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INTRACELLULAR DISTRIBUTION OF SEROTONIN IN THE CENTRAL NERVOUS SYSTEM AND IN THE HEART OF *HELIX POMATIA*

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According to the generally accepted assumption the transmitter molecules are stored in the nerve terminals and synaptic vesicles. The supposition of GERSCHENFELD (1963) according to which in the central nervous system of Molluscs the dense-core vesicles are the storing places of monoamines is supported by results obtained morphologically (ZS.-NAGY, 1968; COTTRELL and OSBORNE, 1970; JOURDAN and NICAISE, 1970; PENTREATH et al., 1973) as well as by differential and gradient centrifugation (HIRIPI et al., 1973).

The histochemical investigations performed on the heart of *Helix* (COTTRELL and OSBORNE, 1969) and of *Lymnaea* (S.-RÓZSA and ZS.-NAGY, 1967) revealed the localization of monoamines in the neuronal elements and muscle cells. The localization of serotonin in the neuronal elements is confirmed also by autoradiographic examinations carried out on the heart of *Aplysia* (TAXI and GAUTRON, 1969).

Although the physiological and pharmacological examinations performed on the nervous system and heart of *Helix pomatia* (S.-RÓZSA and PERÉNYI, 1966; S.-RÓZSA, 1969; GERSCHENFELD, 1973) support the transmitter role of serotonin, its exact localization in these tissues is yet unknown. Since the nervous tissue can be separated by careful homogenization and subsequent centrifugation into different fractions (WHITTAKER, 1965; 1971) we set the target in our present work to investigate the subcellular localization of serotonin in the nervous and heart tissue of *Helix pomatia* by simply applying this method.

Material and method

For the examinations the cerebral ganglion, the suboesophageal ganglion ring and the heart tissue of *Helix pomatia* were used. During the preparation the tissues were collected in ice-cold physiological solution, they were washed, then after blotting with filter paper their wet weights were taken and homogenization was carried out in 0.2 M iso-osmotic sucrose. Prior to homogenization the tissues were cut up, then in a glass potter (clearance 0.1 mm; 3000 rev/min; 15 strokes) they were homogenized in such volume of sucrose that 5 per cent homogenizate were obtained. The differential and gradient centrifugation was carried out by slightly modifying WHITTAKER's method (1965). The

primary fractions were obtained by differential centrifugation as follows. The nuclear fraction (Nuc) was separated by centrifuging at 900 g for 10 min, the mitochondrial fraction (Mit) at 11,000 g for 60 min, the microsomal fraction (Mic) at 100,000 g for 60 min. The residual supernatant represented the soluble (S) fraction. The subfractions of the mitochondrial fraction were obtained by centrifuging on a sucrose gradient. The mitochondrial fraction was resuspended in iso-osmotic sucrose by careful manual homogenization such that 1 ml of suspension was equivalent to 500 mg of original tissue. 1 ml of the suspension was layered onto a discontinuous sucrose gradient, which was prepared in the case of the ganglion from 1—1 ml of 2.0 M; 1.5 M; 1.2 M; 0.8 M sucrose solutions *(Fig. 1A)*, while in the case of the heart from 1—1 ml of 2.0 M; 1.7 M; 1.5 M; 1.2 M; and 0.8 M sucrose solutions *(Fig. 1B)* before using up, and was stored at 0°C for 1 hr. The centrifuging was carried out at 50,000 g for 2 hr. The fractions were obtained with slicing. Beckman Spinco ultracentrifuge (Model L50; rotors SW 25.1 and L50) was used. The procedure was carried out at 4°C. Small aliquots of the fractions were used for the determination of proteins (LOWRY et al., 1951) as well as for electron microscopic examinations.

For estimating serotonin the normality of the fractions was adjusted to 0.4 by $HClO_4$ and they were diluted to 5 ml then were rehomogenized. After keeping at 0°C for 30 min the homogenizate was centrifuged, and the serotonin content of the supernatant was estimated by the method of SNYDER et al. (1965). The RSA of a fraction was calculated as the percentage of the total recovered protein found in the same fraction.

Samples taken from the fractions for electron microscopic analysis were diluted when necessary to the concentration of iso-osmotic sucrose, then centrifuged at 100,000 g for 1 hr. The pellet was fixed in glutaraldehyde diluted with 3% tap-water or by *Helix* physiological solution for 2 – 18 hr at room temperature, or at 4°C. After a short washing the pellet was postfixed in 2% OsO_4-collidine at 0°C for 30 min. Following the dehydration the pellets were embedded in Araldite. Sections were cut on an LKB Ultrotome III, micrographs were taken on a TESLA type BS 413 A electron microscope. The sections were stained with uranyl acetate and lead citrate (REYNOLDS, 1963).

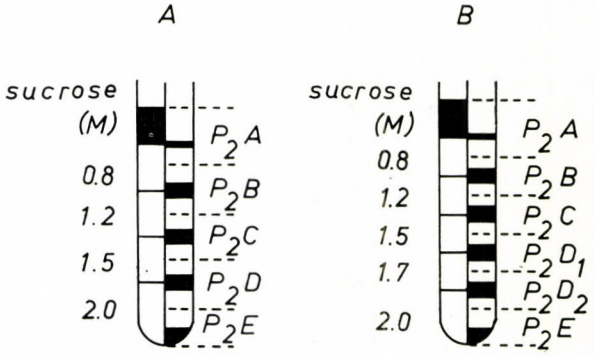

Fig. 1. Arrangement of the gradients before centrifugation (left side) and arrangement of the fractions after centrifugation (right side)

Results

Table I contains the percentage distribution of serotonin in the primary fractions. 70 per cent of serotonin is bound to the tissue elements and 30 per cent of that is present in free form in the soluble fraction. Among the primary fractions of the ganglion the highest concentration of serotonin is found in the nuclear fraction while for the heart in the mitochondrial fraction. The microsomal fraction contains 10—15 per cent serotonin *(Table I)*. The nuclear

TABLE I

Distribution of serotonin in the primary fractions of the ganglion and heart tissue of Helix pomatia

Fractions	Heart	Ganglion
P_1 (Nuc)	24.2	31.9
P_2 (Mit)	43.2	26.5
P_3 (Mic)	14.7	10.45
S (Soluble)	28.6	31.6

fraction of the ganglion contained nuclear fragments, numerous unhomogenized cell fragments, free dense-core vesicles, granules, granular elements of the endoplasmic reticulum, glycogen granules and mitochondria. The dominant structure of the P_2 fraction *(Fig. 2)* is the synaptosoma, in addition a great number of dense-core vesicles, granules of other type, mitochondria and vesicular forms of the granular endoplasmic reticulum also occur. In some areas unique or fascicled collagen fibrils can be found. The glycogen granules or rosettes are uniformly distributed. In the P_3 fraction uniformly distributed free ribosomes and vesicular membrane fragments of smooth surface can be seen *(Fig. 3)*. In the deeper region of the pellet numerous dense-core vesicles also occurred. Elements of the granular endoplasmic reticulum are encountered only rarely *(Fig. 4)*.

In the nuclear fraction of the heart collagen fibrils to a high extent, numerous fibrous and filamentous structures presumably constituting the contractile elements, mitochondria, membrane fragments of unknown origin can be observed. In some areas tubular elements are fairly visible, rather resonably the elements of the sarcoplasmic reticulum. In the mitochondrial fraction nerve endings containing dense-core vesicles or elementary and glycogen granules could be observed. This fraction is characterized by a great number of free dense-core vesicles or granules, glycogen granules. Mitochondria, elements of the granular endoplasmic reticulum, smooth membrane elements are also abundant *(Fig. 5)*. The structure of the microsomal fraction: the upper layer of the pellet generally is characterized by a dense, fibrous fundamental structure containing vesicles of small and larger size, membrane fragments, sometimes mitochondria and granules. Sporadically some collagen fibrils occur too *(Fig. 6)*. The denser layer of the pellet contains numerous rounded, smooth-surfaced membrane profiles as well as a great number of elongated tubular formations, which might be considered to be the constituting elements of the sarcotubular system. The numerous free ribosomes are uniformly distributed *(Fig. 7)*.

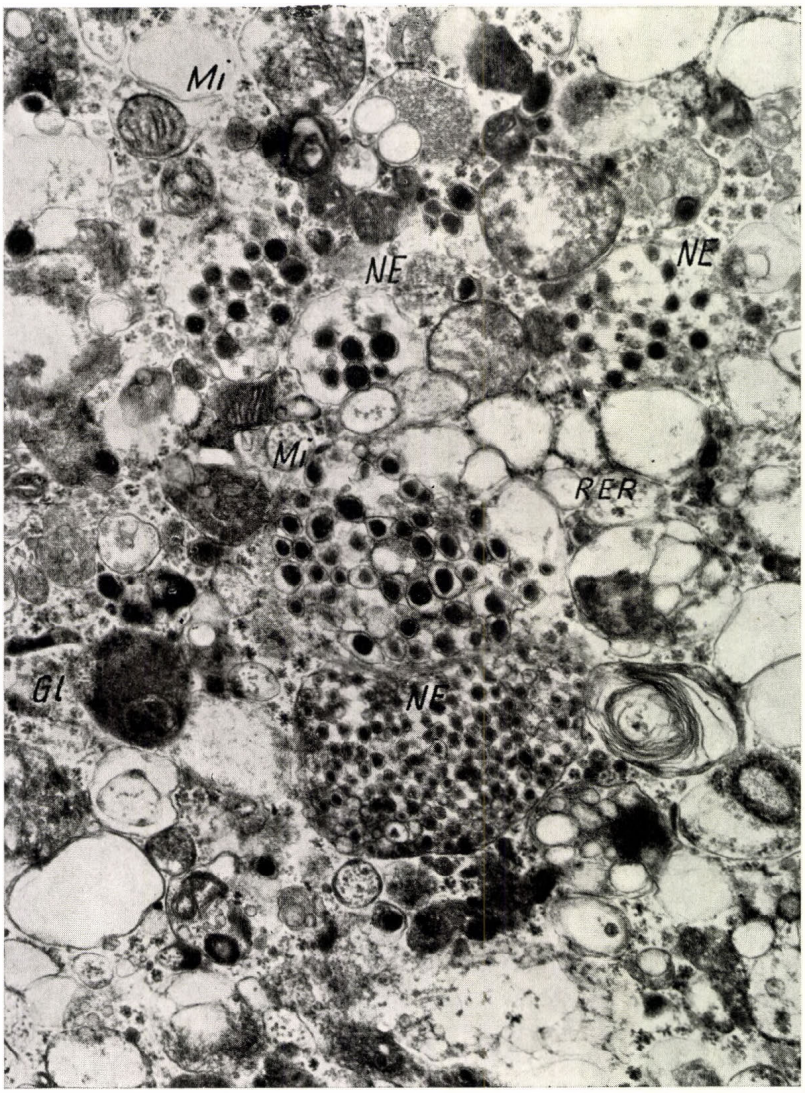

Fig. 2. Electron micrograph of the mitochondrial (P_2) fraction of the ganglion. NE — nerve endings, Mi — mitochondria, GL — glycogen granules or rosettes, RER —granular endoplasmic reticulum. ×22,000

Table II shows the percentage distribution and RSA values of serotonin found in the subfractions of the mitochondrial fraction. The 17 per cent serotonin content of the P_2A subfraction is practically not bound to tissue elements but it is present in free state in the supernatant fluid. The protein concentration of this fraction is minimal and when measuring serotonin in the supernatant, and in other part of the fraction containing tissue elements separately, the quantity of serotonin is found to be 15 per cent in the super-

