saprophytic microorganisms is inversely proportional with Secchi transparency, while it is directly proportional with the organic material content. During the time investigation the quantity of total microbial plankton behaved similarly, only with the exception, that on the 22nd September we measured the highest of all, although at this time, Secchi transparency did not decrease and the chemical oxygen

demand did not increase either. All these facts apparently prove that with the disturbance of sediment, besides the great organic material increase in the water column, a significant quantity of bacteria passed from the sediment into the water. Furthermore, on the effect of the increased quantity of organic material content of the water column owing to the proliferation of bacteria even after the lag-phase the bacterium content of the water increases.

Simultaneously with the short periodic investigation of section G we carried out similar examinations in a site situated in front of our Researh Institute. On the 19th September, as the result of a very high wind Secchi values are extremely low here, too, but subsequently to this date they gradually increase, and the repeated decrease which was observed in section G from the 24th September, here, it was not observed *(Fig. 2)*. Accordingly

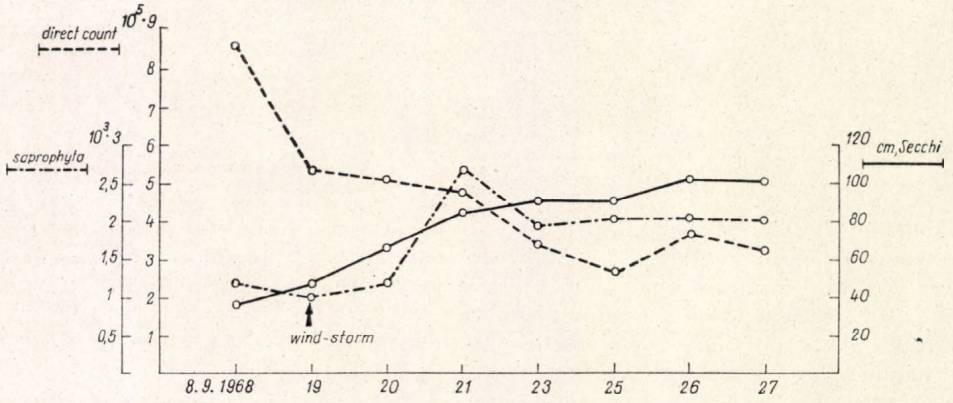

Fig. 2. Short periodic investigations in the open water in front of our Research Institute between 5th and 13th February, 1969

the quantity of the total microbial plankton simultaneously decreases as Secchi transparency increases, disregarding the slight increase which occured on the 26th September. However, the quantity of saprophytic microorganisms subsequent to the storm reach its maximum only after a two-days lag-phase, which is undoubtably the result of an intensive proliferation initiated by the agitation of mud occuring in the water.

Between the 5th and 12th February, 1969 we carried out investigations under the ice in front of our Research Institute. During the whole time of our investigation Secchi transparency was very large (270 cm) of the respective water column *(Fig. 3)*. The quantity of phytoplankton in the different water layers was rather significant $0.9-2.0 \cdot 10^4$/ml, and this value suffered no major changes. The stock of phytoplankton almost exclusively consisted of 4—5 μ big Chlamydomonas, which stains blue with the DE NOYELLES method, accompanied by a few Diatoms and other flagellates. The winter stock of phytoplankton, secured from under the ice, whose larger part comprised the tiny Chlamydomonas belong to μ-algae (RODHE, 1955; LUND, 1961; PENNAK, 1968). It is a well-known fact, that the μ-algae especially in the oligotrophic lakes are significant in the winter plankton (RODHE, 1955; PENNAK, 1968). According to LUND (1961) the more oligotrophic a lake the more important is the role of μ-algae in it. In the water examined by the above

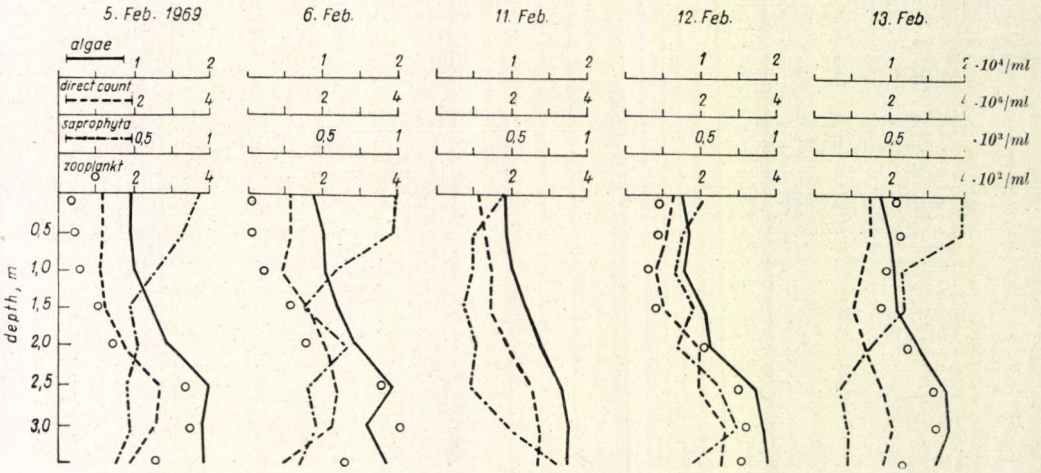

Fig. 3. Vertical short periodic investigations in the open water in front of our Research Institute between 5th and 13th February, 1969

mentioned authors the quantity of μ-algae was the largest during the time of their development 11—14.5 · 10^6/l. In Lake Balaton, during the investigated period the volume of the μ-alga population in the surface layers (0—1.5 m) 11 · 10^6, while in the bottom water (2.5—3.5 m) 18—18 · 10^6/l yielded good values. This particular, inverted stratification is also characteristic for the μ-algae.

The vertical distribution of the total microbial plankton was similar to that of phytoplankton. In the bottom water, the values surpassing 2 · 10^6/ml during the whole period of investigation hardly ever changed. In the surface layers the quantity of the total microbial plankton was 1—1.5 · 10^6/ml. The majority of the stock comprised a coccus form whose size was below 1 μ, which did not develop on the applied sodium-caseinate agar. The fact, that the distribution of this form was similar with the distribution of μ-algae refers to the relation of these two organisms. In spite of the large number of μ-algae and the great quantity of the total microbial plankton biomass the number of saprophytic organisms was low, and their vertical distribution did not follow that of the former. Generally, it was in the surface and bottom layers where maximum were reached.

The large biomass of nutritional organisms brought with it the formation of a significant zooplankton stock, which mainly consisted of the cold-loving *Cyclops vicinus* ULJ. and *Eudiaptomus gracilis* (G. O. SARS), accompanied by a few examples of *Daphnia hyalina* var. *galeata* (G. O. SARS) species. The majority of the stock comprised juvenile exemplars and females with eggs. The zooplankton primarily concentrated in the warmer bottom water layers rich in bacteria and μ-algae.

All this indicates that during winter, in the water of Lake Balaton covered by ice a stabile stratification occurs, thus, the role of short periodic changes is smaller. The strikingly high number of winter total microbial plankton in our opinion is connected with the mass proliferation of μ-algae. The great proliferation of μ-algae observed during the time of investigation is quite

unknown in Lake Balaton. This may be explained, partly by the lack of winter examinations, and partly by the fact, that the membrane filter suitable for the counting of μ-algae was not in use in the algological investigations of Lake Balaton. The generally used Utermöhl technique and the microscope are not suitable for the quantitative determination of μ-algae (RODHE, 1955; BERNHADR et al. 1967). Parallel with the open water investigations carried out under the ice cover, we did short periodic examinations in the reedery in front of our Research Institute *(Table 1)*.

TABLE 1

Time of investigation

	February, 5th	6th	11th	12th	13th
Phytoplankton 10^3 specimens/ml ...	2.1	2.2	2.8	2.1	1.9
Total microbial plankton, 10^6 specimens/ml	1.19	1.18	1.05	0.98	1.05
Saprophytes 10^3 specimens/ml	2.3	2.4	1.5	1.9	2.4
Zooplankton specimens/ml	110	90	84	98	110

The mass proliferation of μ-algae in the open water was completely lacking from the reedery. The phytoplankton stock which is significantly smaller than that of the open water consisted in Diatoms and spores with thick cell walls, and cysts. The quantity of the total microbial plankton was likewise smaller than in the open water, and the period under investigation showed no significant quantitative difference. On the contrary, the number of saprophytic microorganisms in the reedery was more than 10 times higher. The coccus form, smaller than one micron, was dominant in the open water was not found in the reedery, and the large-sized filament forms are dominant in the plankton. At any rate, in comparing the food supply of the open water and the water in the reedery, the latter was worse, which is not favourable for the formation of a large zooplankton stock in the shallow, cold-water reedery.

The daily investigations carried out between the 8th and 12th April showed no stabile vertical stratification as was observed in the winter under the ice cover *(Fig. 4)*. On the 9th April Secchi transparency decreased from 100 cm to 60 cm and consequently, the vertical distribution in the examined period continuously changed. During the investigation, the total microbial plankton and phytoplankton quantity displayed no significant variation, contrary to this, the quantity of saprophytic organisms increased in the total water mass two days after the decrease in Secchi transparency. Pronounced quantitative change occured with regard to the state in February, under the ice cover. The quantity of phytoplankton decreased, from among the 4—5 μ Chlamydomonas species only examplars existed, and the majority of algae was represented by Diatoms. Like it happened with phytoplankton, the quantity of the total microbial plankton decreased from $2 \cdot 10^6$/ml counted in February to $0.1 \cdot 10^6$/ml. The quantity of saprophytes compared to the state under ice, hardly changed.

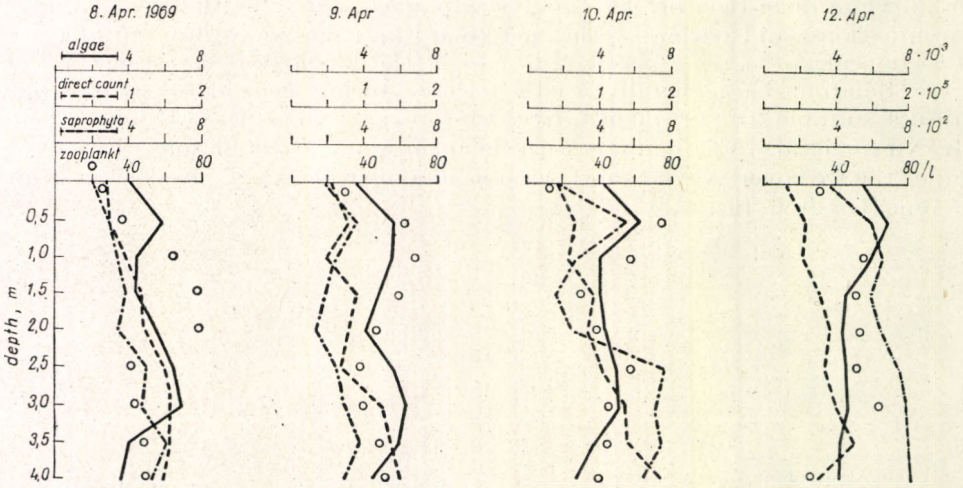

Fig. 4. Vertical short periodic investigations in the open water in front of our Research Institute between 8th and 12th April, 1969

Between the 28th April, 1969 and the 7th May with the increase in water temperature during the examined short period the quantity of the total microbial plankton increased more than four times of its initial value *(Fig. 5)*, and taking the state observed between the 8th and 12th April as basis, this change was ten times bigger. By the increase in temperature the number of saprophytic organisms increased, too. Between the 13th and 16th August *(Fig. 5)* with a high water temperature the quantity of the total microbial plankton, during the whole period of investigation, was around $0.5 \cdot 10^5$/ml, the number of saprophytes also decreased (200/ml). No significant short periodic change occured. These results indicate that on the effect of sediment disturbance besides the changes occurring in Secchi transparency and COD, the rapidly changing water temperature also may cause the significant short periodic changes of the saprophytic and total microbial plankton.

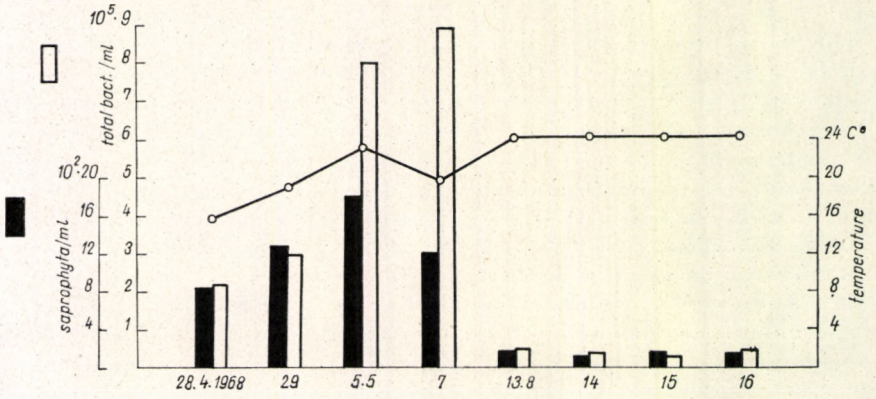

Fig. 5. Short periodic investigations in the open water in front of our Research Institute between 28th April and 16th August, 1969

The short periodic investigations carried out between the 18th and 27th September, 1969 on section G and in the open water stretching in front of our Research Institute *(Figs. 1* and *2)*, indicate that in the different basins of Lake Balaton the order of magnitude of the short periodic changes is not identical. In a section of Lake Balaton, which is the richest in nutritive materials, in Keszthely-Bay, our short periodic investigations also prove this *(Table 2)*.

TABLE 2

Time of investigation

	21st May	24th June	27th June	4th July
Secchi, cm	72	25	64	52
Total microbial plankton 10^5 specimens per ml	5.2	6.7	9.2	6.8
Saprophytes 10^2 specimens per ml	1.9	3.2	4.4	1.3
Water temperature °C	16	21	22	24.5

On the 24th June as the result of a very high wind Secchi transparency decreased to less than its half. The effect of the disturbance in the sediment occurred only three days later with a significant measure. The number of the saprophytic organisms is higher as the result of organic materials getting into the water from the sediment, this number at times attained the hundredfold of its initial value. The organic material which was introduced into the water medium was quickly consumed and by the 4th July the number of saprophytes was again rather low. The very fast proliferation of the saprophytic organisms and their rapid decrease in number prove that the sediment of Keszthely-Bay contains a large quantity of organic material and that the water possesses an intensive self-purifying capacity. All these emphasize that the sediment of the lake, whose water is frequently disturbed, has a close relationship with the water.

In comparing the data of the short periodic examinations with the investigational result obtained by recent monthly analysis (Oláh, 1969a, b), it becomes clear that in the seasonal dynamics of the microbial plancton of Lake Balaton the spring and summer maximum and the summer-late summer minimum seem to be the most significant values, which besides Lake Balaton, are also applicable to the similarly shallow Velence Lake of large water area (Oláh and Vásárhelyi, 1970). We need further data to generalize the significant maximum formed during winter time.

Summary

1. The short periodic changes of the saprophytic and total microbial plancton varied according to seasons: (a) in winter, under the ice the short periodic change was not significant; (b) in spring, with a temperature rise a significant short periodic change was observed even without a decrease in Secchi transparency (c) while in summer, with a decrease in Secchi transparency

in Keszthely-Bay the number of saprophytes increased by several order of magnitude, the, without a Secchi decrease we measured no significant short periodic change; (d) in autumn, the sudden decrease in transparency brought about pronounced short periodic changes. Consequently, we attached importance in the short periodic changes to temperature and to the wind disturbing the sediment.

2. In winter, under the ice we found a stabile stratification and in the bottom layer the quantity of the total mikrobial plankton ($2 \cdot 10^6$/ml) surpassed the values measured in spring, summer and autumn. The high number of bacteria bears a close connection with the winter formation of the μ-algal stock exceeding even $19 \cdot 10^6$/l. All these conditions made possible the development of a significant stock of zooplankton. The mass proliferation of μ-algae in the reedery was lacking and the quantity of the total microbial plankton was likewise smaller, consequently, the number of zooplankton is also low.

3. The seasonal short periodic investigations, beside the first description of high winter values, support the previously observed spring maximum an summer-late summer minimum in the seasonal dynamics.

Acknowledgmenet

Author is indebted to RÉKA VÁSÁRHELYI who was kind enough to supply me with the data of examination carried out on section G (18th—27th September, 1968); to Dr. J. PONYI, head of department for determining crustaceans and for his invaluable criticism while this paper was under preparation; and to Dr. GIZELLA TAMÁS for her altruistic help in identifying the various species of algae.

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RÖVIDPERIÓDUSÚ VÁLTOZÁSOK A BALATON MIKROBIÁLIS PLANKTON MENNYISÉGÉBEN

Oláh János

Összefoglalás

1. A szaprofita és teljes mikrobiális plankton rövidperiódusú változásai évszakonként különbözőek: *a)* télen, jég alatt nem mértünk jelentős rövidperiódusú változást; *b)* tavasszal a hőmérséklet emelkedésével Secchi átlátszóság változás nélkül is jelentős rövidperiódusú változás volt; *c)* nyáron Secchi átlátszóság változással a Keszthelyi-öbölben több nagyságrenddel változott a szaprofiták száma Secchi változás nélkül nem mértünk jelentős rövidperiódusú változást; *d)* ősszel az átlátszóság hirtelen változása jelentős rövidperiódusú változásokat eredményezett. A rövidperiódusú változásban a hőmérséklet és az üledéket felkavaró szél játszanak fontos szerepet.

2. Télen, jég alatt stabil rétegezettséget találtunk és az alzati rétegben a $2 \cdot 10^6$/ml-t elérő teljes mikrobiális plankton mennyiség a tavaszi, nyári és őszi értékeket is meghaladta. A magas baktériumszám a $19 \cdot 10^6$/l-es nagyságot is meghaladó μ-alga állomány téli kifejlődésével kapcsolatos. Mindez jelentős zooplankton állomány kialakulását tette lehetővé. Az μ-algák tömeges elszaporodása a nádasban hiányzott, és a teljes mikrobiális plankton mennyisége is kisebb, ennek megfelelően a zooplankton szám is alacsonyabb volt.

3. Az évszakonkénti rövidperiódusú vizsgálatok a magas téli értékek első leírása mellett megerősítik a szezonális dinamikában korábban megfigyelt tavaszi maximumot és a nyári-nyárvégi minimumot.

КОРОТКИЕ ПЕРИОДИЧЕСКИЕ ИЗМЕНЕНИЯ В КОЛИЧЕСТВЕ МИКРОПЛАНКТОНА БАЛАТОНА

Я. Олах

1. Короткие периодические изменения сапрофитного и тотального микробного планктона проявляют сезонную изменичивость: *а)* в подлёдных условиях зимы периоди ческие изменения незначительны; *б)* с ростом темперетуры весной они проявляют значительные размеры даже в отсутствие снижения показателя прозрачности Secchi; *в)* летом, одноаременно со снижением показателя прозрачности Secchi в Кестхейском заливе, число сипрофитов увеличивается на несколько порядков, тогда как в отсутствие снижения показателя Secchi мы не наблюдали заметных которких периодических изменений; *г)* осенью резкое снижение прозразности находится в связы с выраженными короткими периодическими изменениями. Для коротких периодических изменений представляются важными температура и ветер, вызывающий взмучивание осадков.

2. В подлёдных условиях зимы обнаруживается устойчивая стратификация, когда в придонном слое количество тотального микробного планктона ($2{,}10^6$/млЗ) превышает значения, отмеченные весной, летом и осенью. Высокое число бактерий коррелирует с распределением мелких хламиномонад (μ-algae), количество которых зимой в придонном слое превышает $19 \cdot 10^6$ л. Все эти условия делают возможным развитие значительных запасов зоопланктона. В тростниковых зарослях массового развития μ-algae не наблюдали, и, соответственно, количество тотального микробного и зоопланктона было меньше.

3. Исследование коротких периодических изменений, а также первое описание высоких зимних значений, подтверждают прежние наблюдения относительно весеннего и максимума летного-позднелетнего минимума в сезонной динамике.

ANNAL. BIOL. TIHANY | 37 | 209—222 | HUNGARIA 1970

MEASUREMENT OF THE REDUCING ABILITY OF NATURAL WATERS AND SEDIMENTS: A SIMPLE LIMNOLOGICAL METHOD

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Received: 20th February, 1970

The first redox measurements were carried out in limnology by KARSINKIN and KUZNETSOV (1932), and eversince this first attempt redox potential came into the foreground of interest (KUZNETSOV, 1935; PEARSAL and MORTIMER, 1939; HUTCHINSON et al. 1939; DEEVEY, 1941; ALLGEIER et al. 1941). The classical work of MORTIMER (1941—1942) once again called the attention of researchers to the redox processes (ZOBELL, 1946; HAYES et al. 1958; GORHAM, 1958) instead of pondering upon certain material migrations occurring on the borderline of mud and water, and to some relationships existing between the former and certain redox changes.

At this time, it has been pointed out by HAYES and his collaborators (1958) that in carrying out redox measurements unexpected difficulties may arise, quite recently STUMM (1967) passed severe criticism as to the practicability of directly measured redox potential in natural, mixed systems. Contrary to this, at the same time, BORCHARD (1967) and WAGNER (1967) state that the directly measured redox potential within a natural, mixed system yields valuable and practicable information. RABOTNAVA (1957) gives a very detailed analysis on the properties of the redox potential of biological objects, and establishes that it differs in many respects from the properties of the conception used in chemical sciences. WHITFIELD (1969) considers the problems arising in connection with redox measurements from the point of view of a limnologist, and in order to describe the distribution of reduced and oxidized sediments with good efficiency he uses the following "operational parameter" E_h.

Consequently, it is reasonable and important at the same time to know and carry out measurements as regards to the momentary redox state of waters and sediments from the point of view of limnology. This is supported by the ever increasing number of measurements carried out far and wide (DRABKOVA, 1966; ROMANENKO, 1966; MIHAYLENKO, 1967; PATRICK and TURNER, 1968; KJENSMO, 1968; WHITFIELD, 1969).

However, besides measuring the momentary redox state, we think that to measure the intensity of redox possesses occurring in natural waters and sediments are also deserve attention. In order to study the redox processes occurring in the Hungarian lakes with a great stretch of water and in shallow water the measurement of the reducing ability of water and mud is especially suitable, where owing to continuous atmospheric oxygen supply the momentary redox measurements are insufficient to survey the quantitative relationships of the process. We have found no reference in the literature as to the measurement and to the potential reduction ability of natural waters and

sediments, thus, its order of magnitude may only be calculated from the degree of oxygen consumption. Accordingly, we have elaborated for a direct measurement a simple new method, which is based on the redox changes of a closed system containing natural substrate.

Description of method

The oxygen has a very important role in establishing the redox state of natural waters and sediments as it generally has in the redox processes. The reducing ability of the examined water and sediment was measured during longer or shorter periods of incubation time depending on the sample by excluding the atmospheric oxygen supply and terminating the process of photosyntesis.

The graduated vessel *(Fig. 1)*, a 250 ml glass container of 6.5 cm diametre fitted with a ground glass or rubber stopper. The measuring and reference electrodes built into the stopper were immersed throughout the analysis into the liquid to be measured. By completely excluding oxygen diffusion we could not solve the problem of coupling the reference electrode's agar bridged or its simplified variety in the measuring space (KOVÁCS and MATKOVICS, 1954). We incubated our samples at 25 °C, at the dark parallels the measuring vessel has been covered with aluminium sheet or with thick black paper. The light parallel has been illuminated by 5000 Lux. The measuring vessel was filled excluding all bubbles and the samples were saturated with oxygen by bubbling through them air at 25 °C before filling up.

Throughout our investigations we used electrodes manufactured by Radelkis (Electrochemical Instruments, Budapest). The measuring electrode is a smooth platinum sheet with a surface area of 2×0.5 mm^2. It has been washed before application in cronic-sulphuric acid many times, followed by a careful rinsing in distilled water. The measuring electrodes were calibrate by ZOBELL's solution (1964). Our reference electrode was a saturated calomel electrode. The measurements were carried out in a Beckman GS-type pH

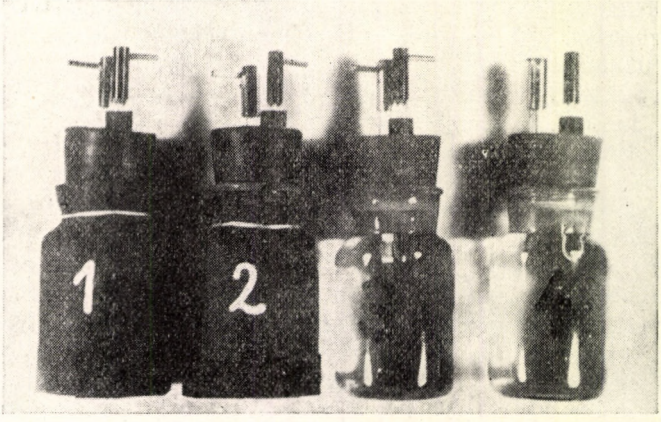

Fig. 1. Measuring vessels for incubation in darkness and in light

measurer and to the values thus obtained we added 250 mV and so the final values were given in E_h. According to the purpose of the analysis we carried out 1—6 measurements a day. When the measurement closely followed the setting up of the investigation quite frequently the significant drift greatly hindered read off, which, however, after a period of 120 min, but mostly after 20 min, ceased.

Working with closed systems the repeated measurements could be reproduced with an exactness of ± 10 mV.

Our investigations were carried out with sample waters and sediments from Lake Balaton and from the Inner Lake of Tihany. The water samples were taken by the help of FRANCEV's sampler (KUZNETSOV, 1962) from a depth of 50 cm disregarding vertical sampling, while the mud samples were taken partly by using the Ekman-Birge dredge, and partly by a mud borer. To determine the reducing ability of the sediment we placed some 50 g moist mud into the measuring vessel then at 25 °C oxygen-saturated lake water was layered on it. Then by differential filtration using Soviet and Oxoid filters (pore size: 100 μ, 6 μ, 0.5 μ) we were able measure separately the role of zoo-, phyto- and bacterioplanktons in the reducing ability of the lake.

The oxygen concentration during the investigation was determined by the Winkler method. The saprophytic microorganisms were counted on the sodium-caseinate agar for this medium proved to the most efficient in the case of water from Lake Balaton (OLÁH and VÁSÁRHELYI, 1970). The total quantity of microbial plankton was determined by RAMUZOV's direct method (1932) and likewise was the quantity of phytoplankton determined by the help of a membrane filter.

When the lake water was incubated in light the redox potential within the measuring vessel remained unchanged for quite a long period of time, or it increased *(Fig. 2)*. Even during a 42-day incubation, this was the longest, no decrease in redox potential was observed. Long lasting incubations generally brought about an increase in the redox potential of the sample, especially when the water sample was taken near the substrate. When the same samples were incubated in darkness — i.e. excluding photosynthesis — depending on the origin of the sample, after a longer or shorter period of time the redox potential decreased. This phenomenon may be called the darkness-induced reducing ability of the sample. The main characteristics of the curve obtained during incubation in darkness *(Fig. 2)*: time requirement until the decrease in redox potential (1); length of duration of the decrease itself (2); and the redox potential value characteristic for the state of equilibrium (3).

By excluding the continuous oxygen supply in the measuring vessel gradually results in the total consumption of oxygen *(Fig. 3)* which in turn set off further processes causing an even more pronounced decrease in redox potential set in (1), consequently depends primarily on the intensity of oxygen consuming processes. The significant decrease in redox potential coincides with the complete disappearance of oxygen (HUTCHINSON, 1957), thus, we obtain data as to the rate of oxygen consumption by observing the darkness-induced reducing ability, and furthermore, we may obtain information on the biochemical oxygen demand (BOD) of natural waters and sediments. By knowing the oxygen content of an oxygen saturated water at a given temperature and the volume of the measuring vessel we can calculate the quantity of the oxygen used up during a given period of time.

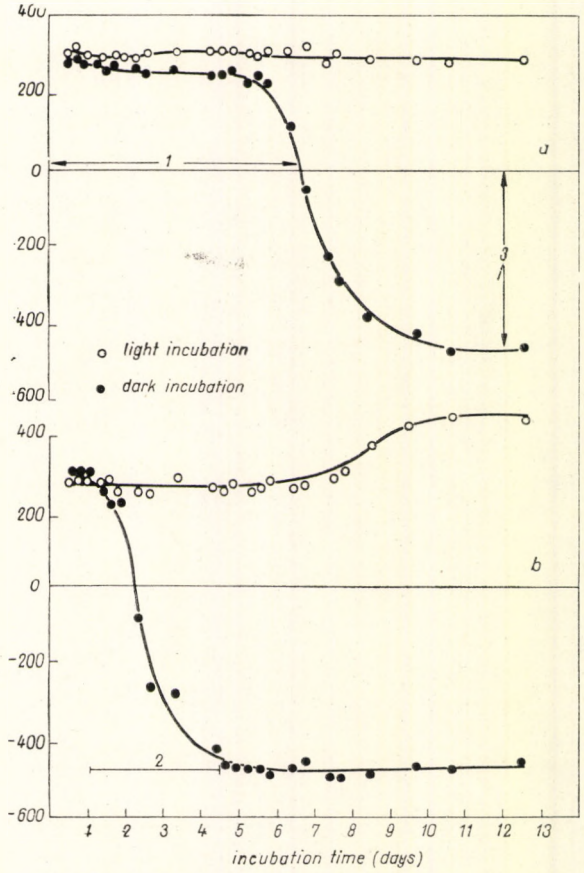

Fig. 2. Changes in redox potential during incubation in darkness and in light (a) surface water, (b) bottom water from the Inner Lake of Tihany

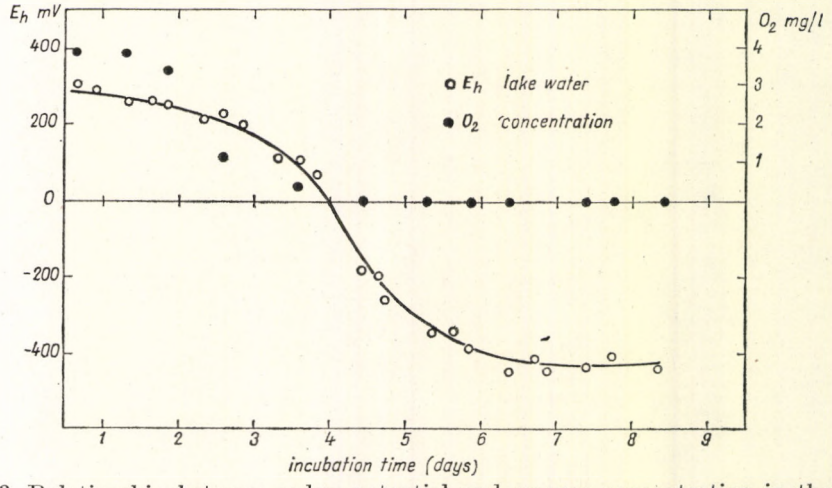

Fig. 3. Relationship between redox potential and oxygen concentration in the water of the Inner Lake of Tihany

The redox potential, however, independently from oxygen concentration, sufferes further changes under the influence of several other factors *(Fig. 3)*. From this fact it follows that by simply measuring the concentration of oxygen we do not get a picture true to reality as regards actual redox state. In the oxygen-free period according to the state of equilibrium the redox potential (3 at any rate depends on the special biological and chemical composition of the sample. The length of the decreasing part (2) on the curve, on the other hand, depends additionally on the change in oxygen concentration and on the chemical and biological composition of the sample.

Some typical experiments with the method and the interpretation of results

On the 23rd May, 1969 we measured the reducing ability in the water full of reed fragments in a reedery in Lake Balaton in front of our Research Institute. A decrease was observed in the redox potential in the lotic zone the open water after the 8th day, while this decrease occurred in the littoral, lenitic zone separated from the open water already on the 2nd day. Here, the

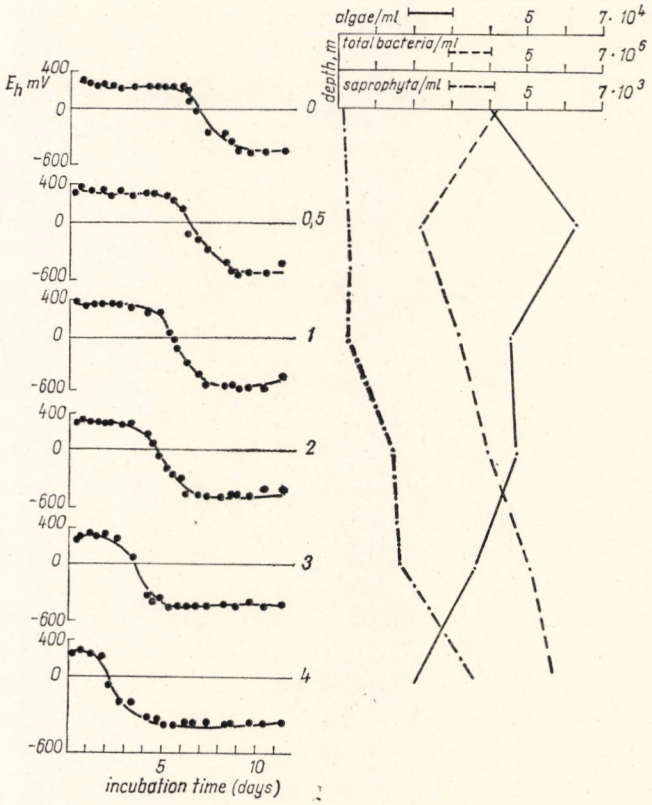

Fig. 4. Relationship between the reducing ability of various water layers and the vertical distribution of plankton in the Inner Lake of Tihany

value for the state of equilibrium was 300 mV, while for the lotic zone this value was only 450 mV. We found no significant difference in the reducing ability between the vertical samples originating from within the reedery and from the open water. A decrease in the redox potential was observed in the reedery on the 6th day, while in the open water on the 18th day.

On the 12th June, 1969, the reducing ability of the water in the strongly eutrophic Inner Lake of Tihany significantly differed verically, too *(Fig. 4)*. Redox potential decrease was measured in the bottom water on the 3rd day, while in the surface water on the 8th day. The time required for the decrease in redox potential from the surface water proceeding downward is gradually shortening by 1 day per metre. Parallel with this, the total quantity of microbial plankton gradually increases towards the bottom from $2 \cdot 10^6$/ml to $6 \cdot 10^6$/ml. The number of saprophytic organisms increased with a similar tendency from 200/ml to 3800/ml. On the contrary, the quantity of phytoplankton decreased toward the bottom. In the surface water the reducing ability is wellnight the same down to a depth of some 50 cm, it is probable that the sudden decrease in the microbial plankton at a depth of 50 cm is compensatedby the maximum number of phytoplankton existing here.

The darkness-induced reducin ability of the water from the Inner Lake of Tihany displayed marked changes according to seasons. The biggest was (4 days) on the 30th July and 3rd September, while the smallest was (9 days) on the 4th December (see *Fig. 5)*.

The vertical examination of the Inner Lake of Tihany proves that the reducing ability of water and sediment is primarily determined by the quantity and quality of living organisms. By differential filtering the effects of individual

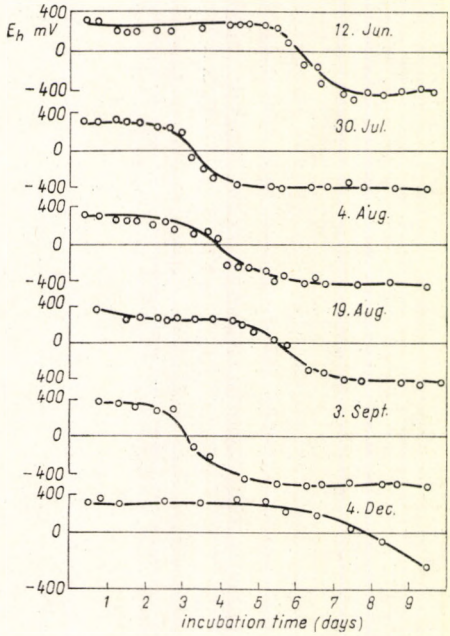

Fig. 5. Seasonal changes in the reducing ability of the water deriving from the Inner Lake of Tihany

components may well be separated. Significant difference was observed between the reducing ability of the filtered and unfiltered water of the Inner Lake of Tihany and that of Lake Balaton. The 6 μ filtering *(Fig. 6)* of the water of Lake Balaton shifted the decrease of the redox potential by a day and a half, and the so obtained state of equilibrium hardly showed any difference from

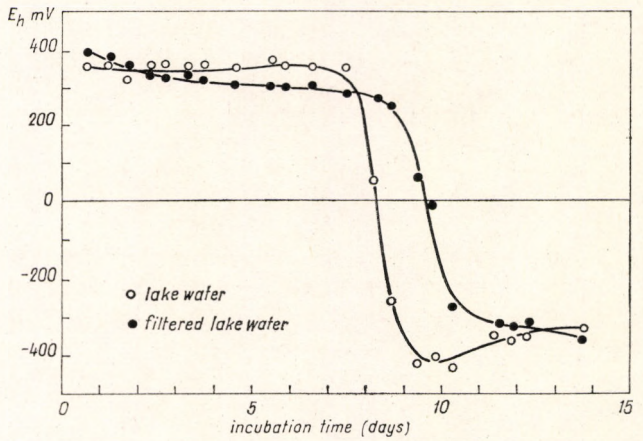

Fig. 6. Reducing ability of filtered and unfiltered water of Lake Balaton

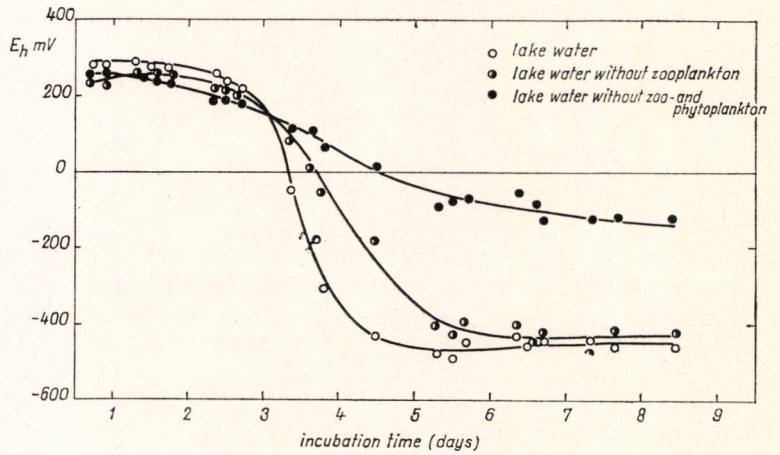

Fig. 7. Reducing ability of filtered and unfiltered water of the Inner Lake of Tihany

that of the unfiltered water. Examining the same in the Inner Lake of Tihany on the 30th July, 1969, the 6 μ filtering *(Fig. 7)* shidted the decrease of the redox potential by three days, on the other hand, the so acquired state of equilibrium was 300 mV more positive than that of the unfiltered water. The separation of zooplankton shifted the decrease of redox potential only by on day, and the so gained state of equilibrium hardly differed from the previous state. Consequently, in the case of the Inner Lake of Tihany the phytoplankton play an important role in the formation of redox potential corresponding to the oxygenfree equilibrial state.