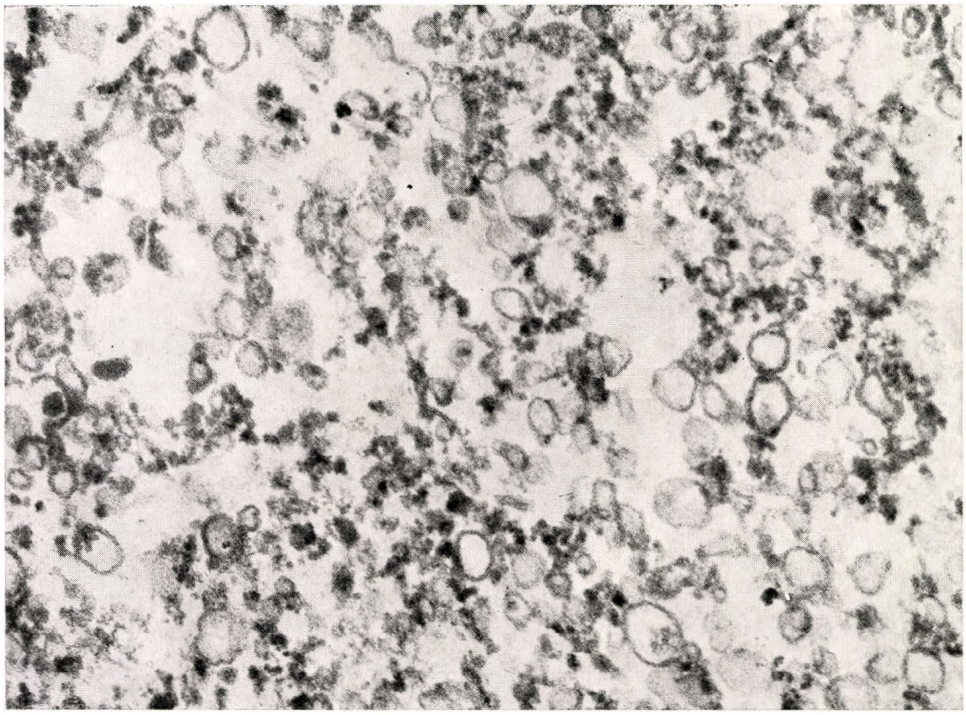

Fig. 3. Electron micrograph of the microsomal (P_3) fraction of the ganglion. ×35,000

natant fluid while only 1—2 per cent in that part, which contains tissue elements. The 10—12 per cent serotonin content of subfractions P_2B and P_2E has a low RSA value. 60 per cent of the serotonin content of the mitochondrial fraction is present in subfractions P_2C and P_2D displaying a RSA of more than 1.0.

In the case of the ganglion according to the electron microscopic examinations the main components of subfraction P_2A were the larger and smaller membrane profiles. Dense-core vesicles and granules are also observed sporadically. Fraction P_2B is characterized by a very great number of free dense-core vesicles and granules. There are many smooth membrane profiles, which partly might have been depleted synaptosomes. In some places intact nerve endings could be seen containing vesicles of different type. The elements of the endoplasmic reticulum carrying ribosomes occurred, though rarely. The predominant structure of the fraction P_2C is the synaptosoma *(Fig. 8)*. The vesicule population of the nerve endings is of heterogeneous appearance *(Fig. 9)*. Beside the typical dense-core vesicles, its elements and neurosecretory granules sometimes terminals could be seen, which might be considered to be of cholinergic nature. This subfraction is characterized by a mass of free dense-core vesicles, granules. Mitochondria can be seen both in free form and in nerve endings and the same arrangement is found concerning the glycogen granules. Elements of the granular endoplasmic raticulum also

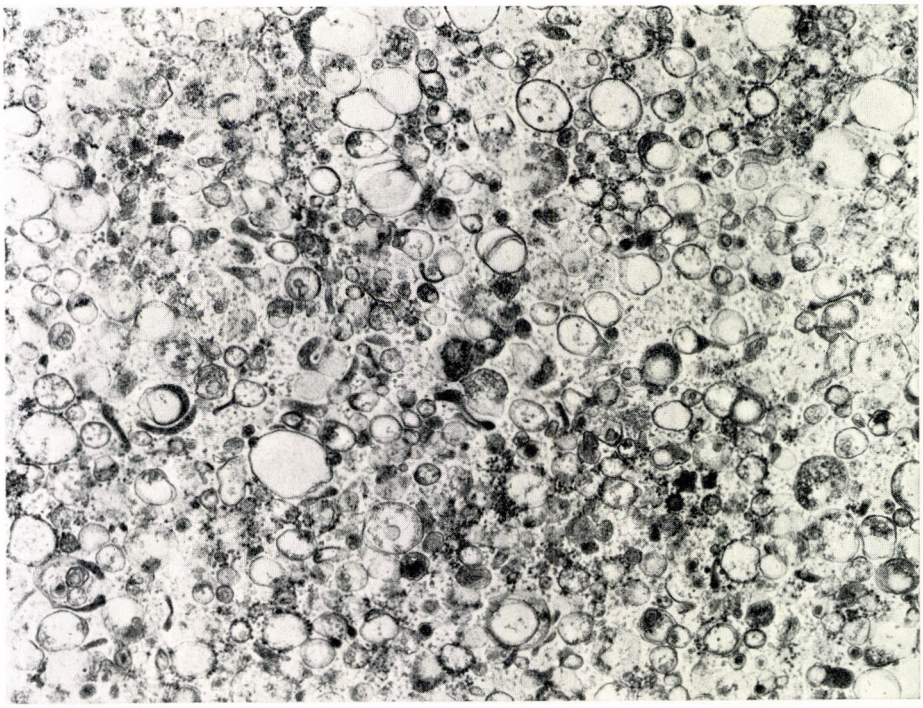

Fig. 4. Electron micrograph of the microsomal (P_3) fraction of the ganglion. ×35,000

TABLE II

Distribution and RSA of serotonin in the subfractions of the mitochondrial fraction obtained from the ganglion of Helix pomatia

Fractions	5HT %	RSA
P_2A	17.1	—
P_2B	12.4	0.84
P_2C	25.8	1.48
P_2D	34.2	1.22
P_2E	10.4	0.38

occur. Subfraction P_2D shows an accumulation of more damaged nerve endings as compared to those of P_2C, most of them are full of dense-core vesicles *(Fig. 10)*. Among the numerous free vesicles glia granules are also abundant. Elements of the granular endoplasmic reticulum are frequently encountered. Glycogen granules can be found in large quantities similarly to those of P_2C. Mitochondria occur to a lesser extent. Fraction P_2E contains sporadically nerve endings, free dense-core vesicles and vesicles of other type. Elements of the reticulum with ribosomes and numerous collagen fibrils are also characteristic here.

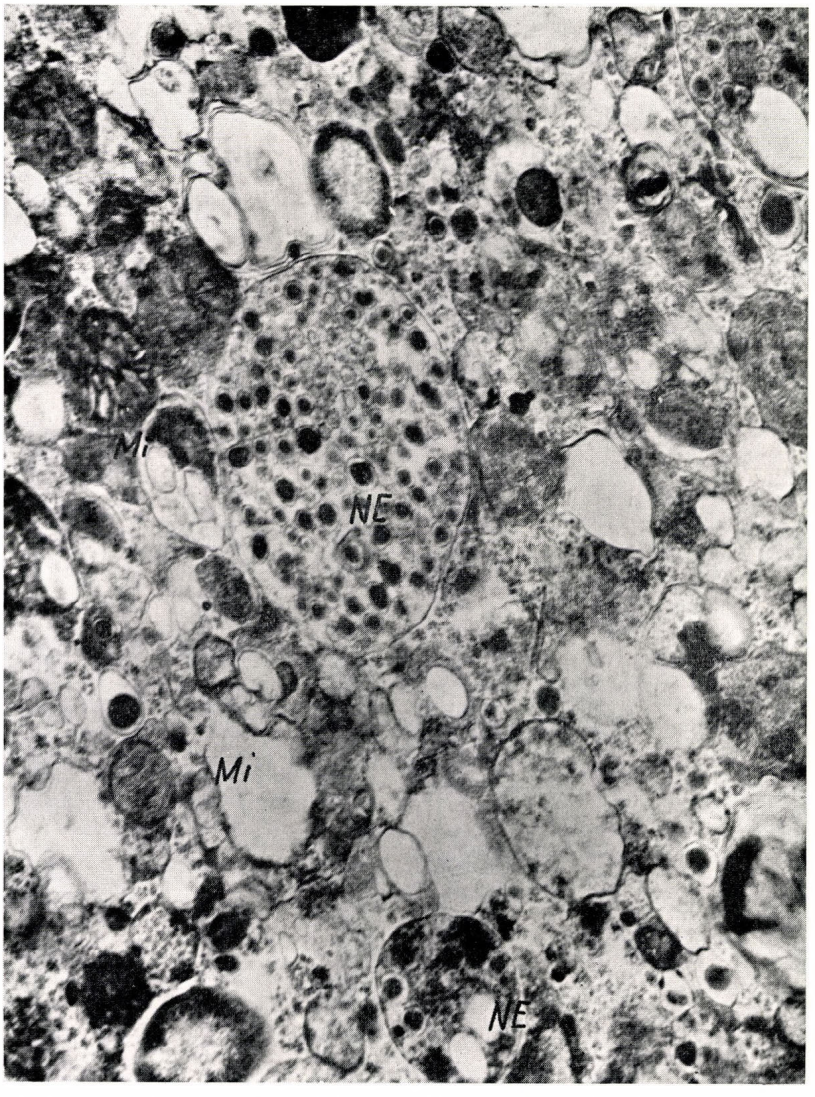

Fig. 5. Electron micrograph of the mitochondrial (P_2) fraction of the heart. NE — nerve endings, Mi — mitochondrium. ×25,000

As far as the heart is concerned, the preliminary investigations showed that when for the separation of fractions the same gradient was used as in the case of the ganglion, fraction P_2D consisted of two fractions of different density, which could be separated to subfractions P_2D_1 and P_2D_2 by increasing the quantity of the gradients in such a way that the RSA value of the more dense subfraction P_2D_2 showed a considerable increase as compared to the RSA of the fraction P_2D. In the case of the ganglion, the separation

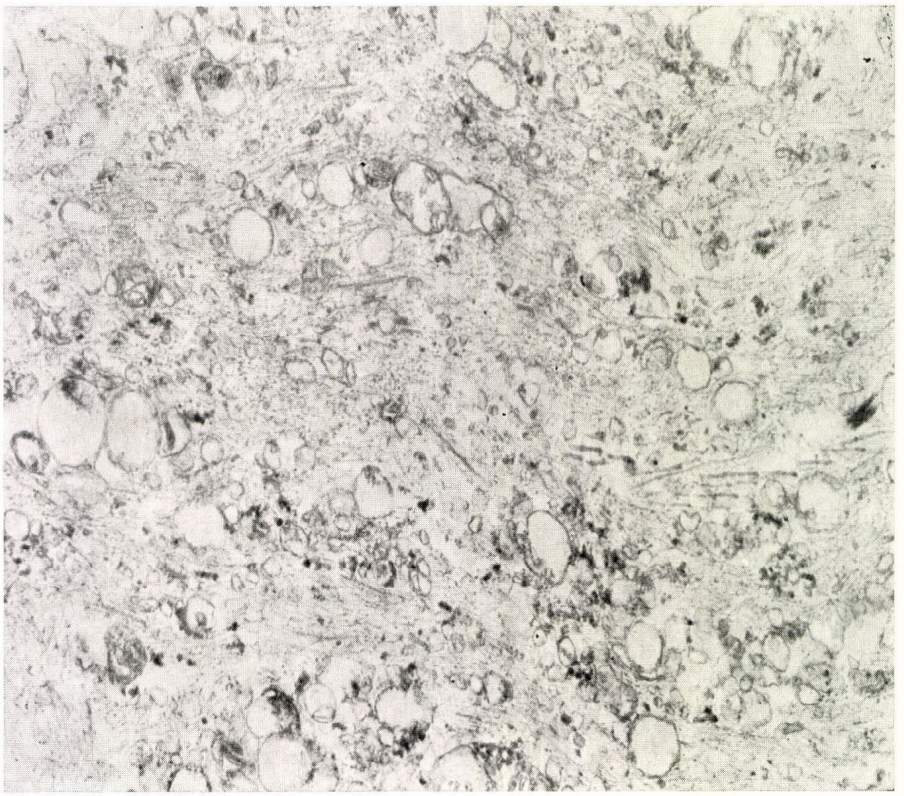

Fig. 6. Electron micrograph of the microsomal (P_3) fraction of the heart. Smooth membrane profiles in the typical filamentous fundamental structure. ×22,000

of the fraction P_2D in the same way did not result in any fraction having higher RSA value. Similarly to that of the ganglion, the subfraction P_2A contained only a few tissue elements, and its serotonin content of 9 per cent was practically present dissolved in the supernatant fluid.

Subfractions P_2B and P_2C contain minimal amount of serotonin with low RSA. The 20 per cent serotonin content of the fraction P_2D_1 has nearly identical RSA value with that of the subfraction P_2D of the ganglion. The highest concentration of serotonin is present in subfractions P_2D_2 and P_2E. However, in fraction P_2E serotonin is bound with a low RSA, while in fraction P_2D_2 with a very high RSA *(Table III)*.

According to the ultrastructural analyses, subfraction P_2A consists of smooth membrane fragments and empty membrane vesicles. Fraction P_2B is similar to P_2A, but here a few vesicular elements can be found within the empty membrane profiles now and then indicating their synaptosomatic origin. Some free dense-core vesicles frequently with eccentric core can also be seen as well as a number of mitochondria. Subfraction P_2C is characterized by a great number of mitochondria as an exclusive structural component in some places *(Fig. 11)*. In addition, smooth membrane fragments, dense-core

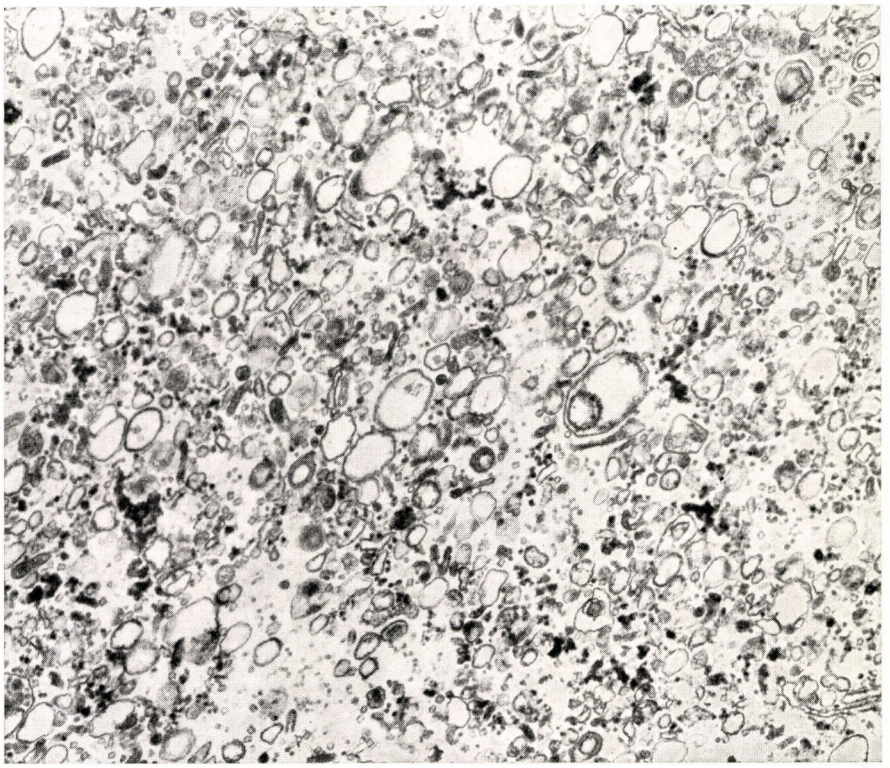

Fig. 7. Electron micrograph of the microsomal fraction of the heart. The frequent tubular formations presumably correspond to the elements of the sarcotubular system. ×30,000

vesicles of relatively frequent occurrence and granules are also seen. The predominant components of fraction P_2D_1 are the mitochondria occurring en masse cohered completely in some places. Rarely a few nerve endings with dense-core vesicles and generally free granules of major size can be seen *(Fig. 12)*. Subfraction P_2D_2 is the only one, where besides a rather large quantity of mitochondria often encountered nerve endings in shrunken state

TABLE III

Distribution and RSA of serotonin in the subfractions of the mitochondrial fraction obtained from the heart tissue of Helix pomatia

Fractions	5HT %	RSA
P_2A	9.00	—
P_2B	4.28	0.79
P_2C	10.00	0.40
P_2D_1	20.60	1.16
P_2D_2	25.20	3.17
P_2E	26.80	0.73

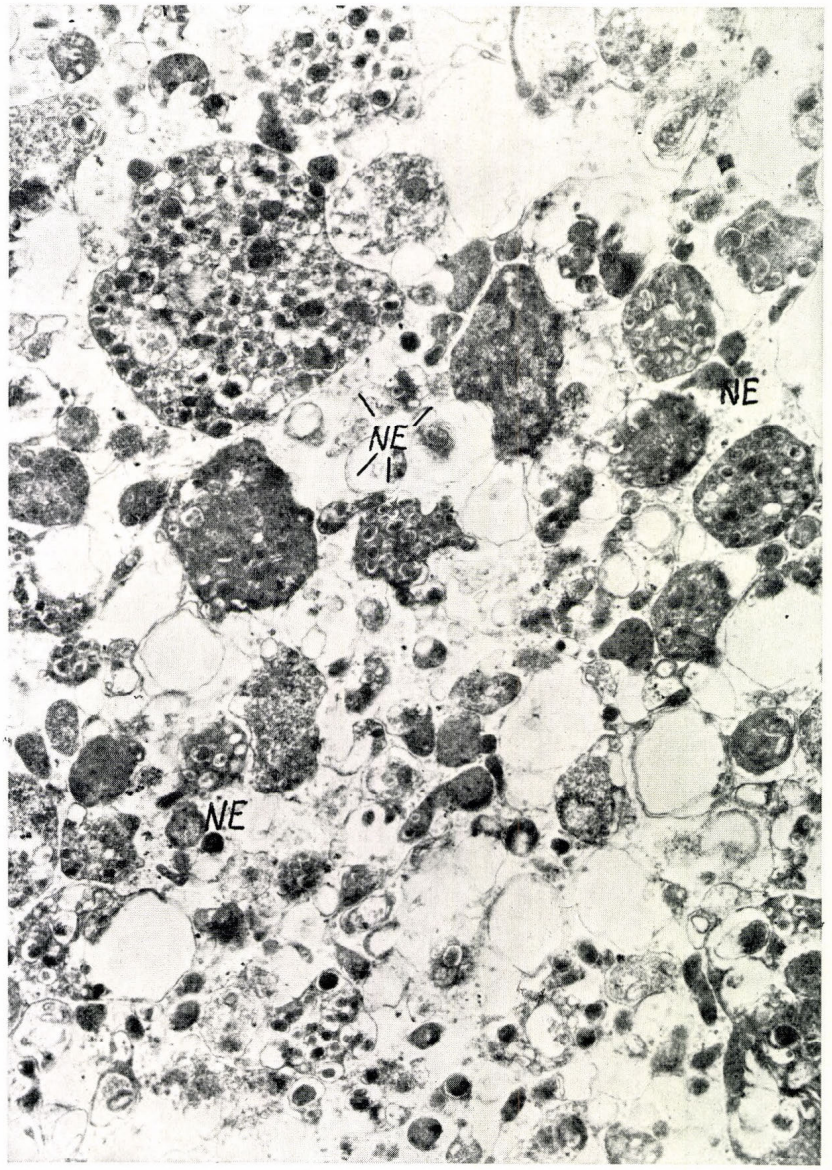

Fig. 8. Electron micrograph of the subfraction P_2C of the ganglion. Nerve ending — NE — is the predominating structure. ×24,000

are characteristically observed *(Figs. 13–14)*. The synaptosomes contained dense-core vesicles mixed with empty ones or elementary neurosecretory granules. Membrane profiles containing no vesicular component frequently occur, but the glycogen granules within them unequivocally indicate their synaptosomatic origin. Freely occurring glia (interstitial) granules are also

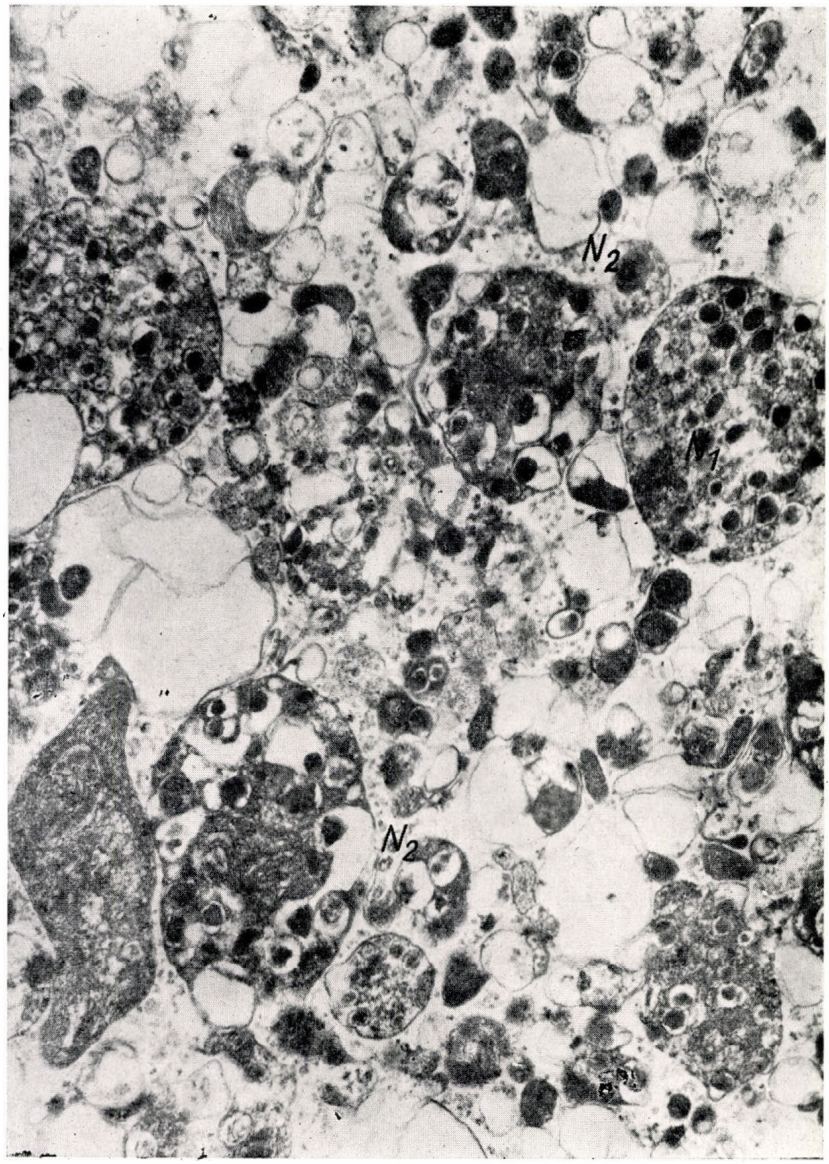

Fig. 9. Electron micrograph of the subfraction P_2C of the ganglion. Nerve endings containing vesicles of different type (N_1, N_2). ×35,000

characteristic. Sometimes the nerve endings are completely filled with an intensively dense medium. Collagen fibrils appear in groups. Subfraction P_2E contains sporadically occurring mitochondria, elementary collagen fibrils, smooth membrane profiles and membrane fragments.