On the 16th April, 1969, we measured the reducing ability of sediments coming from different depths from the reedery of Lake Balaton in front of our Research Institute. The best reducing ability was displayed by the upper, active layer containing the highest number of bacteria. The decrease in redox potential occurred already two days after incubation. A longer period of time is needed for the decrease in redox potential in deeper lying layers. This, however, does not increase parallel with depth. For example, the decrease requires three days in a layer lying at 12—15 cm deep, while this decrease is attained

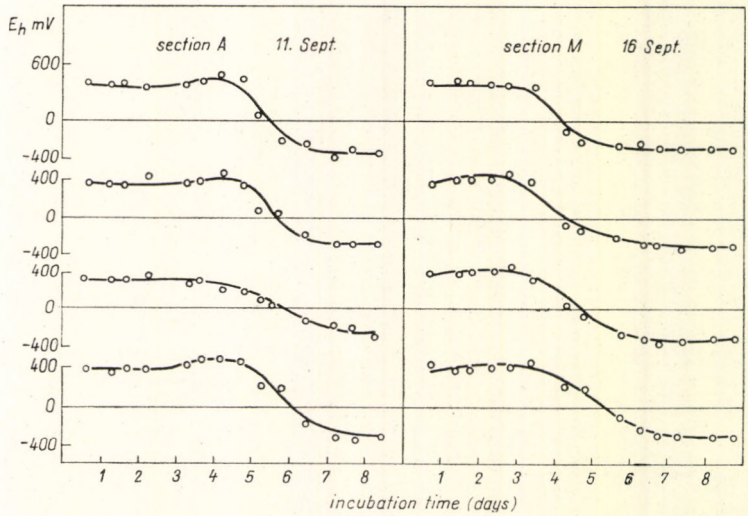

Fig. 8. Reducing ability of mud examined at 4 sites each of sections "A" and "M" (Lake Balaton)

only after six days at a depth of 9—12 cm. This indicates, that the reducing ability of the sediment in a reedery has a definite stratification. The reducing ability of the various layers increases parallel with the quantity of the particulate organic material. Thus, layers displaying a very strong reducing ability are at the same time the accumulational zones of reed detritus.

The reducing ability of the sediment is dependent to a great extent on the origin and age of the detritus. The reducing ability of the mature detritus (RODINA, 1964) of a sediment in a reedery, is greater than that of open-water sediment; at the same time, the reducing ability of young detritus originating from a reedery (RODINA, 1964) is smaller still than that of open-water sediment.

We analyzed the reducing ability of 5 standard sections of Lake Balaton (TAMÁS, 1967) on the 11th April, 1969 but found no great divergencies. A decrease in redox potential occurred the quickest in the sediment deriving from section M (Keszthely-Bay). On the 11th September, 1969, we took 4 samples each from sections M and A and measured the darkness-induced reducing ability of the sediment *(Fig. 8)*. The similarity in the measured reducing ability of sites lying closely to one another within one profile confirm the exactness of the method in investigating sediments. The time requirement for the decrease of redox potential in section M was 5 days, while the same for section A

was 7 days. Which prove the great reducing abilit yof the sediment deriving from Keszthely-Bay (section M).

The darkness-induced reducing ability of natural waters and sediments may be used in studying the processes of mineralization and by their help we are able to measure the influence of various materials exerted on redox potential. Under aerobe condition the mineralization of organic materials is proportional with oxygen consumption. From the quantity of consumed oxygen, and from the rate of consumption we may conclude as to the intensity of mineralization. On the other hand, under anaerobe condition the mineralization products exerting effect on the redox potential is dominant.

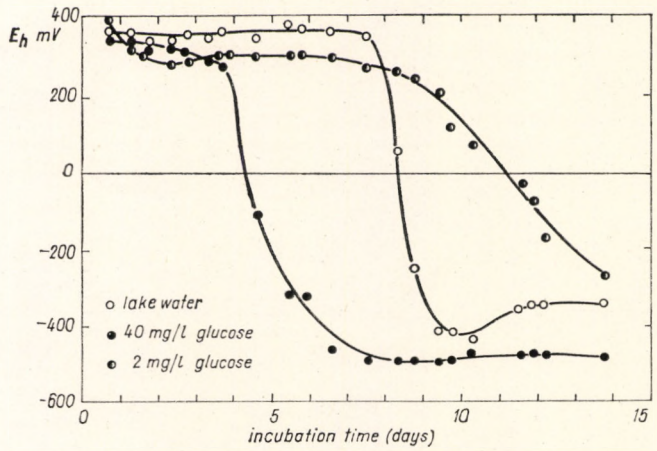

Fig. 9. Influence of glucose on the reducing ability of water deriving from Lake Balaton

When giving to the water of Lake Balaton a 40 mg/l end-concentration glucose solution *(Fig. 9)*, we find that the length of time required for a decrease in redox potential is 4 days shorter. Using a 250 ml measuring flask at 25 °C with a 100% saturation the consumation time of 2 mg oxygen decreases to its half. Carrying out the same experiment with the water of the Inner Lake of Tihany, whose time requirement for a decrease in redox potential is shorter than that of Lake Balaton, under similar conditions with a 40 mg/l end-concentration glucose solution the length of time required for a decrease in redox potential is only one day shorter. Thus, by adding glucose the required time for a decrease in redox potential for the water of Lake Balaton and the Inner Lake of Tihany becomes balanced. On the effect of glucose the redox potential corresponding to the equilibrial state both in the cases of Lake Balaton and in the Inner Lake of Tihany, decreases by nearly 100 mV. On the 19th August, 1969 the lake water of the Inner Lake of Tihany free of phyto- and zooplankton the time required for a decrease in redox potential lengthened by 2 days to the unfiltered water. Comparing it to the one measured on the 30th July the redox potential corresponding to the state of equilibrium it became more positive only by 50 mV. This clearly indicates that even within a season in a lake the role of individual components change in the formation of the potential reducing ability. When we added to the filtered lake water

of the Inner Lake of Tihany a 40 mg/l end-concentration glucose solution we obtained a result very similar to that of the unfiltered water. Consequently, at the time of examination the role of bacteria was decisive in the process.

It was interesting to note, that giving to the water of Lake Balaton and to the Inner Lake of Tihany a 2 mg/l end-concentration glucose solution caused a 4 and 1 day shift in the decrease of redox potential. When the same concen-

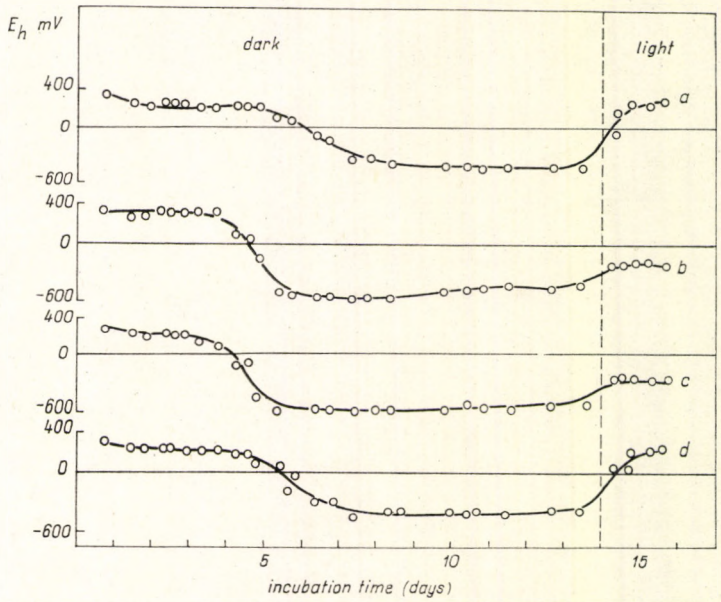

Fig. 10. Influence of glucose and darkness, then of light incubation on the reducing ability of water deriving from the Inner Lake of Tihany.
(a) lake water + 2 mg/l glucose; (b) lake water + 20 mg/l glucose; (c) lake water + 40 mg/l glucose; (d) lake water

tration was given to the water filtered through a 6 μ filter deriving from the Inner Lake of Tihany the length of time required to cause a decrease in redox potential, doubled. Comparing the redox potential corresponding to the state of equi ibrium to the control a 200 mV higher positivity was measured, while the same compared to water to which a 40 mg/l end-concentration glucose solution was added this value reached 400 mV. So far we have no explanation to the phenomena accompanying the addition of glucose with low concentration.

If the sample gaining the equilibrial state characteristic for oxygen-free condition, is placed in light *(Fig. 10)* its redox potential attains a value of the corresponding initial state. In the case of samples filtered through 6 μ sieve — without phytoplankton — reoxidation, naturally, cannot be effected. It is interesting, that after adding a 20 and a 40 mg/l end-concentration glucose solution disregarding a slight increase in redox potential the sample is not reoxidized. On the other hand, using a low concentration of glucose solution the process of reoxidation passes freely.

Summary

In order to measure the potential reducing ability of natural waters and sediments we employed a simple, direct method, which is based on the changes in redox potential of closed systems containing natural substrate. The measuring vessel is a 250 ml graduated glass container fitted with a ground glass stopper or made of rubber into which reference and measuring electrodes are built. By discontinuing the atmospheric oxygen supply, and by excluding photosynthesis because of incubation in darkness, the redox potential decreases in the measuring vessel containing natural substrate. Information received during measurement: 1. time requirement for redox potential decrease; 2. length of decrease; 3. redox potential value characterizing the state of equilibrium.

Some typical experiments exemplifying the applicability of the method brought the following results:

1. The supertrophic water of the Inner Lake of Tihany displays a greater reducing ability than that of Lake Balaton, and further, this reducing ability shows significant changes as regards seasons.
2. In the reedery of Lake Balaton the reducing ability of the water in the lotic zone is smaller than in the lenitic zone, and in both cases the reducing ability is higher than that of the open water.
3. The reducing ability of the water deriving from Lake Balaton does not change vertically, at the same time, in the Inner Lake of Tihany the reducing ability towards the bottom increases. The growth showed a direct relation to the quantitative distribution of the total and saprophytic microbial plankton.
4. The influence of bacterio-, phyto- and zooplankton on the reducing ability of the water of Lake Balaton and the Inner Lake of Tihany is different.
5. The sediment in the reedery of Lake Balaton with regard to reducing ability shows a stratification. The highest reducing ability is displayed by the upper, active layer, which contains the highest percentage of bacteria.
6. The reducing ability of the young detritus is smaller than that of the mature detritus.
7. The reducing ability of the sediment in section M in Lake Balaton is greater than in section A.
8. By measuring reducing ability we may also obtain data as to the intensity of mineralizational processes.
9. If the sample gaining the equilibrial state characteristic for oxygen-free condition, is placed in light, its redox potential attains a value of the corresponding initial state.

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TERMÉSZETES VIZEK ÉS ÜLEDÉKEK REDUKÁLÓ KÉPESSÉGÉNEK MÉRÉSE: EGY EGYSZERŰ LIMNOLÓGIAI MÓDSZER

Oláh János

Összefoglalás

A természetes vizek és üledékek potenciális redukáló képességének közvetlen mérésére egy új, egyszerű módszert alkalmaztunk, amely a természetes szubsztrátumot tartalmazó zárt rendszer változásain alapszik. A mérőedény egy 250 ml-es üvegedény, melynek becsiszolt üveg vagy gumi dugójába mérő és referenciaelektródák vannak beépítve. A levegőből az oxigénutánpótlás megszűnése és a sötét inkubálás folyamán a fotoszintézis kizárása a természetes szubsztrátumot tartalmazó mérőtérbe a redoxpotenciál csökkenéséhez vezet. A mérés során kapott információk: 1. a redoxpotenciál csökkenéséhez szükséges idő; 2. a csökkenési szakasz nagysága; 3. a beálló egyensúlyi helyzetet jellemző redoxpotenciál érték.

A módszer felhasználási lehetőségeit bemutató néhány típuskísérletből a következők állapíthatók meg:

1. A szupereutróf Belső-tó vize a Balatonénál nagyobb redukáló képességgel rendelkezik, és a redukáló képesség szezonálisan jelentős mértékben változik.
2. A Balaton nádasában a víz redukáló képessége a lotikus zónában kisebb, mint a lenitikus zónában, és mindkét helyen nagyobb a nyíltvízben mért redukáló képességnél.
3. A Balaton vizének redukáló képessége vertikálisan nem változik, ugyanakkor a Belsőtóban a fenék felé a redukáló képesség növekedett. A növekedés a teljes és szaprofita mikrobiális plankton mennyiségének eloszlásával egyenes összefüggést mutatott.
4. A Balaton és Belső-tó vizének redukáló képességében a bakterio-, fito- és zooplankton hatása eltérő.
5. A Balaton nádasüledéke redukáló képességét tekintve határozott rétegezettséget mutat. Legnagyobb redukáló képességgel a felső legtöbb baktériumot tartalmazó, aktív réteg rendelkezik.
6. A fiatal detritusz redukáló képessége kisebb, mint az idős detrituszé.
7. A Balaton M szelvényén az üledék redukáló képessége nagyobb, mint az A szelvényen.
8. A redukáló képesség mérésével a mineralizációs folyamatok intenzitásáról is adatok nyerhetők.
9. Az oxigén nélküli redox egyensúlyra beállt mintát fényre helyezve a redoxpotenciál közel a kiindulási értékre áll vissza.

ИЗМЕРЕНИЕ РЕДИЦИРЮЩТЕЙ СПОСОБНОСТИ ПРИРОДАХ ВОД И ОСАДКОВ: ПРОСТОЙ ЛИМНОЛОГИЧЕСКИЙ МЕТОД

Я. Олах

Для измерения потенциальной редицирующей способности природных вод и осадков разработан простой прямой метод, основанный на изменениях окислительно-восстановительного потенциала в замкнутых системах, содержащих природный субстрат. Измерительным сосудом служит градуированный стеклянный контейнер на 250 мл, снабженный нижним стеклянным краном или резиновой пробкой, в которую вмонтированы измеряющий и референтивный электроды. Исключением доступа атмосферентивный электроды. Исключением доступа атмосферногокислорода и исключением фотосинтеза (инкубация в темноте) достигается снижение окислительно-восстанавительного потенциала. В ходе измерения можно получить сведения о: 1. времени, необходимом для снижения потенциала; 2. размере снижения; 3. значениепотенциала после установления равновесия. В нескольких типичных экспериментах, дающих представление о возможностях метода, получены следующие результаты:

1. Супертрофическая вода Внутреннего Озера (Тихаиь) проявляет более высокую редуцирующую способность, чем вода Балатона, и её способность проявляет значительные сезонные изменения.

2. В тростинковых зарослях Балатона редуцирующая способность воды из лотической зоны меньше, чем способность воды открытой части озера.

3. В озере Балатон вода не проявляет вертикальной изменчивости по своей редуцирующей способности, тогда как во Внутреннем Озере (Тихань) способность увеличивается от поверхности к дну. Этот рост прямо соответствует количественному распределению общего сапрофитного микропланктона.

4. Влияние бактерио-, фито- и зоопланктона на редицирующая способность воды двух упомянутых озер различно.

5. Тростниковые осадки Балатона по своей редуцирующей способности стратифицированы. Наивышей способностью обладает самый верхний, активный слой, содержащий больше всего бактерий.

6. Редуцирующая способность молодого детрита меньше чем зрелого.

7. Редуцирующая способность осадков озера Балатон больше в разрезе М, чем в разрезе А.

8. Посредством измерения редуцирующей способности можно также получать сведения об интенсивности минерализационных процессов.

9. Если образец, дотигший состояния равновесия в отсутствие доступа кислорода, поместить на свет, его окислительно-восстановительный потенциал сдвигается к исходному значению.

ANNAL. BIOL. TIHANY | 37 | 223—234 | HUNGARIA 1970

COMPARATIVE BACTERIOLOGICAL INVESTIGATION OF THREE SHALLOW HUNGARIAN LAKES WITH DIFFERENT TROPHIC LEVELS

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Received: 20th February 1970

The quantity of the saprophytic and total bacterioplankton depends on the nutritional supply of lakes (KUZNETSOV, 1952; RAZUMOV, 1962; RODINA and KUZMITSKAYA, 1963; THSHERBAKOV, 1967) and so it can be used for studies of trophic processes. The results of researches carried out in Lake Balaton (OLÁH, 1969 a, b) show that the comparison to deep stratified lakes do not give appropriate information on its trophic state. We know little about the bacterological conditions of our shallow extensive lakes like Lake Balaton, therefore bacterological investigations of our shallow lakes with different trophic levels and the comparison of results are advisable.

Material and methods

The samples were taken from reeds and open water sections of the lakes at 52 sites from 12th—28th, August 1969. Lake Balaton: 12 sites were examined *(Fig. 1)* in open water at 200 m distance from each other in the standard section at Balatonfüred—Zamárdi (OLÁH, 1969 b). Vertical samples were taken at the 12th site. Inner Lake of Tihany: It is a highy eutrophic "small pond" according to the chemical and biological examinations (VARGA, 1937; MÓRICZ, 1938; JACZÓ and MANN, 1940; PONYI and TAMÁS, 1964). Ten sites proceeding from the reedeny toward the centre of the open water *(Fig. 2)* were examined and vertical samples were taken at the 10th site. Velence Lake: it is a shallow eutrophic natron lake covered mainly with reeds (DONÁSZY, 1953; CSAJÁGHY, 1953; DVIHALLY, 1960). The water regions surrounded with reeds are greatly isolated. To compare with Lake Balaton and Inner Lake we had t oexamine at least 6 reeds and open water sections *(Figs. 3, 4, 5)*. Vertical samples were taken from open water in section A, B and F and from reeds in section F.

Water samples were taken by FRANCEV's sampler (KUZNETSOV, 1952) from a depth of 50 cm and transferred into sterile, 250 ml glasses with glas stopper except the vertical samples and the 1st site of reeds-open water section in Lake Balaton. Applying RAZUMOV's direct method (1932) the amount of total bacterioplankton was calculated from the samples. The saprophytes were counted on sodium-caseinate agar. Incubation took place at 25 °C and the number of colonies was counted on the 10th day at a magnification of $\times 10$. Pouring was made in one hour after the collection. Besides counting the saprophytic and total bacterioplankton to estimate the differences among

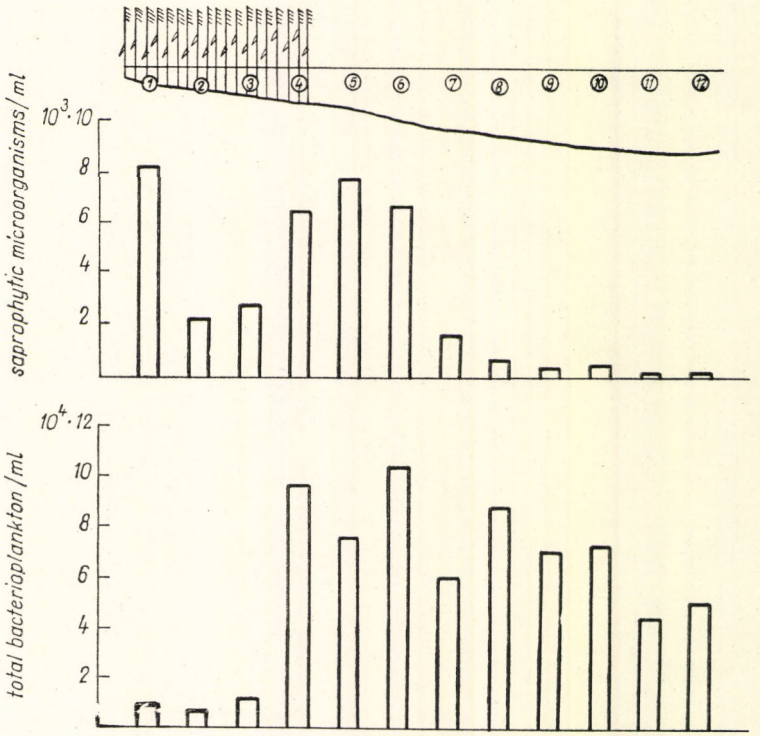

Fig. 1. The quantity of saprophytes and total bacterioplankton in the reeds-open water section in Lake Balaton

the isolated water regions of the Lake additional samples were taken in 6 open water sections: the quantity of dissolved organic nitrogen easily assimilable for microbes expressed in pepton was determined with the method of Bringman Coli biomass' index and the chemical oxygen demand was stated with $KMnO_4$.

Results

In Lake Balaton the quantity of total bacterioplankton in the reeds-open water section was below $1 \cdot 10^5$/ml and in the reeds only about $1 \cdot 10^4$/ml. The distribution of saprophytes was inverse. Their number decreased in open water from site 7 (1700/ml) to 11 and 12, 200/ml and the greatest value was measured in the reeds. The number of saprophytes was greater in the littoral zone of reeds in the narrow stripe of shallow water and in the broader zone of water adjoining the open water than in the open water or in the centre of the reeds. ($7—8 \cdot 10^3$/ml). The number of saprophytes was in the littoral (8200/ml) near the figure of total bacterioplankton (9200/ml).

In the section of Inner Lake *(Fig. 2)* the amount of total bacterioplankton was $7—9 \cdot 10^6$/ml in the littoral and it decreased to $3—6 \cdot 10^6$/ml moving off. The distribution of saprophytes was unequal, the greatest value was in the littoral stripe ($27 \cdot 10^3$/ml).

The distribution of saprophytes in the reeds-open water sections *(Figs. 3, 4)* in Velence Lake was similar to that of in Lake Balaton. In all the 6 sections the greatest values were in the reeds. The most significant difference in the saprophytes between the reeds and the open water was in sections B and D. The different quantity of saprophytes characterised the reeds of the

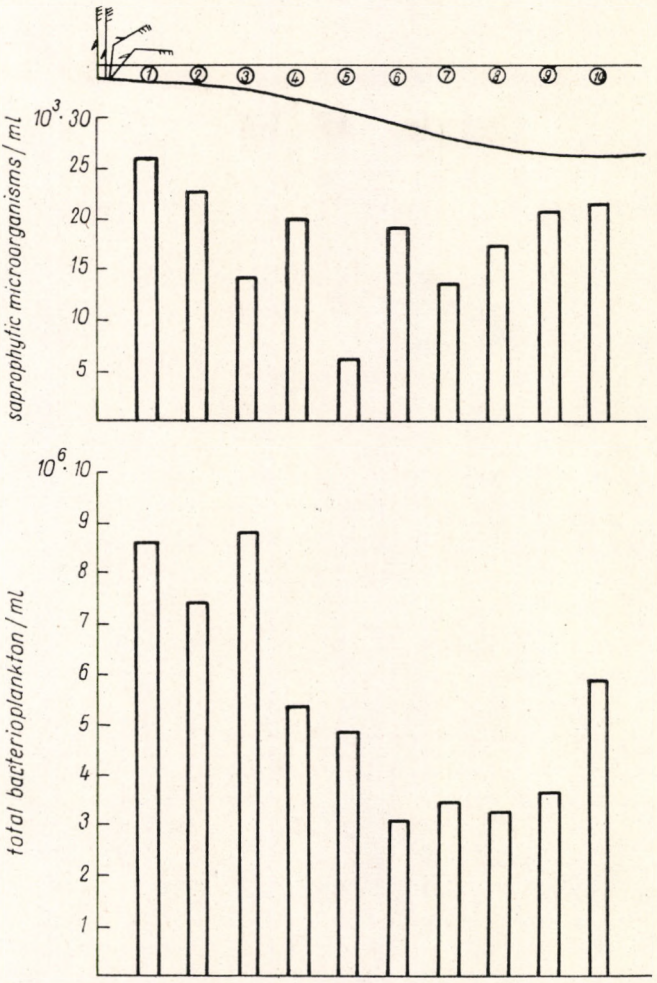

Fig. 2. The quantity of saprophytes and total bacterioplankton in the reeds fragment-open water section in Inner Lake

sections too. The amount of saprophytes was 12—14 · 10^3/ml in the reeds of section B and 4.5 · 10^3/ml in that of section A and E. The greatest amount of saprophytes (18.5 · 10^3/ml) was found in the reeds of section D, the lowest amount (2.5 · 10^3/ml) was found in the reeds of section F. In open water of the sections the number of saprophytes was lower. It was in the sections A, B, E, and F between 5 · 10^2—3.5 · 10^3/ml, higher values (3.5—10 · 10^3/ml were reached only in sections C and D. The distribution of the total bacterio-

plankton did not give the same proportions between reeds and open water like saprophytes. The distribution similar to Lake Balaton was detected only in section D. The amount of total bacterioplankton in the reeds was $2 \cdot 10^5$/ml and in the open water higher ($4.3-7.6 \cdot 10^5$/ml). In other cases equal distribution was measured (B, E, F) or the differences of the sites (A, C) did not reflect the proportions of reeds and open water.

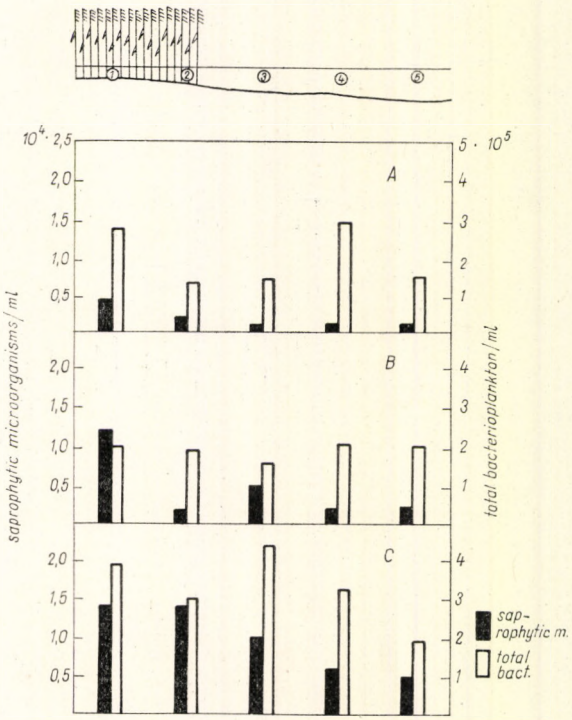

Fig. 3. The quantity of saprophytes and total bacterioplankton in reeds-open water sections A, B and C in Velence Lake

In summarizing the data on the quantity of saprophytes and total bacterioplankton in each section *(Fig. 5)* the water-regions strongly separated by reeds differ from each other referring to the bacterioplankton. The amount of bacterioplankton is the greatest in section C and D according to the amount of saprophytes and total microbioplankton, too. Section A, B and E are in the next order of magnitude. Section F has an outstanding low amount of total bacterioplankton ($3 \cdot 10^4$/ml) among the water-regions separated by reeds. With the decreasing number of total bacteria in the same order mentioned above, the Secchi transparency increases. In sections C and D it was 20—30 cm and in the section F 190 cm.

In the open water sections of Velence Lake the chemical oxygen demand did not show significant differences *(Fig. 6)*. The Bringmans Coli-biomass index did not follow the great differences observed in the quontity of the total bacterioplankton. The Coli-biomass index in section F containing an extremely

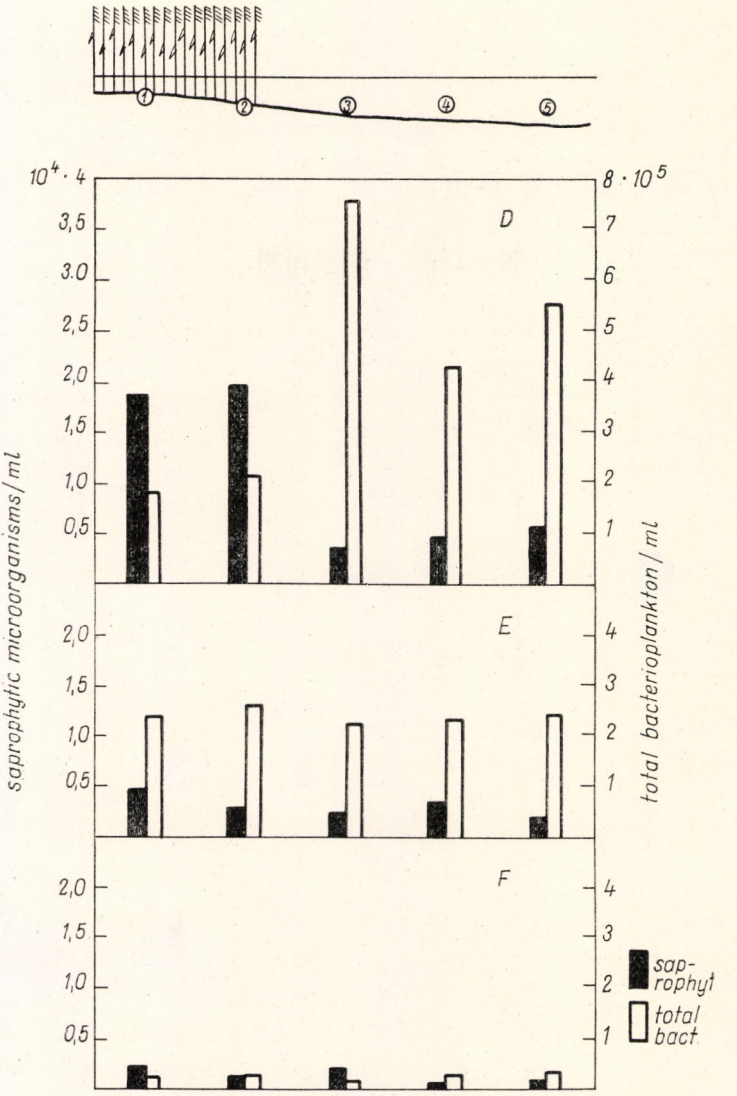

Fig. 4. The quantity of saprophytes and total bacterioplankton in the reeds-open water sections D, E and F in Velence Lake

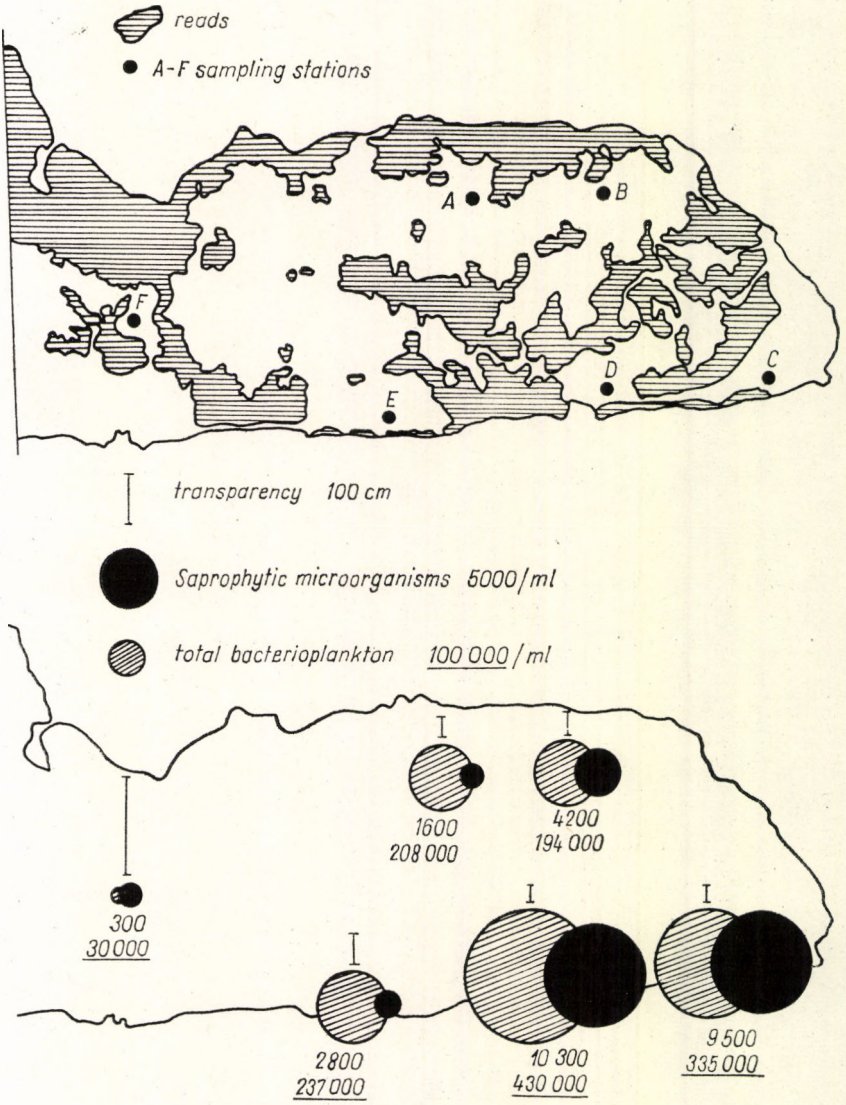

Fig. 5. The average amount of saprophytes and total bacterioplankton at six sites observed in Velence Lake

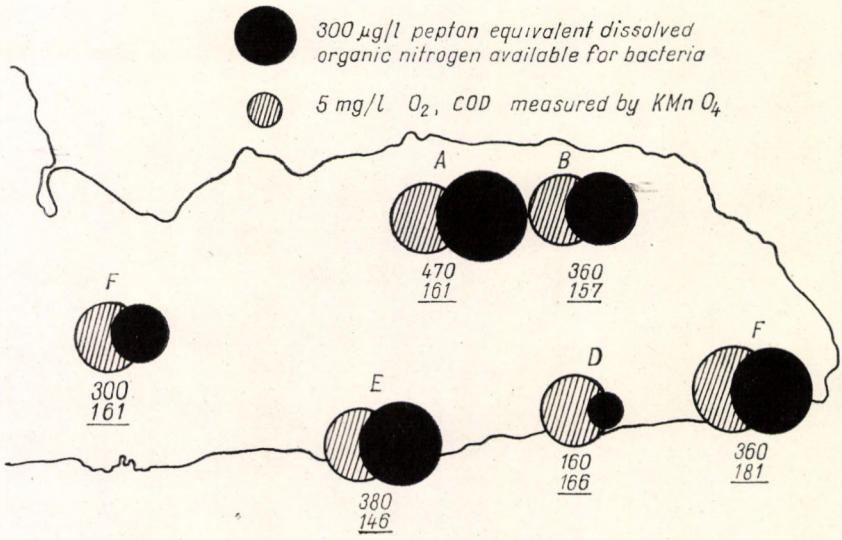

Fig. 6. The amount of dissolved organic nitrogen in pepton equivalency measured with Bringman's Coli biomass index and the chemical oxigen demand measured with $KMnO_4$ at six sites observed in Velence Lake

low amount of bacterium comes close to that of in sections A, B, C and E. In section D containing the highest amount of bacterium the biomass index was low.

The vertical distribution of total bacterioplankton and saprophytes in the open water of Lake Balaton was not homogeneous *(Table 1)*. The amount

TABLE 1

Depth (m)	*Number of individuals per ml*	
	saprophytes	total bacterioplankton
0.0	180	51 000
0.5	400	67 000
1.0	440	50 000
1.5	440	90 000
2.0	580	98 000
2.5	290	71 000
3.0	180	118 000
3.5	210	93 000

of saprophytes immediately on the surface and in the deeper layers was lower. The amount of total bacterioplankton in the deeper layers was higher. Maxima of the saprophytes and the total microbioplankton were found in the layer above the bottom in Velence Lake *(Table 3)* and in Inner Lake *(Table 2)*. In the layer close to the mud in Inner Lake the amount of saprophytes decreased suddenly.

TABLE 2

Depth (m)	*Number of individuals per ml*	
	saprophytes	total bacterioplankton
0.0	21 000	5 900 000
0.5	17 000	5 900 000
1.0	16 000	4 600 000
1.5	23 000	5 800 000
2.0	16 000	5 900 000
2.5	19 000	5 400 000
3.0	36 000	5 400 000
3.5	30 000	6 900 000
4.0	6 000	9 300 000

TABLE 3

Depth (m)	*Number of individuals per ml*							
	A		B		F		F reeds	
	saproph.	total bact.	saproph	total bact.	saproph.	total bact.	saproph.	total bact.
0.0	760	158 000	2 000	210 000	320	32 000	2 350	34 000
0.5	8 750	412 000	3 180	452 000	1 790	29 000	1 930	37 000
1.0	3 860	187 000	10 000	756 000	1 260	111 000	7 690	163 000
1.5	8 770	140 000	12 400	393 000				

Discussion

The quantity of bacterioplankton is characteristic for lakes with different trophic levels (KUZNETSOV, 1952; RAZUMOV, 1962; THSERBAKOV, 1967; ROMANENKO, 1969). However, the orders of magnitude were studied in deep stratified lakes and our data (OLÁH, 1969 a, b) show, that these cannot be applied in the case of the extensive shallow Lake Balaton. E.g. the order of magnitude $0.05-2.3 \cdot 10^6$/ml obtained in 1966—68 agrees on that of the oligotrophic and mesotrophic lakes.

At present investigations the quantity of total bacterioplankton in reeds and open water section in Lake Balaton was very low similar to the oligotrophic lakes ($1 \cdot 10^5$/ml). The low value $9.4 \cdot 10^3$/ml of the reeds is not frequent in the oligotrophic water either. It is very striking, that the amount of total bacterioplankton in Velence Lake between the state of eutrophy and "senescence" (LINDEMAN, 1942) did not exceed the order of oligotrophic lakes. The amount of total bacterioplankton in littoral-open water section of Inner Lake was $3-9 \cdot 10^6$/ml. This order is corresponding or rather superior to the values weighed in eutrophic lakes (KUZNETSOV, 1952; THSHERBAKOV, 1967). The low values of the detailed survey in August in Velence Lake prove the possibility of strong reduction the quantity of the total bacterioplankton similar to that of Lake Balaton in the seasonal dynamics. Such low values were not weighed in the shallow Inner Lake whose small size differs from Lake Balaton and Velence Lake. Although, summer minima are well known

(HENRICI, 1938; POTAYENKO, 1968), data of such decrease in summer were not found in the literature referring the meso- and eutrophic lakes. The extraordinary low value in August measured in the two lakes was observed in the 1966—68 summer irregularly. The average of the results of 15 year's investigation in Coli content (PAPP, 1969) refers also to a great reduction in August in both lakes. Considering the short-term investigations in the 1968—1969 years (OLÁH, 1970) the strong reduction in the quantity of bacterioplankton in late summer in our extensive but shallow lakes seems to be general. The exhaustion of nutritive substances due to the oxygen saturation and the high temperature in the constantly moving water is the main factor in this process.

In Velence Lake isolated by reeds differently the quantity of saprophytes and total bacterioplankton changed in inverse ratio to the Secchi transparency. In section F in the dark brown water with transparency near 200 cm the lowest value of saprophytes and total microbioplankton was measured. At the same time, in the grey turbid water with Secchi transparency 20—30 cm in the section C and D the highest values were found. Probably the different separation of water regions and the different cover of plants at the bottom influence the size of Secchi transparency. The section F is the mostly isolated by reeds and its bottom was covered with thick reed-grass and Cladophora. At the bottom in section D and C a cover of aquatic vegetation was not detected. In these parts of the lake the hight bacterial content at the mud-water interface has an effect on the great quantity of bacteria in the water column through the frequent disturbance of shallow water. This is indicated also by the fact that there were no great differences in quantity of chemical oxygen demand and in that of pepton equivalent dissolved organic nitrogen available for bacteria in the regions of the lake isolated by reeds.

The data of our survey in the 8 reeds-open water sections in Lake Balaton, Velence Lake and Inner Lake make possible the generalization of the results of our seasonal survey in 1968 in the same sections in Lake Balaton (OLÁH, 1969 b).

The amount of saprophytes are always higher in the reeds than in the open water. Due to the introduction of *Ct enopharyngodon idella Cuv.* et VAL. in the Inner Lake the reeds were dead and the amount of the saprophytes did not increase significantly toward the littoral zone.

In the section reeds-open water in Lake Balaton the amount of saprophytes has the biggest value at the line of reeds-open water. This characteristic distribution was also discovered in May and August 1968 on the reeds-open water section. The cause of this distribution of saprophytes may be the moving water washes away the broken fragments of detritus out of the reeds and keeps floating in contrast with quiet water of the reeds, moreover, gives appropriate nutritive material and surface for the bacteria population in the water in front of the reeds.

The distribution of the total bacterioplankton in the reeds-open water sections is different. There is no difference in quantity among the reeds and open water sections in Velence Lake except in section D. At the same time, the amount of the total bacterioplankton decreased significantly in the reeds on the reeds-open water section in Lake Balaton and in section D of Velence Lake. Although in the seasonal survey in 1968 generally a greater amount of the total bacterioplankton was stated in the reeds (OLÁH, 1969 b), this considerable decrease, occurred in late August-early October of the same year,

too, and its reason is, very probably the sedimentation of the particular nutritive substances in the stagnant water and the exhaustion of the dissolved organic nutritive substances in the water.

Summary

1. The quantity of both the saprophytes ($5-27 \cdot 10^3$/ml), and total bacterioplankton ($3-8.8 \cdot 10^6$/ml) in Inner Lake is in accordance with the data in literature referring to eutrophic lakes. However, the extremely low quantity of the bacterioplankton in the extensive and shallow Lake Balaton and Velence Lake does not reflect the real trophic state.

2. The considerable decrease of the quantity of the total bacterioplankton measured is not known in the mesotrophic and eutrophic lakes. This significant decrease in summer and late summer period is an important feature of our shallow extensive lakes.

3. The quantity of saprophytes and total bacterioplankton in the parts isolated with reeds in Velence Lake is different and it is in inverse ratio to the Secchi transparency. Thevalues of COD and Bringman's Coli biomass test have little alteration.

4. We can state on the examination of the 8 reeds-open water sections that the quantity of saprophytes in the reeds is always greater than in the open water. The following periodic phenomena had been observed are characteristic to the reeds-open water sections in Lake Balaton: a) the highest amount of saprophytes is at the border of reeds-open water. b) the amount of the total bacterioplankton in the reeds can decrease to a low value ($1 \cdot 10^4$/ml).

5. The vertical surveys show the possibility of the formation of the bacterioplankton stratification in our shallow lakes like Lake Balaton besides the heterogeneous vertical distribution.

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HÁROM SEKÉLY, KÜLÖNBÖZŐ TROFITÁSÚ MAGYARORSZÁGI TÓ ÖSSZEHASONLÍTÓ BAKTERIOLÓGIAI VIZSGÁLATA

Oláh János és *Vásárhelyi Réka*

Összefoglalás

1. A Belső-tóban mind a szaprofiták ($5—27 \cdot 10^3$/ml), mind a teljes bakterioplankton mennyisége ($3—8{,}8 \cdot 10^6$/ml) az eutróf tavakra vonatkozó irodalmi adatokkal összhangban van. Ezzel szemben a sekély, nagykiterjedésű Balaton és Velencei-tó a vizsgált periódusban az irodalomban ismert nagyságrendek oligotróf tavaihoz hasonló alacsony baktériumtartalma nem tükrözi a reális trofikus állapotot.

2. A mezo- és eutróf mély tavakban nem ismert a teljes bakterio-plankton jelen munkánkban mért nagyarányú csökkenése. Ez a nyári-nyárvégi erőteljes csökkenés sekély, nagykiterjedésű tavaink fontos sajátossága.

3. A nádasokkal különböző mértékben izolált Velencei-tavi tórészletekben a szaporfiták és a teljes bakterio-plankton mennyisége is eltérő, a Secchi-átlátszósággal fordított arányban áll. A COD és a Bringman Coli-biomassza teszt értékei kevéssé változnak.

4. A 8 nádas-nyíltvíz szelvény alapján megállapítható, hogy a szaprofiták száma a nádasban mindig nagyobb, mint nyíltvízben. A balatoni szelvényre időszakonként jellemzőek a következő, korábbi években is megfigyelt jelenségek: *a)* a legmagasabb szaprofita szám a nádas—nyíltvíz határon alakult ki; *b)* a nádas vizében a teljes bakterioplankton mennyisége $1 \cdot 10^4$/ml-re lecsökkent.

5. Vertikális vizsgálataink azt mutatják, hogy az egyenetlen vertikális elterjedésen túl sekély vizeinkben, így a Balatonban is kialakult határozott bakterioplankton rétegződés.

СРАВНИТЕЛЬНО БАКТЕРИОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ ТРЁХ МЕЛКИХ ВЕНГЕРСКИХ ОЗЕР С РАЗЛИЧНЫМИ ТРОФИЧЕСКИМИ УСЛОВИЯМИ

Я. Олах и *Р. Вашархейи*

1. По количеству тотального бактериопланктона ($3—8{,}8 \cdot 10^6$/мл) и сапрофитов ($5—27 \cdot 10^3$/мл) Внутреннее Озеро (Тихань) соответствует озерам, называемым в литературе эутрофическими. Напротив, исключительно низкое количестью бактериопланктона в обширных и мелких озерах Балатон и Веленце не отражает действительной трофики этих озер.

2. Значительное снижение тотального бактериопланктона, наблюдаемое в озерях Балатон и Веленце летом и в конце лета, не известно для мезотрофных и эутрофных озер и является важной чертой упомяниутых обширных мелких водоемов.

3. Количество сапрофитов и тотального бактериопланктона в разных частах озеро Веленце, изолированных тростниковой растительностью, различно и находится в обратном отношении к величение показателя. Значения биомасс COD и Coli, по Bringman, проявляют небольшие изменения.

4. На основе изучения 8 разрезов, включающих тростниковые заросли и открытую воду, можно заключить, что количество сапрофитов в зарослях всегда выше чем в открытой воде. Для таких разрезов озера Балатон характерны следующие периодические явления: *а)* самое высокое содержание сапрофитов- на границе зарослей и открытой воды; *б)* количество тотального бактериопланктона в зарослях может падать до весьма низкого значения ($1 \cdot 10^4$/мл).

5. Вертикальные исследования показывают, что в мелких озерах, подобных Балатону, помимо неоднородности вертикального распределения бактериопланктона может иметь место его стратификация.

ANNAL. BIOL. TIHANY	37	235—246	HUNGARIA 1970

COMPARATIVE NUTRIENT AGAR STUDIES ON THE QUANTITATIVE SURVEY OF SAPROPHYTIC WATER MICROORGANISMS

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(Received: 20th February 1970)

One of the most important endeavours of present-day research in water microbiology is to elucidate the dynamics of saprophytic and total microbial associations in waters of various trophism (OCEVSKI, 1966; DAUKSTA, 1967; OVERBECK, 1968 a, b; RHEINHEIMER, 1968; POTAYENKO, 1968; DAUBNER, 1969; DEUFE, 1969; FONDEN, 1969; ROMANENKO, 1969, and others).

The majority of water microbiologists employs RAZUMOV's direct method (1932) in order to make quantitative surveys of total microbial flora living in waters and in sediments, consequently, the obtained results are well comparable. To standardize the counting of saprophytes would also be desirable (BABENZIEN, 1962). The nutrient agar used in hygienic practice is quite inadequate for counting the autochton, slowly growing water inhabiting bacteria (STRZELCZYK et al. 1967) thus, there is an ardent quest to find new nutrient media and the line of suggested and applied nutrient agars has not yet come to an end (HESSE and NIEDNER, 1898; FRED et al. 1924; STARK and MCCOY, 1938; OPPENHEIMER and ZOBELL, 1952; FERRER et al. 1963; STRZELCZYK et al. 1967; MELCHIORRI-SANTOLINI and CAFARELLI, 1967; FONDEN, 1967, 1968) which makes comparative study extremely difficult.

It is important to choose the right composition of the nutrient agar in the process of plate-pouring and spreading when determining the number of saprophytes, however, besides this, a large number of other factors may also influence the number of bacteria appearing on the agar-slide (CARLUCCI and PRAMER, 1957; JONES and JANNASH, 1959; BUCK and CLEVERDON, 1960; GUNKEL et al. 1960; FONDEN, 1967; CLARK, 1967; STRZELCZYK et al. 1968).

To take into consideration all the above presented facts we thought important to try and apply some new nutrient agar in order to count the saprophytic microorganisms in connection with the new and detailed microbiological investigation of Lake Balaton. To this effect we have carried out a comparative research on the basis of 12 nutrient agars, relying partly on literary data and partly on our own resources; our samples were taken from Lake Balaton, from the Inner Lake of Tihany, from Velence Lake, and from River Danube and cultured accordingly.

Material and method

The samples from Lake Balaton have been taken at some 500 metres from the shoreline in front of our Research Institute, the other sample taking localities, the Inner Lake of Tihany and River Danube, were in the littoral

TABLE 1

The composition

		Nutrient agar I.a	Nutrient agar I.b	Complex agar II.	TAYLOR's agar III.	Sodium caseinate agar IV.
Distilled water,	ml	—	1000	1000	1000	1000
Lake „		—	—	—	—	—
Tap „		1000	—	—	—	—
Agar	g	15	15	15	15	15
K_2HPO_4	g	—	—	0.5	0.2	0.2
Na_2HPO_4	g	—	2.0	—	—	—
KNO_3	g	—	—	0.2	—	—
$NaNO_3$	g	—	—	—	—	—
$MgSo_4 \cdot 7H_2O$	g	—	—	0.2	0.05	0.2
$(NH_4)2So_4$	g	—	—	—	—	—
$FeSO_4 \cdot 7H_2O$	g	—	—	—	—	trace
$FeCl_3$	g	—	—	trace	trace	—
NaCl	g	—	3.0	—	—	—
Glucose	g	—	—	0.5	—	1
Glycerin	ml	—	—	—	1	—
Soluble starch	g	—	—	—	0.5	—
Bacto pepton	g	5	10	—	0.5	—
Bacto beef extract	g	3	1.5	—	—	—
Bacto yeast extract	g	—	—	0.05	—	—
Sodium caseinate	g	—	—	0.1	0.5	1.0
Mud extract	ml	—	—	—	—	—
Powder alga	g	—	—	—	—	—

Note: X.a — Lake water agar of Balaton; X.b — Lake water agar of Lake Belső

zone *(Table 3)*. While the mud-samples of Velence Lake originate from the yellow-coloured oxidized micro-zone and from the black-coloured reduced zone. The watersamples have been taken by the help of a Francev sampler (KUZNETSOV, 1952) into a sterile, 250 ml flask from a depth of 50 cm. The mud-samples have been secured by using an Ekman-Birgs drege and from the so obtained mud-blocks by the help of instruments cauterized in alcohol flame from inside we took our samples. The samples have been elaborated in the following hour of collecting from sites Lake Balaton and Inner Lake while samples originating from River Danube on the next day. The mud from Velence Lake has been stored in a refrigerator for a longer period of time.

The total microbial plancton quantity has been determined by RAZUMOV's direct method (1932).

The slides were sealed from 10—10 ml of 42 °C nutrient agar, whose composition is shown in *Table 1.* Our pulverized alga-nutrient medium have been obtained from the laboratory mass-culture of *Scenedesmus obtusiusculus* CHOD. The desiccated alga fragments were ground in a ball and tube mill. To the mud-extract agar 800 g mud from Lake Balaton was boiled in 2 litre Balaton water, then following sedimentation it was passed through filter paper. Considering the low number of bacteria in Lake Balaton, 0.1 and 1 ml lake water without dilution were inoculated, while in case of the Inner Lake of Tihany and River Danube the degree of dilution was 10 and 100-times, respectively. In the case of mud-sampes coming from Velence Lake we made dilutions of 10-, 100- and 1000-times. In the case of sealings we had 2 controls

of the mediums

JENSEN's agar V.	OPPENHEIMER's agar VI.	Fe-peptone agar VII.	Powder alga agar VIII.	Mud extract agar IX.	Lake water agar		KRASHENINIKOVA's agar XI.
					X.a	X.b	
1000	—	—	—	—	—	—	400
—	1000	—	1000	—	1000	1000	500
—	—	1000	—	—	—	—	100
15	15	15	15	15	15	15	15
0.5	0.1	—	—	—	—	—	0.01
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
—	—	0.1	—	—	—	—	0.005
—	trace	0.1	—	—	—	—	—
trace	—	—	—	—	—	—	—
—	—	—	—	—	—	—	0.05
1	—	0.1	—	—	—	—	—
—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—
—	5	5	—	—	—	—	—
—	—	—	—	—	—	—	—
—	1.0	—	—	—	—	—	—
0.2	—	—	—	—	—	—	—
—	—	—	—	1000	—	—	—
—	—	—	5	—	—	—	—

without inoculum and 3—5 parallels. The slides were incubated at 25 °C and as the colonies appeared they were counted under 10-times of magnification on the 1st, 2nd, 3rd, 6th, 12th, 14th, 32nd and 36th day. The results obtained have always been referred to 1 ml and 1 g of the original sample.

Results and discussion

It was striking to observe that the bacterioplankton quantity of Lake Balaton and that of the Inner Lake of Tihany shows significant difference *(Fig. 1)*. The total microbial plankton quantity (nearly $12 \cdot 10^6$/ml) of the Inner Lake of Tihany when compared to the data found in literature (ROMANENKO, 1969) shows a pronounced degree of eutrophism. Likewise is the number of saprophytes examined on every nutrient agar greater, more than one order of magnitude, than the values obtained for samples taken from Lake Balaton.

The comparison of the examined nutrient agars *(Fig. 1)* unambiguously prove that in hygenic practice as well as in water microbiology the media used are inadequate for bacterial cultures, and only a low number of colonies develop. Sealings of various samples originating from different waters, and taken at different times, the proportion of bacteria developing on various culture media varies in every case, but we never obtained favourable culture values on nutrient agar.

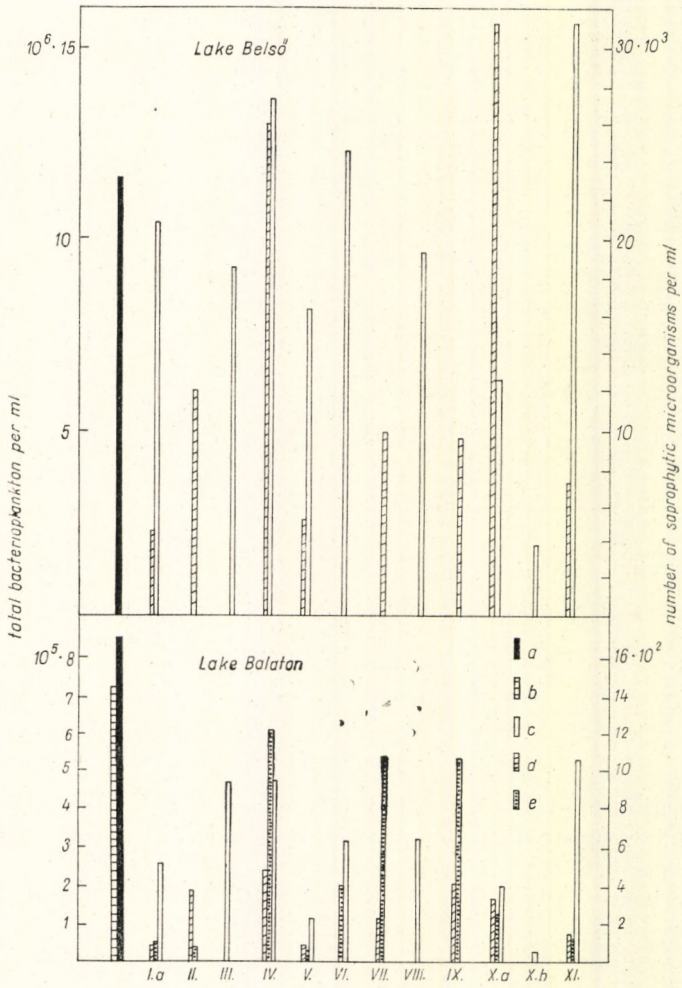

Fig. 1. Appearing colonies on the examined nutrient mediums and the total quantity of the microbial plankton in plate pouring carried out in different times from Lake Balaton and from the Inner Lake of Tihany. Total microbial plankton: (a) 19th VIII, 1968; (b) 8th IX, 1968. Plate pourings: (c) 6th VIII, 1969; (d) 19th VIII, 1968; (e) 8th IX, 1968

From among the culture media rich in organic materials the one containing sodium-caseinate appeared to be the most effective, for the largest number of colonies developed in this type of culture media. The OPPENHEIMER medium and the iron-peptone agar are also better for bacterial cultures than the nutrient agar, although, the proportions vary in the case of individual sealings. We examined three culture media rich in organic material and yielding high values for a period of one week by daily sealings *(Table 2)* using an inoculum originating from the water of Lake Balaton.

The sodium-casenate medium in this particular case also has yielded the highest value. The modified iron-peptone agar proved to be only half as efficient as regards the quantity of bacteria, than the former. The OPPENHEIMER medium has yielded the owest values in every case of examination.

TABLE 2

Media	*Time of plate pouring*						
	1968 IX. 19	20	21	23	25	26	27
	number of individuals/ml						
IV	1013	1200	2730	1950	2088	—	2000
VI	325	253	810	690	510	450	520
VII	594	560	1320	710	1600	110	—

In examining the water samples taken from River Danube in order to indicate the pollution we have shown the oxygen consumption of samples measured with $KMnO_4$ *(Table 3)*.

It is interesting to note that in one instance in the most polluted water sample on the sodium-caseinate agar we obtained a low value. On a small decrease of pollution the sodium-caseinate agar again yielded the highest values and the samples taken from the comparatively pure water of River Danube in both cases the biggest number of bacterium colonies developed on the sodium-caseinate agar.

TABLE 3

Media	*Time of plate pouring and Origin of samples*			
	22. X. 1969 Megyer	22. X. 1969 Nagymaros right shore	4. XI. 1969 Branch of River Danube at Soroksár Quay at Ferencváros	5. XI. 1969 Petőfi bridge leftshore
	number of individuals/ml			
I/a	—	—	5 310	2 870
I/b	12 350	10 850	8 960	20 220
VI	19 050	35 660	30 190	24 800
IV	41 280	29 370	37 000	14 180
Oxygen consumption O_2 mg/l	6.7	7.7	12.1	13.8

A significant difference has been observed between these two nutrient agars, too. Nutrient agar containing various salts has yielded higher values.

The highest number of bacterium colonies developed again on the sodium-caseinate agar from samples originating from the oxydized microzone and the reduced zone of Velence Lake *(Table 4)*.

Both on the OPPENHEIMER agar and on the sodium-caseinate agar the samples taken from the oxidized microzone yielded more than twice as many bacteria than from samples originating from the reduced zone. At the same

TABLE 4

Media	*Origin of samples*	
	Oxydized microzone, yellow mud	Reduced zone, black mud with a seell of H_2S
	number of individuals/ml	
I/b	3 684 000	3 477 000
VI	8 156 000	3 408 000
IV	13 210 000	5 240 000

time we have not found such a significant difference on nutrient agar, thus, when we work with nutrient agar only in the examined mud zones, we find the quantity of saprophytes well-nigh the same.

From all the above it issues that from the already employed media containing much organic materials the sodium caseinate agar appears to be the most favourable for counting saprophytic microorganisms especially for samples taken from Lake Balaton, and for other types of waters as well. In the case of strongly polluted waters it is well advisable to use nutrient agars containing a variety of salts.

In Poland, STRZELCZYK and his collaborators carried out investigations in 1967 on Lake Jeziorak and found that the sodium-caseinate agar did not give as high a value as the iron-peptone agar, for the latter yielded the highest values. Notwithstanding, in Hungary and elsewhere the sodium-caseinate agar has gained a wide acceptance for the examined waters (FRED. et al. 1925; FRED and WAKSMAN, 1928; STARK and MCCOY, 1938; TAYLOR, 1940; POTTER and BAKER, 1956, 1961; COLLINS and WILLOUGHBY, 1962; WILLOUGHBY and COLLINS, 1966).

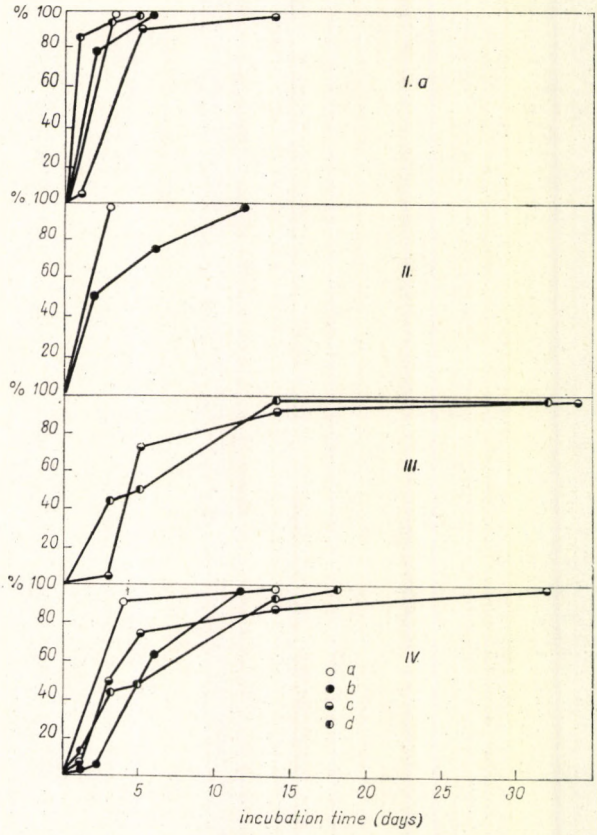

Fig. 2. Nutrient media marked I.a, II, III, IV showing the dynamics of appearing colonies in plate pouring carried out in different times from Lake Balaton and from the Inner Lake of Tihany. Lake Balaton: (a) 19th VIII, 1968; (b) 8th IX, 1968; (c) 6th VIII, 1969. Inner Lake of Tihany: (d) 6th VIII, 1969

The other advantage of the sodium-caseinate agar yielding higher number of bacteria that the bacterium colonies appearing on the slide are small, they are clearly delineated from one another, as compared to those cultured on nutrient agar whose colonies are large, spreading fast and becoming liquefied, consequently, they render counting difficult. The cromogenic bacteria also develop on the sodium-caseinate agar in a higher percentage (STRZELCZYK et al. 1967).

The medium containing lake water and algal powder yielded a better result when inoculated with the water of Lake Balaton than the same procedure carried out on nutrient agar. Somewhat smaller values were obtained when inoculated with the water of the Inner Lake of Tihany. Here, the colonies were small and easy to count. Surrounding a number of colonies appearing 4—6 days after inoculation, on a dark green agar slide, striking light zones may be perceiveable. By counting the colonies having lighter zones we might get some information to the quantity of microorganisms probably taking part in the decomposition of pigment materials.

The dynamics of the development of the final number of bacterial colonies on media enriched with organic materials *(Figs 2* and *3)* is rather

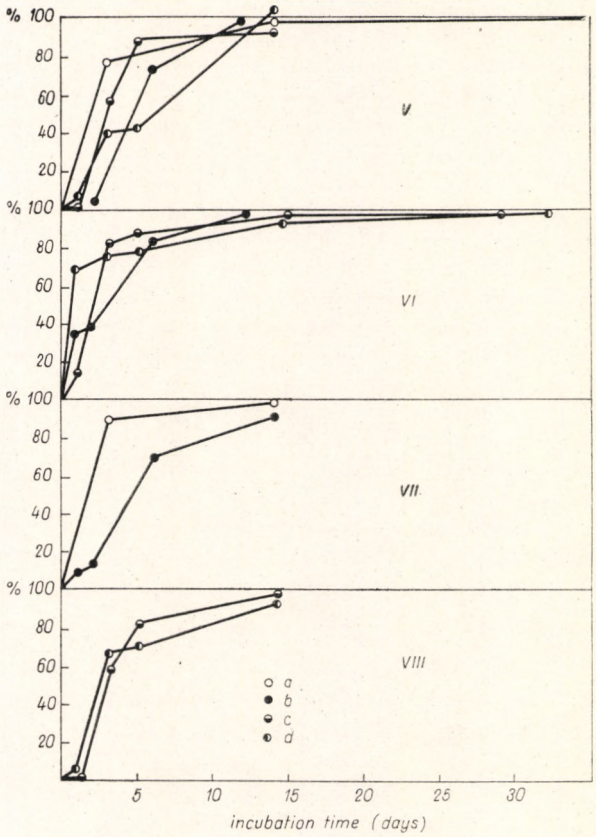

Fig. 3. Nutrient media marked V, VI, VII, VIII showing the dynamics of appearing colonies in plate pouring carried out in different times from Lake Balaton and from the Inner Lake of Tihany. For symbols see *Fig. 2*

variable. The fastest rate of growth has been observed on nutrient agar. On the fourth day 85% of the final number of bacterial colonies appear. On the other media, the rate of growth is slower, and by the 14th day 95—100% of the final number of bacterial colonies appears.

The other group of examined media not enriched with organic materials, but consisting of natural substrate or containing salts is shown in *Table 1.* On these culture media far many colonies developed than on the nutrient agar rich in organic materials, and quite frequently they yielded the highest values among all the culture substrates examined *(Fig. 1).* It was quite surprising that when Balaton water was inoculated on the lake-agar deriving from the supertrophic water of the Inner Lake of Tihany we obtained 10-times less number of bacterial colonies, while inoculated with the water of the Inner Lake of Tihany this number was only 4 when using lake-agar deriving from the water of Lake Balaton. The cause of this phenomenon may be sought for in the high algal content of the Inner Lake of Tihany whose colour is permanently of a green hue. In preparing culture substrates, as the result of repeated boiling, a large quantity of organic material as well as materials inhibiting the growth of bacteria become dissolved. An extravagant increase in organic materials, as has already been seen, may inhibit the growth of a part of the bacterial flora inhabiting the water.

The development of the final number of colonies on the lake water and mud-extract agars containing merely natural substrate as well as on oligocarbophilic medium, is slower than on substrates enriched with organic materials *(Fig. 4).*

The majority of colonies appearing on the agar-slides containing natural substrate develops on the borderline of the agar and glass. This peculiar phenomenon may be due to surface effects (ZoBell, 1943). The colonies are frequently small, indistinct, spotlike, difficult to perceive and thus counting is hindered. The development of colonies is very slow, and besides the saprophytic colonies other colonies also appear on the agar slide. For this particular cause the agarized nutrient media containing natural substrate are used only for special investigations. For example, the nutritive material supply of a lake may be calculated with their help quite easily, as well as the developmental rate of the natural microflora, etc. The MPN procedure was combined with a membrane filtering by Melchiorri-Santolini and Cafarelli (1967), where the lake waters was used for nutritive solution, receiving a higher number for bacteria than in nutritive solution enriched with organic materials.

When comparing the properties of nutritive media, on the one hand, those which has been enriched and examined by us, and on the other hand, those containing the natural substrate taking into consideration the properties of bacteria regarded to be autochton and zymogen as established by Winogradsky (1932), the following conclusion is well justified: that zymogen bacteria are able to develop on nutrient media enriched with organic materials, while media containing only natural substrate is good for autochton bacteria only. Gibson (1957) says that the Winogradsky's classification of soil bacteria is also valid for water inhabiting microorganisms.

We should, of course, bear in mind that only a supposed parallelism exists, for Winogradsky's classification is rather general, as the above presented results conclusively prove that on various culture media, how divers representatives of natural microbial associations may develop. The difference

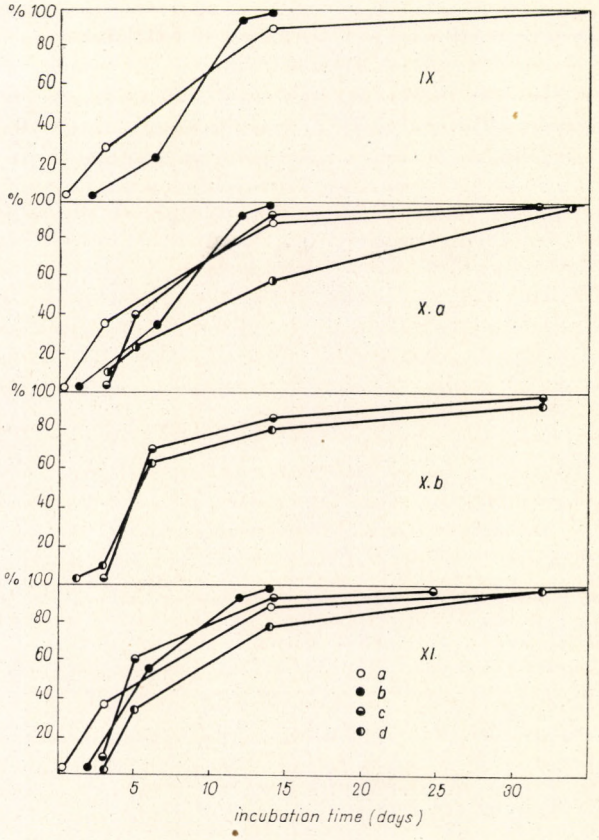

Fig. 4. Nutrient media marked IX, X.a, X.b, XI showing the dynamics of appearing colonies in plate pouring carried out in different times from Lake Balaton and from the Inner Lake of Tihany. For symbols see *Fig. 2*

between the result obtained by plate pouring and direct counting is mainly due to a high degree of selective capacity of the various nutrient media than to the high number of dead organisms counted on the membrane filter, for the quantity of dead organisms, measured by any type of method, compared to the total number hardly reaches a mere 20% (RAZUMOV, 1962).

Summary

1. Significant differences have been observed between culture media used by us for counting saprophytic microorganisms.

On the basis of results obtained by comparative analysis, for the waters in Hungary the sodium-caseinate agar proved to be the most suitable for counting saprophytic microorganisms. This medium yielded the highest number for bacteria, the colonies were small, did not liquefy and the chromogenic bacteria occur in them in greater numbers.

2. It was interesting to note that on nutrient media containing only natural substrates and salts a large number of bacteria were able to develop. The slow rate of growth of the colonies and the difficulty in their counting make them suitable, primarily, for "special" investigations.

3. The authors in studying the microflora of waters by direct and indirect methods found that significant differences exist between the results of the two methods, which, according to them, are mainly due to the selecting property of the employed nutrient media as against the supposed high percentage of dead organisms counted on the membrane filter.

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ÖSSZEHASONLITÓ TÁPTALAJ-TANULMÁNYOK A SZAPROFITA VIZI MIKROORGANIZMUSOK MENNYISÉGI FELMÉRÉSÉRE

Oláh János és *Vásárhelyi Réka*

Összefoglalás

1. A szaprofita mikroorganizmusok számlálására általunk felhasznált táptalajokon nagymértékben eltérő eredményeket tapasztaltunk. Az összehasonlító vizsgálataink eredményei alapján a nátrium kazeinátos táptalaj bizonyult a legalkalmasabbnak a szaprofita mikroorganizmusok számlálására.

A legmagasabb baktériumszámot adta, a telepek aprók, nem összefolyók és közöttük a kromogén baktériumok is nagy számban szerepelnek.

2. A csak természetes szubsztrátumokat és sókat tartalmazó táptalajokon meglepően sok baktérium képes fejlődni. A kolóniák lassú növekedése és számlálásuk nehézkessége miatt azonban elsősorban „speciális" vizsgálatok elvégzésére alkalmasak.

3. A szerzők a vizek mikroflórájának direkt és indirekt módszerekkel történő tanulmányozása során a két módszer eredményei közötti nagy különbségeket — szemben a membrán filteren számolt elhalt sejtek feltételezett magas százalékával — elsősorban az alkalmazott táptalajok szelektáló hatásának tulajdonítják.

СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ПИТАТЕЛЬНЫХ СРЕД ДЛЯ КОЛИЧЕСТВЕННОГО ИЗУЧЕНИЯ САПРОФИТНЫХ ВОДНЫХ МИКРООРГАНИЗМОВ

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1. В развитии сапрофитных микроорганизмов на используемых нами различных питательных средах наблюдались различия. На основе сравнительных исследований самой лучшей питательной средой, для подсчёта микроорганизмов, оказалась питательная среда содержащая казеинат натрия. Эта питательная среда дала самое большое число бактерий. Маленкие колонии не сливаются и между ними в большом числе находятся хромогенные бактерии.

2. На питательных средах содержащих только натуральные субстраты и соли развивается удивительно много бактерий. Из за медленного роста колоний и трудного подсчёта они пригодны в основном для специальных исследований.

3. Авторы обнаружили при изучении водной микрофлоры прямыми и косвенными методами значительные различия между результатами двух методов. Эти различия трактуются как результат того, что использованные среды обладают селективными свойствами.

ANNAL. BIOL. TIHANY | 37 | 247—279 | HUNGARIA 1970

KLADOCERA TANULMÁNYOK A BALATONON
IV. SZUBFOSSZILIS* MARADVÁNYOK BALATONI ÜLEDÉKEKBEN II.

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Érkezett: 1970. február 28.

E tanulmány folytatása SEBESTYÉN: „Kladocera tanulmányok a Balatonon" sorozat IV. fejezete szubfosszilis kladocera maradványokkal foglalkozó I. részének (SEBESTYÉN, 1969 a: 247, 255). A soron levő fajok: *Eurycercus lamellatus*, *Camptocercus rectirostris*, *Acroperus harpae*, *Leydigia leydigi*, *L. acanthoceroides* továbbá a Balatonból eddig ismert öt *Pleuroxus* faj: *P. truncatus* [syn. *Peracantha truncata*], *P. laevis*, *P. aduncus*, *P. trigonellus* és *P. uncinatus* v. *balatonicus*.

A tanulmány felépítése azonos az említett I. részben követett menettel: (SEBESTYÉN, 1969a, 235, 251—252)

Eurycercus lamellatus O. F. MÜLLER 1785
1—2 ábra, 1—4 kép.

I. a. Balatoni recens adatok

DADAY (1897) csak a Kis-Balatonból sorolja fel, ahol 1891-ben „rengeteg tömegben" gyűjtötte, 1904-ben a Balatonból nem ismert fajok listájában szerepel.

HANKÓ (1925) planktonmintáiban PONYI (1965) találta.

KOTTÁSZ (1933, E-K-S, 1937: 12 B táblázat) planktonból és homokos nádas partok közeléből említi.

A negyvenes években a tihanyi Kisöböl és Gödrös növényzettel benőtt területéről, főként Myriophyllumosból van feljegyezve (SEBESTYÉN, 1948: 110, *2—3 ábra, táblázat)*. Lásd még SEBESTYÉN, 1965: *I. B. táblázat.*

A hatvanas évekből (SEBESTYÉN, nem közölt adatok): Tihany, Gödrös, fonalas moszatok között (1964. V. 25., 1965. VII. 25.), nádmozaik, bevonattal.

Balatonfüred, nádas közelében (1965. VI. 25.) Vízmélység 265 cm.

Keszthely (1964. IX. 16. M 28/1 sz. minta), strand hídja melletti vegyeshínárosból.

PONYI, 1956: 115, 1957: 1., *III. táblázat*, parti hínárosban, *Myriophyllum* állományban (Aszófői patak torkolata, Fűzfő kikötő, Csóka-partok III. táblázat) és nádasban (Balatonudvari, kevés, 1962 : 135).

I. b. Elterjedés, lelőhelyek

* A sorozat I. részében használt „Szubfosszilis . . ." kifejezés helyett — a lektoráló KRETZOI MIKLÓS professzor szíves javaslatára — a megfelelőbb „Negyedkori . . ." megjelölést e dolgozatban, technikai okokból, még nem használhattam.