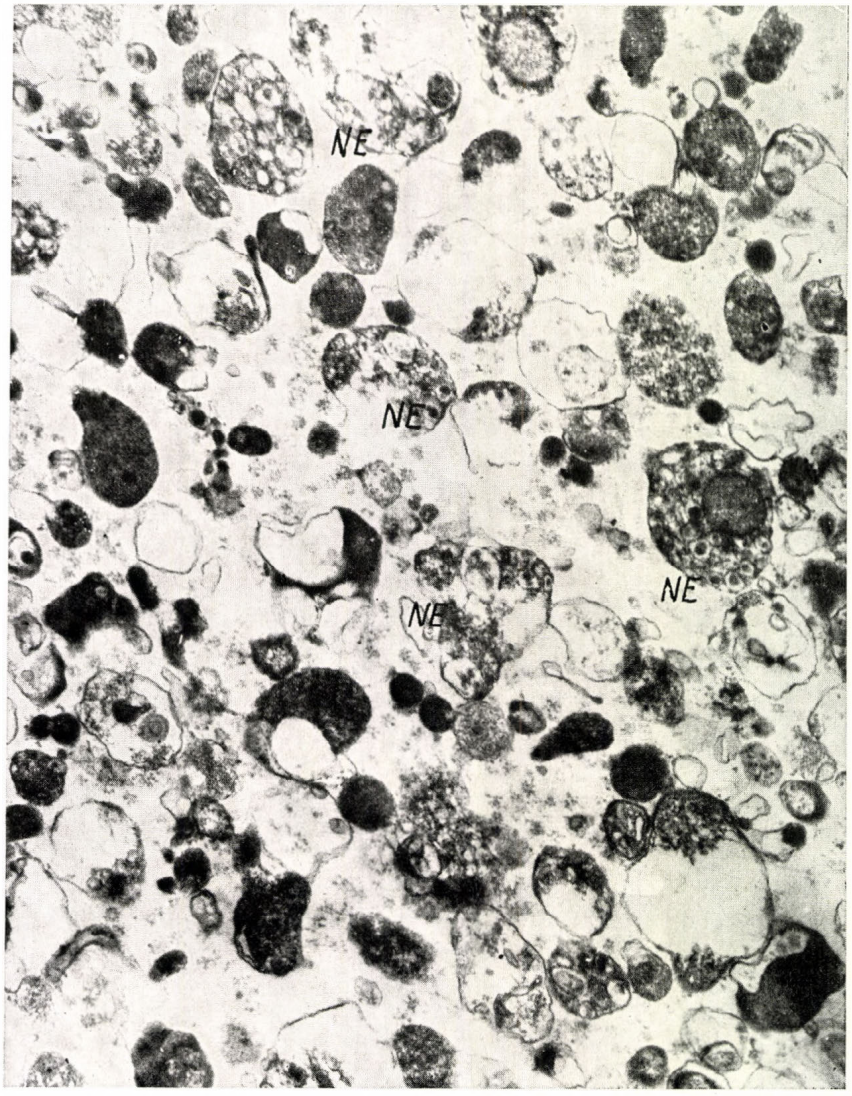

Fig. 10. Electron micrograph of the subfraction P_2D of the ganglion. NE — nerve ending. ×31,500

Discussion

Our results show that after the primary fractionation of the homogenizate obtained both from the ganglion and from the heart 70 per cent of serotonin is bound to the tissue elements. It is in agreement with the ratio of free 5HT to bound 5HT described for the mammalian brain (WHITTAKER, 1965; 1971). However, it is conspicuous, that the percentual serotonin content of the nuclear fraction in *Helix* is a relatively high value, especially as far as

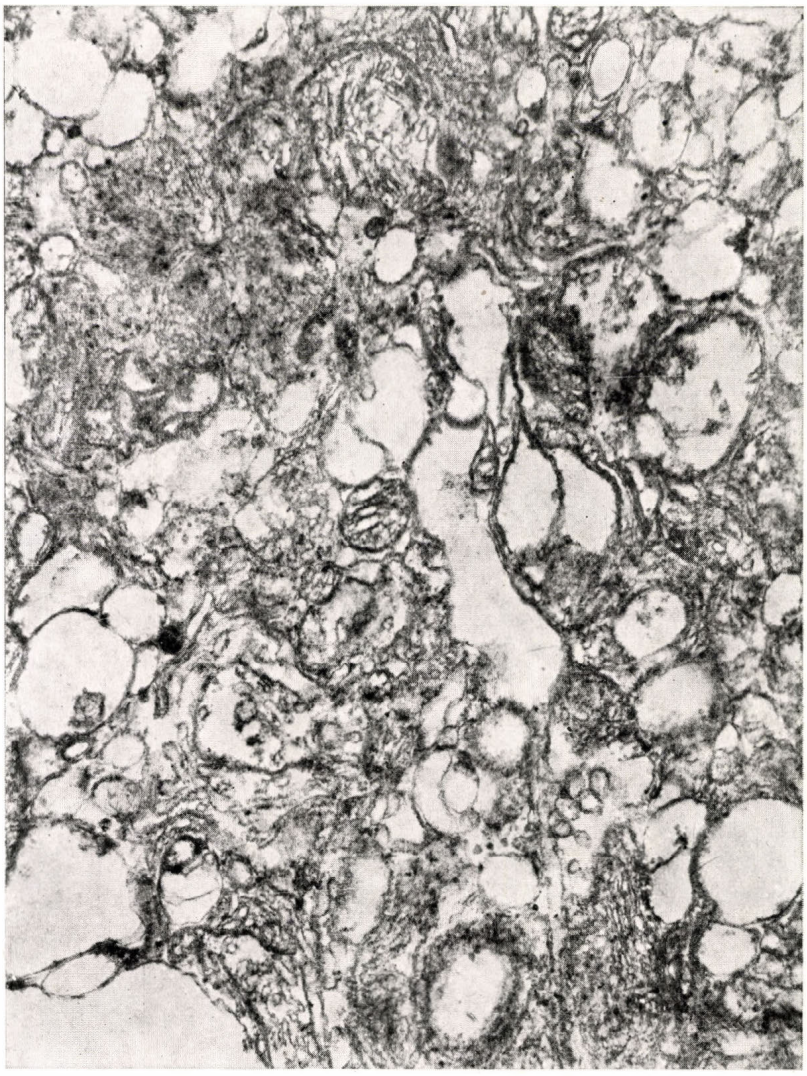

Fig. 11. Electron micrograph of the subfraction P_2C of the heart. ×35,000

the ganglion is concerned. The high serotonin concentration of this fraction is accounted for the difficulty to homogenize both the ganglion for its strong connective tissue and the heart for its fibrous structure, thus, the frequent occurrence of unhomogenized cellular particles, supported by the morphological analyses, increases the serotonin content of this fraction. Though the serotonin content in the nuclear fraction decreases by using improved and more powerful homogenization, this decrease in fact causes an increase in the percentual serotonin content of the soluble fraction but not in the mitochondrial

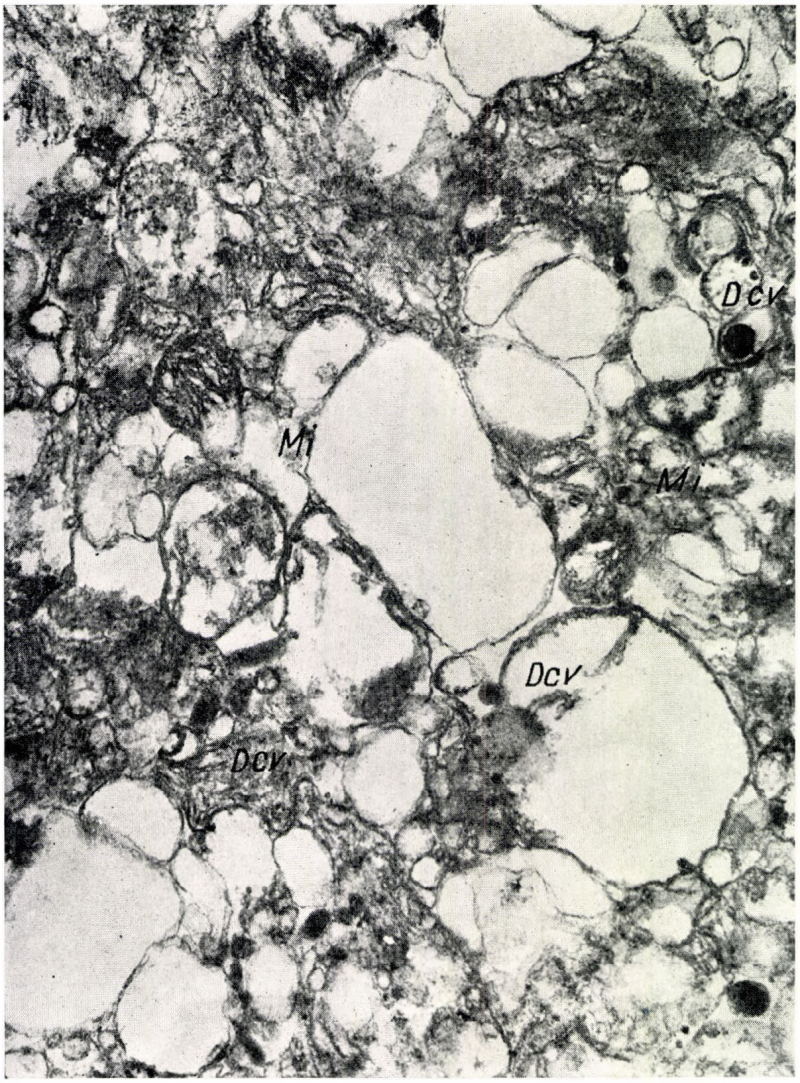

Fig. 12. Electron micrograph of the subfraction P_2D_1 of the heart. DCV — dense-core vesicle, Mi — mitochondrium. ×35,000

one. In the case of the ganglion, the high percentual serotonin content may be due to the fact that the elements of connective tissue sedimented in the nuclear fraction contain serotonin in a considerable concentration (JUORIO and KILLICK, 1972).

Since the serotonin content of the microsomal fraction is relatively low and the mitochondrial fraction contains those tissue elements, which may take part in the storage of the transmitter, the subfractions of the latter fraction were investigated.