Alámerült és emersus (NEGREA) növényzet között, tavak parti övében, sekély parti vízben, kis vizekben (LILLJEBORG, K. BERG, SCOURFIELD és HARDING), az angol tóvidék sok tavában, chárásokban (FLÖSSNER, WAGLER), folyóvízben (ZEMP, SMIRNOW) és mocsarakban is (ZEMP).

Fonalasmoszatok szövedékében legtöbbnyire a fiatalja található. A fonalakra antennájával és a héj szélével tapad (MEUCHE, 1939: 444, *11 táblázat)*.

FLÖSSNER abba a kladocera csoportba sorolja, mely sűrű makrovegetáció állományokban és növényzetnélküli fenéken is megél, de nem ubiquista. Synekológiai szempontból az *Eurycercus lamellatus*—a csoport névadója (1964: 68, 85, 87—88).

Ekológiájáról részletesen SMIRNOW tájékoztat. Elterjedése a Volga rezervoirjaiban növényzettel benőtt területekre szorítkozik. Abundanciája valószínűleg a frissen elhalt növényi részek baktériumtáplálékától függ. Detritus táplálék egymagában nem biztosítja a populáció jólétét (SMIRNOV, 1962). Táplálkozásában detritusznak és baktériumoknak van szerepe. A meleg időszak előrehaladt szakaszában iszapos üledékben való tartózkodásának alapja a baktériumgazdagság. Kultúrákban baktérium-táplálékon hosszú ideig megél, szaporodik (utalás RODINA, 1950-re).

I. c. Tavi üledékek felületi rétegéből a külsőváz részei előkerültek a Madison tavakból (FREY, 1960 b), a Mississippi völgy egy szakaszában levő tavak közül az északi fekvésűekben folyamatosan, a déliekben meglehetősen szórványosan fordul elő (DE COSTA, 1964). Szerző északi fajnak minősíti. Indiana állam északi részében levő tavak mindenikében gyakori (MUELLER, 1964).

Szubfossilis előfordulás

II. d. Általában:

FREY, 1958 *(3—4 táblázat)* maradványok előfordulása irodalom alapján leírás, ábrázolás (235—239 c, *19—27 ábra.* — 1959: 33. o. *3—4 ábra*, — 1962. a: *3—4 ábra.* — 1962 b: 1139, *1, 11, 20 ábra.*

Lásd még GOULDEN, 1964: 20.

II. e. Európa.

ZEMP, 1941: 61, 67. *Táblázat.* Wauwiler See tavikréta rétegben és kultúrrétegben **P S C A.****

Längsee FREY, 1955: **P.C. A_1** *III. táblázat.* Wallesen FREY, 1958: *1—2. táblázat* **S P H C** A^1, Alleröd II a, II b, *1—2. ábra*, üledékben C^{19}**I** 11 300 év a leggyakoribb. Nincs adat arra, hogy az *Eurycercus glacialis* előfordult volna e tóban.

Schleinsee FREY, 1961: *2. ábra.* VIII—X, kevés. Herning szelvény. FREY, 1962 b: *1—2. táblázat, Fig. 3.* (spektrum). Előfordult az Eemi interglacialisban, Post Eemiben is.

Leggyakrabban **P H S** (l. még GOULDEN, 1964: 20).

Esthwaite Water. GOULDEN, 1964: *2—3. táblázat 5—6. ábra.* Csaknem valamennyi mintában: II—V ill. VI—VII b pollen-zóna. A populáció relatíve kicsiny, legmagasabb értékek a II. alján levő rétegek közepénél. Ma is él e tóban.

** A szövegben nagybetűkkel jelölöm a külső váz részeit: P = utópotroh, C = ennek végkarma, H = fejpajzs, S = héj, E = ephippium, A = antenna.

II. f. Lake Nojiri TSUKADA (1967). A Late glacialistól egészen a korai hamurétegig fordul elő, maximum a korai jégkorszak utáni (RI, R II) rétegek határán.

II. g. Magas hegyi tavak

Lake Zeribar MEGARD, 1967: 184—185. o. *Fig. 2—3.* palinológiai adatok alapján megállapított klímával egybeeső kladocera zónákban. A zónában (22,600 ± 500 év, 17 m alatt) a következő *B* zóna közepéig 8100 ± 160. A populáció kicsiny, valamivel a *Leydigia leydigi* elmaradása előtt tűnik el. Az *A* zónában kozmopolita és északi fajok vannak, hasonlóan a Wallensen és az Esthwaite Water Late glacialis Alleröd intervallumának üledékeiben (l. fentebb). Ebben a korszakban az európai tavakban *E. lamellatus* mellett *Chydorus sphaericus*, *Alona rectangula*, *Acroperus harpae*, *Graptoleberis* volt gyakori (v.ö. SEBESTYÉN, 1969: 238. o.). a fajok száma (13—14) egyidejűleg azonos volt. E fajok északi földrajzi elterjedése arra mutat, hogy viszonylag intoleransok a meleggel szemben (v.ö. *Leydigia leydigi* fejezetét).

Whimpy Lake DECOSTA, 1968: *4. ábra*, (%-os összetétel, spektrum). Fajunk a Late Glaciális alján a *Leydigia leydigi* és *Alona quadrangularis*t követően jelenik meg. A Chydoridae zónák határai nem esnek szükségszerűen össze a pollenzónákéval. Az **I. b** zónában kevés. Az *Alonella nana*val együtt „minor" elem (412. o.). — A spektrum szerint van kevés adat a **II . b.**-ből is. **III.**-ból nincs.

II. h. Trópusi kistavakból nincs adat.

II. i. Balaton *(2. táblázat)*.

Valamennyi minta pozitív, a két legújabb kivételével (N° 1,20). Összesen 110 drb. *E. lamellatus*-maradvány került elő. Az előfordulás gyakorisága megfelel — kor szerint — a Wallensen és az Esthwaite Water adatainak.

A 160. sz. mintában (Ia pollenzóna) 78 *E.l.* maradvány. Minőség szerinti megoszlás: **S** = 37, **P** = 17, **H** = 3, **C** = 3, A = 5, egyéb = 13.

E mintában a Cladocera fajok gyakorisági megoszlása: 22—24%—98%: *Acroperus*, *Eurycercus*, *Alonella nana*, *Alona affinis*. 2.15—1.19%: *Camptocerus*, kis *Alona*, *Chydorus sphaericus*, *Alonella excisa*, *Alona quadrangularis*. A következő fajok %-os gyakoriságértéke kisebb 1%-nál: *Oxyurella tenuicaudis*, *Graptoleberis*, *Pleuroxus uncinatus*, *Chydorus piger*, *Alanella rostrata*, *Sida*. Összesen 15 faj, a két kis *Alonát* összevonva.

Camptocercus rectirostris SCHOEDLER 1862
syn. *Lynceus macrurus* (FISCHER) 1848
5. kép

I. a. DADAY (1897) RICHARDS adatára (Keszthely) és saját feljegyzésére (Tihany, Balatonfüred, Badacsony) utal. KOTTÁSZ planktonmintából és *Potamogeton* állomány közeléből említi. MESCHKAT (1964) füredi nádas bolyhos bevonatában találta tiszta- és zavarosvizű területeken (491, 492). További adatok: negyvenes évekből: Tihany, Gödrös, növényzet nélküli területek (SEBESTYÉN, 1948), tihanyi Kisöböl, 1943. X. 17, 1947. X. 29. *(rajzok)*. Hatvanas évekből: Gödrös, nádmozaikos szélvíz, 1965. VII. 14., VII. 25. Tihanyi kisöböl, két nádas közötti homokos terület, 1963. IX. 25. XI. 5. neuszton. Balatonfüred, strand előtt (1963. VII. 18.) (fejpajzs). Keszthely, parti hínáros, a strand hídja és nádas közelében (1964. IX. 16.).

I. b. Lelőhelyek általában: növényzettel benőtt parti területeken, fenék közelében (LILLJEBORG, BERG) közönséges. SCOURFIELD és HARDING, meglehetősen ritka. FLÖSSNER és WAGLER gyakori. Hegyipatakok partiövében is, valamint növényzettel benőtt kistavak iszapjában (NEGREA, 1966). FLÖSSNER szerint Chárásokban, parti Myriophyllumosban, növényzet nélküli vagy sűrűn benőtt területeken egyaránt előforduló fajok csoportjába tartozik. Synekológiai szempontból az *Eurycercus* csoportba sorolja, Cladocera taxocenozis tekintetéből a *Chara*-mezők csoportjába (FLÖSSNER, 1964: 51, 68, 88).

I. c. Maradványok felületi üledékekből.

Madison tavak, FREY, 1960 b.

A Mississippi völgy 45 tavából 39-ben. Eurytop. %-os előfordulás legmagasabb értéke Luisiana állam két tavában (pH 6,8, 6,4) (DECOSTA, 1964).

Indiana állam északi részének három tavában (MÜLLER, 1964.).

Szubfosszilis maradványok

II. d. Általában.

FREY, 1958: Előfordulás. Maradványok ismertetése.

FREY, 1959: 34. o., *9—11. ábra*, fejpajzs és ábrázolása.

FREY, 1960: 693, *7—8. ábra*.

FREY, 1962 b: *8—11. ábra*.

GOULDEN, 1964: 22. o. irodalmi adatok.

II. e. Európa.

Wauwiler-See. ZEMP, 1941: Csak a tavikréta rétegben, ritkán. **S P C.** *C. lilljeborgi* kevésbé gyakori. **S P C.**

Längsee FREY, 1955: 156. **S P C.**

Wallesen FREY, 1958: Legtöbb maradvány **S H C P** a Late Glacialisból (Lower Alleröd II. a.). *Fig. 42*, 44—48. Az európai populáció fejpajzsa némileg különbözik egy Indiana állambeli tó szubfosszilis populációjáétól.

Schleinsee FREY, 1961: *2. ábra*, VI-x pollenzóna valamennyi mintájában.

Herning szelvény. FREY, 1962 b: *1. Táblázat*, valamennyi Eemi és Posteemi rétegben.

Esthwaite Water, GOULDEN, 1964: 22. o. *2—3. Tábl., 5—6. ábra.* **C H S P** maradványok. Leggyakoribb az Allerödben és a Pre-borealban, a populáció ezután lecsökken, kieséssel, II—V. pollenzónában, VI—VII. b-ben folyamatosan.

II. f. Lake Nojiri. TSUKADA, 1967: Populáció a korai Postglaciálistól (R I) az első hamuesőig (R II). Ezután az *Eurycercus lamellatus*sal egyidőben eltűnik.

II. g. Magas hegyi tavak.

Lake Zeribar. MEGARD, 1967: 185—186. Csupán a *C. australis* fordul elő, mely morfológiailag kevéssé különbözik a *C. rectirostris* s. st.-tól, állatföldrajzi elterjedése más.

Whimpy Lake, DECOSTA, 1968: *4. Ábra*. Egyetlen adat a II. B Cladocera zónából.

II. h. Laguna de Petenxil, GOULDEN, 1966 a: 92. Egy trópusi *Camptocercus* fordul elő.

II. i. Balaton. Mint láttuk, balatoni recens előfordulásáról kevés adatunk van. Figyelmet érdemel az is, hogy FLÖSSNER szerint (1964, 51, *2. Táblázat, 2. Ábra)* fajunk parti vizek lakója és nem mozgékony szervezet a helyváltoztatást illetően. Tavunkban szélesebb körű elterjedésére lehetne következtetni, a parti öv habitatjai nincsenek kellő mértékben átkutatva. Sztenotop fajnak látszik, ekologiai valenciájának ismeretében valószínűleg több lelőhelyről kerülhet elő és habitatja is jobban körülírható.

A balatoni populáció morfológiai szempontból a *Camptocercus rectirostris* s. str.-nak felelhet meg. Kevés számú szubfosszilis maradványa (**H C P**) két holocén mintából hiányzik. A jégkorszak végén, e korból származó minták adataiból következtetve, gyakorisága magasabb lehetett, mint a holocénban.

Acroperus harpae BAIRD 1835 syn. *A. leucocephalus* KOCH
3. ábra, 6—7. kép

I. a. DADAY (1897) Balatonfüredről említi. HANKÓ planktonmintáiból PONYI (1965) sorolja föl. KOTTÁSZ (1933, E-K-S, 1937) planktonmintáiba is bekerült néhány példány. *Potamogeton* „sziget" környékén, a Remete-barlangok táján nádas mellől, homokos nádas partoknál is följegyezte.

Harmincas évek további adatai: Tihany, Kisöböl, rengeteg (1937. VII. 29.), *Myriophyllum* között (VIII. 12.). A Kisöböl és Gödrös habitatjaiban (Balatoni Faunakatalógus).

A negyvenes évek adatainak feldolgozása 1944—1948 évek adataira támaszkodik (SEBESTYÉN, 1948). ENTZ (1947) megállapította, hogy ősz elején a Kisöböl *Myriophyllum*-állománya jóval gazdagabb *Acroperus*ban, mint a *Potamogeton*, a litorális öv hínárosainak *Acroperus*-népessége sűrűbb, mint a szemilakusztrikus állományoké. Vízmélység szerint a felső vízréteg látszik a legkedvezőbbnek.

A hatvanas évek főként üledékminta-sorozatok részben feldolgozott adatai a Kerekedi öbölből, Balatonfüredről a strand közeléből és Keszthelyről származnak.

PONYI nyár derekán leginkább *Ceratophyllum*osban találta több lelőhelyen (1965, 1957), s nádasból is említi (1962).

eI. b. Honos nagy tavak parti övében, kis vizekben (LILLJEBORG, WAGLER, K. BERG, ZEMP), növényzet között, szemilakosztikus állományban is (SCOURFIELD és HARDING), továbbá be nem nőtt területeken. Kora tavasszal tychoplanktikus is lehet (FLÖSSNER, 1964).

Utóbbi szerző a „Stechling-See- Gebiet" tavaiban jellegzetes habitatjaként a tavirózsa-hínáros *Myriophyllum* és *Stratiotes* állományt jelöli. Chárásokban, más macrophytaállományokban és iszapban is domináns tagnak minősíti.

Románia számos tavában, különösen a Duna árterületén és a delta tavaiban (NEGREA, 1966 139—140) él. Különböző alakjainak rendszertani jelentősége nincs. MEUCHE (1939: 444. *11. Tábl.):* algaszövedékben meglehetősen gyakran, de kevés egyedszámban találta.

I. c. A külső váz (vedlett) részeit megtalálták a Madison tavak mindenikében (FREY, 1960 b), az Indiana állam északi részében levő három tóban

(MUELLER, 1964). Jelenlétét a Mississippi-völgy tavaiban DECOSTA mutatta ki 14 tóból: északi fekvésűekben folyamatosan, déliekben igen szórványosan fordult elő.

Szubfosszilis előfordulás

II. d. Általában. FREY, 1958: Maradványok előfordulása az irodalom szerint. Ismertetés. Ábrázolás, l. még GOULDEN, 1964: 20.

II. e. Európa, Wauwiler See. ZEMP, 1941. Maradványai kultúrrétegekben gyakoriak, tavikrétában ritkán fordulnak elő.

Längsee. FREY, 1955: **S H P C.**

Wallensen. FREY, 1958: Leggyakoribb a II a és II b pollenzónában, III-ban szórványosan.

Schleinsee. FREY, 1961: Maradványok valamennyi elemzett mintában (VII—X). Leggyakoribb a X pollenzónában.

Herning szelvény. FREY, 1962 b: 1139. o., *14—32. Ábra.* **H S** leggyakoribb. Valamennyi Eemi és Posteemi mintában változó abundanciában.

Esthwaite Water. GOULDEN, 1964: **S H P.** A tó fejlődése alatt végig megvolt. Legnagyobb abundanciája az Alleröd ben és a Pre-Boreálisban, lecsökkent a Borealisban és az Atlanticumban. Ezután máig kis populációban állandóan jelen van.

II. e. Lake Nojiri. TSUKADA, 1967: Jelen van a Late Glacialistól, a két hamueső között is, a tó egész történetében.

II. f. Magashegyi tavak. Whimpy Lake. DECOSTA 1968: *4. Ábra* (spektrum). A II Chydoridae zónában honosodik meg e tóban. Előfordulása sporadikus, és abundanciája rendkívül alacsony, jelenléte növényzet térfoglalására utal. A faj északisága összeegyeztethető récens és szubfosszilis előfordulásával.

Lake Zeribar. MEGARD, 1967: Szerző közel 23 ezer évnek megfelelő üledékrétegeket elemezve, eurytopikus fajnak tartja. Valemennyi zónában előfordul — *A*-ban kevés, változó abundanciával, jelentősebb lesz a *B* zóna tetejétől, *C* zóna közepétől abundanciája csökken. 15 000 BP-től 5000 BP-ig felmelegedés van, de a hőmérséklet nem lehetett magasabb a mainál. Azoknak a fajoknak egyike, melyek európai vizekben az Alleröd ben nagy abundanciával voltak jelen, megvannak a Lake Zeribar A zónájában. A klíma ekkor olyan lehetett, mint a Wallensenben és az Esthwaite Waterban: hűvösebb, mint ma, a zóna tetejének megfelelő időben száraz, a diatomeákról ítélve a víz alkalikus, és gyakorlatilag *Chara* az egyetlen növény, mint első submersus forma.

II. h. Trópusi — kistavakból nincs adat.

II. i. Balaton. A feldolgozott mintákból összesen 140 maradvány került elő, legtöbb a jégkorszakvégi mintákból: 160. sz. mintából 87 drb, 140 számúból 49. **S** = 74, **H** = 33, **P** = 29. Holocén mintákból néhány fejpajzs és több héj van feljegyezve. A héjak identifikálása nem minden esetben biztos. A 160. sz. mintában növényi törmelék dominál, 140.-ből néhány *Chara*-termés van feljegyezve.

Az *Acroperus harpae* eurytherm volta mellett (kifejezett hidegtűréssel) szólna egyfelől a jégkorszakvégi mintákban gyakorisága, másrészt pedig tömeges előfordulása a nyáron erősen felmelegedő Balaton vizében.

Leydigia leydigi (SCHOEDLER) 1863
syn. *Alona leydigii* SCHOEDLER *Leydigia quadrangularis* (LEYDIG)
4. Ábra

I. a. DADAY Balatonfüredről és Keszthelyről említi, utalva RICHARD és FRANCÉ gyűjtéseire, majd Siófokról is (1888, 1894, 1897, 1903, 1904).

Újabb adatok, hatvanas évek: PONYI (1963): Keszthelyi öböl, nagyobb tömegben csak egyik nyári mintában. PONYI (1966): u.a. öböl középső, legmélyebb részén.

SEBESTYÉN mintasorozatából:

1. Balatonfüred és Tihany között, fele úton 1965. VI. 25. nyíltvíz; iszap, m = 365 cm — Együttes: *L. acanthocercoides, Alonella rostrata, Monospilus, Sida, L. leydigi, Alona* (SEBESTYÉN, 1965: *IB táblázat).*

2. 1965. VII. 23. Tihany, Kisöböl bejárata, nyíltvíz jellegű terület, m = 240 cm. *L. leydigi, Macrothrix laticornis, Alonella rostrata, Pleuroxus uncinatus, Monospilus (Collotheca sessilis* epibionttal).

3. Balatonfüred, 1965. X. 8. Horgászóhely, nádas: m = 170 cm. A szűredék nádkaparékhoz hasonló. Két élénk vörös színű *L. leydigi* példány. Együttes: *Pleuroxus uncinatus, Iliocryptus*, három élénk karminvörös példány. *Alona affinis.*

4. Balatonfüred, 1966. VII. 12. Nádas előtt, ritka hínáros (*Pot. perf.*). *Leydigia leydigi, L. acanthocercoides, Macrothrix, Alona, Pleuroxus.*

I. b. Kifejezetten fenéklakó, iszapba rejtőzködik (KURZ, LILLJEBORG, WAGLER, SCOURFIELD és HARDING). Parti iszapos területeket kedvel, kemény alzatot bevonó vékony iszaprétegben (FLÖSSNER). Jég alól is előkerült (LILLJEBORG). Szerző balatoni példányai vörös színűek. Erre vonatkozóan lásd még LILLJEBORG megjegyzését. Hemoglobin jelenléte a környezet O_2-szegénységére utal (GOULDEN, 1966, SARS után). Behatol nagyobb mélységbe (LILLJEBORG) és a litorális övön túli területekre (MUELLER, 1964: 26).

I. c. Maradványok felületi üledékekből:

FREY, 1960 b: Mendotaban a három leggyakoribb faj között. Indiana állam 3 tavából MUELLER (1964) mutatta ki. 3. DECOSTA, (1964): 45 tó közül csupán négyben nem találta maradványait. Az Illionisbeli Carbon Lakeban (pH 7,8.) a Chydorida-maradványok közel fele e fajhoz tartozik. Szerző eurytopnak minősíti (vö. MEGARD, 1967: 185).

Szubfosszilis maradványok

II. d. Általában. FREY, 1959: 37, *15—18. ábra* **H**,
1962 a: *15—18. ábra* **H**,
1962 b: 1139, *21. ábra* **H**,
1960 b: 695, *4—5. ábra* **S C.**
GOULDEN, 1966 a: **S H P**, 101. 1966 b: 387—388.

II. e. Európa. Schleinsee, FREY, 1961: VII. VIII—IX. határán, X. pollenzónákban, kevés, igen szórványos.

Herning, Eemi interglaciális. FREY, 1962 b.

II. f. Lake Nojiri. Nincs adat.

II. g. Hegyi tavak. Dead Man Lake (MEGARD, 1964: *1. táblázat, 20—21. ábra*). A fajszegény, egy rétegben legfeljebb 4—5 Chydorida fajt tartalmazó

Pleistocén I—V. Chydorida zóna mindenikében előfordul: Az I.-ben zónajelző a *Chydorus sphaericus*-szal, a IV. zóna kis *Alona-Leydigia leydigi* zónának van jelölve. Előfordul a felületi rétegekben is, hiányzik az ún. fekete üledék többi, Chydorida fajokban gazdag rétegeiben.

Szerző szerint a zónák a *Chydorus sphaericus* és kis-*Alonák* kombinációja egymással és a *Leydigi leydigi*vel vagy nagy-*Alonák*kal váltakozva. A zónális tagoltság nem látszik összefüggésben lenni klimatikus vagy egyéb külső tényezőktől indikált változásokkal.

Lake Zeribar, MEGARD, 1967: *2—3. ábra.* Az A és B zónákban a *L. leydigi* nem éri el a *L. acanthocercoides* abundanciáját. Az a körülmény, hogy a B zónában az *Eurycercus lamellatus* eltűnése után csakhamar eltűnik, s jelenléte egybeesik a *L. acanthocercoides* megjelenésével, a klímával fokozatos kedvezővé válására enged következtetni (12 000—5000 BP között). Ez pedig, a *L. leydigi* mai elterjedését figyelembe véve, arra utalhat, hogy fajunk intoleráns a meleggel szemben, de arktikus állapotokat sem tűr.

A *L. acanthocercoides* tárgyalásánál látni fogjuk, hogy e tóban a chydorida-zonáció változása összhangban van a palinológiai alapon megállapított klímaváltozással.

Whimpy Lake. DECOSTA, 1968: *4. ábra.* Szerző a tó üledékében 4 Chydorida zónát ismer fel, melyekben általában egy trópusi kis *Alona* és két *Chydorus* sp. dominál. A L. *1.* általában igen kis populációban szerepel, de az I. zóna alján fajunk és az *Alona quadrangularis* abundanciája is elég magas. A későbbi rétegekben szórványos.

II. h. Trópusi kistavak. Laguna de Petenxil, GOULDEN, 1966 a: *2—3. táblázat, Fig. 3.* és Aguada de Santa Ana de Vieja, GOULDEN, 1966 b. Előbbiben igen kevés és szórványos *L. leydigi* mellett a *L. acanthocercoides* és *L. parva* maradványai vannak jelen. Utóbbit DADAY írta le Paraguayból, több adata nem ismert. Az Aguada üledékeiből a *Leydigiopsis megalops* Közép- és Dél-Amerika humid tropikus régiójában honos faj maradványai kerültek elő. E faj ekológiai igénye hasonló a *L. leydigi*éhoz. Spektrumában mind a rétegek szerinti előfordulás, mind az abundancia hasonló a *L. leydigi*éhoz.

II. i. Balaton. Csak 4 Holocén mintából kerültek elő maradványai, legtöbb az Atlanticumból. Valamennyi **H.** A mért hét **H** hossza 250—309 μ. középérték 271.5. u.

Leydigia acanthocercoides (FISCHER) 1854
5. ábra, 8—10. kép

I. a. DADAY korábbi balatoni vonatkozású munkáiban (1888, 1897, 1918*) *Alona acanthocercoides* FISCHER néven említi, későbbi (1904) dolgozatában *Leydigia acanthocercoides* FISCHER néven szerepel. A DADAYtól *A. balatonica* névvel új fajként leírt Chydorida azonosnak látszik a *L. acanthocercoides* FISCHER-rel (SEBESTYÉN 1965: 203, 220).

E faj balatoni elterjedését l. SEBESTYÉN, 1947, 1948, 1965. A hatvanas évek eddigi nem közölt adatai (Balatoni Fauna katalógus, vázlatok, vázlatos

* A „*Fauna Regni Hungariae*" = „*A magyar Birodalom Állatvilágá*"-ban (1918) a krustáceák jegyzékét DADAY J. állította össze. Irodalmi listájában a szerző legkésőbbi munkája 1894-ből származik. A millennium alkalmából összeállított listának Arthropoda része 1900-ban már készen volt, s meg is jelent. (Előszó kelte: 1918. II. 31.)

feljegyzések, minták elemzése S. O.) megerősítik azt a korábbi megállapítást, hogy a *L. acanthocercoides* a Balaton egyik leggyakoribb fenéklakó Chydoridája (SEBESTYÉN, 1965: 202). A populáció életpályájának rövid aktív szakasza kihatásaként évi átlagban ez nem tűnhet ki (SEBESTYÉN, 1965: 220, l. még GOULDEN, 1966: 397). Egyéb adatok a Balatonból: PONYI (1956, 1957), hínárvizsgálatai során a Sióban *Myriophyllum* bevonatában találta szórványosan, továbbá 1963: 115) a Keszthelyi-öbölben a *L. leydigi*val azonos üledék mintákban és mennyiségben. Planktonmintáiba is bekerült (PONYI, 1968: 171).

SEBESTYÉN (1964, *2—3. táblázat)* horizontális planktontanulmánya keretében vertikális vízoszlop szüredékében találta planktonrákok és más iszaplakók között. Pl.:

a) DNy tórész 648 ab sz. minta, 1958. VII. 7. *b)* Keszthelyi-öböl 694. sz. minta, 1958. IX. 30. *c)* Keszthelyi-öböl bejárat, 786. ab sz. minta. 1958. VI. 9. *d)* Keszthelyi-öböl közepe, 785. ab minta.

I. b. Limicol faj (KURZ, 1878). Iszapos fenéken él (WAGLER, NEGREA). Homokkal kevert iszapos fenéken (LILLJEBORG). Lehatol nagyobb mélységbe is (LILLJEBORG, WAGLER). E meglehetősen ritka fajról nincs adat az angol tóvidék tavaiból (SCOURFIELD és HARDING), NEGREA, 1966), a vizsgált tavak egy részéből sorolja fel. ZEMP K. BERG és MEUCHE munkáiban nem szerepel.

I. c. Levedlett vázmaradványai tavak felületi üledékében: FREY, (1960 b, Madison tavak), DECOSTA (1964). Utóbbi a Mississippi-völgy 45 tava közül a délies fekvésűekben folyamatosan, az északi szakaszon szórványosan megtalálta. Déli fajnak minősíti.

Szubfosszilis maradványok

II. d. Általában.

FREY, 1958: *4. táblázat.*

Maradványok leírása és ábrázolása (FREY, 1959: 37 és 1962 a: *19—20. ábra* **H,** 1960 b: 695, *6. ábra,* 1962 b: 1139, *8. ábra.*

II. e. Európa.

Längsee, FREY, 1955: *III. táblázat* **P.**

Schleinsee. FREY, 1961. Az üledék felső rétegeiben (X pollenzóna), kevés, szórványos. Korábbi rétegekben nem találta.

Herning. FREY, 1962 b: Eemi interglaciális két mintájában.

II. f. Lake Nojiri. TSUKADA, 1967: a Late glaciális rétegek aljától folyamatosan az Early Post-glaciális és Late Postglaciálison át, a jelent is beleértve.

II. g. Hegyi tavak. Whimpy Lake. DECOSTA, 1968: Az előforduló fajok többségét tevő oly formák közé tartozik, melyek kevés adattal vannak képviselve (Post-glaciális II, III zóna).

Lake Zeribar Irán. MEGARD, 1967: Hosszú geológiai kort (±23 000 év) és a tó egész történetét magábanfoglaló tanulmányból határozottan kitűnik a Balatonban is előforduló két iszaplakó *Leydigia*-faj eltérő hőigénye (186. o.). A legrégibb *A* zónában (fanélküli steppe, száraz hideg klíma, 23 000 B.P.—12 000 B.P.) csak sporadikusan van jelen. Abundanciájának állandó emelkedésével zónajelző lesz, mutatva a Chydorida együttesben bekövetkezett lényeges változást (felmelegedés), elérve az összes kladocerák között a legmagasabb értéket. *B* zónában, noha alacsony abundanciával, már trópusi fajok is jelen vannak (12 000 B.P.—5000 B.P.). A *C* zónában (5000-től) még

mindig a leggyakoribb kladocera, jóllehet az abundancia csökken. Ekkor a máig is tartó tölgyerdő uralkodása veszi kezdetét.

II. h. Trópusi kistavak. A Laguna de Petenxil kladocerái nagyrészt Dél-Amerikában elterjedt fajok (GOULDEN, 1966). A két hazai *Leydigia* mellett a DADAY-tól Paraguayból leírt *L. parva* is előfordul. A furat spektrumjában a *L. parva* és *L. acanthocercoides* spektruma nagyjából azonos. A *B*-ben kitűnik a *L. leydigi* alárendelt szerepe a *L. acanthocercoides*-hez és a *L. parva*-hoz viszonyítva.

La Aguada de Santa Ana de Vieja. GOULDEN, 1966 a: Kevés adattal van képviselve mind a minták számát, mind az abundanciát tekintve.

II. i. Balaton. A B 28 furat feldolgozott mintáiból 91 maradvány került elő (37 **P**, 21 **S**, 17 **H** és 16 **C** + E + héj széle). Maradványok a 120. sz. és valamennyi Holocén mintában voltak. Előfordulásuk gyakorisága részben e faj délies affinitásával, másrészt a récens előfordulás gyakoriságával van összhangban (vö. SEBESTYÉN, 1968: 220—221).

Pleuroxus truncatus (O. F. MÜLLER) 1785
Syn. *Peracantha truncata* (O. F. MÜLLER) 1785
(l. EDMONDSON, 1959: 649, SMIRNOV, 1966: 62)

I. a. Tavunkból egyedül KOTTÁSZ jegyezte föl: tihanyi vizekből gyűjtött nyári mintákból *Potamogeton* és nádas szomszédságából. Nyíltvízi planktonmintáiba is bekerült, bár nem oly gyakran, mint *Sida* (KOTTÁSZ, 1933, ENTZ, KOTTÁSZ és SEBESTYÉN, 1937).

I. b. Tavak parti övében, kisvizekben, tócsákban és mocsarakban fordul elő növényzet között, ill. ily területek iszapjában (LILLJEBORG, K. BERG, WAGLER, SCOURFIELD és HARDING, ZEMP: Európa, Szibéria). Gyakorisága algaszövedékben a *Chydorus sphaericus*é után következik. Abiotikus alzat vékony moszatbevonatából régebben ismert (LANGHANS, 1911, MEUCHE után). Előfordulása a mész-szegény Pinnseeben — egyedüli kladoceraként — tömeges (MEUCHE). Az angol tóvidék alacsony fekvésű tarn-jaiból (magas összion-tartalom [SMYLY, 1958], GOULDEN, 1964 után), Dánia számos vizéből (lakes, ponds, K. BERG) ismert. Erősen savanyú vizeket — WAGLER szerint — kerüli. (l. még NEGREA, 1966) Fitofil apróságfaló. A Stechling-See vidékén növényzet között parti és nyíltvízi területeken nagy tömegben él. *Sida*hoz, *Polyphemus*hoz, *Alonopsis elongata*hoz hasonlóan leginkább elszakad litorális területektől. Alzatához nem ragaszkodik erősen. Ekológiailag átmenetet képez eulemnetikus formákhoz (FLÖSSNER, 1966). Az Esthwaite Waterben planktonban is (GOULDEN, 1964).

Szubfosszilis maradványok

II. d. FREY, (1958): Előfordulás, maradványok leírása és ábrázolása **H** kivételével. **H** jellemzése, ábrázolása, l. FREY (1959): 39—40. FREY, (1962 b): 1141, 22—50. ábra. GOULDEN, 1944: 29, I. táblázat.

II. e. Wauwiler See, ZEMP (1941): Tavikréta- és kultúrrétegekben, nem ritka. **S** mind hím.

Längsee, FREY, (1955): III. táblázat **S.**

Wallensen, FREY (1958): **S** (legtöbb), **P H C** E. *1—2. táblázat.* Főként a Late Glaciálisban, hasonlóan a *Pleuroxus trigonellus*hoz.

Schleinsee. FREY (1961): Előfordulás kevés és szórványos, folyamatosan a VII—IX.-ben, legtöbb a IX. alján, X.-ben is.

Herning. FREY (1962 b): *1—2. táblázat*, általában kevés, szórványosan. Legtöbbje **S**, egyetlen **H**, az Eemi interglaciálisban.

Esthwaite Water, GOULDEN (1964): Először az Atlanticumban (VII) jelenik meg, innen folyamatosan, — ma planktonban is.

II. f—h. Lake Nojiriből, hegyi tavakból és trópusi vizekből nincs adat.

II. i. Szubfosszilis balatoni adat nincsen.

A *Paracantha truncata* alakja, főként idény szerint változó (LILLJEBORG, K. BERG). Habitatjának ekológiai jellegéről, ill. e faj ekológiai valenciájáról még nincs egységes képünk. FLÖSSNER adatai felkeltik az érdeklődést ilyen vonatkozásban (l. **I. b**). A szubfosszilis maradványok legnagyobb része héj, mely a faji felismeréshez elegendő, de vékony és többnyire gyűrött állapotban kerül elő (FREY, 1958: 256).

Paleolimnológiai adatok arra engednek következtetni, hogy a hideget tűri (Wallensen), de általában melegebb klíma (interglaciálisok) kladocera faunájához tartozik, *Pleuroxus* fajokhoz hasonlóan.

Pleuroxus laevis G. O. SARS 1861*
6—7. ábra

I. a. DADAY 1904-ben (p. 56, 94) a Balatonból nem ismert fajok között sorolja föl.

SEBESTYÉN (1948) a tihanyi Gödrösben (mocsaras jellegű szélvíz, *Butomus umb.* pH 8,06) talált egy petés nőstény példányt (h = 660 ω, **S** = 550 μ) *Alonella excisa* és *A. exigua* társaságában (1947. X. 22.).

PONYI (1962) kevés példányt jegyzett fel, nádasból *(Scirpeto-Phragmitetum fontinalosum [hydrocharetum])*.

I. b. Svédországban stb. szórványos. Általában kis vizekben, nagyvizek parti övében, folyók lagunáiban honos, dús növényzet között (LILLJEBORG). Széltében elterjedt, nem közönséges, iszap és növényzet között élő faj. Az angol tóvidékről és Dániából kevés adat. — WAGLER szerint ritka. NEGREA (1964, 1966) a vizsgált hét tó közül kettőből említi ezt a nagy tavak parti övében, zavaros vízű kis vizekben honos holarktikus fajt. ZEMP szintén úgy ismeri, mint nagy tavak partján és kis vizekben élő formát. A németországi Stechling-See vidékén igen szórványosan fordul elő mind növényzettel benőtt, mind növényzet nélküli habitatokban, de nem ubiquista. Az üledék felületén levő detrituszgazdag rétegben (ooze) különböző növényi cenozisokban honos. Synekológiai szempontból a *Simocephalus* csoportba tartozik, melynek habitatja „Laubdy in Erlenbrüchen" (FLÖSSNER, 1966).

I. c. Külső vázának maradványait a Mississippi-völgy E—D szakaszában tavi üledékek felszíni rétegében keresve, 45 tó közül csak háromból említi DECOSTA)1964), *Pleuroxus hastatus*. H = 6,5 7,1, 7,7). Az USA-ban ritka (EDMONDSON, 1959).

* LILLJEBORG szerint a *Pleuroxus laevis* azonos a *P. hastus*-sal. Utóbbi néven hazánkból régóta ismert (DADAY, 1904: 94). — *A. P. laevis* G. O. SARS 1861 és *P. hastatus* G. O. SARS 1962 fajok azonosságára l. MEGARD, 1967: 186. jegyzet.

Szubfosszilis előfordulás

II. d. Általában.

Frey, (1958): Zemp-re utal, ami azt jelenti, hogy régebbi adat e faj szubfosszilis maradványairól nincs az irodalomban.

H leírása és ábrázolása. Frey (1959): 39, *55. ábra.* (1962a): *55. ábra.*

II. e. Európa Wauwiller-See. Zemp, 1941: Tavikréta rétegben, héj, ritka; Schleinsee, Frey (1961): VIII—IX—X pollenzónában, végig, kevés. Esthwaite Water, Goulden, (1964): *1. 3. táblázat.* 29. o. Kevés adat, bár több mint akár *P. trigonellusból*, akár *P. uncinatusból.* Első megjelenése az Atlanticumban. VII a-ban szórványos, VII b-ben a *P. trigonellus* helyébe lépve jelenléte meglehetősen folyamatos, különösen e zóna felső részén. **H S P.**

II. f. Lake Nojiri. Tsukada (1967): Szórványos adatok a korai Post-Glaciálistól (RI) végig.

II. g. Magashegyi tavak. Lake Zeribar, Megard, (1967): Noha a *B* és *C* Chydorida zónában maximális sűrűséget elért fajok legtöbbje déli, két kozmopolita is előfordul: *Alona affinis* és *Plauroxus laevis.* Utóbbiak abundanciája (az üledék cc $\times$ 10^4-ben adva) általában alacsony. A *P. laevis* legmagasabb értékei idején a *Camptocercus rectirostrisé* is magas értékű, *Leydigia acathocercoides* pedig hiányzik vagy csak kevés.

Whimpy Lake. (DeCosta, 1968): E faj maradványainak jelenléte csak egy rétegből van feltüntetve (II b. zóna, Postglaciális környezet), alacsony értékkel.

II. h. Trópusi kis tavakból nincs említve.

II. i. Balaton. Egyetlen P került elő az Atlanticusból. Egy **S** a felületi rétegből kérdéses (rajz). A balatoni előfordulás mind récens mind szubfosszilis adata igen kevés ahhoz, hogy értelmezni lehessen tótörténeti szempontból. Elterjedésére vonatkozó récens adatokból sztenotópiára lehet következtetni.

Pleuroxus aduncus (Jurine) 1820
8—10. ábra

I. a. Daday 1904-ben (94. o.) a Balatonból nem ismert fajok között sorolja föl.

Balatoni előfordulása Tihanyban a Gödrös előtti nádas széléről van először jelentve (Sebestyén, 1948: 111. o. *táblázat).* Itt a keskeny nádas és a partvonal közötti mocsaras jellegű szélvizekben korhadó nádmozaik, nádtörmelék és különböző mocsári növények között ismételten találta a szerző (1945. VIII. 8., XI. 6., 1946. V. 28.). Az őszi mintákban petés és ephippiumos nőstények meg hímek voltak. Előkerült még a füredi nádasból (1949. X. 25.) és a tihanyi Kisöbölből (1953. VI. 25, 1965. VII. 27, VIII. 3.).

Füredi vizek *Ceratophyllum*-állományából valamint nádasok mindhárom asszociációjából Ponyi jegyezte fel (1965, 1957, 1962).

I. b. Európai szerzők szerint általában közönséges faj, mocsarakban, kis vizek, tavak parti övében s folyókban is él (Lilljeborg, K. Berg, Zemp, Flössner). Algabevonatban nem ritka (Meuche, 1939). Dániában nem közönséges (K. Berg). Svédországban nem hatol annyira északra, mint a *P. trigonellus* (Lilljeborg). Romániában általában ritka (Negrea, 1966). (USA nyugati részén, növényzet között és pool-okban, Ward és Whipple 2^d). A németországi Stechling-See vidékén határozottan fitofil, kis tavakban

bevonat — és detritus-gazdag növényzet között, melyek növénycenológiai szempontból tavirózsa hínárosokba sorolhatók. Határozottan kerüli a *Potametum lucentis* társulás habitatjait. Alzathoz szorosan ragaszkodó szemilakusztris forma. Synekológiai szempontból az *Acroprrus* csoporthoz tartozik (FLÖSSNER, 1964).

I. c. A külső váz részeit a Mendota tavak négy egységének felületi üledékében FREY gyakorinak találta. Szerző felhívja a figyelmet a fejpajzs rostrális részének rövidségére mindkét nemen, más északamerikai *Pleuroxus*-fajokéhoz viszonyítva (FREY, 1960 b: 696, *II. táblázat, 20—25. ábra* — **H S**).

Szubfosszilis maradványok

II. d. Általában kevés irodalma van.
FREY (1958): *4. táblázat.*
(1959: 39 **H** leírása
(1962 b): 1141, *54. ábra* (**H**)
(1960 b) l. fentebb.

II. e. A Wauwiler See hajdani tó üledékéből ZEMP (1941) e faj héját és postabdomenjét mint gyakori elemet jegyezte fel a tavikréta- és kultúrrétegből.

A dániai hajdani tó (Herning) Eemi interglaciális rétegében egyetlen **S** (FREY, 1962).

II. f. Lake Nojiri, nincs adat.

II. g. Hegyi tavak. Lake Zeribar (MEGARD, 1967: *2—3. ábra* spektrum, maradvány per cc üledék $\times 10^3$). Előfordul az *A B C* pollen zónákban (184 o.) kevéssé változó sűrűségben. Szerző e dolgozat paleo-zoogeográfiai fejezetében felsorolja azokat a fajokat, melyek a tó történetének teljes folyamán jelen voltak. A *Pleuroxus aduncus* is csaknem mindig jelen van a tó történetében, de nem hatol annyira északra, viszont (HARDING, 1955, szerint) meglehetősen gyakori a trópusokon. E fajok elterjedése arra utal, hogy meglehetősen közömbösek a klímaváltozásokkal szemben.

II. h. A két hegyi tó üledékéből más *Pleuroxus* fajok maradványait említik a szerzők.

II. i. A feltárt balatoni üledékmintákban nem találtam *Pleuroxus aduncus* maradványt. A balatoni recens populációban előfordulnak hosszú és rövid rostrumú példányok *(10. ábra)*. A ♀ héjának posteoventralis sarkán levő fogak száma változó még ua. példányon is. Pl. 1945. XI. 6. gyűjtött példányon fiatal ♀: 2—2 fog, kifejtett ♀: 1—2, 2—2, 2—3, 3—4 fog (l. még FREY, 1960 b, SMIRNOV, 1966).

A postabdomen széle és distális vége, az antennák és a Md füstszínű, ami erős chitinezettségre utal (hasonlóan az *Alonella rostrata*-ra és *Pleuroxus uncinatus*ra). A táplálkozásban résztvevő lábak egyes sertéi is színesek. Több szerző megjegyzi, hogy a *Pleuroxus aduncus* utópotroha igen hasonló a *P. trigonelluséhoz*. HARDING és SCOURFIELD mutat rá a különbség lényegére: a *P. trigonellus* utópotrohának dorzális szélén fogak vannak, a *P. aduncus*-én sertecsoportok. A rövid, egy síkba eső sorokba tömörült serteegységek bazális végének rövid vonala szöget alkot az utópotroh dorzális szélével. Az egyes sertecsoportok nem simulnak rá az utópotroh oldalára, hanem arra közel merőlegesen helyezkednek el, s az utópotroh laterális nézetében egymást részben fedik. Igy jön létre az a látszat, mintha több fog volna egy csoportban. A tárgylencse fókuszát változtatva, kitűnik a való helyzet. A postabdomen

armatúrájának hasonló elhelyezkedése fordul elő az *Alona rectangulá*-n (récens és szubfosszilis példányok a Balatonból) és egyes esetekben az *Alona quadrangularis* balatoni maradványon.

1945. novemberében gyűjtött példányokból sikerült több mint féléven át nyerstenyészetet fenntartani laboratóriumi körülmények között (Balatonvíz, szobahőmérséklet, táplálék: szárított-porított hínár). A héjak közé bevont zöldalga-fonalakon mászkáltak, s az arra tapadt finom detritusz-törmeléket gyűjtötték össze bonyolult felépítésű lábaikkal. A fölösleget időnként kilökték az utópotroh karmával. Táplálkozás közben az utolsó lábpár és a mandibulák ritmikus mozgása figyelhető meg (lélegzés, felaprózás). A fonalakon lassan egyenletesen haladnak vagy egy helyben vesztegelnek. Úszó mozgásuk a rövid antenna szapora mozgása következtében remegő, de gyors.

Az a körülmény, hogy a *P. aduncus* — az irodalom szerint — kis vizekben és tavak parti övében gyakori fitofil forma, továbbá, hogy szubfosszilis maradványok kevés adata kis vizekre szorítkozik, arra utal, hogy populációja — tavi vonatkozásban — nem lehet népes. Kis terjedelmű parti területeken (tihanyi Kisöböl, Gödrös, nádason belüli szélvíz) vett minták elemzése e faj mozaikszerű előfordulására utal.

Pleuroxus trigonellus (O. F. Müller) 1785
11. ábra

I. a. Daday (1897) a Balatonból és a Kis-Balatonból említi: Balatonfüreden Francé gyűjtötte hínáros partról. Meglehetősen ritka, a Kis-Balatonban gyakoribb.

Tihanyi vizeken *Potamogeton* „sziget"-nél, nádas közelében s nyíltvizen Kottász (1933, E-K-S, 1937) gyűjtötte.

Újabban makrovegetáció nélküli tihanyi vízterület iszapjából (1965. VII. 9. A_1-B_0 között 420 cm víz alól) és (1968. X. 31, vízmélység 270 cm) Tihanyból jövet jóval a füredi strand előtt jutott az iszapmarkolóba. A korábbi minta adatai: tipikus kékesszürke iszap, felületén vékony drap réteg,* t = víz 20 C°, t = levegő 19 C°, Secchi 61 cm. Együttes: *Alonella rostrata, Alona, Pleuroxus*. Utóbbi minta adatai: szürke homogén iszap, felületén vékony drap réteg. H_2S szag. t = víz 10,5 C°, Secchi. 113 cm. Sok kladocera: *Macrothrix laticornis, A rostrata* ♀ ♂, *P. uncinatus* ♀ ♂, *Pleuroxus trigonellus* ♀, ♂ *Monospilus*. Legtöbb a *Macrothrix* és *Pleuroxus*.

I. b. Tavak, lassú folyású vizek csekély mélységében, iszapon, de néhány fonalnyira is (Lilljeborg) (l. Frey, 1960 b). Széltében elterjedt növényzet között és iszapban (Scourfield és Harding). Meglehetősen szaporának tűnik, noha általában kis számban fordul elő (K. Berg). — Gyakori (Wagler). — A Stechling-See vidéki vizekben fenéklakó iliophil forma. A parti öv iszapos üledékét kedvelő, ekológiailag egységes *Alona quadrangularis*-csoport tagja (hasonlóan a *P. uncinatushoz)*. Előfordul homokon, moszatbevonaton, növényzet között is (Flössner, 1964: 58, 69, 74, 90. stb.).

* Ez a tavi üledékek felületén ismételten megfigyelt vékony drap réteg legnagyobb valószínűséggel abból a morzsolt kőzetből (murva) származik, mellyel az utóbbi években — homok helyett — a tó környéki parkok útjait szokták felszórni. Dolomit eredetű. Ez az anyag az utakon nedvesen világos sarat ad, szárazon elhordja a szél, elszórja a környéken, a vízben, beviszi a partközeli lakásokba is.

I. c. A külsőváz részeire vonatkozó adatok felületi üledékekből.

Madison tavak. FREY, 1960 b: Kengosa kivételével mindenik tóban igen kevés.

Indiana állam három tavában, kevés. MUELLER, 1964.

A Mississippi-völgyszakasz feldolgozott tavainak kb. felében van, több déli fekvésűből hiányzik. Legmagasabb %-ban a Lake Geneva-ban (pH 7,1), DECOSTA, 1964.

Szubfosszilis maradványok

II. d. FREY, 1958: Maradványok előfordulására vonatkozó irodalom, **S H P C E** leírása, ábrázolása.

FREY, 1959: 40, 1962 a: *58—59. ábra*, fejpajzs leírása, ábrázolása.

FREY, 1962 b: 1141. 7, 26, *51. ábra.*

GOULDEN, 1964: 28: FREY vizsgálati eredményeinek rövid összefoglalása.

II. e. Európa. Längsee, FREY, 1955: 156, *III. táblázat* (♂ **P**)

Wallensen, FREY, 1958: *1—2. táblázat:* Maradványok és gyakoriságuk *(2. táblázat)*. A talált fajok közöttük a *P. trigonellus*-maradványok spektruma. Maradványok gyakorlatilag hiányzanak a szubarktikus klíma idején (Ia pollenzóna. Older tundra period) és a III pollenzónából (Younger Dryas). Lényegileg az Upper Alleröd (II b) és utóbbi, valamint a III határán, továbbá a III és IV határán (lápos állapot) fordulnak elő maradványai. Az Alleröd intervallumban populációja kicsiny.

Schleinsee. FREY, 1961: VII-ben és a VIII második felében a leggyakoribb Chydoridae. Ezután csökkent és kb. azonos marad a IX—X végéig.

Herning, FREY, 1962 b. A *Pleuroxus*- nem négy, ill. öt képviselőjének *(P. trigonellus, P. aduncus, P. uncinatus, P.* sp. + *Peracantha truncata)* maradványai szerényen vannak képviselve az Eemi interglacial középső és késői rétegeiben. A *P. trigonellus* és a *Peracantha* aránylag a leggyakoribb. *(1—2. táblázat,* **S H** + egyetlen **P,** mindössze 18 db.). Spektrum: *Fig. 3, ábrák: 7, 26, 51.*

Esthwaite Water. GOULDEN, 1964 : A furat mintáinak elemzésekor egyetlen *Pleuroxus* faj maradványát sem találta. A VI—VII. pollenzónában Atlanticum Post-atlanticum *(Table 3.)* **S H P** néhány minta negatív. E periódus spektrumából *(Fig. 6.)* kitűnt, hogy a genus többi tagjával összehasonlítva, a *P. trigonellus* előfordulása a legfolytonosabb, habár nem nagyobb a populáció. Szerző véleménye szerint a *Pleuroxus* fajok *(P. trigonellus, P. laevis, P. uncinatus)*, valamint a *Peracantha* stb. megjelenése az Atlanticumban és Post-Atlanticumban közvetlenül utal a klíma ameliorizációjára.

II. f. Lake Nojiri. Nincs adat.

II. g. Magas hegyi tavak közül csak a Lake Zeribar üledékeiben van adat. MEGARD, 1967: 63-furat *C* zóna: igen kevés. Szerző szerint a *P. trigonellus* annak a négy fajnak egyike, amelynek a tó története különböző szakaszaiban rövid életű populációi fejlődtek *(Oxyurella tenuicaudis, Alonopsis ambigua, Alona quadrangularis, Pleuroxus trigonellus.*

II. i. Balaton. Kevés maradvány **P H S** *(1. táblázat).*

A külföldi szubfosszilis adatok és a balatoni maradványok mérlegelésével hangsúlyozni lehet GOULDEN szavait (1964: 28): „too little is known of its ecological requirements to interpret its abundance in lakes".

1. TÁBLÁZAT

B28 furat kilenc mintájából feltárt és e dolgozatban tárgyalt kladoceramaradványok gyakorisága % értékek az összes kladocerákra vonatkoznak

160	140	120	100	80	60	40	20	1	Minta Sample	
370	330	290	250	208	170	123	60	0—3	mélység depth cm	Fajok Species
I.a	I.b	III—IV	V	VI	VII	VIII	IX	X	pollenzona	
78	15	4	+	8	3	2	—	—	db	*Eurycercus lamellatus*
18.71	6.97	1.02		1.47	0.91	0.44		—	%	
8	5	—	2	1	—	—	1	1	db	*Camptocercus rectirostris*
1.91	2.32		0.72	0.18			0.36	0.15	%	
87	49	3	+	+	+	+	1	+	db	*Acroperus harpae*
20.86	22.79	0.76					0.36		%	
—	—	—	—	8	1	5	—	1	db	*Leydigia leydigi*
				1.47	0.30	1.12		0.15	%	
—	—	5	+	20	11	31	6	18	db	*Leydigia acantho-cercoides*
		1.27		3.67	3.34	6.95	2.19	2.82	%	
—	—	—	—	—	—	—	—	—	db	*Pleuroxus truncatus*
									%	
—	—	—	—	1	—	—	—	1	db	*Pleuroxus laevis*
				0.18				0.15	%	
—	—	—	—	—	—	—	—	—	db	*Pleuroxus aduncus*
									%	
—	—	2	—	1	—	—	—	—	db	*Pleuroxus trigonellus*
		0.51		0.18					%	
2	—	2	—	12	4	1	—	1	db	*Pleuroxus unc. balat.*
0.47		0.51		2.20	1.21	1.12		0.15	%	
—	—	+	—	14	—	—	—	6	db	*Pleuroxus* sp.
				2.57		1.12		0.94	%	

Pleuroxus uncinatus Baird 1850
12—15. ábra, 11—13. kép

I. a. A Magyar Fauna Katalógus (1918) a *Pleuroxus* nemből a *P. trigonellust* és a *P. balatonicust* említi a Balatonból. Az ugyanott felsorolt „*Pleuroxus excisus*" és a „*P. exiguus*" ma az *Alonella* nembe van sorolva. Ennél több a BTTE Fauna kötetében (1897) sincs e nemre vonatkozóan. Daday a *Pleuroxus balatonicusról* Siófokon talált néhány példány tanulmányozása alapján tesz először említést (1884), melyek, miután hímet nem említ, mind ♀-k lehettek. Leírását, rajz nélkül, magyarul és németül adta (1885: 182, 162). Megelőző évben ue. folyóiratban (12. o.) név szerint említi, s megjegyzi, hogy Richardnál (1891) is e néven szerepel. Rajzát 1888-ban teszi közzé (Taf. *1. Fig.* 45—46). Tudjuk, hogy a *P. glaber* és a *P. personatus*, melyekhez

az újonnan leírt kladocerát hasonlónak véli, synonimjai a ma használatos *P. uncinatus* BAIRD elnevezésnek. (l. még LILLJEBORG, 1900: 538). Morfológiájáról, többek között, megjegyzi, hogy a héj posteoventrális sarkában fog nincs, és hogy az utópotroh karma sima (l. FREY, 1965).

SEBESTYÉN (1947) a negyvenes években a Tihany előtti nyíltvíz iszapjában talált *Pleuroxus* példányokat *P. balatonicusnak* véli, azon az alapon, hogy 1944 novemberében Tihany előtt nyíltvízi iszapban és a Kisöböl különböző habitatjaiban lelt hímek utópotroha különbözik európai vizekből ismert más *Pleuroxus*-fajok hímjeiétől abban, hogy az hasonló a ♀ példányokéhoz. SEBESTYÉN is megjegyzi, hogy a ♀ példányok héjának posteoventrális sarkában fog nincsen. 1945. július 6-án fiatal ♀ példányt talált egyetlen nagy foggal (egykorú rajz). E kérdéssel még érdemes foglalkozni (SMIRNOV, 1966: 190).

SEBESTYÉN, 1948. munkájában mind a *P. uncinatust*, mind a *P. balatonicust* felsorolja, ui. 1947. X. 29-én a tihanyi Gödrös táján parti vízben tipikus *P. uncinatus* hímeket jegyzett fel (SEBESTYÉN, 1948. *Táblázat)*.

Több, mint 10 év elteltével R. SRAMEK-HUSEK (1959. 4. 1. in litt) hívja fel a szerző figyelmét arra, hogy a *P. balatonicus* hímjére vonatkozó 1947-ben közölt rajzok valószínűleg androgyn példányokat ábrázolnak.

A hatvanas években számos balatoni példány gondos átvizsgálása után FREY megállapította, hogy gynandromorph jelenségről lehet szó. Tenyészetében ugyanis nem tipikus ♂ példányok vas deferensében kifejlett spermiumot figyelt meg (FREY, in litt.). Véleménye szerint a *„balatonicus"* megjelölés legfentebb varietás értékű lehet, azon az alapon, hogy a balatoni példányok az irodalomban megadott méretnél kisebbek, egy populációban kétféle hímek fordulnak elő, s a ♀ példányokon a héj posteoventrális sarkában nincs fog (FREY, 1965).

Hogy e fajon gynandromorph hímekkel egyidejűleg a *P. uncinatus* BAIRD-re jellemző hímek is előfordulnak a Balatonban, bizonyítja az is, hogy SEBESTYÉN 1963-ban Tihany előtti nyíltvíz iszapjából származott régi minta (No. 51., 1947. X. 29.) újravizsgálása során a *P. uncinatusra* tipikus hímeket talált (12. *ábra)*. L. még SMIRNOV, 1967: 567, 570).

A következőkben a balatoni recens populáció megjelölésére a *Pleuroxus uncinatus* BAIRD 1850. v. *balatonicus* DADAY 1884 megjelölést használom.

Fenéklakó kladocerákra vonatkozó újabb mintasorozataim (1963—1969, M 1—65, kb. 250 minta) legtöbbje *Pleuroxusra* pozitív volt (részletes feljegyzés, együttesek és habitatok vázolása, vázlatok kéziratban). Leggyakoribb a *Pleuroxus uncinatus* v. *balatonicus*. Igen kevés adat vonatkozik *P. aduncusra*, *P. trigonellusra* és *P. laevisre*.

Pleuroxus uncinatus balatonicust a Balatonban a következő helyekről gyűjtöttem:

Tihany előtti (a Tihany félsziget K. partja előtt) nyíltvíz iszapja (A_1 gyűjtőhely a horizontális planktonvizsgálatokban: SEBESTYÉN, 1964). A negyvenes évek iszapmintáinak is gyűjtőhelye (SEBESTYÉN, 1947).

A_1-től a Kisöböl tengelyében a part felé menve, még nyíltvíz jellegű területen (öböl bejárata), két kisnádas között, az öböl változatos habitatjaiban, parti detritusz-túrzásban, szélvízi neusztonban (SEBESTYÉN, 1965: 192—193). — Tihany, Gödrös táján, nádasmenti szélvizek és üledékük.

Balatonfüred, nádas közelében, strand előtt.

Balatonfüred—Tihany, fele úton: tipikus nyíltvízi üledék.

Keszthelyi öböl: parti szélvizek, a strandtól ÉK-re.

Planktonmintába is bejut: kis területnek megfelelő vertikális vízoszlop-szűrés (SEBESTYÉN, 1964: 232—233, *2—3. Táblázat:* 676. sz. planktonminta, A_1 gyűjtőhely, 694. sz. planktonminta, Keszthelyi-öböl, a Zala beömlése előtt. Tychoplanktikus elemek is felsorolva).

Pleuroxus uncinatus balatonicust tartalmazó üledékminta adatai: 1968. X. 12. Tihanytól keletre az A_1 gyűjtőhely vonalában, m = 355 cm, t víz = 14 C°, szürke kénhidrogénszagú iszap, felületén vékony drap réteg: *P. uncinatus balatonicus* nagyon sok ♀ ♂, *Alonella rostrata* petés és *ephippiumos* ♀, nagy *Alona* egy-egy, *Monospilus, Ectinosoma abrau, Micronecta, Turbellaria cocon, Botryococcus.*

1968. X. 12. Balatonfüred Tihany között fele után, v > 3 m. Makrovegetáció nincs: *Pleuroxus* ♀, nagy *Alona*, kis-*Alona, Monospilus, Alonella rostrata, Macrothrix laticornis—Ectinosoma, Micronecta.*

I. b. Holarktikus (NEGREA), széltében elterjedt gyakori faj, főként tavak, de kisvizek iszapos fenekén is (LILLJEBORG, K. BERG, SCOURFIELD és HARDING, ZEMP, NEGREA). Chárásokban detrituszban gazdag iszap-padokon (FLÖSSNER). — Az üledék homokos voltára valamint makrovegetáció jelenlétére vonatkozóan eltérőek a megállapítások (LILLJEBORG, ZEMP, FLÖSSNER). — Iliobiont, KURZ a limicol fajok közé sorolja — *Pleuroxus (Rhyophilus) glaber* SCHOEDLER néven. Lehatol nagy mélységbe is (LILLJEBORG, FLÖSSNER). FLÖSSNER az *Alona quadrangularis* ekológiailag jól körülhatárolható, egységes csoportjában sorolja. A parti öv iszapos fenekű habitatjára jellemzőnek ítéli. Iszapos üledék taxocoenozisában négy subdomináns faj közé helyezi.

I. c. É-Amerikai tavak felületi üledékéből *P. uncinatus* maradványok nincsenek említve.

Szubfosszilis maradványok

I. d. Általában.

FREY, 1959, 1962 a: *56—57. ábra* ♀ ♂ **H** leírása, ábrázolása,

FREY, 1964: 48 Utal KORDE (1956) fejpajzs ábrázolására.

GOULDEN, 1964: Előfordulásának összefoglalása ROSSOLIMOtól kezdve.

II. e. Európa Wauwiler See, ZEMP, 1941. **S**, a posteoventralis sarokban erőteljes fogak. Seekreide és kulturrétegek leggyakoribb maradványai közé tartozik.

Längsee, FREY, 1955: P.

Schleinsee, FREY, 1961: a VIII végén lép fel. A IX—X pollenzónában, lényegesen kevesebb adat, mint a *P. trigonellus*ból. X-ben a legtöbb.

Herning profil, Dánia, FREY, 1962 b: Az Eemi interglacialisból néhány maradvány.

Esthwaite Water, GOULDEN, 1964. Hiányzik a II—V pollenzónából, igen szórványos a VI—VII b-ben. Az előforduló *P.* fajok közül az *uncinatus*ról van legkevesebb adat.

II. f. Lake Nojiri, TSUKADA, 1967: A vizsgált rétegekben — a hamuesőt megelőző időkben is — végig megvan.

II. g—h. Magas hegyi tavakból és trópusi kistavakból más fajok vannak említve.

II. i. Balaton. Az elemzett kilenc mintából három kivételével (20, 100, 140) kerültek elő maradványai. Gyakoriság **H > P > S.**

Bár recens adatok arra utalnak, hogy tavunk egyik leggyakoribb iszaplakó Chydoridája, a maradványok számszerű adatai ezt nem tükrözik vissza. Ezt az ellentmondást talán úgy lehetne magyarázni, hogy fajunk maradványai nem annyira időtállóak, mint más Chydoridae fajokéi. Kérdés, hogy lehet-e ezt a *P. uncinatus balatonicus* hidrofil voltával kapcsolatba hozni?

A Wauwiler See kivételével, a tekintetbe vett európai tavak üledékeiben (Esthwaite Water, Herning) fajunk kevesebb maradvánnyal van képviselve, mint e genus más tagjai. A japáni Lake Nojiri üledékeiben is a *P.* u. maradvány kevesebb, mint a *P. laevis*-é.

A Late Pleistocenből előkerült maradvány (két fejpajzs és egy héjpár, utóbbi qualitativ mintából) talán kevés ahhoz, hogy messzemenő következtetéseket lehessen levonni, bár, mint a faj létezésének bizonyítéka, jelenléte esetleg felmelegedésre utalhat.

Feltűnően kiugrik aránylag sok adatával a 80. sz. minta (Atlanticum, **H P S**), mely mintából csak az a két *Pleuroxus* faj maradványa hiányzik, melyekről egyetlen mintában sem volt pozitív adat.

Hím fejpajzs a 120 mintából került elő (a jégkorszak vége, Holocén kezdete).

Összefoglalás

E tanulmány folytatása Sebestyén (1969 a) hasonló tárgyú dolgozatának. A most feldolgozott Chydoridák: *a) Eurycercus, Camptocercus, Acroperus, b)* két *Leydigia* faj, *c)* öt *Pleuroxus* faj.

A tanulmány felépítése azonos a sorozat I részében közölt menettel (234—235, 251—252).

Az *a)* csoport tagjai makrovegetációhoz kötött formák. Gyakori jelenlétük jégkorszakvégi mintákban alámerült növényzet általánosabb elterjedésére utalhat. A *Leydigia* és *Pleuroxus* genus tagjai között iszaplakók *(Leydigia leydigi, Pleuroxus uncinatus)*, továbbá növényzettel benőtt területek iszapjában élő, bár úgylátszik ekológiai igény szerint nem egységes sztenotop formák vannak. Ez magyarázza, a *Pleuroxus uncinatus* kivételével, recens állományok, maradványok előfordulására vonatkozó adat kevés számát. Az *Acroperus harpae* és a *Pleuroxus truncatus* szemilakusztrikus hínárosokban is élő, sőt talán planktofilnek minősíthető tagok.

Az északias *Eurycercus lamellatus* maradványai gyakoriak jégkorszakvégi mintákban. A délies *Leydigia acanthocercoides* az életpálya aktív szakaszának megrövidülésével hidegebb klímához alkalmazkodhat.

Pleuroxus fajok megjelenése külföldi irodalom szerint felmelegedés jele. A *P. trigonellus* legrégibb maradványai a 120. mintából valók *(Pinus)*, a *P. uncinatus*éra már a tőzegréteg alakulását megelőző korszakból van adat. Ez arra is utalhat, hogy az ágascsápú rákok az életpálya rövidsége és a terjeszkedési lehetőségeik következtében gyorsan reagálnak klímaváltozásokra. A jégkorszakvégi mintákból az akkori kladocera fauna egyhangúsága tűnik ki. A lombosfák megjelenésével jellemezhető Atlanticum időszak (80. sz. minta), a sorozat első részében tárgyalt fajokhoz hasonlóan, itt is kitűnik fajokban és egyedekben való gazdagságával.

A balatoni maradványok jelenléte az eddig nyert adatok alapján általában összhangban látszik lenni a külföldi irodalom megállapításaival.

A táblázat a balatoni üledékekből eddig feltárt maradványok számszerű előfordulását és százalékos relatív abundanciáját mutatja.

Eredeti c.l. rajzok és mikrofotók.

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CLADOCERA STUDIES IN LAKE BALATON IV.
SUBFOSSIL REMAINS IN THE SEDIMENTS OF LAKE BALATON II.*

O. Sebestyén

This paper is the continuation of a study on both recent and subfossil Cladocera. The species are dealt with are: a) *Eurycercus lamellatus, Camptocercus rectirostris, Acroperus harpae* b) two species of the *Leydigia* and c) five species of the *Pleuroxus* genera.

Structure of this paper is the same as that of part I. of the series (SEBESTYÉN, 1969a: 234—235, 251—252). Data in *Table I* in the previous work concern to this part too. Some morphological data regarding to Lake Nojiri missing from that *Table* are to be found now on this page *(References)*. *Table I* in this paper gives the numerical data of occurrences of remains for the species mentioned above, being recovered from nine samples of core B 28.

Acroperus harpae, Leydigia acanthocercoides and *Pleuroxus uncinatus* v. *balatonicus* are the most common members in the present biota of this lake, all forming large populations. The other members of groups a) and c) seem to have a stenotopic nature. They occure in various habitats within stands of macrovegetation. However their distribution seems to be rather mosaic like, having very likely different ecological demand.

Scarcity of data on the recent occurrence of *Leydigia leydigi* may be found an explanation in the cryptic mode of life of this species. Being a true limicol form (KURZ) it is buried in the mud and able to endure depletion of Oxygen in the presence of haemoglobin. However its population hardly can be large, concluding from the few remains recovered.

Judging from the related literature both *Acroperus* and *Pleuroxus truncatus* (syn. *Peracanthe truncata)* occur in semilacustris stands of submerse vegetation too. *Acroperus* usually develops large population in certain part of the lake in the appropriate

* Lake Nojiri's data missing from Table 1. SEBESTYÉN, 1969: area 3.9 km², mean depth 20.8 m. Lake formed by damming of a valley by volcanic mudflows or lahars (HORIE, 1962; 196, 219).

E munkából pótolva a Lake Nojiri adatai, melyek, sajnálattal, kimaradtak SEBESTYÉN, 1969b *I. táblázatából:* A 3.9 km² terjedelmű és 20.8 m középmélységű tó vulkáni iszaptól elzárt völgyben keletkezett. (HORIE, 1962: 196, 219).

season. *Pleuroxus truncatus* has only been recorded from this lake by one scientist. Its population can't be large. *Pleuroxus laevis*, *P. aduncus* and *P. trigonellus* are seemingly stenotopic too having different ecological demands. *P. aduncus* seems to be most common among them. It inhabits littoral habitats in marshy situation. It propagates well in raw culture under laboratory condition when fed by detritus. It is tolerant to high concentration of salts (RUTTNER-KOLISKO, 1966: *Verh. Internat. Verein Limnol.* 16: 529).

Pleuroxus trigonellus is known from the lake since DADAY's time as a "rare" from. Recent data on its occurrence are also scarce. Indeed, it is very little known of its habitat in this lake. In spite of having records of both recent and subfossil occurrences of *P. laevis*, no more can be said of this species.

Considering the frequency and large population of *P. uncinatus* v. *balatonicus* (see FREY, 1965, SMIRNOV, 1966) more remains might be expected. This may be due to the delicate nature of the exoskeleton with the exception of the postabdomen. It is one of the few species which populate the eprofundal of muddy nature in Lake Balaton.

The extensive littoral zone of Lake Balaton with luxurious growth of *Phragmitetum* and the increasing stands of submerse vegetation offer a great variety of habitats for Chydorids. This is true even in the case of small territories exemplified by "Gödrös" and "Kisöböl" off Tihany. A thorough study in the various seasons promise more data even in our time of increasing human influence.

It seems that the northern affinity of *Eurycercus lamellatus* is mirrored by the frequency of the remains in Lake Balaton in the Late Pleistocene samples. It has to be kept in mind, however, that this large species is distinguished by a good preservation of the most various parts of the exoskleton.

Leydigia acanthocercoides although a southern form, has the shortest period of the active life in our lake, in comparision to the other Chydorids. Presence of remains in the Late Pleistocene samples (Ia) may suggest an incidental amelioration of the climate (GOULDEN).

ИЗУЧЕНИЯ КЛАДОЦЕР ОЗЕРА БАЛАТОН. IV. СУБФОССИЛЬНЫЕ ОСТАТКИ В ОСАДКАХ ОЗЕРА БАЛАТОН (II)

О. Шебештен

В настоящей статье, являющейся продолжением исследования современных и ископаемых кладоцер, рассматриваются следующие формы: *а) Eurycercus lamellatus*, *Camptocercus rectirostris*, *Acroperus harpae*, *б)* два вида *Leydigia*, и *в)* пять видов рода *Pleuroxus*.

Структура статьи такая же, как в первой публикации этой серии. (SEBESTYÉN, 1969а: 234—235, 251—252). Данные Таблицы 1 предидущей работы относятся и к настоящей статье. Некоторые недоставщие в этой таблице данные по морфологии озера Балатон можно найти здесь на стр. 00. Таблица 1 данной статьи содержит данные о нахождении остатков упомянутых видов в девяти образцах пробы B28.

Acroperus harpae, *Leydigia acanthocercoides* и *Pleuroxus uncinatus* v. *balatonicus* являются самыми обычными компонентами современной фауны озера и образуют большие популяции. Другие члены групп *а)* и *б)* как будто имеют стенотипическую природу. Они обнаруживаются в различных местах обитания, что определяется характером макровегетации, и их распространение имеет довольно мозаичный характер.

Особенности образа жизни *Leydigia leydigi*, возможно, объясняют причину малочисленности данных о современном состоянии этого вида. Этот вид обитает в иле и способен переносить недостаток кислорода благодаря присутствию гемоглобина. Судя по малому числу остатков, вряд ли популяция этого вида может быть большой.

Acroperus обычно даёт большие сезонные популяции в некоторых частях озера. *Pleuroxus truncatus* лишь однажды был отмечен одним автором, и популяции этого вида не могут быть значительными. *Pleuroxus laevis*, *P. aduncus* и *P. trigonellus* повидому тоже стенотиппы и проявляют разные экологические потребности. Найболее обычным среди них кажется. *P. aduncus* неселяющий литторальные места обитания болотного типа. Он хорошо разводится в лабораторных условиях при кормлении детритом и устойчив к высокой концентрации солей.)

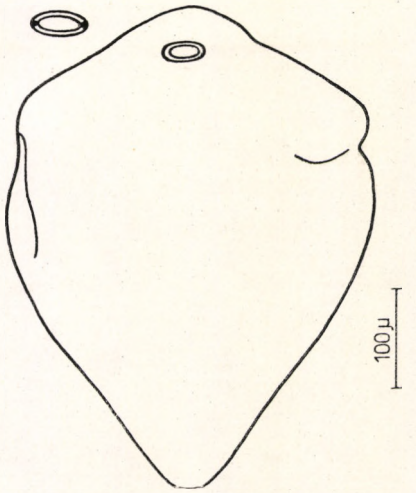
Pleuroxus trigonellus со времени Дадаи известен как «редкая» форма для озера. Современные нахождения его тоже немногочисленны, и очен мало известно о местах его

обитания в озере. Несмотря на присутствие того вида в современной и субфоссилной фауне, мало что можно сказать об этом виде.

Судя по частоте нахождения и большим популяциям *P. uncinatus* v. *balatonicus* (см. Frey, 1965, Smirnov, 1966) можно было бы ожидать более частых остатков. Их редкое нахождение, возможно, объясняется хрупкостью наружного скелета.

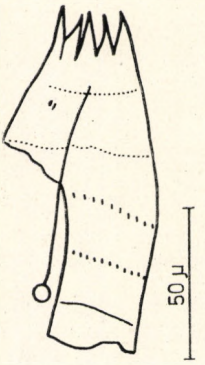
Существование в Балатоне литторальной зоны с пышным ростом *Phragmitetum* и другой растительности предоставляет большой выбор мест обытания для хидорид. Частая встречаемость остатков *Eurycercus lamellatus* в позднем плейстоцене, возможно, овязано с хорошей сохранимостью экзоскелета этого крупного вида, но, может быть, отражает его северную принадлежность.

Leydigia acanthocercoides, будучи южной формой имеет самый короткий период активной жизни в озере, по сравнению с другими хидоридами. Присутствие остатков этого вида в поздном плейстоцене (1a), возможно, указывает на временное потепление климата (Goulden).