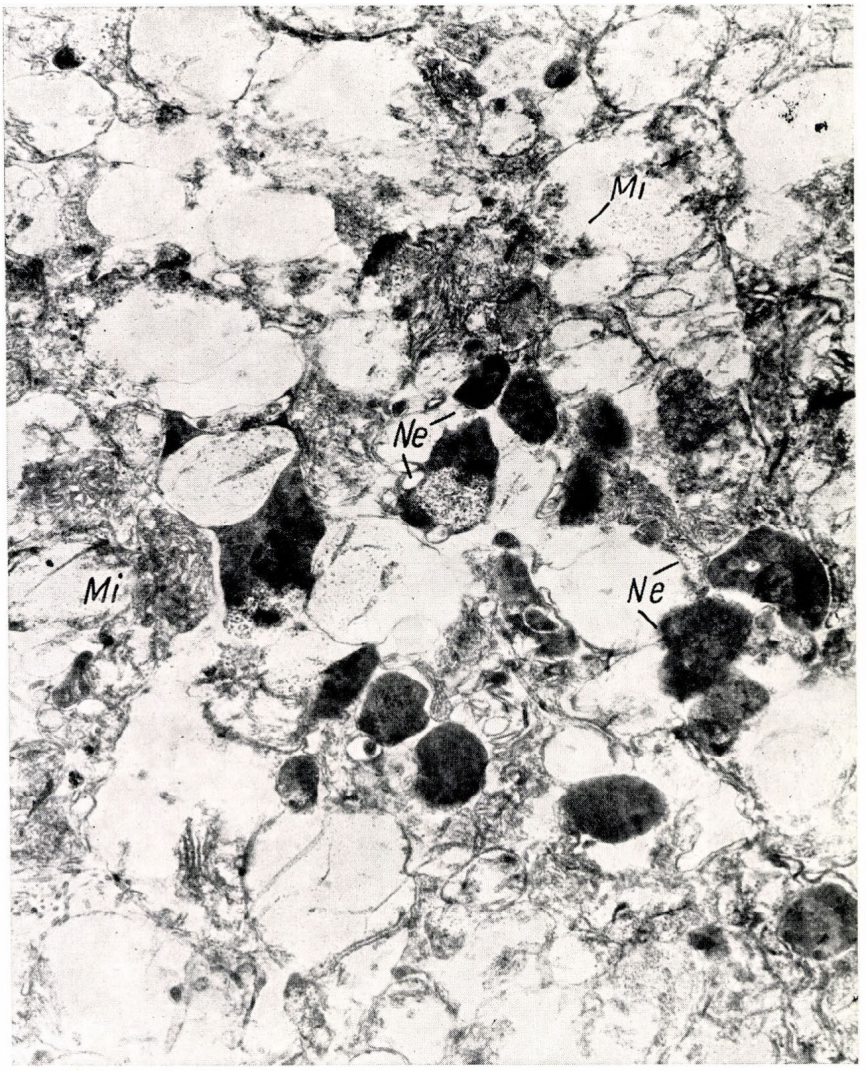

Fig. 13. Electron micrographs of the subfraction P_2D_2 of the heart. NE— nerve ending, Mi — mitochondrium. ×22,000

In the case of the ganglion, among the mitochondrial subfractions, P_2C and P_2D have the highest 5HT content with highest RSA, while subfractions A, B and E contain a lower quantity of serotonin with low RSA value. On the basis of ultrastructural analyses we may suppose that subfractions C and D may bind 5HT more specifically than the other three subfractions. Since nerve endings and free dense-core vesicles occur in fractions C and D in the greatest number, it is obvious to suppose that these structures take part in the binding of 5HT. It is in agreement with the results of our previous investigations performed on mussel (HIRIPI et al., 1973), where the primary binding of 5HT

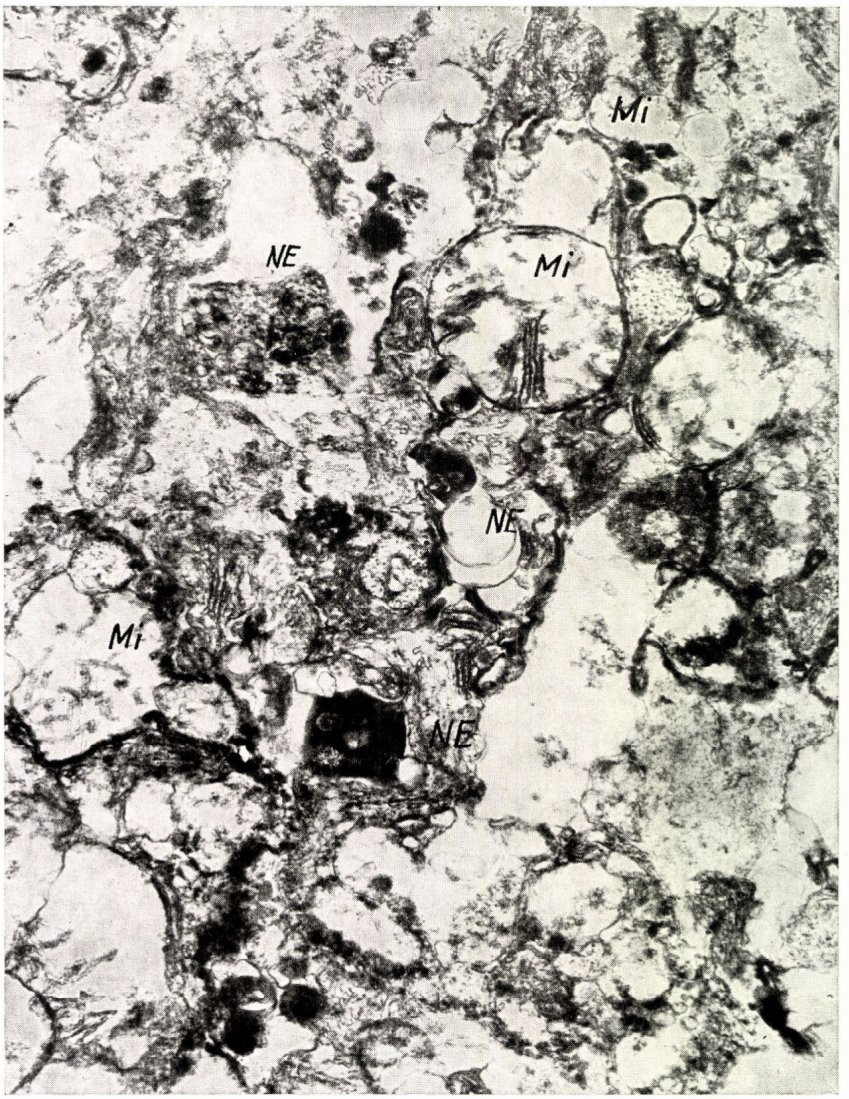

Fig. 14. Electron micrograph of the subfraction P_2D_2 of the heart. NE — nerve ending, Mi-mitochondrium. ×22,000

in synaptosomes and vesicles was also observed. The vesicular binding of 5HT in the central nervous system of the snail is suggested also by normal electron microscopic, autoradiographic and electron-hystochemical examinations (Cottrell and Osborne, 1970; Jourdan and Nicaise, 1970; Pentreath and Cottrell, 1973; Pentreath et al., 1973; Weinreich et al., 1973).

Among the mitochondrial subfractions of the heart, subfraction P_2D_2 shows a significantly high RSA of serotonin. The electron microscopic anal-

yses revealed that this is the only subfraction in the heart, where a large quantity of nerve endings occurs. On the basis of this fact, it can be supposed that serotonin is bound to the nerve endings also in the case of the heart. In the heart of *Aplysia* the examination of the accumulation of radioactive serotonin by electron-microscopic autoradiography also indicates the 5HT content of the nervous elements (TAXI and GAUTRON, 1969). However, it is conspicuous, that the 5HT content and RSA in subfraction P_2D_1 of the heart is hardly lower than those of the subfractions P_2C and P_2D containing mainly synaptosoma. Considering the almost exclusive mitochondrium content of subfraction P_2D_1 of the heart the possibility of 5HT binding also to the mitochondria cannot be disregarded.

Though the RSA value characterizing the serotonin content of the subfraction P_2E is low, the fact cannot be neglected, that its percentual serotonin content corresponds to that of fraction P_2D_2. Since this fraction consisted mainly of elementary collagen fibrils, the possibility should not be excluded that the muscle cells may store a considerable amount of the serotonin. This confirms the results of the histochemical investigations showing localization of serotonin in the muscle elements (S.-RÓZSA and ZS.-NAGY, 1967; COTTRELL and OSBORNE, 1969).

Summary

Examination of the subcellular localization of serotonin in the nervous and heart tissue of *Helix pomatia* by differential and gradient centrifugation revealed that

1. In the primary fractions of the ganglion and heart homogenizates 70 per cent of 5HT is present in bound, 30 per cent in free form. In the ganglion the 5HT content of the nuclear fraction is relatively high.

2. In the nervous tissue the highest proportion of bound 5HT is contained by the dense-core vesicles of the synaptosoma.

3. 5HT is primarily localized in the nerve endings also in the case of the heart and mitochondria also contain 5HT in a considerable quantity.

4. In the heart 5HT is also localized in the muscle elements.

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SZEROTONIN INTRACELLULÁRIS MEGOSZLÁSA *HELIX POMATIA* KÖZPONTI IDEGRENDSZERÉBEN ÉS SZIVÉBEN

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Összefoglalás

Szerotonin szubcelluláris lokalizációjának vizsgálata differenciál és gradiens centrifugálással *Helix pomatia* ideg és szívszövetében igazolta, hogy

1. A ganglion és szív homogenizátum primér frakcióiban az 5HT 70%-a kötött, 30%-a szabad formában van jelen. Ganglionban a nuclearis frakció 5HT tartalma relatíve magas.

2. Idegszövetben a kötött 5HT a legnagyobb hányadát a szinaptoszóma dense-core vezikuláii tartalmazzák.

3. Az 5HT a szív esetén is elsődlegesen idegvégződésekre lokalizált, de emellett a mitochondriumok is jelentős mennyiségű 5HT-t tartalmaznak.

4. Az 5HT szívben az izomelemekre lokalizáltan is megtalálható.

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THE UPTAKE KINETICS OF SEROTONIN, DOPAMINE AND NORADRENALINE IN THE PEDAL GANGLIA OF THE FRESH-WATER MUSSEL (*ANODONTA CYGNEA* L., PELECYPODA)

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Serotonin, dopamine and noradrenaline are putative transmitters not only in the central nervous system of vertebrates but in the invertebrate animals, too (GERSCHENFELD, 1973). The amine accumulation as a possible mechanism for the inactivation of synaptically released monoamines were extensively studied in the vertebrates (IVERSEN, 1970, 1971; SNYDER et al., 1970).

The monoamine oxidase responsible for the enzymatic inactivation of the monoamines is not present in the nervous system of some molluscs (CARDOT, 1964; MCCAMAN and DEWHURST, 1971), while in others, it has weak activity (HIRIPI and SALÁNKI, 1971; MARSDEN, 1972; JUORIO and KILLICK, 1972). In molluscs the uptake system may be an important mechanism in the inactivation of monoamines and could substitute the monoamine oxidase in the inactivation process.

Earlier, the accumulation of serotonin was shown in the CNS of *Helix pomatia* and in the giant serotonin-containing neurones (PENTREATH and COTTRELL, 1972; 1973; OSBORNE and NEUHOFF, 1974).