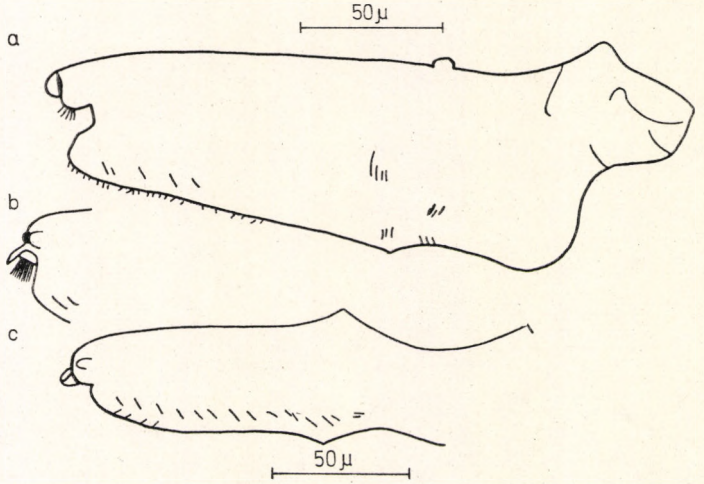
1. *Eurycercus lamellatus* fejpajzs B 28. 160 No 1115

Fig. 1. Eurycercus lamellatus Headshield B28/160 No 1115



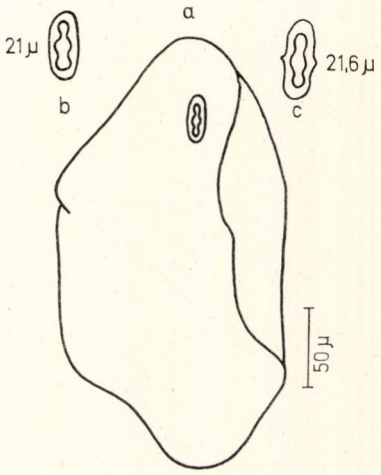
2. *Eurycercus lamellatus* antennula B 28/140 No 1040 l. érzőserte tövét

Fig. 2. Eurycercus lamellatus Antennule B 28/140 No 1040 showing pore of location of sensory bristle



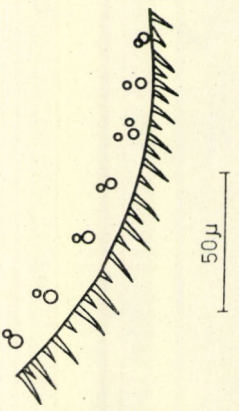
3. *Acroperus harpae* utópotroh B 28/140 a) ♀ No 947 c) ♂ No 1018 b) ♂ utópotroh vége erősebb nagyításban

Fig. 3. Acroperus harpae Postabdomen B 28/140 a) ♀ No 947 c) ♂ No 1018 b) end of postabdomen of ♂ in larger magnification



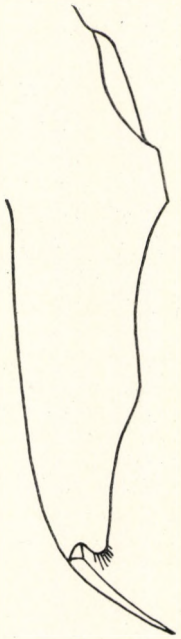
4. *Leyidigia leyidigi* a) fejpajzs B 28/40 No 394, b) c) porusok erősebb nagyításban

Fig. 4. Leydigia leydigi a) Headshield B 28/40 No 394, b), c) pores in larger magnification



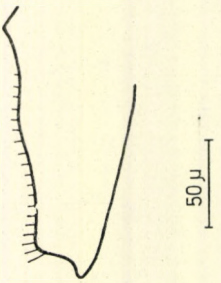
5. *Leydigia acanthocercoides* héj szélének részlete B 28/120 No 912

Fig. 5. Leydigia acanthocercoides margin of postabdomen, detail B 28/120 No 912



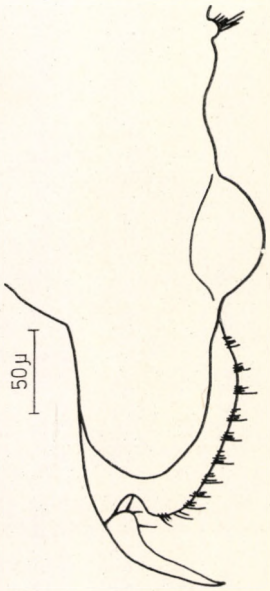
6. *Pleuroxus laevis* potroh ♀ Tihany, Gödrös 1947. X. 22. (e példány héjának hossza 550μ)

Fig. 6. Pleuroxus laevis Postabdomen ♀ Tihany, Gödrös, 1947. X. 22 (l = shell of same specimen = 550 μ)



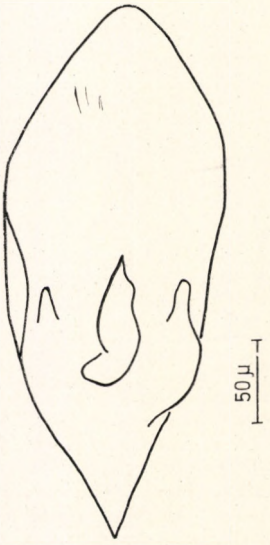
7. *Pleuroxus laevis* utópotroh ♀ B 28/80 No 520

Fig. 7. Pleuroxus laevis Postabdomen ♀ B 28/80 No 520



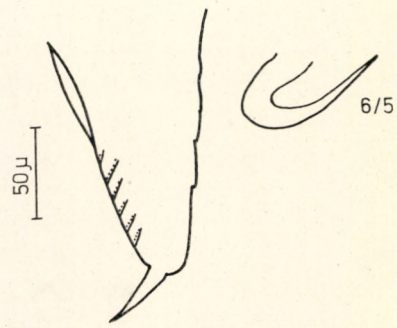
8. *Pleuroxus aduncus* utópotroh ♀ 1945. XI. 6. Tihany, Gödrös (fiatal példány kultúrából, 1946. I.)

Fig. 8. Pleuroxus aduncus Postabdomen ♀ 1945. XI. 6. Tihany, Gödrös (young specimen from culture, 1946. I.)



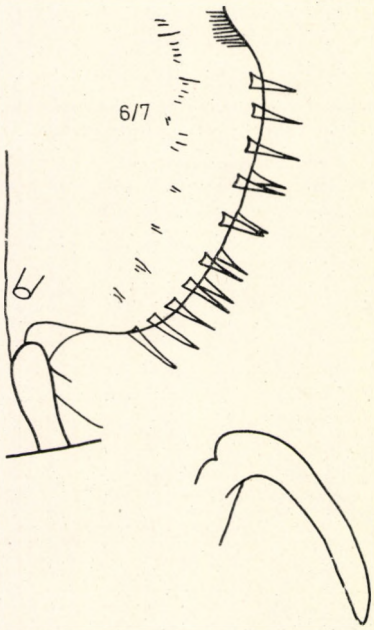
10. *Pleuroxus aduncus* fejpajzs kultúrából (Tihany, Kisöböl, 1966)

Fig. 10 Pleuroxus aduncus Headshield from culture (Tihany, Kisöböl, 1966)



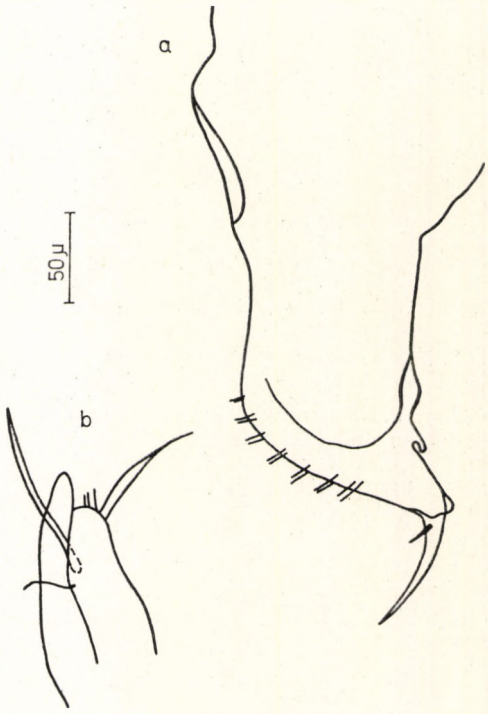
9. *Pleuroxus aduncus* ♂ utópotroh és hím kampó. Gödrös, 1945. X.

Fig. 9. Pleuroxus aduncus ♂ Postabdomen and hook Gödrös, 1945. X.



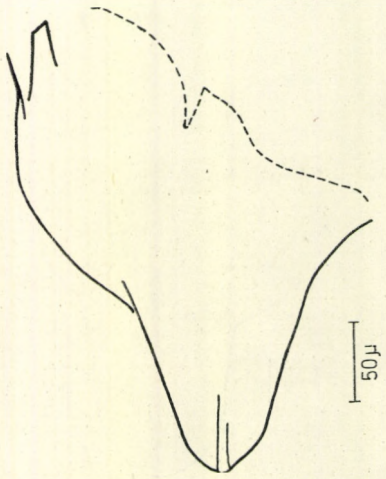
11. *Pleuroxus (trigonellus?)* Tihany = Bf feleúton. Utópotroh distális vége és ♂ kampó Gynandromorph (?) (e példány héjának hossza 431 μ). 1965. X.

Fig. 11 Pleuroxus (trigonellus?) distal part of postabdomen Gynandromorphs specimen (l-of shell ± 431 μ)



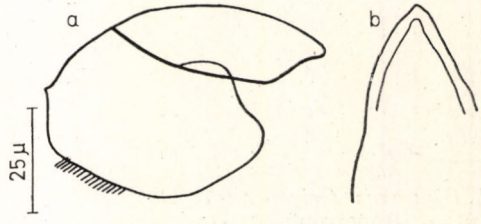
12. *Pleuroxus uncinatus* (?) ♂ a) utópotroh, b) rostrum vége és antenulla, Gödrös, 1947.X.29

Fig. 12. Pleuroxus uncinatus (?) a) Postabdomen b) end of rostrum and antennule. Gödrös, 1947. X. 29.



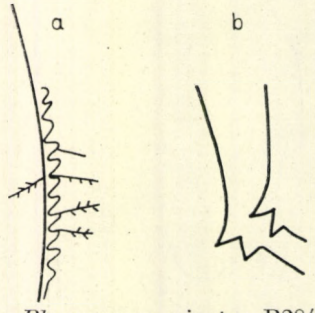
13. *Pleuroxus uncinatus* fejpajzs rostrális vége, ♂ B 28/120 No 910a

Fig. 13. Pleuroxus uncinatus rostral end of headshield ♂ B 28/120 No 910a



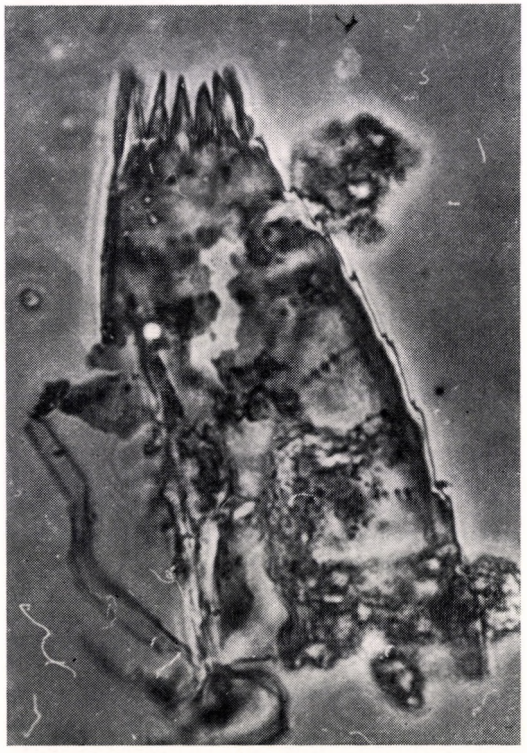
14. *Pleuroxus uncinatus* a) ♂, b) rostrum vége erősebb nagyításban. Balatonfüred, 1965.X.

Fig. 14. Pleuroxus uncinatus a) ♂ b) end of rostrum in larger magnification Balatonfüred, 1965. X.



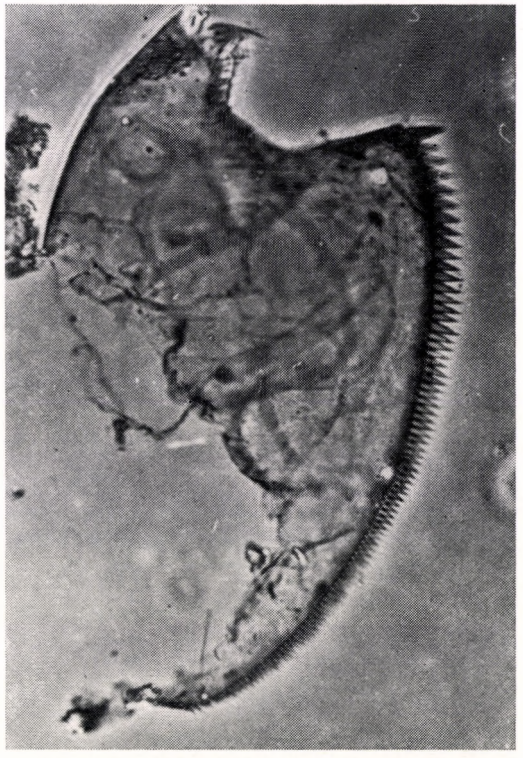
15. *Pleuroxus uncinatus* B28/160 No 1270. a) a héj fűrészes szélének részlete, b) héj hátsó sarkának armatúrája, sósavval kezelt mintából (e példány héjának hossza 478μ)

Fig. 15. Pleuroxus uncinatus B 28/160 No 1270 a) detail of serrated margin of shell with plumous setae, b) posterior-ventral corner of shells (from sample treated with HCl (l of shell of specimen =478 μ)



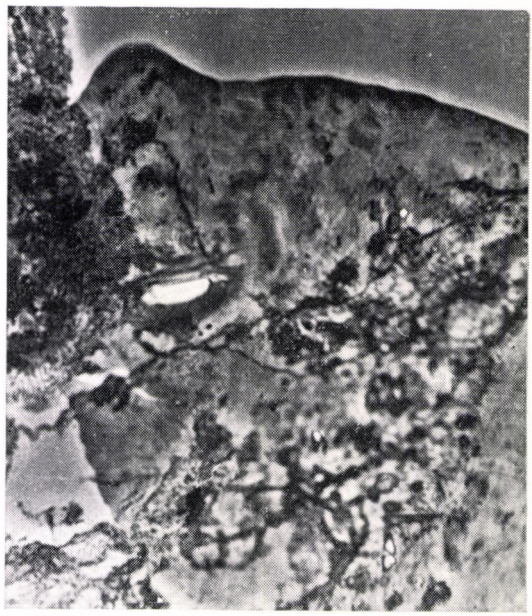
2. kép. Eurycercus lamellatus, első antennula. 160. minta. No 1163

2. *Eurycercus lamellatus* Antennule Sample 160. No 1163



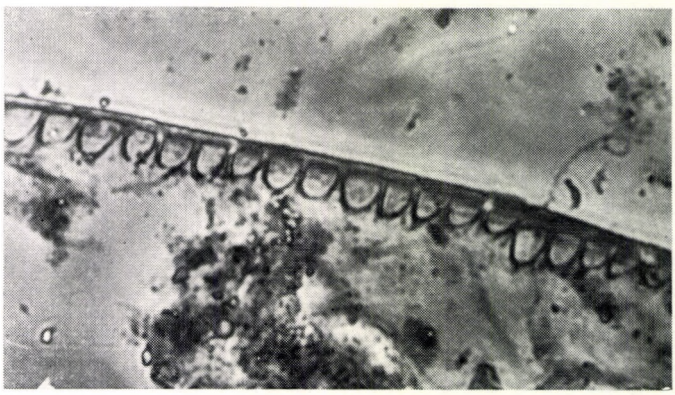
1. kép. Eurycercus lamellatus, utópotroh. 160. minta. 250 μ. No 1160

1. *Eurycercus lamellatus* Postabdomen Sample 160. No 1160



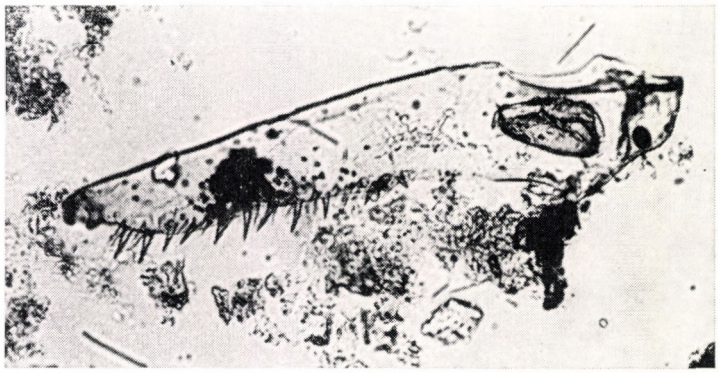
3. kép. Eurycercus lamellatus, fejpajzs distális vége a porussal (töredék). 160. minta. porus h = 30 μ. No 1169

3. *Eurycercus lamellatus* Fragment of headshield, length of pore 30 μ. Sample 160. No 1169



4. kép. Eurycercus lamellatus, héj szélén a serték csatlakozási helye. 140. minta. h = 7—8 db $\pm$54 μ, No 1057

4. *Eurycercus lamellatus* Margin of shell showing articulations for the plumose setae. 5—6 scallops measure $\pm$54

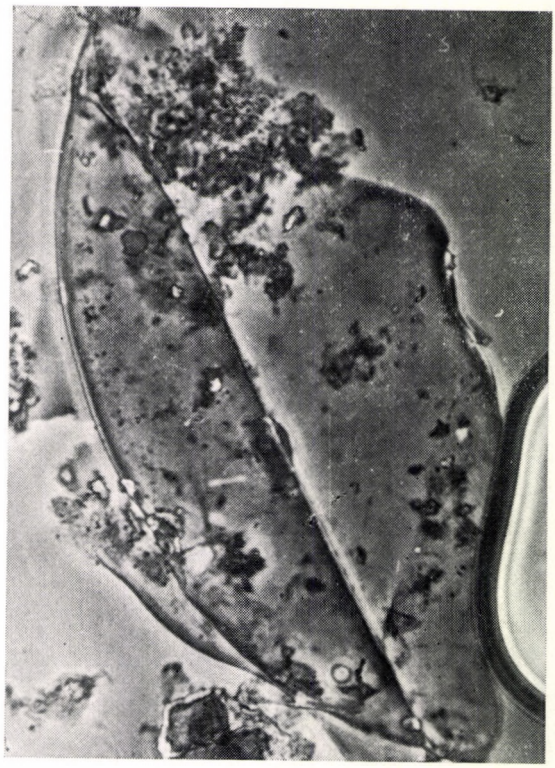


5. kép. Camptocercus rectirostris, u. potroh. 140. minta. h = 338 μ. No 1238

5. *Camptocercus rectirostris* Postabdomen l=338μ. Sample 140. No 1238.

6. kép. Acroperus harpae, utópotroh. 160. minta. h = 135 μ. No 1159

6. *Acroperus harpae* Postabdomen l = 135 μ. Sample 160. No 1159

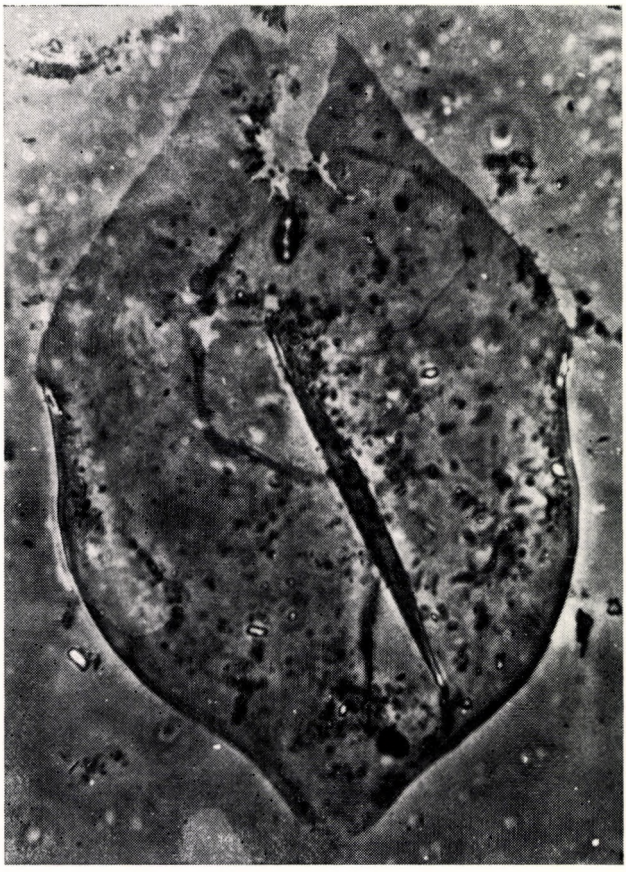


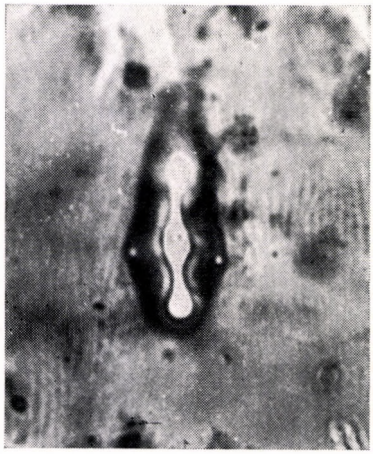
7. *kép. Acroperus harpae* BAIRD, a háti részen felébe hajtott és összetapadt fejpajzs egyik oldalsó lebenye részben felhajolva. 140. minta. h = 396 μ. No 1143

7. *Acroperus harpae* Headshield, partly folded l = 396 μ. Sample 140. No 1143

8. kép. Leydigia acanthocercoides, fejpajzs ♂ (?). 40. minta. h = 206 μ. No 349b

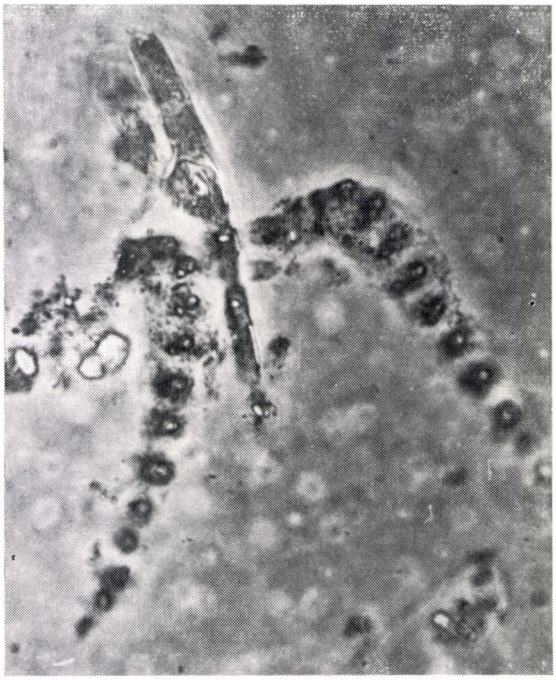
8. *Leydigia acanthocercoides* Headshield ♂ l = 206 μ. Sample 40. No 349b





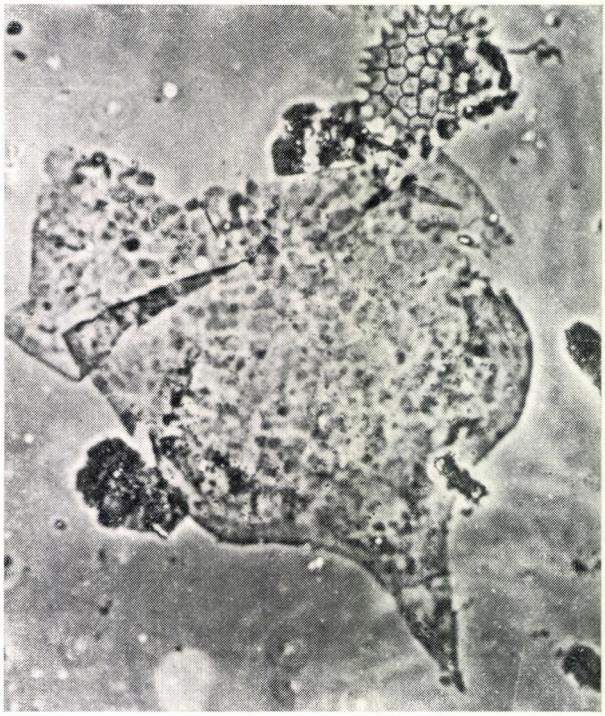
9. kép. Leydigia acanthocercoides, a 8. képen ábrázolt fejpajzs porus-rendszerének nagyított részlete

9. *Leydigia acanthocercoides* Part of headshield showing pores in larger magnification of No 8



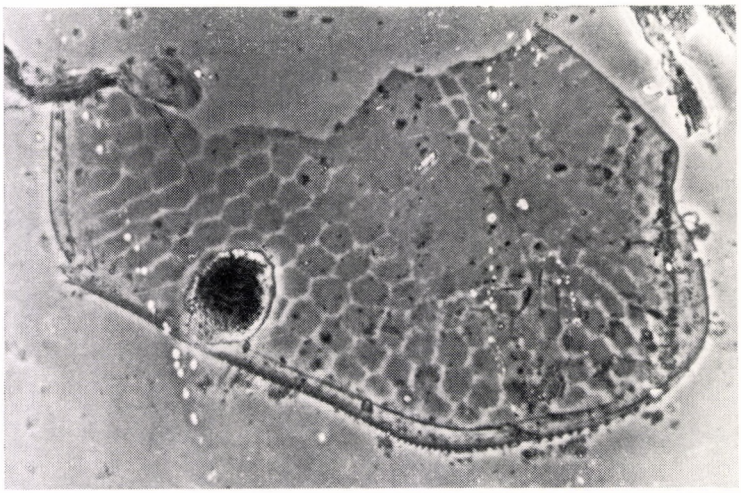
10. kép. Leydigia acanthocercoides, utópotroh maradványa a dorzális szegélyből megmaradt részletekkel. 1. minta. h, szegély = 130 μ. No 607

10. *Leydigia acanthocercoides* Remains of postabdomen 1 = 130 μ. Sample 1. No 607



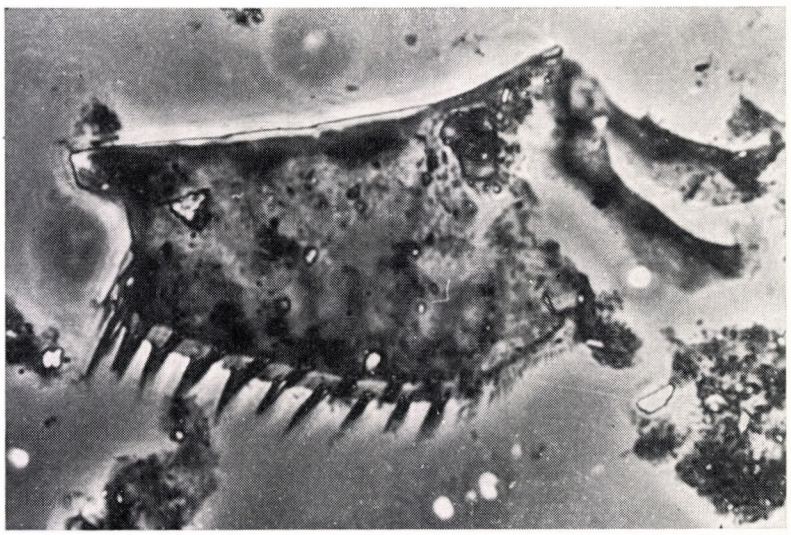
11. kép. Pleuroxus uncinatus, fejpajzs rostrális vége, 80. minta, sz. a fornix mögött ± 230 μ

11. *Pleuroxus uncinatus*, rostral part of headshield widht behind the fornices ± 230 μ. Sample 80. No 504.



12. kép. Pleuroxus uncinatus, héj töredék. 80. minta. h = 571 μ. No 494

12. *Pleuroxus uncinatus* Fragment of shell l = 571 μ. Sample 80. No 494



13. kép. Pleuroxus uncinatus, utópotroh. 80. minta. h = ventrális rész, 128 μ

13. *Pleuroxus uncinatus* Postabdomen l, ventral part = 128 μ. Sample 80.

ANNAL. BIOL. TIHANY | 37 | 281—289 | HUNGARIA 1970

CALCAREOUS MICROFOSSILS IN THE SEDIMENTS OF LAKE BALATON

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Received: 28 February, 1970

Method

All samples from sediments of Lake Balaton analysed as far (SEBESTYÉN, 1969) bubble up when treated with diluted HCl. In the procedure applied to recover chitinous remains of Cladocera, most part of the inorganic sediments could be eliminated with treatment of HCl (FREY, 1961). In order to recover microfossils of calcareous nature a simple sedimentation method has been applied with good results.

Equipment: Zeiss Stereomicroscope S. M. XX, one larger and few smaller watchglasses (diameter ±10 cm resp. 70 mm), pipette of various size, Leonard pinzette, vials, distilled water and alcohol, 50%.

Procedure: 0.1—0.2 ml sediment is transferred into the larger watchglass containing about 10 ml water. Dispersion of the solid particles is aided by a jet of water through a pipette. Larger particles settle quickly. The cloudy supernatant containing minute particles having the size of "ultratripton" should be decanted promptly with care and saved for further examination.

Fresh water being added, subsamples should be transferred into small watchglasses. For microscopical investigation (epi-illumination, 10—25× magnification) solid particles of organogenic nature (valves of both Ostracods and glochidia, *Pisidium*, fruit of Characeae etc.) could be removed by pipette or pinzette to a series of small watchglasses containing water. (Text *Table 1)*

[1] TABLE 1

Calcareous microfossils in the 9 samples of the sediment B 28 (Balatonboglár—Révfülöp, ZÓLYOMI) *and in the sample No 145 of the sediment B 24 (Keszthely—Gyenesdiás,* ZÓLYOMI). *The depth of the samples can be seen in Table 2*

Remains	Sample 160.	140.	145. B.24	120.	100.	80.	60.	40.	20.	1.
Characa	+	—	+	—	—	—	—	—	—	—
Ostracoda	+	+	++	++	+	+	+	+	++	+
glochidium	+	—	—	+	—	—	+	—	++	+
Pisidium	—	—	—	—	—	—	—	—	—	+
snail (csigahéj)	—	—	—	+	—	—	—	—	—	—

Valves of Ostracods and glochidia (as well as headshields and shells of cladocera) are usually filled with minute inorganic particles (carbonates, quartz) seldom with organic detritus. All these usually settle on the bottom of the watchglass concave surface facing upward. Microfossils containing

carbonates being extremely fragile, should be transferred with care into water or alcohol. Large particles of inorganic nature may be tested for carbonates by diluted HCl. Insoluble particles have not been tested for quartz yet. Mica ranging in length from 70 to 200 μ and black sphaerules of pyrite (VALLENTYNE, 1963) ranging in length from 16 to 22 μ) are also present.

Part of the newly dispersed solid particles of the supernatant saved at the beginning of the procedure are soluble in diluted HCl, most of the rest of the particles of brownish tint are evidently of organogenic nature. Observing this phase of the procedure through microscope (epi-illumination), it could be established that the minute inorganic particles are usually attached to the organic ones.

In the text *Table 1* distribution of the calcareous microfossils to be seen.

All investigations carried out as far have only a preliminary value. If volume or weight of the initial sample is known the selected remains result quantitative data.

Remains of glochidium larvae of Unionidae (Mollusca)

Using the method described above remains of glochidia larvae have been recovered from several samples of ZÓLYOMI's core B 28 bored at the middle of the profile Balatonboglár-Révfülöp. Samples 40 (New Holocene), 100 (older Holocene) and 140 (Late Pleistocene) yielded only negative results (Text *Table 2*). Glycerine gelatine is appropriate for making slides for microscopic investigation. It seems that polyvinil lactophenol dissolves the substance of the glochidia.

During the selection of the remains it could be established that specimens of glochidia belong to two size orders. All with the exception of two half valves were filled with inorganic particles *(Fig. 1—2)*.

In the identification of subfossil glochidia BRODNIEWICZ's method has been followed (1968: *Figs 1—3, Tables 1—2*, photos and explanation of the photos). Dr. BRODNIEWICZ distinguishes subfossil glochidia at the generic level by the size (length, hight, the proportion of these) and the form of the valves. Specimens measuring above 300 μ belong to the genus *Anodonta*, those measuring about 200 μ to *Unio*. Glochidia of *Anodonta* being fairly equilateral are that of *A. cygnea*, those being rather assymetrical of *A. complanata*.

According to this key, subfossil glochidia recovered from the sediments of Lake Balaton so far, belong very likely partly to *Unió*, partly to *A. cygnea* and *A. complanata. (Figs. 1—6, microphoto 1—4, Table 3)*. Valves of the remains of *A. cygnea* have a yellowish tint all the rest of the remains are colorless.

Although from samples yielding only negative results more subsamples have been investigated in comparison with those having positive data, analysation should be continued. Nevertheless the negative results of sample 140 core B 28 and sample 145 core B 24 being pollenstatistically of the same age (Late Pleistocene previous to peat formation, SEBESTYÉN, 1967: 313) may mirror the real situation.

The presence of glochidia of Unionidae recovered from several samples of core B 28 is significant in that sense that they prove that in the corresponding age Unionidae inhabited the Lake perhaps since its pre-Balaton period in the Late Pleistocene (SEBESTYÉN, 1969: 296). Their presence suggests also the

TABLE 2

Distribution of the remains of glochidia of Unionidae in the sediments of Lake Balaton from the samples shown in Table 1

Sample	Depth, cm	*Unio*	*Anodonta*	Total
1	0—3	+	+	3
20	60	7	53	60
40	123	—	—	—
60	170	?	+	2
80	208	+	—	1
100	250	—	—	—
120	290	?	6	7
140	330	—	—	—
145	318—320	—	—	—
160	370	?	+	3

TABLE 3

Dates on the sizes of the remains of glochidia of Unionidae

Sample	No of sketshes	No of praep.	*Unio*		*Anodonta*		
			length μ	hight μ	lenght μ	hight μ	sp
1	1278/a	656			326	291.5	compl. F
	1279	656	209.8	198.2F	338	291	
20	1276/a	658	186.5	174.9F			
	1276/b	658	186.5	186.5			
	1276/c	658	198.2	198.7			
	1277/a	657			349.8	338.1	cygn. F
	1277/b	657	186.5	186.5	+		cygn.
	1277/c	657			326	338.1	cygn.
	1266/d	657			+		cygn.
	1277/e	657			+		cygn.
	1277/f	657			314.9	314.9	cygn.
	1280/a	654			338.1	338.1	cygn. ? R
	1281/b				326	303.2	cygn. R
	1282				279.8	291.5	cygn. ? R
	1283				338.1	326.7	cygn. R
	1284				+	338.1	cygn. R
60					338.1		cygn.
80		659	204.5	180.7			
120	1275/a	653			361	338.3	cygn. ?
	1275/b	653			326	320.7	compl. F
	1275/c	653			326	326.7	cygn. ?
	1269/a	653			326	291	cygn. R
	1269/b	653			+	291.5	cygn. R
	1275	653			326	391	
160	1274	652			338.1	303.1	compl. F

+ = not measurable R = cam. luc. sketch F = microphoto

Fig. 1. Anodonta cygnea, inside of empty valve showing scar of the adductor muscle. Sample B 28, 20. Specimen 1281b For size see *Table 3*

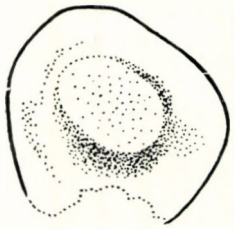


Fig. 2. Anodonta cygnea, inside of empty valve showing scar of adductor muscle. Sample B 28,20. Specimen 1284. For size see *Table 3*

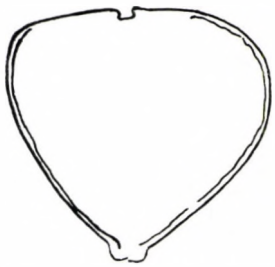


Fig. 3. Anodonta cygnea?, right valve filled with carbonate particles. Specimen No 1280a. For size see *Table 3*

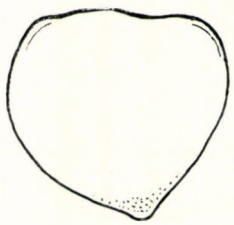


Fig. 4. Anodonta cygnea?, left? valve, Sample B 28,20. Specimen 1282. For size see *Table 3*

Fig. 5. Anodonta cygnea, left valve, Sample B 28,20. Specimen No 1283. For size see *Table 3*

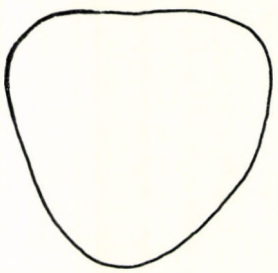
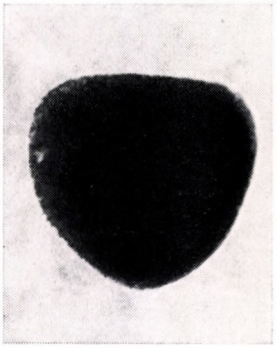
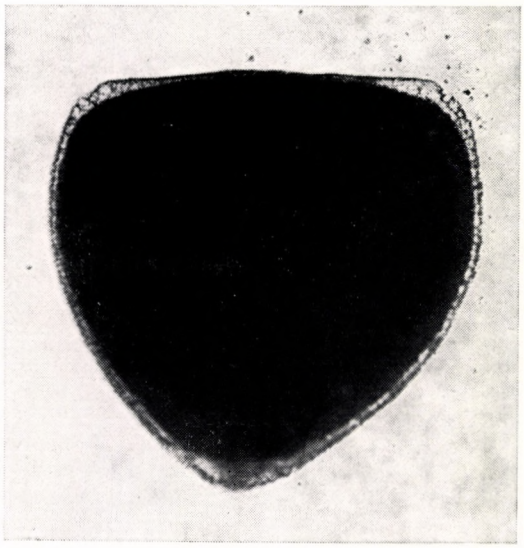


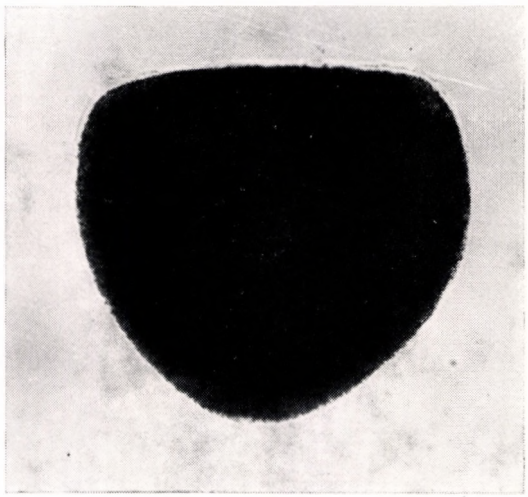
Fig. 6. Anodonta cygnea, half valve sample B 28,120. Specimen 1269a. For size see *Table 3*



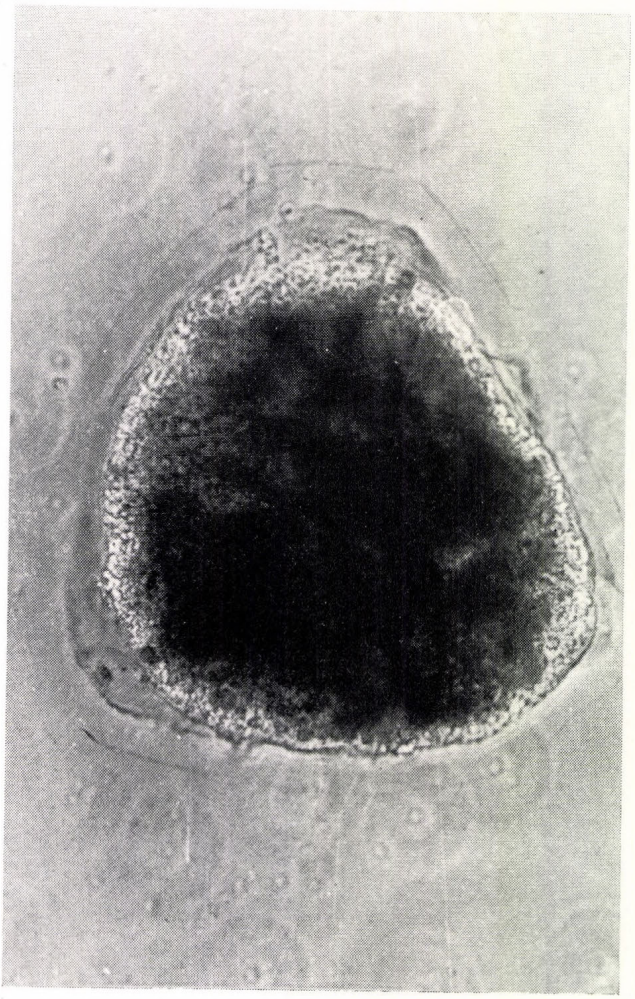
M.ph. 1. Unio sp. Sample B 28,20. Specimen 1276. Length 186.5 μ, height 175 μ



M.ph. 2. Anodonta cygnea right valve. Sample B 28,20. Specimen 1277 a Length 350 μ, height 338 μ



M.ph. 3. Anodonta complanata ?, right valve. Sample B 28,1. Specimen 1278. Length 326 μ, height 291.5 μ



M.ph. 4. Anodonta complanata left valve. Sample B 28,160. Specimen 1274, Length 338 μ, height 303 μ

presence of fish because, as it is well known, the glochidium larvae of Unionidae being released by the mother mussel lead a parasitic mode of life on fish before assuming the final free mode of life. (LUKACSOVICS and LÁBOS, 1965).

From samples of L. LÓCZY's several borings in the sediments of Lake Balaton, specimens of subfossil *Unio* and *Anodonta* and other molluscs had been recovered (LÓCZY, 1916: 536, 630, 638). It is a question, however, whether the depth of the layers being positive for mussels could be compared with those layers of nowadays borings.

Malakological investigation of ZÓLYOMI's borings of Lake Balaton is in progress by a specialist. Results of investigations may throw light on the problem.

Specimens of *Unio* with larvae being in the glochidium stage have been found in the middle of the thirtieth at the Tihany Ferry, at the time of the mussel disaster in Lake Balaton. Free larvae might be caught occasionally in planktonnet. Shells have been recovered from recent surficial mud samples.

In the rescent biota of Lake Balaton the following species and varieties of Unionidae are present:

Unio crassus RETZ. *bosnensis* Mlldff. frm. *serbicus* DROUET "frequent" (SOÓS, 1943: 425).

Unio tumidus solidus ZEL. "common" (SOÓS, 1943: 426).

Unio pictorum L. subsp. *balatonicus* KSTR. (ibidem p. 426) "very common".

Unio pictorum L. subsp. *platyrchynchus* ROSSM. Fauna Catalogue, Balaton and specimens, Tihany.

Anodonta (Pseudoanodonta) complanata ZEL. Fauna Catalogue, Balaton and specimens, Tihany.

Anodonta cygnea L. "generally distributed in Lake Balaton" (SOÓS, 1943: 428).

Anodonta cygnea L. frm. *cellensis* SCHRÖTER Fauna Catalogue, Balaton, and specimens, Tihany.

Anodonta cygnea L. frm. *piscinalis Nillson* (syn. *A. balatonica* HAZAY) (SOÓS, 1943: 428).

Other remains of calcareous nature

Remains of Ostracod valves have been recovered from all samples investigated. Such material is studied by a specialist.

Considering the through study on the Characeae in Lake Balaton and surrounding (FILARSZKY, 1931) it would be of interest to know the connection between the Characeae flora in the past and present. (see Text *Table 1).*

Remains of *Phacotus lenticularis* (EHRBG/STEIN/Chlorophyta, Volvocales) are to be expected from the Balaton sediments. Remains of this minute organism (20 μ) could be recovered by the described method although a propriate larger magnification should be applied. There are several records of the presence of *Phacotus lenticularis* in the plankton of our lake (KOL, 1939; in ENTZ—KOTTÁSZ—SEBESTYÉN, 1937; HORTOBÁGYI, 1939: 335). R. FRANCÉ found the calcareous shells of it off Tihany, Balatonfüred and Keszthely. He recorded its presence in the plankton too (FRANCÉ, 1894, 1897). The calcareous remins of *Phacotus lenticularis* are well known from sediments rich in carbonates (FREY, 1964: 16).

Summary

Using a simple sedimentation method, described here microfossils of calcareous nature have been recorded from the sediments of Lake Balaton. From nine samples of core B 28, had been analysed for cladocera and *Pediastrum* (Late Pleistocene, Holocene) remains of glochidia of Unionidae, valves of Ostracods, oosporangia of Characeae have been recovered. The glochidia belong to the genera of *Unio* and *Anodonta*, inhabiting the lake at present. Ostracod remains are being studied by a specialist. Subfossil glochidia of Unionidae have been recorded so far only in Poland. Remains of the calcareous shells of *Phacotus lenticularis* are to be expected.

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MÉSZTARTALMÚ MIKROFOSSZILIÁK A BALATON ÜLEDÉKEIBEN

Sebestyén Olga

Összefoglalás

Egyszerű ülepítő eljárással fel lehet tárni a Balaton karbonátokat tartalmazó üledékeiből oly mikrofosszíliákat, melyek karbonátokat tartalmaznak. ZÓLYOMI B. B 28 furata *Pediastrum*-okra és Cladocerákra elemzett kilenc mintájából (Late Pleistocén, Óholocén, Újholocén) és a B 24 furat 145. mintájából (Late Pleistocén) sikerült a részletesen ismertetett eljárással balatoni üledékekből Unionida glochidium kagylósrák és Chara-oo sporangium maradványokat feltárni.

A glochidium-maradványok oly kagylók *(Unio, Anodonta)* lárváinak maradványai, melyek ma is tagjai a tavi biotának. ZÓLYOMI említett és más furataiból a Mollusca és Ostracoda maradványokat szakemberek dolgozzák fel. Glochidium-maradványok édesvízi üledékeiben való előfordulására a közölteken kívül csak lengyelországi adatok vannak (BRODNIEWICZ) a szakirodalomban. Várhatók még a *Phacotus lenticularis* maradványai.

МИКРОСКОПИЧЕСКИЕ ИЗВЕСТКОВЫЕ ИСКОПАЕМЫЕ В ОСАДКАХ БАЛАТОНА

О. Шебештен

С помощью описанного в статье простого седиментационного метода в осадках Балатона обнаружены микроскопические ископаемые известковой природы. В девяти образцах пробы B28, проанализированной на присуствие кладоцер и Pediastrum (поздний плейстоцен, голоцен), найдены остатки глохидий унионид, раковинки остракод, и ооспорагии харовых. Глохидии принадлежат родам Unio и Anodonta, которые обитают а озере и в настоящее время. Остракод надлежит определить специалисту. До сих пор субфоссильных глохидий унионид обнаруживали только в Польше. Можно ожидать, что в осадках будут также обнаружены известковые остатки микроскопической зеленой водоросли Phacotus lenticularis.

ANNAL. BIOL. TIHANY | 37 | 291—308 | HUNGARIA 1970

THE QUANTITATIVE PROPORTIONS OF ROTIFERA PLANKTON IN LAKE BALATON, IN 1967

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(Received: 28th February, 1970)

A number of papers have been published — mainly the result of occasional investigations — on the wheelanimalcules inhabiting the water of Lake Balaton, which provide valuable information on species living in various parts of the lake in different biotopes or habitats (VARGA, 1938; 1939; 1941; 1944—45; 1957). The elaboration of Rotifera plankton of the lake is given in three papers based on several years of systematic work (ENTZ et al. 1937; SEBESTYÉN et al. 1951; SEBESTYÉN, 1953), the studies make reference to the seasonal changes of species inhabiting the open water stretching before the Tihany Peninsula. Horizontal investigations carried out simultaneously for the whole area of the Lake Balaton have not yet been done up to this date, our present paper endeavours to supply data to this effect.

The rapid development of the shoreline: erections of houses, increased cultural actual activities, higher degree of water polution, shoreline arrangements all contributed to certain changes taking place in the aquaeous habitat, consequently, we may justifiably suppose, that since the last Rotifera investigations in 1951 both the number of individuals and species suffered changes. Furthermore, the question arose whether the results obtained previously for the water stretches before Tihany Peninsula also hold good for other parts of Lake Balaton.

In order to give exhaustive answers to these questions, and to see clearly the conditions issuing from the great fish stock destruction which occured in 1964 in Lake Balaton, since 1965 we have collected plankton samples both for quantitative and qualitative investigations (P.-ZÁNKAI and KERTÉSZ, 1967). In our present study, we made our quantitative analysis on Rotifera collected in 1967.

Material and method

Sample taking was done between May and October once a month at 5 places of the depth longitudinal axis of the Lake Balaton and at 3—3 places of its transversal section. The places of sample taking in each section were 2000-2500 m apart from each other *(Fig. 1)*. (For detailed description of the sections see SEBESTYÉN, 1960, p. 118.)The samples were taken by the help of FRIEDINGER apparatus from the depths of 0.3, 1, 2, 3 and when it was possible from 4 metres. The one litre water samples taken from the different depths were poured together in order to obtain better values for average,

subsequently, the mixture was preserved with formalin, after deposition the surplus water was removed by VOLK filtration (SEBESTYÉN et al. 1951). After determining the volume of the condensed sample, one third and one fourth of it were examined, of which we pipetted 1, 2 or 4 ml of sample quantity at a time into 60×30 mm counting dish, then in turn each sample was counted under a magnification of ×130. This procedure depending on the good parallels was repeated 3—6 times. The obtained results were calculated for one

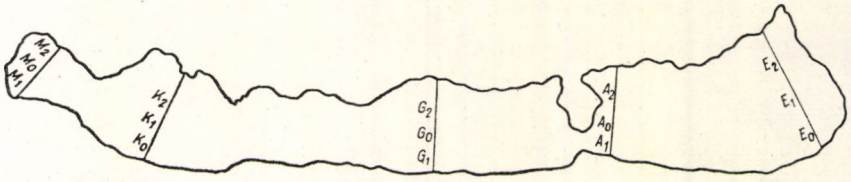

Fig. 1. Sketch of Lake Balaton showing the sites of sample taking

litre of water. Those species which were difficult to determine we cleared on separate slides during counting, of course, after sufficient preparation procedures we determined them.

At the time collecting we made ample notes as to the transparency, temperature of the water as well as to weather conditions.

Results

We found great differences between Keszthely-Bay ("M") and the other parts of Lake Balaton when taking the sum of individual numbers per litre collected at 3—3 points of the 5 sections separated from one another by different distances during the 6 months of investigation *(Table 1)*.

TABLE 1

Quantitative distribution of total Rotifera in the sections of the Lake Balaton (individual per litre)

	M	K	G	A	E
V	340	290*	711	765	1125
VI	167	330	496	163	324
VII	143	654	549	489	436
VIII	262	725	843	408	413
IX	35	139	170	219	234
X	207	169	308	215	323
	1154	2307	3077	2259	2855

* The sample taken at point marked K_0 was broken, thus, the sum is the result of 2 parallels only.

In each of the four sections twice ("K" and ' A") and three times more Rotifera plankton was present than in the water of Keszthely-Bay.

If we add up the individual numbers per litre at each point of the section referring it to the whole period of investigation then we find that between the

collecting sites near the south and north shores and those situated in the middle axis of the Lake Balaton i.e. sections "M" and "A" the difference is comparatively small, in the case of the others, i.e. sections "G" and "E" the two point near the shore show somewhat greater similarity. Generally, excepting section 'M", the central points of all others sections display higher values *(Table 2)*.

TABLE 2

Quantity of total Rotifera at the different collecting sites of the sections with reference to the whole period of examination

M_1	M_0	M_2	K_0	K_1	K_2	G_1	G_0	G_2
460	325	369	672	1061	574	957	1191	929
		A_0	A_0	A_2	E^0	E_1	E_2	
		678	814	767	728	1327	800	

The average values of individual number per litre of the total Rotifera of each section show that the populations increase twice or three (?) times *(Fig. 2)*. In May, the Rotifera plankton density is high in all the 5 sections this is the time when the highest number of individuals occur in sections "M" "A" and "E". The population greatly decreases in June, then in July, ex-

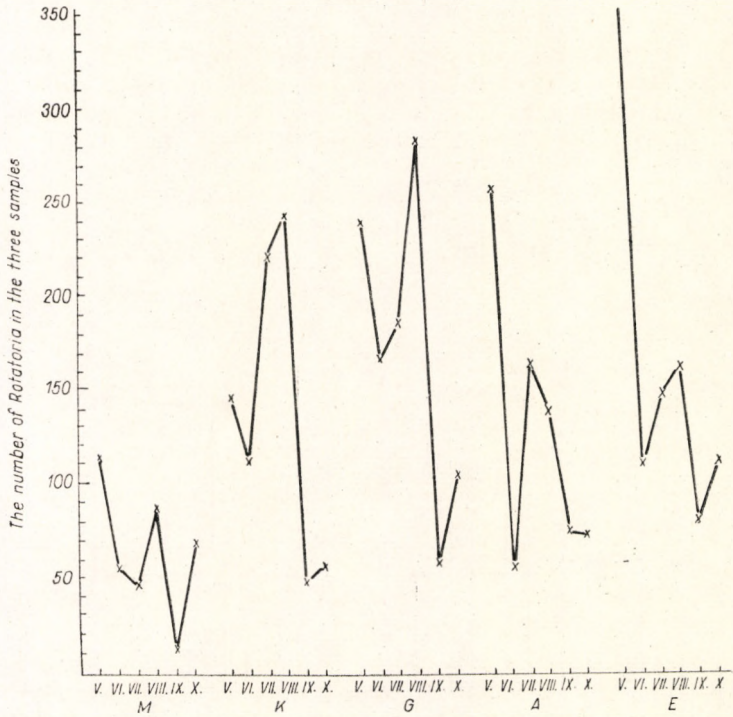

Fig. 2. Monthly change of the specimens per litre values of the total Rotifera shown in the average for the three sites of each section

cepting section "M" the population again gradually increases, and in August the late summer maximum is formed, which value is well below that of the spring results, excepting sections "K" and "G". The lowest individual number of populations was recorded in September, except in section "A", however, in October some slight or bigger increase could be observed.

During the collecting period 24 species, varieties and forms have been determined. From among them 4 species *(Keratella cochlearis, Polyarthra vulgaris, Keratella C. tecta, Keratella quadrata)* occurred in every section and during the whole period of investigation, 1 species was lacking in Keszthely-Bay during the whole period. These 5 species consequently may be regarded

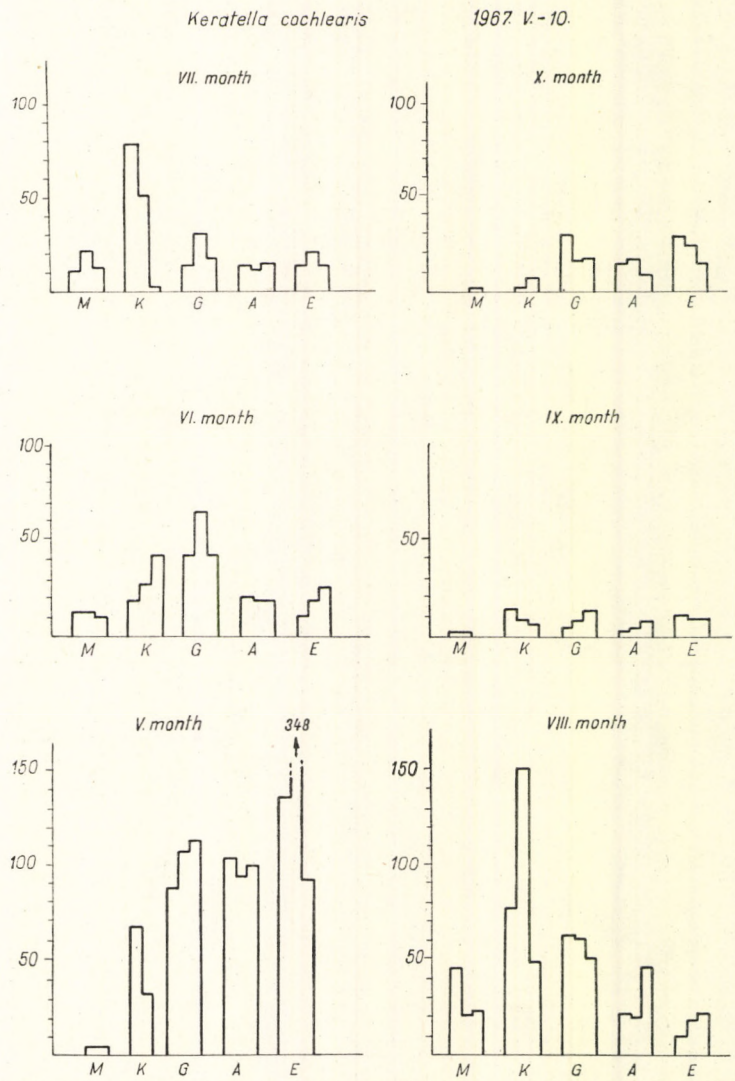

Fig. 3. Monthly change of the number of individuals per litre of *Keratella cochlearis* at 3-3 sites of each section

as the main components of the Rotifera plankton of Lake Balaton from early spring to November. Because each these species is capable for very rapid proliferation (SEBESTYÉN et al. 1951) we thought important to treat them in more detail concerning their population changes with regard to months both within each section — following the order of chronology — and their interrelationships.

Keratella cochlearis GOSSE *(Fig. 3).* In section "M", after a very small individual number in May, the population increases gradually until August, reaching a value of 1 specimen per litre, and the same number may be observed in October, too. In section "K" (?) and "G" the population increases twice, once in May and once in August. In the case of section "A" but especially in

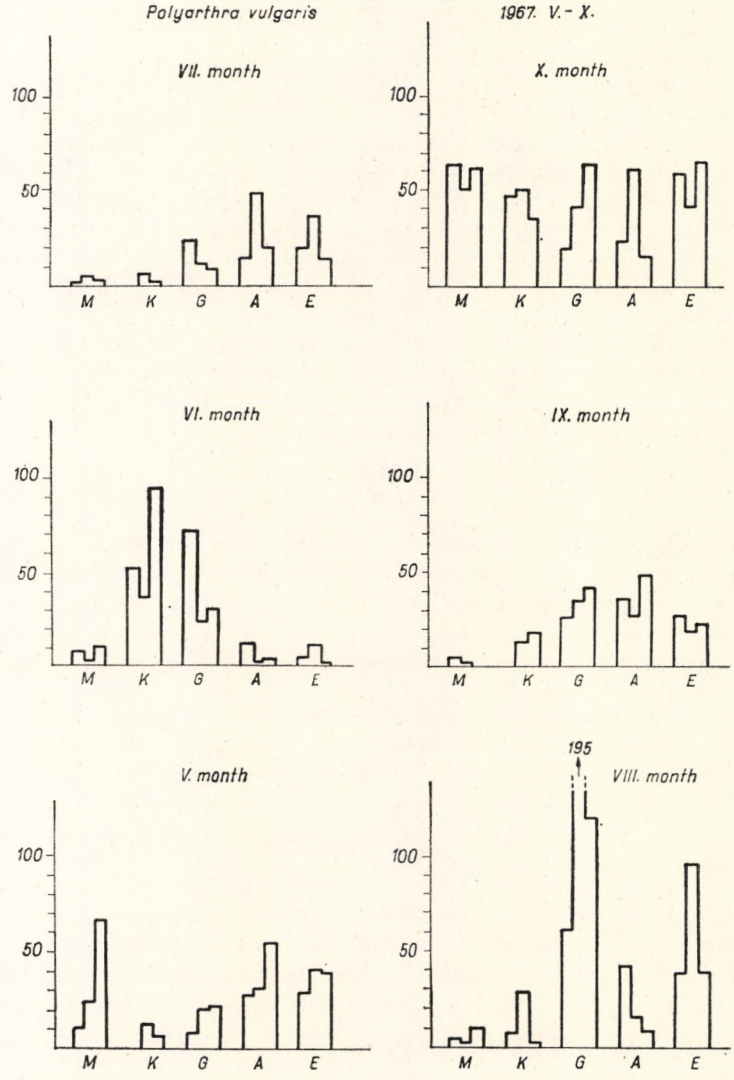

Fig. 4. Monthly change of the number of individuals per litre of *Polyarthra vulgaris* at 3-3 sites of each section

the case of section "E" after May the number of individuals suddenly drops and only a slight increase occurs in October, which cannot be called a second maximum by any rate.

Comparing the populational changes observed in all sections, it becomes clear that in May the litre density of *Kochlearis* gradually increases from sections "M" to "G", the value of "A" is identical with that of "G", while the value of section "E" is extremely high as regards number of individuals. In June, from section "G" and in July from section "K" a gradual decrease could be observed proceeding towards the two ends of Lake Balaton. In August, excepting section "E" the population density increases in all cases, in this month the highest number of individual was counted in section "K", which in September decreases suddenly and showed a well-nigh similar distribution in the open water of the Lake Balaton. In October its quantity again increases between sections "G—E".

Polyarthra vulgaris CARLIN *(Fig. 4)*. In May, and in June and October, in sections "M" and "K" the two increases in population may well be observed. In the case of "E" and "G" it so appears that three increases are present in population, but the quantitative differences between the autumnal values are not very convincing. In sectio "A" disgarding the low value in June, the population density is even during the whole period of investigation.

Comparing the results of the examined sections in May we see that as regards species the poorest parts of the Lake Balaton are in the middle ("K" and "G"). The proportions change in the following months so that the middle areas become the richest in the number of individuals. During July—September the high values of population density occur in sections "G" and "E" while in October, the number of individuals is evenly distributed at a high level over the whole area of the open water.

Keratella cochlearis tecta GOSSE *(Fig. 5)*. In sections "M" and "K", this species shows a characteristic late summer (August) development, its population density gradually increases from May until August, then subsequent to this month a pronounced drop may be observed. In section "G" the population seemingly appears with a double increase. In section "A", similarly to sections "M" and "K", it shows a characteristic maximum in August. In section "E" a distinct maximum in population density did not occur in any of the months throughout the whole period of investigation — except in May — the density is even.

With regards to its horizontal distribution this is a characteristically summer developing species, its highest individual number is found in the central parts of the southwestern basin. In the months of spring and autumn its distribution is very nearly even, excepting Keszthely-Bay.

Keratella quadrata MÜLL. *(Fig. 6)*. In section "M", it appears with an even density between May and the autumnal months, in September and October, however, its number decreases to one specimen per litre. In the other four sections the maximum of development is in May. In October, from quantitative point of view in the Rotifera plankton play an inferior role.

As regards horizontal distribution, it may be established that in May its population from section "M" to "E" gradually increases, in June—September, it plays a significant role only in the southwestern basin.

Pompholyx sulcata HUDSON *(Fig. 7)*. In the northeastern basin section "A" and "E" and in section "G" its population density, from May until July, slowly increases, then in August suddenly drops. In larger number they were

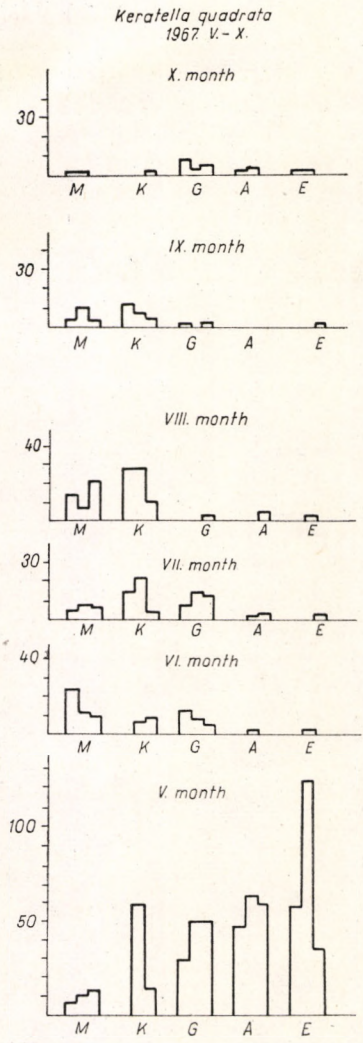

Fig. 5. Monthly change of the number of individuals per litre of *Keratella cochlearis* tecta at 3-3 sites of each section

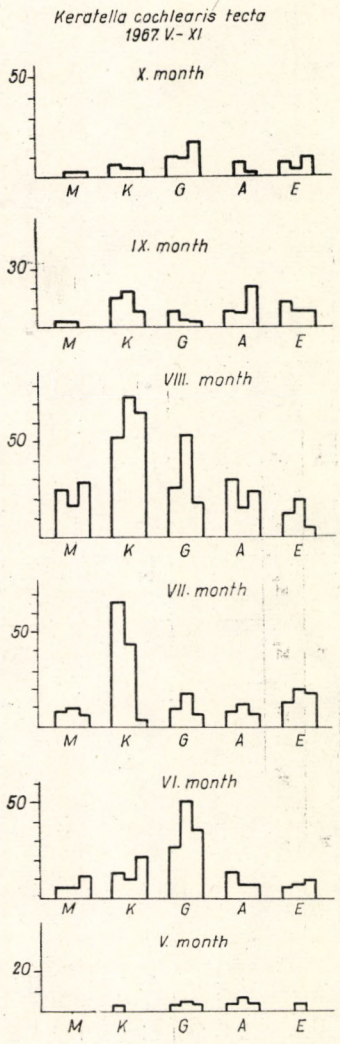

Fig. 6. Monthly change of the number of individuals per litre of *Keratella quadrata* at 3-3 sites of each section

only collected in the area limited by sections "G"—"E". As regards its distribution in the Lake Balaton, its population gradually increases from section "G" towards east, except in October, when the species is evenly distributed in all the three sections; a further exception is the collecting site marked A_0 in July where the highest value was obtained for the whole period of investigation (150 individuals per litre).

Other species. The five species discussed above occurring most frequently are accompanied by some further 19 species *(Table 3)* which, however, with

TABLE 3. QUANTITATIVE DATA OF THE NUMBER OF INDIVIDUALS PER LITRE OF INFREQUENT SPECIES

Species	Data of collection	M			K			G			A			E		
		M_1	M_0	M_2	K_0	K_1	K_2	G_1	G_0	G_2	A_1	A_0	A_2	E_0	E_1	E_2
Asplanchna girodi DE GUERNE	V. 16—18												1		2	
	VII. 19—20											1			2	1
	X. 23											3		1	1	1
Brachionus angularis GOSSE	V. 16—18						2			2			1		2	
	VI. 20			1												
	IX. 19							1								
Brachionus sessilis VARGA	V. 17												1			
	VII. 18—19	4					1	1	10	8	13	9	12	9	5	5
	VIII. 15—16		1		4	38	4	15	6	17	2	8	2	1	5	10
	IX. 19—20		1						1			2		2		
Cephalodella catellina (MÜLL.)	IX. 19							1								
Cephalodella gibba (EHRBG.)	VII. 18						2									
	VIII. 15—16			2								1				
Collotheca balatonica VARGA	V. 18														2	
	VI. 20, 26								1			1		1	8	
	VII. 19—20								6	2		4		1		
	VIII. 15—16							4	6	1	10	7	4	2	1	1
	IX. 20														1	
	X. 23													1		
Collotheca sp.	VI. 26											2	9	3		
	VII. 19—20							3	4	6	6	3		3		
	VIII. 16											6	2			
	IX. 20										1					1
	X. 23											3		4		4
Conochilus unicornis ROUSSELET	V. 16—17	157	38	4		7	6	5	1		3	1				
Filinia longiseta (EHRBG.)	V. 17												1			

Kellicottia longispina (KELLICOTT)	V. 16—18					42	26	26	51	78	27	46	24	57	34	24
	VI. 20, 26			1		1	1	3	2	7	1			3		5
	VII. 18—19	2			6	12	1	4		11			1			2
	VIII. 15	2	1		7	10	1		2	3						
	IX. 19—20	1		3	12		7	2	3	1	2			2	2	1
	X. 17, 23		1	2	2	6	1	14	4	5	2	6	1	6	3	1
Keratella cochlearis macracantha f. *micracantha* LAUTERBORN	V. 16								1							
	VI. 20, 26								1					1		
	VII. 18		1	1	6											
	VIII. 15		1													
	X. 17									5						
Notholca squamula (MÜLL.)	X. 23														1	
Polyarthra major BURCKHARDT	X. 16—17	9	5	3	2	1	1									
Synchaeta oblonga EHRBG.	VI. 20	8	16	19	3	4	1									
	VII. 18—19	5	7	6	55	56	13	2	6	3						
	VIII. 15	2		4			4									
	IX. 19		1													
	X. 23, 26	1									2					
Trichocerca pusilla (JENNINGS)	VI. 20			1												
	VII. 18	4	7		69	89	29	81	63	41	5	9	4	3	5	3
	VIII. 15	5	6	14	22	51	15	19	29	6	28	1	6		11	
	IX. 19—20						1		2		1	2		2	2	4
	X. 23											1	1		1	
Trichocerca rousseleti (VOIGT)	VI. 20, 26		1								1				2	
	VII. 18	2	7	3	6	9										
	VIII. 15		2	4	2											
	IX. 19								1							
Trichocerca stylata (GOSSE)	VII. 18	1														
Trichocerca tenuior (GOSSE)	X. 23											1				
Trichocerca tigris (MÜLL.)	V. 17											1				
	VIII. 16											3				
	X. 13												1			

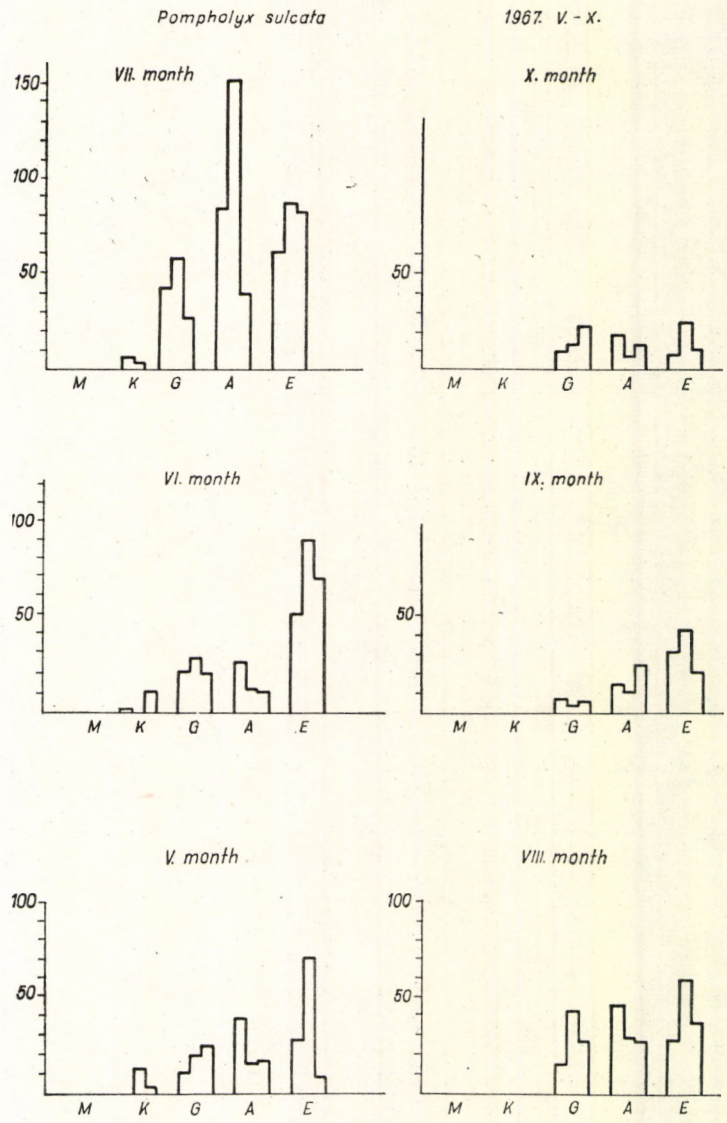

Fig. 7. Monthly change of the number of individuals per litre of *Pompholyx sulcata* at 3-3 sites of each section

regard to their number of individuals play only an inferior role in the Rotifera plankton population of Lake Balaton. The majority of these species (11 species) belongs to the plankton, some inhabits the littoral zone (4 species), others inhabit both the littoral zone and the open water, consequently, they may not be considered exclusively the inhabitants of either habitat (4 species). The appearance of a larger number of individuals in the case of a few species is rather limited to one or two months, while the others occur only sporadically.

The occurence of these species in the various parts of the Lake Balaton supposedly indicates certain changes which have taken place in the quality and composition of the water.

Discussion

On the basis of earlier investigations (SEBESTYÉN, 1953) it was expected that the Rotifera plankton of Lake Balaton for the whole area of the lake in various periods of time displays a great variability in the appearance of species, in distribution and also in the disappearance of species.

The Rotifera plankton investigations carried out from May, 1967 until October, show that as regards the total number of individuals there are two maxima being valid for the whole open water area of Lake Balaton, these maxima occur in May, July and in August. Owing to the lack of early spring samples (March and April) — whose collection due to technical difficulties were postponed — a supposition may be staged that the high number of individuals observed by us in May (first maximum) might perhaps have developed in one of the earlier months. However, quantitative investigations carried out in recent years (SEBESTYÉN et al. 1951; SEBESTYÉN, 1953) prove that neither in March nor in April occurred any high value as regards the number of individuals, furthermore, investigations carried out over a period of seven years also prove that in April of several years a very low value was yielded (15 specimens per litre). In May, on the other hand, in several years a high value for Rotifera plankton was recorded. Investigations of other lakes (KREUTZER, 1934; CARLIN, 1943; HUTCHINSON, 1967; EINSLE, 1967) also make reference that these plankton in the whole year show their highest values in spring-early summer months. Data referring to Lake Balaton show that the species giving the significant mass of Rotifera plankton, those which have been examined by us too *(Keratella cochlearis, Keratella quadrata, Polyarthra vulgaris)* occur in larger or smaller number of individuals throughout the whole year. On the basis of the above thus we may conclude that in spite of the lack of early spring collectings, the maximum occurring in May is valid for the total Rotifera plankton of Lake Balaton.

The results obtained by hydrobiological investigations on several lake in Denmark by NYGAARD (1938) prove conclusively, that in a moderately eutrophic lake the absolute plankton maximum is under normal circumstances in August—October, while the second maximum occurs either in April or in May. The absolute plankton minimum occurs in May—June, rarely at the end of August. The total Rotifera plankton of Lake Balaton also has two maxima, accordingly, our lake also belongs to the group of moderately eutrophic waters. The composition of Chrustaceous plankton as well as the dynamics of population refer to the fact that Lake Balaton, which in 1951 was regarded to be on the borderline of oligo and eutrophic lakes on the basis of investigations carried out that time (SEBESTYÉN, 1953) today, we consider it moderately eutrophic in character.

The peak values of Rotifera plankton appearing in great masses in May and in August can be brought into connection with two ecological factors:

1. The rise in temperature brings about the disappearance of cold stenotherm species *(Filinia longiseta, Notholca squamula)*, and the proliferation of

eurytherm species which until that time occured only in small numbers, and also the appearance of warm stenotherm species *(Pompholyx, Trichocerca, Brachionus sessilis)*. These observations conform to the opinions of other authors who examined the relationship existing between the increase in population and temperature.

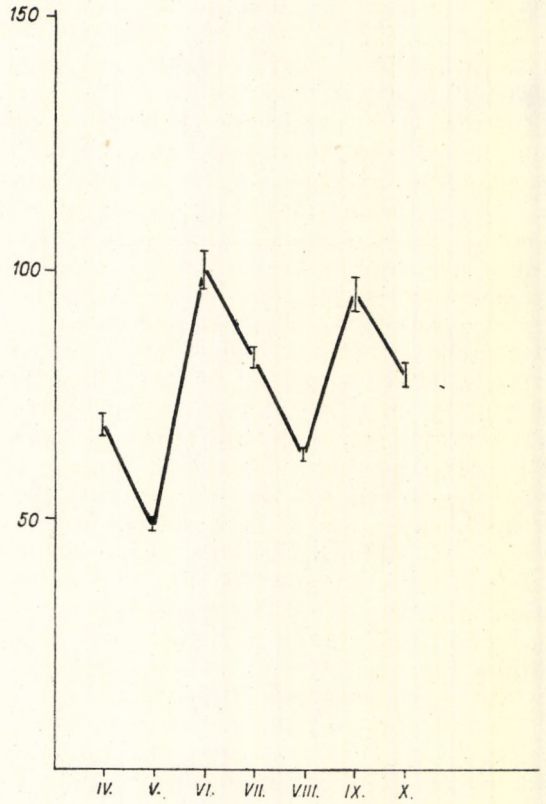

Fig. 8. Monthly change of transparency measured by a Secchi disc in the average of the sample taking places of each section. The scattering of points are shown by the standard divergence from average

2. The bulk of foodstuff of Rotifera consists mainly of algae, bacteria and detritus fragments of about 10 μ in size. Comparing the quantitative increase of bacterial plankton taken at the same time when our collectings were done, with the seasonal changes of total Rotifera individuals we could establish relationships as regards individual sections. Thus, in the case of section "M", Oláh (1969) has shown that parallel with the Rotifera maximum an increase in bacterioplankton was present. Similar foodstuff enrichment could be established both in the case of section "A" and "E" in the maxima occurring in May. In other cases, a quantitative increase of bacterioplankton was closely followed in the next month by an increase in Rotifera population (in the case of sections "G" and "E" in July).