In the central nervous system of *Aplysia* and *Helix* (ASCHER et al., 1968) and *Aplysia* (CARPENTER et al., 1971) the accumulation of serotonin and dopamine was demonstrated and it was suggested that the accumulation is partly due to a specific and partly to a non-specific process.

According to kinetic analysis a high and a low affinity transport system is responsible for the monoamine accumulation in the nervous system of vertebrates. Such type of investigations on molluscs was carried out only for dopamine (MYERS and SWEENEY, 1973).

Earlier data (HIRIPI et al., 1973; SALÁNKI et al., 1974) support the transmitter role of 5HT, DA and NA at interneuronal level in the central nervous system of *Anodonta*. In this work we have studied the accumulation of these amines in the pedal ganglia of *Anodonta cygnea* from a kinetic point of view.

Material and method

In our experiments specimens of the fresh-water mussel *(Anodonta cygnea* L.*)* weighing 200–300 g were used.

After dissection the pedal ganglia were stored in ice-cold physiological saline (MARCZYNSKI, 1959). About 6—10 mg pedal ganglia from three animals was preincubated for 10 min at 25°C in 1 ml physiological saline solution, it was followed by a 30 min incubation in the presence of 0.1—100.0 μM ^{14}C amines (^{14}C-5HT — 57 mCi/mmol; ^{14}C-DA — 57.3 mCi/mmol; ^{14}C-NA— 52.0 mCi/mmol; Amersham).

At the end of the incubation the ganglia were washed in 20 ml ice-cold physiological solution, blotted dry on filter paper, weighed and homogenized in 2 ml aethanol. Homogenate and the aliquot from the incubation medium diluted in 10 ml BRAY's solution were counted in Nuclear Chicago liquid scintillation spectrometer. The counting efficiency was monitored by the addition of internal standards and appropriate corrections were made. The tissue/medium ratios (T/M) and the rate of the uptake was calculated by the method of SHASKAN and SNYDER (1970).

Results

Our present results demonstrated that the pedal ganglia of *Anodonta* are able to accumulate ^{14}C-serotonin, ^{14}C-dopamine and ^{14}C-noradrenaline from the medium. The accumulation for all amines were linear for at least 30 minutes, so the incubation was carried out for 30 minutes. The tissue/medium ratios are near the same for all the amines at 0.1 μM medium amine concentration and the values 11.08, 11.62 and 10.4 were found for serotonin, dopamine and noradrenaline, respectively. For kinetic analysis the T/M ratio was estimated with concentrations of amines ranging from 0.1 μM to 100 μM. The T/M ratios of the amine decreased with increasing amine concentration in the media for all amines *(Table I)* and it was shown that monoamine accumulation in the ganglia is a saturable process.

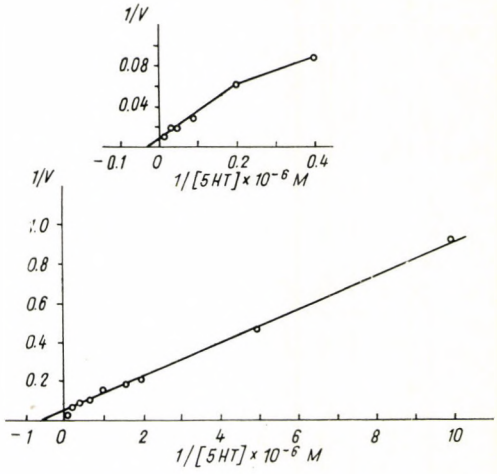

Fig. 1. Reciprocal values of serotonin accumulation plotted against the reciprocal 5HT concentration. V = nmol/g tissue/30 min

In order to evaluate the kinetics of the amine accumulation, reciprocals of the amine accumulation rate calculated from the T/M ratios and amine concentration were analysed by Lineweaver-Burk plots. For all amines, the

TABLE I

Tissue/medium ratios for ^{14}C-amine accumulation

Amine conc. (μM)	Tissue/medium ratios		
	5HT	DA	NA
0.10	11.08	11.62	10.40
0.20	10.65	—	—
0.25	—	9.85	9.93
0.50	9.38	9.68	8.65
0.63	8.42	—	—
1.00	7.08	8.39	6.28
1.45	6.57	—	—
1.45	6.57	—	—
2.00	—	—	5.20
2.45	4.65	—	—
3.30	—	4.87	—
5.00	3.21	3.88	4.50
10.00	—	2.54	4.02
12.00	2.91	—	—
20.00	—	—	2.56
25.00	—	1.68	—
28.00	1.91	—	—
50.00	—	1.47	1.94
56.60	1.52	—	—
100.00	—	1.04	—

experiments resulted in plots that could not be described by single straight lines but which could be resolved into two straight line components *(Figs 1—3)*. This shows that the accumulation process has two components with different affinity.

The values of K_m and V_{max}, the parameters of accumulation were estimated graphically and presented in *Table II*. The K_m and V_{max} values

TABLE II

Kinetic constants for the accumulation of ^{14}C—5HT, ^{14}C—DA and ^{14}C—NA

Amine	High affinity system (uptake$_1$)		Low affinity system (uptake$_2$)	
	K_{m_1} (M)	V_{max_1} (nmol/g/min	K_{m_2} (M)	V_{max_2} (nmol/g/min)
5HT	1.66×10^{-6}	0.66	4.00×10^{-5}	4.74
DA	2.50×10^{-6}	0.95	3.33×10^{-5}	3.70
NA	1.33×10^{-6}	0.55	1.66×10^{-5}	3.33

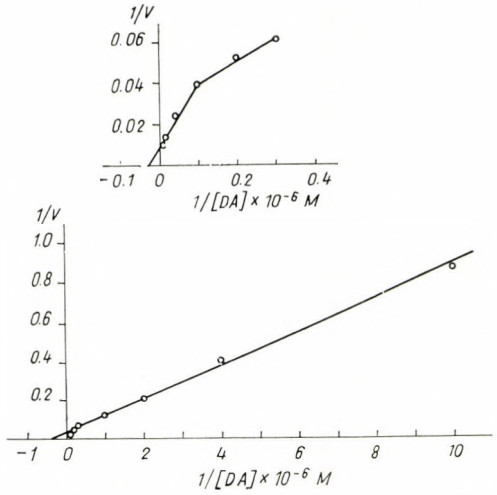

Fig. 2. Reciprocal values of dopamine accumulation plotted against the reciprocal DA concentration. V = nmol/g tissue/30 min

for the higher affinity component (uptake$_1$) have been referred to as K_{m_1} and V_{max_1} and that of the lower (uptake$_2$) as K_{m_2} and V_{max_2}. The K_{m_1} values are almost the same for all amines. The V_{max_1} values for serotonin and noradrenaline are about the same, however, for dopamine it is higher by 50—80% than for the previous two.

The K_{m_2} values representing the lower affinity system are at least 10 times higher than the K_{m_1} values and the values for NA are the lowest, while for 5HT the highest. The V_{max_2} values are about the same for NA and DA, whereas for 5HT it is higher by some 40%.

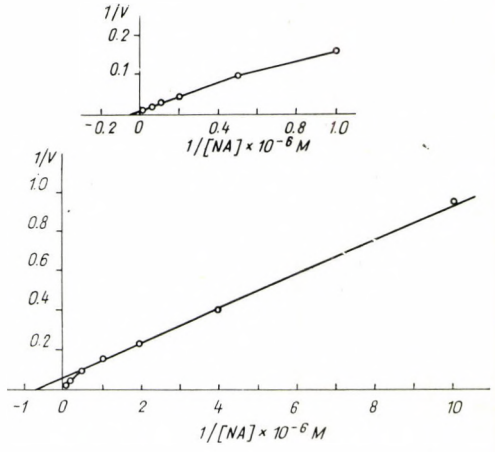

Fig. 3. Reciprocal values of noradrenaline accumulation plotted against the reciproca NA concentration. V = nmol/g tissue/30 min

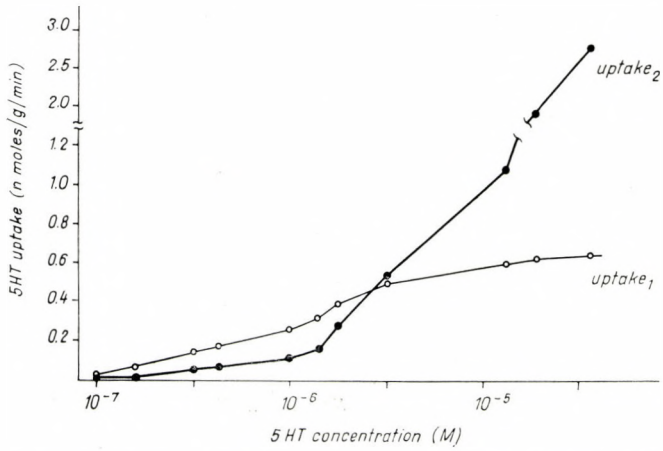

Fig. 4. Rate of 5HT accumulation by $uptake_1$ and $uptake_2$ at varying 5HT concentrations

The MICHAELIS–MENTEN equation was used to calculate the relative contributions of uptake 1 and 2 to the total accumulation of the amines at a variety of amine concentrations *(Figs 4–6)*.

At low 5HT concentration between 10^{-7} and 10^{-6} M $uptake_1$ had 2–3 times the rate of $uptake_2$. At a concentration of 5×10^{-6} M the rate of uptake 1 and 2 was about the same, while an additional increase in the concentration brought about a higher rate for the $uptake_2$ and the rate of $uptake_1$ approximates the limiting value. A concentration of 5×10^{-5} M $uptake_2$ had a rate 4 times that of $uptake_1$ *(Fig. 4)*. For DA in a concentration range of 10^{-7} to 8×10^{-6} M the $uptake_1$ has a higher rate than $uptake_2$, whereas from the concentration of 10^{-5} M the situation is reversed *(Fig. 5)*.

For NA in a concentration range of 10^{-7} to 10^{-6} M the rate of $uptake_1$ is higher than that of $uptake_2$, however, the differences are less than the

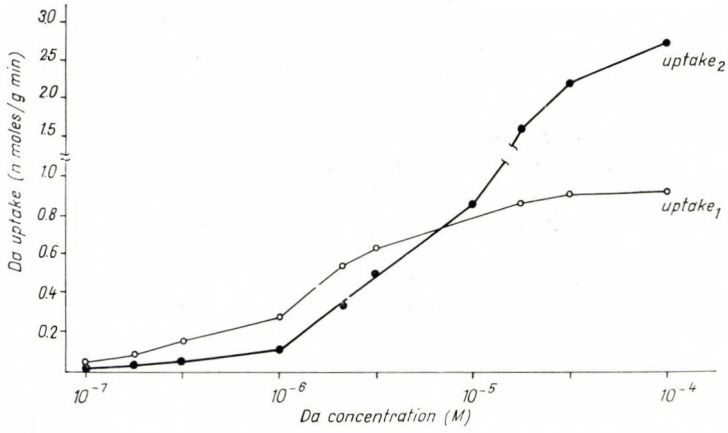

Fig. 5. Rate of DA accumulation by $uptake_1$ and $uptake_2$ at varying DA concentrations

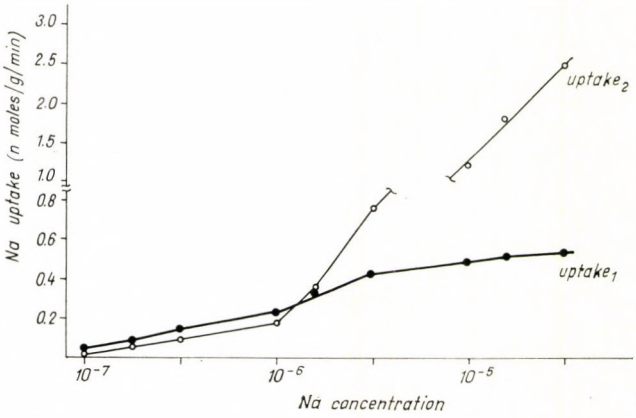

Fig. 6. Rate of NA concentration by uptake$_1$ and uptake$_2$ at varying NA concentrations

values for DA and 5HT. The values of the two rates increase parallel up to a concentration of 10^{-6} M, but at a higher concentration the rate of uptake$_2$ has higher values than those of uptake$_1$, therefore uptake$_2$ contributes a greater proportion to the total NA accumulation *(Fig. 6)*.