Comparing the maximal density of the population with the transparency data measured at the same time *(Fig. 8)* — which, in fact, are nothing else

than floating seston being proportional to their quantity and refer to the accessible food supply changes for Rotifera — relationships can be established between them. In May and in August, when transparency is very small for all the section in other word, the foodstuff concentration was high, the Rotifera population showed a spring maximum. In June and in September, we observed the exact reverse of the above. Numerous literary data (NÁDAY, 1914; SEBESTYÉN et al. 1951; EDMONDSON, 1960, 1965; HUTCHINSON, 1967) put forth the supposition of existing relationship between the magnitude of Rotifera population and the quantity of foodstuff.

Species occurring in the biggest number of individuals of the Rotifera plankton in Lake Balaton also occur in the majority of European freshwaters, therefore, they are regarded less sensible to the chemical and other conditions of the water. The seasonal changes in their population in the various waters are frequently diverging, so much so, that they may even display differences in the same lake year after year.

In examining the seasonal changes of the *Keratella cochlearis* population certain authors found two maxima. Thus, for example, EINSLE (1967) in Mindelsee, where the summer temperature of the water does not exceed 20 °C, recorded one maximum in the middle of April, and another one at the end of September. On the other hand, RUTTNER (1930) could not establish a regular periodicity in the seasonal changes of population. KREUTNER (1934) from Lake Sulan in Silezia for two years secured samples every 14 days, and making analysis he found that in both years the maximum occurred in May. In the summer-autumn period one or two population increase may also occur. In Lake Balaton, in the years of 1936—1937 no spring maximum could be shown, while in 1938 definite maxima occurred in May and in September. In later years, the maximum number of individuals appeared in January, February and in September (SEBESTYÉN, 1953). Comparing our results with the most recent data of investigations (number of individual layers per litre, taken as mean values) it appears that the result obtained for section "A" in the examined six months decreased in the light of values received in 1951 (in 1951: 58 specimens per litre; in 1967: 16 specimens per litre).

Polyarthra vulgaris like in other water often comes second after *K. cochlearis* (KOCH ALTHAUS, 1963; EINSLE, 1967; NIPKOW, 1952). In the lakes of Switzerland two cycles of proliferation have been observed one in the beginning of July and the other one in October (NIPKOW, 1952). In Lake Balaton, in 1967 the population of *P. vulgaris* increased in spring and autumn only in sections "M" and "K", which may perhaps conform to two cycles of proliferation. The unequivocal increase in population occurred only in October in every section. However, recent investigations embracing a number of years (SEBESTYÉN, 1953) show that there is a slow population increase in summer (July and August), and one in late autumn. Certain modifications taking place in the population dynamics of the species (maximum divergencies) can supposedly be explained by the changes occurring in the food supply of the lake. Paying due attention to the changes in the number of individuals per litre, and comparing them to the results obtained in the years of 1947 and 1951 we find a thinning in population in the case of this species.

Species *Keratella cochlearis tecta* was regarded on the basis of earlier investigations (SEBESTYÉN, 1958) carried out in the lake stretching before Tihany Peninsula, to be an autumnal form, for the biggest number of individ-

uals was found between August and October. However, in 1967 the highest number of individuals was observed in the months of June—August. Further investigations are needed to decide whether it is a characteristic summer or autumn developing species. Comparing the population data with that of years 1947 and 1951 we find a decrease.

Species *Keratella quadrata* on the basis of literary data (SEBESTYÉN et al. 1951; HUTCHINSON, 1967; EINSLE, 1967) during the whole year may sporadically occur, still it is characteristically a species developing in spring. In Lake Balaton, taking into consideration earlier results generally it yields a maximum in May. Investigations carried out in deep lakes (KOCH ALTHAUS, 1963) point out that simultaneously with a rise in temperature the specimens of the species retreat into deep water layers. In Lake Balaton, it seems to bear up well to the higher temperature (19 °C) of the water.

Species *Pompholyx sulcata* in the literature is generally mentioned as a summer species, in Lake Balaton, too, it yields its maximum in July, August (SEBESTYÉN, 1953). In 1967, the biggest population density was in July, whose values for the number of individuals well surpassed the data of earlier years.

Comparing the results of 1930s and 1940s it appears (SEBESTYÉN, 1953) that the population of the species *Keratella cochlearis, K. c. tecta* and *Polyarthra* in the recent years increased in Lake Balaton. Our present investigations in connection with these species ascertained a decrease in the population. In order to decide whether the population of these three species really shows a decreasing tendency or the values obtained were characteristic only for 1967, further investigations are needed covering the whole area of the lake. About the probable cause of this change and about the phenomenon itself we must say a few words because the great decrease observed in section "A" in 1967 compared to the data of 1951 is very significant.

	1951, SEBESTYÉN	1967, P.-ZÁNKAI—PONYI	Decrease
Keratella cochlearis	58 sp/l	16 sp/l	3.6×
Keratella cochlearis tecta	53 sp/l	13 sp/l	4.0×
Polyarthra vulgaris	46 sp/l	23 sp/l	2.0×

sp = number of specimens.

The numerical data are the calculated mean values from 4—4 sections of the identical four months.

Comparing the average values of other sections e.g. "K" for the very same species of period of time with the data obtained by Sebestyén in front of Tihany we can see that the decrease of *K. cochlearis* and *K. c. tecta* is only 1.6×, while the same for *Polyarthra* the values for specimens per litre are the same. The example described above and the previously presented data suggest two conclusions.

1. The changes and dynamics in the zooplankton conditions of Lake Balaton can hardly be elucidated on the basis of even detailed and regular investigations carried out in one section, because at the same time, in the different water areas the population of Rotifera and Crustacea (PONYI, 1968) also differ from each other.

2. Certain Rotifera species — if not to such a great extent as in section "A" — decreased in some degree in every section when compared to the values of 1951. Perhaps one of the causes may be attributed to the wide-scale application of DDT from years 1957—58 in the lake and its environment (cf. PONYI et al. 1968).

The role of Rotifera in saprob-system is not great (LIEBMANN, 1962), still it appears that certain species groups with regard to trophism may be significant (KOCH ALTHAUS, 1963; BERZINS, 1949; LILIEROTH, 1950). Many of these species also occur in Lake Balaton, some of them in small *(Brachionus angularis)*, others in large population density *(Pompholyx sulcata)*. *Trichocerca pusilla* which is regarded to indicate the onset of eutrophic processes by many authors, in the central sections of the lake its population is also very high. These facts also refer (cf. also p. 301) to the moderately eutrophic condition of the lake.

Summary

1. With regard to the quantity of the total Rotifera significant difference occurred between Keszthely-Bay and the open water areas of the lake. The average values per section during the whole period of investigation in Keszthely-Bay yielded 64 sp/l, while for the other sections ("K"—"E") this value fluctuated between 125 and 171.

2. The number of individuals of the total Rotifera plankton in every section increase twice (perhaps three times?).

3. The 24 species determined during the investigations, including varietas and forms too, five species *(Keratella cochlearis, K. c. tecta, K. quadrata, Polyarthra vulgaris* and *Pompholyx sulcata)* can be regarded as the main components of Rotifera plankton in Lake Balaton. From among these species the population of *K. quadrata* and *P. sulcata* increases once in the whole area of the lake. *K. cochlearis* and *K. c. tecta* show two maxima in the middle part of the lake ("K"—"G"), while towards the two ends of Lake Balaton they show only one maximum. *Polyarthra* shows clear population increase twice only in Keszthely-Bay, while in the other parts of the lake apparently it shows three maxima. The development of maxima generally may be placed in the month of May, and in August and October.

4. The quantitative change of the Rotifera plankton is inversely proportional with Secchi (transparency); i.e. apparently it is directly proportional with the concentration of the formed foodstuff.

5. The population changes and dynamics of Rotifera plankton cannot be elucidated even by detailed and regular investigations if it is restricted to only one section, because investigating the different areas of the lake in the same time, the development of Rotifera population may differ from one another.

6. The double population increase of the total Rotifera plankton and the proliferation of species indicating eutrophic processes apparently prove that Lake Balaton may be ranged among the moderately eutrophic waters.

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A BALATON ROTATÓRIA PLANKTONJÁNAK MENNYISÉGI VISZONYAI 1967-ES ÉVBEN

P.-Zánkai Nóra és *Ponyi Jenő*

Összefoglalás

1. Az összes Rotatória mennyisége szempontjából, jelentős különbség mutatkozott a Keszthelyi-öböl és a tó nyíltvízi területei között. Az egész vizsgálati időszak szelvényenként átlagértékei db/lit.-ben kifejezve a Keszthelyi-öbölben 64, a többi szelvényben („K"–„E") 125–171 között változtak.

2. A teljes Rotatória plankton egyedszáma az összes szelvényeken kétszer (esetleg háromszor?) emelkedik.

3. A vizsgálatok alkalmával talált 24 faj, varietas és forma közül 5 faj *(Keratella cochlearis, K. c. tecta, K. quadrata, Polyarthra vulgaris* és *Pompholyx sulcata)* tekinthető a Balaton plankton Rotatóriai fő alkotóelemeinek. E fajok közül a *K. quadrata* és a *P. sulcata* népessége a tó egész területén egyszer emelkedik. A *K. cochlearis* és *K. c. tecta* a tó középső tájain („K"–„G") kettő, míg a Balaton két vége felé egy maximumot mutat. A *Polyartha* csak a Keszthelyi-öböl és környékén mutat határozottan 2 népességemelkedést, míg a tó többi területein úgy tűnik, hármat. A maximumok kifejlődése általában május, ill. augusztus és október hónapokra tehető.

4. A Rotatória plankton mennyiségének változása fordítottan arányos a Secchi-átlátszósággal, azaz úgy látszik, egyenesen arányos a formált táplálék koncentrációjával.

5. A Balaton Rotatória népességének változása és dinamizmusa egyetlen szelvény részletes és rendszeres kutatása alapján nem ismerhető meg, mivel közel azonos időben vizsgálva a tó különböző területeit, a Rotatóriák népességének kifejlődése egymástól eltérhet.

6. Az össz-Rotatória plankton népességének kétszeri emelkedése, valamint az eutrófizációt jelölő fajok elszaporodása alapján feltételezhetően tavunkat a mérsékelten eutróf jellegű vizekhez kell sorolni.

КОЛИЧЕСТВЕННОЕ ИССЛЕДОВАНИЕ ПЛАНКТОННЫХ КОЛОВРАТОК В ОЗЕРЕ БАЛАТОН В 1970 ГОДУ

П.-Занкаи Нора и *Е. Пони*

1. В отношении общего числа коловраток отмечены значительные различия между Кестхейскими заливом и открытой частью озера. Средние для всего периода исследования значения на разрез соответствуют в Кестхейском заливе 64 вида/л, тогда как в других разрезах («К» — «Е») значения колеблются между 125 и 171.

2. Число особей общего планктона коловраток в каждом разрезе увеличивается в 2, а возможно и в 3 раза.

3. Определяющими в течение периода исследований являлись 24 вида, включая разновидности и формы; из них пять видов *(Keratella cochlearis, K. c. tecta, K. quadrata, Polyarthra vulgaris, Pompholyx sulcata)* можно считать главными компонентами планктона коловраток Балатона. Из них по всей области озера однажды увеличивается популяция *K. quadrata* и *P. sulcata*. *K. cochlearis* и *K. c. tecta* проявляют два максимума в средней части озера («К» — «G»), а в направлении к двум концам озера они проявляют только один максимум. *Polyartha* проявляет чёткий рост популяции дважды только в

Кестхейском заливе, тогда как в других частях озера этот вид как будто имеет 3 максимума. Их развитие обычно происходит в мае, в августе и в октябре.

4. Количественные изменения планктона коловраток находятся в обратной зависимости от изменений показателя прозрачности по Secchi, то есть по-видимому в прямой зависимости от концентрации образующихся кормовых веществ.

5. Изменения популяции и динамика планктонных коловраток не могут быть выяснены на основе даже детальных и регулярных исследований, если они ограничены только одним разрезом, потому что в разных частях озера развитие популяции протекает различным обрезом.

6. Двойное увеличение популяции общего планктона коловраток и пролиферация видов, характерных для эутрофических процессов, вероятно свидетельствуют о том что Балатон может быть отнесен к разряду умеренно эутрофных озер.

CHRONICLE

1969 was the first year in a period of a three year's research plan. The two scientific departments continued investigations as it was scheduled.

1. *Department of Experimental Zoology* has started its three year's plan within the main topic "Investigations of physiological and morphological specificites of neurohumoral regulation in invertebrates".

2. Investigations of the *Department of Hydrobiology* concentrated on the main theme "Hydrobiological investigations of Lake Balaton".

Dr. OLGA SEBESTYÉN, former head of the Department of Hydrobiology, now retired, continued her work on the field "Paleolymnological investigations on microfossils of Lake Balaton sediments". In 1969 Dr. OLGA SEBESTYÉN participated in the work of the Editorial Board of *Annal. Biol. Tihany* for the last time; she resigned on the grounds of her advanced age. Dr. OLGA SEBESTYÉN has been working since 1948 with the Editorial Board and took part in the editing of 19 volumes. She played a prominent part judging and evaluating the papers on hydrobiological topic. Her altruistic help is gratefully acknowledged hereby.

Results of the work performed by the members of the two Departments were published partly in *Annal. Biol. Tihany* **36,** and partly in various Hungarian and foreign periodicals (see *Annal. Biol. Tihany*, 1970, **36,** p. 318).

The Institute's permanent staff 54 persons were divided as follows: 19 scientific research workers, 14 technical assistants, 6 administrative and 15 other workers.

The following changes took place in the scientific staff of the Institute: CS. CSUKÁS biologist-geographer assistant scientific research worker left for the Brewery, Debrecen on the 16th of May; K. BIRÓ biologist-chemists assistant scientific research worker left for Medical Plant (Herb) Research Institute, Budapest on the 21st of September; K. ELEKES biologist-geographer on the 1st of August and T. KISS biologist-chemists on the 1st of September were appointed as assistant scientific research workers to the Department of Experimental Zoology.

Inland study trips

K. BIRÓ assistant scientific research worker was working from the 1st of January untill the 10th of March at the Zoological Department of Eötvös Loránd University in Budapest; Dr. I. ZS.-NAGY scientific research worker visited the Institute of Pathological Anatomy of the Medical School in Pécs from 21st to 25th of April. Dr. E. LÁBOS scientific research worker spent two weeks in the Institute of Biophysics of Medical School in Pécs.

Travels abroad

P. Biró assistant scientific research worker was working from the 17th of June to the 17th of August in Poland — in the Department of River Menagement of the Institute of Inland Fisheries (Zabieniec) and in the Department of Ichthyology of the Institute of Inland Fisheries (Olstyn—Kortowo).

L. Hiripi assistant scientific research worker spent four weeks in the Physiological Institute of Czechoslovakian Academy of Sciences, Prague (Czechoslovakia).

I. Kiss assistant scientific research worker started his six-month long study trip in Physiological Institute of the Ukraine Academy of Sciences, Kiev (Soviet Union) on the 4th of September.

Dr. P.-Nóra Zánkai was working in Borok (Soviet Union) at the Institute of Freshwater from the 2nd of September until 28th of October, following she visited the Natural History Museum in Moscow from 29th of October until 26th of November.

Dr. J. Ponyi head of the Department of Hydrobiology attended the International Congress on the Pollution of Water in Prague (Czechoslovakia) on 21—26 April; following the invitation he worked in the Mineralogical-Petrographical Institute of Heidelberg University (West-Germany) from 30th of May until 27th of August.

Dr. Katalin S.-Rózsa senior scientific research worker visited the Soviet Union from the 18th of October to 1st of November attending the Second Congress of Soviet Biochemists held in Tashkent; than she left for Czechoslovakia, and she stayed there between 10th of November and 5th of December, where she visited the Physiological and Entomological Institutes of the Czechoslovakian Academy of Sciences (Prague) and the Physiological Institute of the Slovakian Academy of Sciences (Bratislava).

Dr. J. Salánki director of the Institute attended the IBRO Seminar held in Kotor (Yugoslavia) from 30th of June until 11th of July; than attended the Second Congress of Soviet Biochemists held in Tashkent (Soviet Union) from 18th of October until 1st of November.

Dr. B. Entz deputy director of the Institute has finished his work in Ghana on the 1st of June, then he has started his work in the UAR as an expert.

Visiting scientists from inland and abroad

Similarly to the previous years in 1969 also several research workers visited our Institute: The following investigators spent longer time in the Institute:

Dr. K. Richter Zoological Institute Jena (DDR); Dr. Oltean Mircea, Institut de Biologie "T. Savulescu" de l'Academie R. S. R. Bucuresti; Dr. F. Verzár, Institute of the Experimental Gerontology Basel; Y. Robin Collège de France Biochimie Générale et Comparée Paris; Dr. K. Galewski Institutute Zoologica PAN, Warsawa; Dr. V. Novák Entomologisches Institut CSAV, Abteilung für Physiologie, Leiter der Abteilung Praha; I. D. Carthy (London); P. Duchesne (Liege).

Besides the above mentioned research workers the following visitors have spent few days in the Institute:

J. J. Ramanauszkasz (Vilnius); F. Hanson (Texas); Dr. G. Harwich (Berlin); K. A. Vespäläinen (Helsinki); G. Linke (Berlin); A. T. Natarajan (Stockholm); P. Pasik and A. Tauba (New York); F. Makled (Cairo); Ch. Graul Berlin (DDR); M. Gaze (London); Z. Kobilinski (Warsawa); B. Botio (Sofia); P. Drach (Paris); Nikola D. Nikolov (Sofia); L. Vladimir (Praha); H. Whlod (Jena); K. Rostarish (Wroclaw); Professor T. André (Paris); H. E. Lacob (Jena); R. E. Austin (Texas).

The following Hungarian scientists worked here as visiting research workers or visited the Institute by aim of exchanging experiences:

From Budapest:

Prof. Dr. G. Ádám, Department of Comparative Physiology of Eötvös Loránd University; Prof. Dr. E. Biró, Biochemical Department of Eötvös Loránd University; Dr. L. Felföldy, VITUKI; Dr. M. Garamvölgyi, Institute for the Retraining of Physicians; Dr. J. Hámori, Anatomical Department of the University Medical School; Dr. T. Jermy, Scientific Research Institute for Plant Protection; Dr. Z. Kaszab, Hungarian Natural History Museum; Dr. J. Matskási, Hungarian Natural History Museum; Dr. M. Molnár, Hungarian Natural History Museum; Dr. Nagy Tiborné, Botanical Department of Eötvös Loránd University; Dr. M. Nemessuri, School of Physical Training; Dr. L. Perényi, Korányi TBC School; Dr. A. Pásztor, Hungarian Nerve Surgical Institute; Dr. B. Pénzes, Botanical and Zoological Garden; Dr. Á. Soós, Hungarian Natural History Museum; Dr. L. Varjas, Scientific Research Institute for Plant Protection; R. Vásárhelyi, VITUKI; Dr. I. Törő Jr., University Department of Histology and Embriology in University Medical School.

From Debrecen:

Dr. Cs. Hadházi, Anatomical Department of the University Medical School; Dr. I. Fekete, Zoological Department of Kossuth University; Dr. S. Imre, Pathophysiological Department of University Medical School; Prof. Dr. I. Krompecher, Anatomical Department of University Medical School; Dr. Z. Kádár, Kossuth University; Dr. Nagy Endréné, Dermatological Department of University Medical School.

From Szeged:

Prof. Dr. B. Csillik, Department of Anatomy, Histology and Embriology of University Medical School; Dr. L. Erdélyi, Physiological Department of József Attila University; Prof. Dr. O. Fehér, Physiological Department of József Attila University; Dr. J. Megyeri, High School of Pedagogy; Dr. J. Pórszász, University Medical School; Dr. A. Stammer, Zoological Department of József Attila University; Dr. G. Uherkovics, Station for Tisza Research.

From Pécs:

Prof. Dr. K. Lissák, Physiological Department of University Medical School.

From Keszthely:

Dr. G. Borbély, Academy of Agriculture; Dr. Kárpáti Istvánné, Academy of Agriculture.

From Alsógöd:

Dr. G. Szemes, Station for Duna Research.

Valéria Kovács student from the Biological-Geographical Department of Budapest Eötvös Loránd University performed her dissertation thesis submitted for certification at the Zoological Department of the Institute.

During the summer months 17 Hungarian University students joined the Institute's work for 3—4 weeks.

In the 1969 year the *Scientific Council* of the Institute held two sittings where they were informed on the results of the fulfilments of the Institute's year plan and approved the publications submitted for the 36th volume of *Annal. Biol. Tihany.*

Meetings

In the course of the year 1969 nine meetings were held at the Institute:

1. Between the 26th of January the course entiled: „Indentification and significance of Algae" was held with 15 participants by the Lymnological Section of the Hydrobiological Society, Hydrobiological Department of the Institute and the Hydrobiological Committee of the Hungarian Academy of Sciences.

2. From the 29th of January to the 8th of February "Winter School" in the field of nuclear physics was organized by the Central Research Institute of Physics the of Hungarian Academy of Sciences with participation of 59 scientists.

3. Between the 18th and 26th of June "Summer School" in the field of nuclear physics with 45 participants in the organization of the Central Research Institute of Physics of the Hungarian Academy of Sciences.

4. Summer course for the pupils of secondary schools with biological specialization from the 7th to the 17th of July was organized by the Biological Department of the Hungarian Academy of Sciences with 24 participants.

5. Between 17th and 23nd of August retraining course for the teachers of biology in the secondary schools was held with 26 participants by the Biological Department of the Hungarian Academy of Sciences.

6. From the 1st to the 4th of September the "Memory Symposium" was held by the Comparative Physiological Department of Budapest Eötvös Loránd University and the Biological Department of the Hungarian Academy of Sciences with 44 participants.

7. Between the 15th and 20th of September Summer School in the field of astro-physics was held in organization of the Mathematical Department of the Hungarian Academy of Sciences with 40 participants.

8. Between the 28th and 30th of September "Hydrobiological Days" attended by 33 participants were organized by the Hungarian Hydrobiological Society and the Department of Hydrobiology of the Institute.

9. From the 13th of October to the 2nd of November the course "Identification and importance of water animals in the water-menagement" was

held with 28 participants by the Lymnological Section of the Hydrobiological Society, Hydrobiological Department of the Institute and The Hydrobiological Committee of the Hungarian Academy of Sciences.

Improvement in research facilities

The Equipment park was completed among others by Preparative Ultracentrifuge (Beckman Spinco L-50, a USA), Homogenizer (MSE, Typ. 7700, Great Britain), one beam oscilloscope (Typ. EMG-1546), a universial dual beam oscilloscopes (Zeiss NU-2), a binocular microscope (Zeiss SMXX), a universial turn-bench, turner's lathe (EMU-220), a Grinding machine on the tripod (Typ. EVIG CSF64/2), a Digital DC Voltmeter (EMG Typ. TR-1652), a Desk calculator (Hunor 131), a Fraction collector fractiomat (Typ. Labor 59 932), a Micromanipulator (Typ. Brinkman RP-5), a boring machine (42 V), a Biomix homogeniser (Labor), a Rodatest rotary evaporator (KUTESZ).

At the end of year 1969 the Library of the Institute registered 44 178 volumes. Over 610 different periodicals are currently received, among them 12 are "Abstracts".

The Institute's Year Book, *Annal. Biol. Tihany*, Vol. 36. (1969) was sent to 579 Institutes all over the world, in exchange the library received 345 different journals and publications.

KRÓNIKA

Az 1969-es év a soron következő hároméves tervciklus első éve volt. A két Tudományos Osztály munkája a hároméves tudományos tervnek megfelelően folyt.

1. A *Kísérleti Állattani Osztály* „A neurohumorális szabályozás fiziológiai és morfológiai sajátosságainak vizsgálata gerinctelen állatokon" — című témában kezdte meg hároméves tervét.

2. A *Hidrobiológiai Osztály* vizsgálatait a „Balaton hidrobiológia kutatása" c. fő témán belül folytatta.

Dr. Sebestyén Olga nyugalmazott osztályvezető továbbra is aktív tudományos tevékenységet fejtett ki az Intézetben. Munkáját „Paleolimnológiai vizsgálatok balatoni üledékben" c. témakör keretében végezte. Dr. Sebestyén Olga 1969-ben vett részt utoljára az *Annal. Biol. Tihany* szerkesztőbizottságának munkájában; a továbbiakban előrehaladott korára való tekintettel, e megbízatásáról lemond. Dr. Sebestyén Olga 1948 óta folyamatosan tagja volt a szerkesztőbizottságnak, 19 kötet szerkesztésében vett részt. Jelentős szerepe volt a hidrobiológiai tárgyú dolgozatok elbírálásában, értékelésében. Áldozatos munkájáért köszönet illeti.

A két osztályon dolgozó kutatók tudományos tevékenységét tükröző dolgozatok részben az *Annal. Biol. Tihany* **36,** kötetében, részben más hazai és külföldi folyóiratokban kerültek publikálásra (lásd. *Annal. Biol. Tihany* 1970, **37,** 318).

Az Intézet személyi állománya 54 fő, ami a következőképpen oszlott meg, kutató: 19, kutatási segéderő: 14, adminisztratív: 6, egyéb: 15.

Az Intézet kutatói állományában az alábbi változások történtek: Csukás Csaba biológia-földrajz szakos tanár, május 16-án a Debreceni Söripari Vállalathoz, Biró Kálmán biológia-kémia szakos tanár szeptember 21-én a Budapesti Gyógynövény Kutató Intézethez távozott. Elekes Károly biológia-földrajz szakos tanár augusztus 1-én és Kiss Tibor biológia-kémia szakos tanár szeptember 1-én segédmunkatársi kinevezéssel az Intézet Kísérleti Állattani Osztályára került.

Belföldi tanulmányutak:

Biró Kálmán tudományos segédmunkatárs január 1-től március 10-ig az ELTE Állatrendszertani Intézetében (Budapest) dolgozott. Dr. Zs.-Nagy Imre tudományos munkatárs, április 21-től április 25-ig, dr. Lábos Elemér tudományos munkatárs pedig november 10-től november 22-ig a POTE Kórbonctani, illetve Biofizikai Intézetében dolgozott Pécsett.

Külföldi utak:

Biró Péter tudományos segédmunkatárs június 17-től augusztus 17-ig Lengyelországban a Belvízi Halászati Kutatóintézetben (Varsó, Zabience, Olsztyn-Kortovo) dolgozott akadémiai kiküldetésben.

Hiripi László tudományos segédmunkatárs négy hetet töltött Csehszlovákiában akadémiai kiküldetésben a Csehszlovák Tudományos Akadémia Élettani Intézetében, Prágában.

Kiss István tudományos segédmunkatárs szeptember 4-én megkezdte hathónapos tanulmányútját az Ukrán Tudományos Akadémia Fiziológiai Kutatóintézetében, Kievben.

Ponyi Jenőné szeptember 2-től október 28-ig a Boroki víztároló és Belvizekkel foglalkozó Kutatóintézetében dolgozott, majd október 29-től november 26-ig a Moszkvai Természettudományi Múzeumban folytatta tanulmányútját.

Dr. Ponyi Jenő a hidrobiológiai osztály vezetője április 21-től április 26-ig részt vett a Prágában rendezett Vízszennyezési Világkongresszuson (Csehszlovákia), majd május 30-tól augusztus 27-ig Nyugat-Németországban, a Minerológiai-Petrográfiai Intézetben (Heidelberg) dolgozott meghívásra.

Dr. S.-Rózsa Katalin tudományos főmunkatárs október 18-tól november 1-ig a Szovjetunióban tartózkodott, ahol részt vett a Taskentben rendezett II. Össz-szövetségi Biokémiai Kongresszuson, majd november 10-től december 5-ig Csehszlovákiába utazott tanulmányútra, ahol a Csehszlovák Tudományos Akadémia Fiziológiai Intézetét és Entomológiai Intézetét (Prága), valamint a Szlovák Tudományos Akadémia Fiziológiai Intézetét (Bratiszlava) látogatta meg.

Dr. Salánki János intézet igazgató 1969. június 30-tól július 11-ig részt vett a Kotorban (Jugoszlávia) rendezett IBRO szemináriumon, majd a II. Össz-szövetségi Biokémiai Kongresszuson Taskentben (Szovjetunió) október 18-tól november 1-ig.

Dr. Entz Béla igazgatóhelyettes június 1-ig Ghanában dolgozott, szakértői minőségben, majd az év folyamán az Egyesült Arab Köztársaságban kezdte meg két éves szakértői tevékenységét.

Vendégkutatók

Az előző évekhez hasonlóan több külföldi és hazai kutató dolgozott az Intézetben.

Hosszabb időt töltöttek az Intézetben:

K. Richter, Jénai Egyetem; Dr. Oltean Mirceau, Biológiai Intézet (Bukarest); Dr. F. Verzár, Gerontológiai Intézet, Basel; Y. Robin, Colège de France, Paris; Dr. T., Galewski Zoológiai Intézet, Warsawa; P. Duchesne, Anatómiai Intézet, Liège; V. Novák, Entomológiai Intézet, Prága; J. Charty, Field Studies Cauncil, London.

A fentieken kívül pár napos látogatást tettek az Intézetben:

J. J. Ramanauszkasz, Valstybinis Universitetas, Vilnius; Hanson Frank, University of Texas; G. Hartwich, Berlin; K. Vepsäläinen, Genetikai Intézet Helsinki; G. Linke, Universität of Halle/Saale, Berlin; A. T. Natarajan, Stockholm University; P. Pasik és A. Tauba; School of Medicine New York City, U.S.A.; Fathi Makled, University of Kairó; Christa Graul

University of Berlin; M. Gaze,University of London; Kobilinski Zbignum, Lengyel Tudományos Akadémia Warsawa; Botio Betev, Bolgár Tudományos Akadémia Szófia; P. Drach, Paris; Nikola D. Nikolov, Institute of Physiology Sofia; Rank Ettanson, Austin Texas; Vladimir Londa, Entomológiai Intézet Prága; Whlod Herbel, Jena; K. Rostarish University of Wroclav; André Thomas, Faculté des Sciences, Paris; H. E. Lacob Institut F. Mikrobiológica Jéna.

Hazai kutatók közül vendégkutatóként dolgoztak, ill. tapasztalatcsere céljából keresték fel az Intézetet:

Budapestről:

Dr. Ádám György, ELTE Összehasonlító Élettani Intézet; Dr. Biró Endre, ELTE Biokémiai Intézet; Dr. Felföldi Lajos, VITUKI; Dr. Garamvölgyi Miklós, Orvostovábbképző Intézet; Dr. Hámori József, BOTE Anatómiai Intézet; Dr. Jermy Tibor, Növényvédelmi Kutató Intézet; Dr. Kaszab Zoltán, Természettudományi Múzeum; Dr. Matskási István, Múzeum, Állattár; Dr. Molnár Miklós, Természettudományi Múzeum; Dr. Nagy Tiborné, ELTE Növénytani Tanszék; Dr. Nemessuri Mihály, Testnevelési Főiskola; Dr. Perényi László, Korányi TBC Intézet; Dr. Pásztor András, Országos Idegsebészeti Intézet; Dr. Pénzes Bethen, Fővárosi Növény- és Állatkert; Dr. Soós Árpád, Természettudományi Múzeum; Dr. Varjas László, Növényvédő Kutató Intézet; Vásárhelyi Réka, VITUKI; Dr. ifj. Törő Imre, BOTE Szövet- és Fejlődéstani Intézet.

Debrecenből:

Dr. Hadházi Csaba, DOTE Anatómiai Intézet; Dr. Fekete István, KLTE Állattani Intézet; Dr. Imre Sándor, DOTE Kórélettani Intézet; Dr. Krompecher István, DOTE, Anatómiai Intézet; Dr. Kádár Zoltán, KLTE; Dr. Nagy Endréné, DOTE Bőrklinika.

Szegedről:

Dr. Csillik Bertalan, SZOTE Anatómiai Szövet- és Fejlődéstani Intézet; Dr. Erdélyi Lajos, JATE Állatélettani Intézet; Dr. Fehér Ottó, JATE Állatélettani Intézet; Dr. Megyeri János, Tanárképző Főiskola; Dr. Porszász János, SZOTE Sebészeti és Műtéttani Intézet; Dr. Stammer Aranka, JATE; Dr. Uherkovics Gábor, MTA, Tiszakutató Állomás.

Pécsről:

Dr. Lissák Kálmán, POTE Élettani Intézet.

Keszthelyről:

Dr. Borbély György, Agrártudományi Főiskola; Dr. Kárpáti Istvánné, Agrártudományi Főiskola.

Alsógödről:

Dr. Szemes Gábor, MTA, Dunakutató Állomás.

Kovács Valéria, az ELTE biológia-földrajz szakos hallgatója szakdolgozatát az Intézetben készítette.

A nyári hónapokban 17 hazai egyetemi hallgató kapcsolódott be az Intézet munkájába 3—4 hétre.

Az 1969. évben az *Intézet Tudományos Tanácsa* kétszer ülésezett, amikor is meghallgatta az éves tervek teljesítésének eredményét, valamint jóváhagyta az *Annal. Biol. Tihany* 36. kötetének anyagát.

Rendezvények

1969. évben 9 nagyobb rendezvény került lebonyolításra az Intézetben.

1. Január 6—26-ig „Algák felismerése és jelentősége" c. kurzus a Hidrobiológiai Társaság Limnológiai Szakosztályának, az Intézet Hidrobiológiai Osztályának, valamint az MTA Hidrobiológiai Témabizottságának rendezésében, 15 fő részvételével.

2. KFKI Téli Iskola január 29 és február 8 között az MTA Központi Fizikai Kutató Intézetének rendezésében. Résztvevők száma: 59.

3. Június 18—26 Magfizikai Nyári Iskola az MTA Központi Fizikai Kutató Intézetének rendezésében. A résztvevők száma: 54.

4. Nyári tanfolyam a biológiai szakosítású középiskolák diákjai számára július 7—17-ig, az MTA Biológiai Osztályának rendezésében 24 diák részvételével.

5. Továbbképzés biológiát oktató középiskolai tanárok részére augusztus 17—23 között az MTA Biológiai Osztályának rendezésében, 26 fő részvételével.

6. Emlékezésbiológiai Szimpozium szeptember 1—4-ig az ELTE Összehasonlító Élettani Intézetének és az MTA Biológiai Osztályának rendezésében, 44 fő részvételével.

7. Szeptember 15—20 között Asztrofizikai Nyári Iskola 40 fő részvételével az MTA Matematikai Osztályának rendezésében.

8. Hidrobiológus Napok szeptember 28—30 között a Hidrobiológiai Társaság és az Intézet Hidrobiológiai Osztályának rendezésében 33 fő részvételével.

9. „Víziállatok felismerése és jelentősége a vízgazdálkodásban" c. kurzus október 13-tól november 2-ig 28 fő részvételével a Hidrobiológiai Társaság Limnológiai Szakosztályának, az Intézet Hidrobiológiai Osztályának, valamint az MTA Hidrobiológiai Témabizottságának rendezésében.

Kutatási feltételek fejlődése

Az év folyamán beszerzett jelentősebb műszerek: Ultracentrifuga (Spinco **L—50**, USA), Homogenizátor (MSE, Angol), EMG—1546 típusú egysugaras oszcilloszkóp (1 db.), EMG—1552 típusú kétsugaras oszcilloszkóp (2 db.), NU—2 (Zeiss) univerzális kutatómikroszkóp, SMXX (Zeiss) binokuláris mikroszkóp, EMU—200 egyetmes műszerészeszterga, Állványos csiszológép (EVIG Typ. CSF 64/2), Digitális DC Voltmérő (EMG Typ. TR—1652), Hunor 131 számológép, Frakciószedő (Labor typ.: 59932) Fractiomat, Brinkman manipulator (Typ. RP—5), Fúrógép 42 V-os, Biomix homogenizátor (Labor), Rotadeszt rotációs lepárló (KUTESz).

Az évvégi összesítés alapján az Intézeti könyvtár állománya 44 178 egység. Az év folyamán összesen 610 különböző folyóirat és kiadvány járt az Intézetbe, köztük 12 referáló jellegű.

Az Intézeti Évkönyv — *Annal. Biol. Tihany* **36.** kötetét 579 címre küldtük meg, melyért cserébe 345 kiadvány érkezett.

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ZS.-NAGY, I., H. P. von HAHN, F. VERZÁR (1969): Age-related alterations in the cell nuclei and the DNA content of rat tail tendon. — *Gerontologia* **15,** 258—264.

ZS.-NAGY, I., D. A. SAKHAROV (1969): Axo-somatic synapses in Procerebrum of Gastropoda. — *Experientia* **25,** 258—259.

PONYI, J., K. MOLNÁR (1969): Adatok Magyarország halai parazita faunájának vizsgálatához. V. rész. Parazita. Copepodák. — *Parazitologia Hungarica.* (1).

S.-RÓZSA, K. (1969): Theory of step-wise excitation in Gastropod heart. — *Comp. Physiol. of the Heart: Current Trends.* Ed. F. V. MCCANN. *Experientia Suppl.* **15,** 69—77.

S.-RÓZSA, K. (1969): The influence of 5HT on heart phosphorylase activity in the snail *Lymnaea stagnalis* L. — *Life Sci.* I. **8,** 229—234.

SALÁNKI, J., A. GUBICZA (1969): Histochemical evidence of direct neuronal pathways in *Anodonta cygnea* L. — *Acta Biol. Acad. Sci. Hung.* **20,** 219—234.

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A kézirat nyomdába érkezett: 1970. VI. 15 — Terjedelem: 28,35 (A/5) ív
70.69902 Akadémiai, Nyomda Budapest, — Felelős vezető: Bernát György