Discussion

The present study has clearly shown that similarly to that of the vertebrates, the central nervous system of *Anodonta* has an active transport system which is able to accumulate serotonin, dopamine and noradrenaline. In the central nervous system of vertebrates noradrenaline is accumulated by a high affinity (uptake$_1$), whereas dopamine and noradrenaline are accumulated by a high (uptake$_1$) and a low (uptake$_2$) affinity transport system (SHASKAN and SNYDER, 1970; SNYDER and COYLE, 1969; IVERSEN, 1970; 1971). In the central nervous system of *Anodonta* the two uptake systems are operated, however, in *Anodonta* noradrenaline is accumulated not only by uptake$_1$, but also by uptake$_2$ similarly to the peripheral tissues of the vertebrates (IVERSEN, 1970).

Similar data were obtained for the ganglia of *Anodonta* as for the brain slices of rat (SHASKAN and SNYDER, 1970): K_m and rate values of uptake$_1$ are generally smaller by one order of magnitude than those of uptake$_2$. The affinity and the rate of the uptake are lower in the ganglia of *Anodonta* than in the rat brain slices. This can be explained by the species differences and by the physiological temperature (10°C lower in *Anodonta* than in the rat). At the same time, the K_m values measured in the ganglia of *Quadrula pustulosa* (MYERS and SWEENEY, 1973) were near the same as the K_{m1} values measured in the ganglia of *Anodonta*. For the high affinity system the order of the affinity is NA > 5HT > DA, whereas for the low affinity system this order is NA > DA > 5HT.

At low medium amine concentrations (10^{-7} – 10^{-6} M) uptake$_1$ contributes a greater proportion of the total 5HT and DA accumulation into the

ganglia tissue. Since at low concentration for NA the rate of the uptake 1 and 2 and their changes are almost the same, both systems contribute to the total NA accumulation in the same degree. It may well be in connection with the endogenous pool of NA which is 10 times lower than that of the 5HT and DA. It is likely that at low concentrations the NA is accumulated into the DA pool by the $uptake_2$ mechanism.

$Uptake_1$ seems to represent a specific, whereas $uptake_2$ a not specific process in the accumulation. The specific accumulation was shown in *Anodonta* (ELEKES, 1975) by autoradiographic methods. Using the low 5HT concentration where the $uptake_1$ mechanism is predominant, the serotonin is localized in the nerve cells. The autoradiographic accumulation of the dopamine into the nervous structures was found in *Quadrula pustulosa* (MYERS, 1974) and that of the dopamine in *Planorbis* and serotonin in *Helix* (PENTREATH and COTTRELL, 1972; 1973). At the same time using the high concentrations of monoamines in *Helix* the 5HT and DA were taken up by non-nervous structures (ASCHER et al., 1968).

Summary

The kinetic analysis of the ^{14}C-serotonin, ^{14}C-dopamine and ^{14}C-noradrenaline accumulation into the pedal ganglia of the fresh-water mussel *(Anodonta cygnea* L.*)* was shown that

1. Serotonin, dopamine and noradrenaline are accumulated into the ganglia by an active process.

2. The accumulation process has a high ($uptake_1$) and a low ($uptake_2$) affinity component.

3. The K_m and V_{max} values in the case of high affinity system are as follows: for 5HT 1.66×10^{-6} M and 0.66 nmol/g/min; for DA 2.5×10^{-6} M and 0.95 nmol/g/min; for NA 1.33×10^{-6} M and 0.55 nmol/g/min. In the case of low affinity system these values are: for 5HT 4×10^{-5} M and 4.74 nmol/g/min; for DA 3.33×10^{-5} M and 3.7 nmol/g/min; for NA 1.66×10^{-5} M and 3.33 nmol/g/min.

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A SZEROTONIN, DOPAMIN ÉS A NORADRENALIN BEÉPÜLÉS KINETIKAI VIZSGÁLATA TAVIKAGYLÓ (*ANODONTA CYGNEA* L., PELECYPODA) PEDÁLIS GANGLIONJAIBA

Hiripi László, Rakonczay Zoltán és Nemcsók János

Összefoglalás

A ^{14}C-jelzett szerotonin, dopamin és noradrenalin tavikagyló *(Anodonta cygnea L.)* pedális ganglionjába való beépülésének kinetikai vizsgálata során megállapítottuk, hogy:

1. A szerotonin, dopamin és a noradrenalin aktív folyamat közreműködéséve l akkumulálódik a ganglionban.

2. Az akkumuláció egy nagy affinitású ($uptake_1$) és egy alacsony affinitású ($uptake_2$) folyamat közreműködésével valósul meg.

3. Az affinitási konstansok és a maximális sebesség számértéke a nagy affinitású rendszer esetén 5HT-re $1{,}66 \cdot 10^{-6}$M és 0,66 nmol/g/min; DA-ra $2{,}5 \cdot 10^{-6}$M és 0,95 nmol/g/min; NA-ra $1{,}33 \cdot 10^{-6}$M és 0,55 nmol/g/min, mig az alacsony affinitású rendszer esetén 5HT-re $4{,}0 \cdot 10^{-5}$M és 4,74 nmol/g/min; DA-ra $3{,}33 \cdot 10^{-5}$M és 3,7 nmol/g/min; NA-ra $1{,}66 \cdot 10^{-5}$M és 3,33 nmol/g/min értékeknek adódtak.

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ANALYSIS OF THE EFFECT OF IONTOPHORETICALLY APPLIED SEROTONIN AND DOPAMINE ON THE IDENTIFIED NEURONES OF THE CENTRAL NERVOUS SYSTEM OF *LYMNAEA STAGNALIS* L.

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Among the biogenic monoamines, dopamine and serotonin have become the subjects of extensive research in Molluscs in the past decades. Both substances are considered to be possible transmitters in some Gastropod species. Results concerning this topic have been described in GERSCHENFELD's comprehensive study (1973). It has been proved in *Helix*, *Aplysia* and *Cryptomphallus* species by the method of iontophoretic application that there are receptors sensitive to biogenic monoamines on the soma and axon hillock of the giant neurones. ASCHER (1967; 1972) distinguished receptors reacting to dopamine with depolarization and hyperpolarization, while GERSCHENFELD and PAUPARDIN-TRITSCH (1974) postulated six different 5HT receptors.

Conclusions were drawn for the ionic mechanisms underlying the effect of both 5HT and dopamine partly from measuring the reversal potential and partly from investigating the direct ionic dependence of effects. The depolarization caused by both substances is explained generally by an increase in the Na^+-conductance of the membrane (ASCHER, 1972; GERSCHENFELD and PAUPARDIN-TRITSCH, 1974) and no role is attributed to the change in Cl^--permeability, unlike the case of ACh (CHIARANDINI et al., 1967). On every Gastropod species, the inhibitory effect is connected with a change in either K^+- or Cl^--conductance, which is supported among others by the fact, that the reversal potential is always higher than the resting level (GERSCHENFELD, 1973). In contrast with the data of experiments performed on the heart muscle of different invertebrate species (S.-RÓZSA et al., 1973; WILKENS and GREENBERG, 1973) the role of Ca^{2+} is not proved in the realization of the effect of 5HT and dopamine (GERSCHENFELD, 1973).

In our previous experiments (KISS and SALÁNKI, 1971) carried out on *Lymnaea*, different types of cells reacting diversely to dopamine and serotonin were distinguished in such a way that the substances were applied to the surface of the whole ganglion. However, it is essential in order to understand the functional property of the identified cells to separate the synaptic and direct somatic effects of the examined mediators. For this purpose, in the present work, dopamine and 5HT were applied to the surface of neurones by iontophoresis. Our aim was partly to compare the data obtained in such a way with the results of our earlier work. On the other hand, the iontophoretic

application makes a more detailed analysis of the effects possible. Consequently, we tried to determine the ionic dependence of the effect employing ion-free solutions and measuring the reversal potential on certain neurones.

Material and method

Experiments were carried out on the identified neurones located in the abdominal and right parietal ganglia of the isolated nervous system of *Lymnaea stagnalis (Fig. 1)*. All the examinations were carried out at room temperature. Making the preparation and placing it in the experimental chamber were done as described earlier (KISS and SALÁNKI, 1971). Membrane and action potential were recorded by glass microelectrodes of 4—5 MOhm resistance filled with 1 M potassium acetate. For the experiments an NCA 1 amplifier (VÉRÓ, 1974) was used. The membrane potential was measured after the compensation of tip potential of the electrode using a digital voltmeter. The equipment made it possible to set the membrane potential at any desired level.

The current required for the microiontophoresis was obtained from a square-wave generator and the micropipette was filled with 0.1 M serotonin—creatinine sulphate or 0.1—0.5 M dopamine—HCl, respectively. The resistance of the micropipette used for microiontophoresis filled with a substance was not more than 3—5 MOhm. This resistance corresponds to a tip diameter of more than 1 μ. The outflux unavoidable under these conditions was prevented by a constant breaking voltage of similar amplitude but directed oppcsite to that used for the application of substances. During the investigation the preparation was kept in a chamber containing 2.5 ml physiological solution

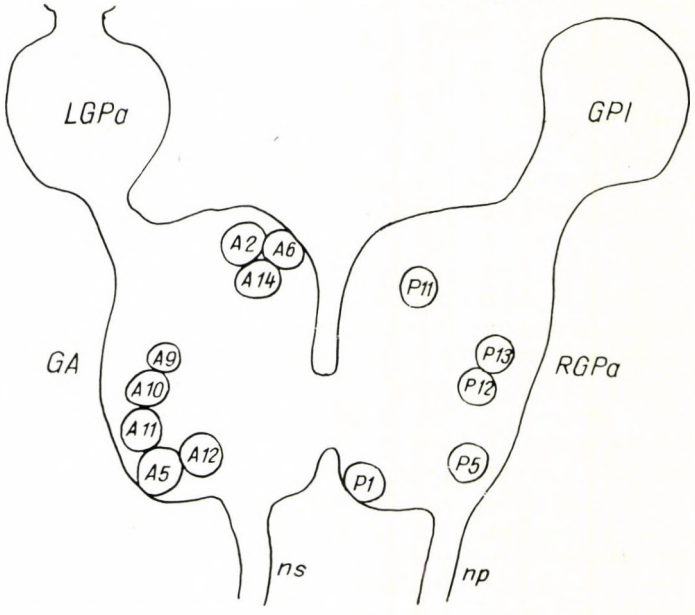

Fig. 1. Scheme of the abdominal and right parietal ganglia with the identified neurons

(KISS and SALÁNKI, 1973). The testing of serotonin and dopamine was done so that the microelectrode used for application was conducted as near as possible to the neuronal membrane in the vicinity of the recording electrode, then after recording the control activity, a positive voltage was connected up the microelectrode instead of the negative breaking voltage. The positive square impulses were applied in series for 15—30 sec. The frequency and impulse duration was selected to reach an operation ratio of 90 per cent. The maximum current used was 200 nA. 4—8 measurements were carried out on every cell.

For determining the reversal potential the substances were applied at different levels of membrane potential.

For preparing Na^+-free solution, NaCl was replaced by Tris-Cl. In the Cl^--free solution Na_2SO_4, K-acetate and Ca-propionate was used instead of Cl^--salts of the ions. The exchange of normal solutions for ion-free ones was made in such a way, that at the first step the whole volume of the physiological solution was sucked out and exchanged, then continuous perfusion was used for a short time to ensure the complete removal of ions.

Results

I. *Effect of 5HT in normal solution*

1. On the majority of the investigated cells (A5, A6, A14, A10, A11, P13, P1) an excitatory reaction was obtained under the influence of 5HT. This reaction could be observed on these neurones from preparation to preparation, although there were some preparations, in which one or another neurone did not respond with an excitation. A11 cell proved to be a peculiar one, since here the excitatory effect appeared only in 50 per cent of the cases, and it actually happened to be inhibited. Nevertheless, it was classified under this group, and not under another category of neurones that show variable reaction (to be discussed later), because the different effects do not alternate randomly, but they show a seasonal variation: the insensitivity of this cell or its reaction directed opposite falls in the summer period.

The excitation resulted in the first place in an increase of the firing rate of the spontaneous activity, a significant depolarization did not develop on any cell or rather could not be estimated with the present technical level. The changes in the firing rate are expressed in the per cent of the control.

The individual neurones have different sensitivity, the slightest effects were obtained on A10 and A11 cells *(Fig. 2a)*. On these two cells partly the increase in the firing rate produced by 5HT is small, and partly there is a notable latency between the start of the iontophoretic current and the onset of the reaction (average 17.2 ± 7.2 and 8.8 ± 4.7 sec, respectively). At the same time, a desensitization during the application scarcely develops. On the other neurones a higher degree excitation was detected *(Fig. 2b)*, in some cases even silent cells could be activated by 5HT *(Fig. 2c)*. It is worthwhile to mention here that this activation realizes at a lower level of depolarization than the threshold of electrical excitability. On these neurones the latency of reaction ranges from 1.9 to 7.7 sec and the majority of them show a marked desensitization during the application of 5HT.

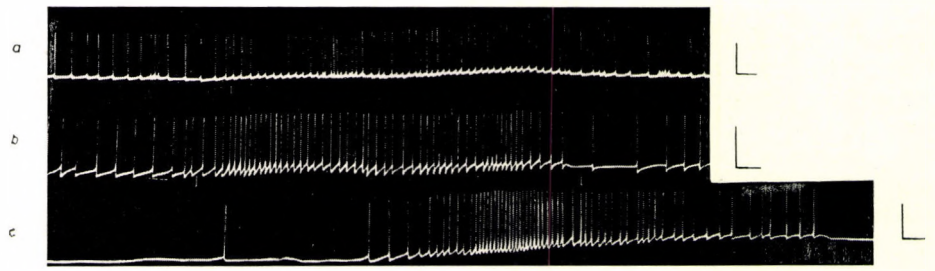

Fig. 2. Excitatory effect of 5HT on different neurones *a)* on A11 cell; *b)* on A6 cell; *c)* activating effect on P13 cell. Calibration: 50 mV; 2 sec. Here and in all of the following figures the two small vertical lines show the onset and offset of the iontophoretical current

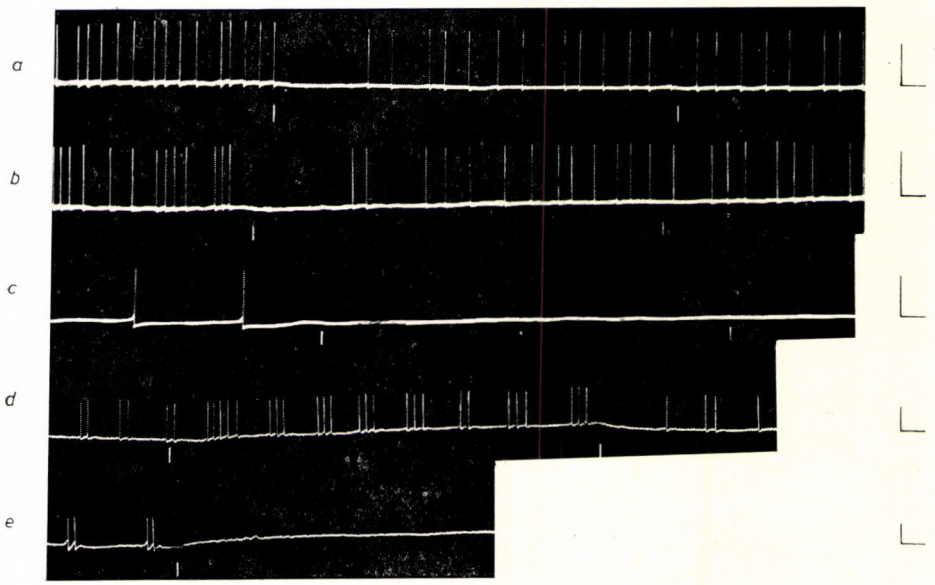

Fig. 3. P12 and P11 neurones react upon 5HT with inhibition. On P12 neurone 5HT inhibits the spontaneous activity *a)* in normal solution; *b)* in Cl^--free solution; *c)* in Na^+-free solution; on P11 neurone the type of 5HT effect is a function of the actual membrane potential; *d)* at a membrane potential level of −52 mV the cell exhibits an excitatory reaction; *e)* at a membrane potential level of −35 mV the activity is inhibited. *Note:* Here and in other cases like this the apparent depolarization accompanying the inhibition is an artifact resulting from the application technics of the substances. Calibration: 50 mV; 2 sec

2. Two neurones were found to be inhibited by 5HT: P11 and P12 cells. The degree of inhibition is significant (41—42%), however, a complete block of the activity ensues only rarely *(Fig. 3a)*.

On P11 cell it is possible to measure directly the reversal potential of the effect, which was found to be -44.7 ± 1.8 mV *(Fig. 3d, e)*.

Hyperpolarizing the cell P12 to −50 mV, the 5HT effect does not reverse.

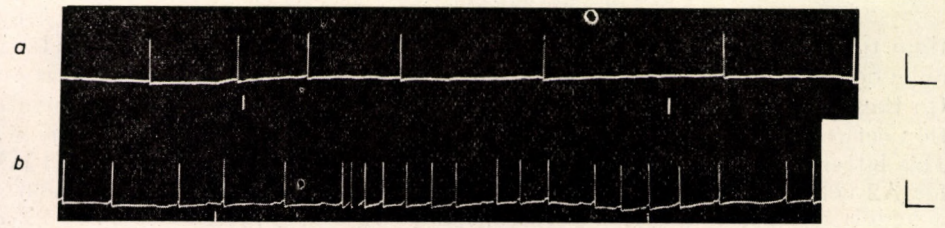

Fig. 4. On neurone A2 the effect of 5HT is varying from time to time. The variability of the effect is connected with the alteration of resting potential. *a)* At the resting potential level of −38 mV 5HT causes an inhibition. *b)* 2.5 mV hyperpolarization reverses the reaction. Calibration: 50 mV; 2 sec

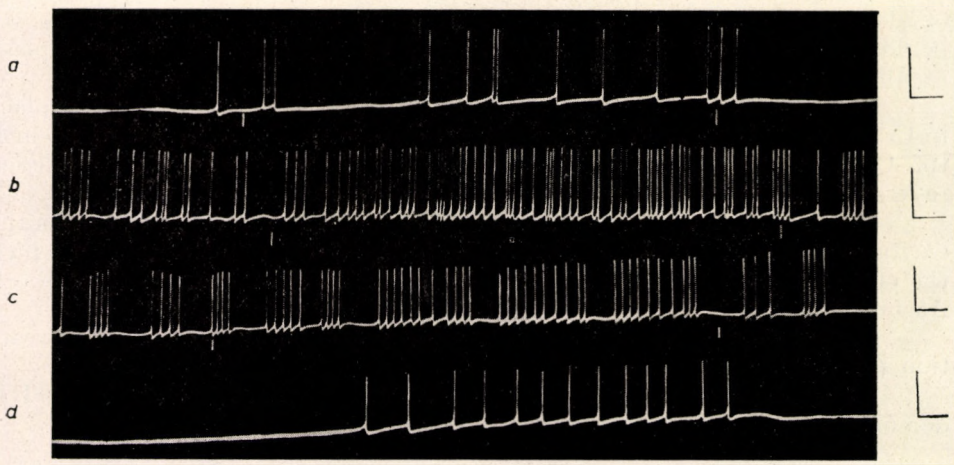

Fig. 5. Effect of Cl^- and Na^+-free solutions on the neurones stimulated by 5HT. *a)* Effect of 5HT on A14 neurone under control conditions. *b)* On the same neurone the excitatory effect of 5HT persists also in Cl^--free solution. *c)* Effect of 5HT on A5 neurone under control conditions. *d)* On the same neurone in Na^+-free solution the spontaneous activity has stopped, but 5HT activates the cell. Calibration: 50 mV; 2 sec

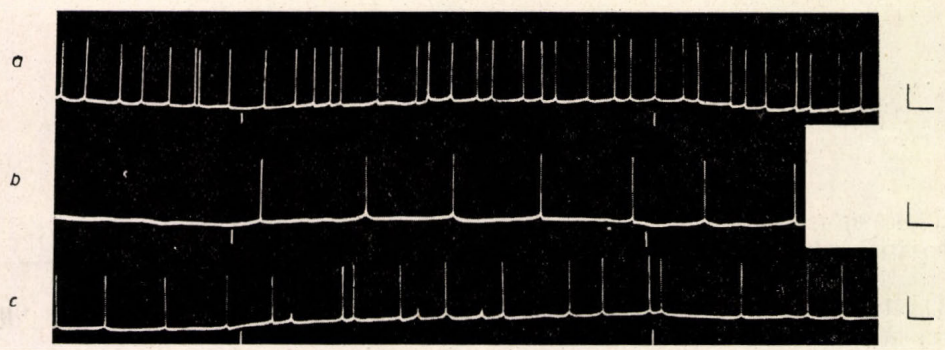

Fig. 6. On Pl neurone the excitatory effect of 5HT is blocked neither by Na^+-free nor Cl^--free solution *a)* control; *b)* Na^+-free solution; *c)* Cl^--free solution. Calibration: 50 mV; 2 sec

3. On A9 and A2 neurones the effect caused by 5HT is variable from time to time, excitation (39%), inhibition (46%) and ineffectiveness (15%) alike occurred in our examinations. They are characterized by desensitization. On these cells, it is obvious to relate the diversity of effects with the correlation between the actual membrane potential and the reversal potential *(Fig. 4)*. This latter was found to be -34.1 ± 1.2 mV for A9 cell and -38.1 ± 2.1 mV for A2 cell. For a comparison the average membrane potential of A9 cell is -32.9 ± 1.9 mV and that of A2 cell is -38.2 ± 1.2 mV.

II. *Effect of 5HT in Na^+- and Cl^--free solutions*

Between the effect of the substance applied in normal and in ion-free solutions only the qualitative difference was estimated: namely, the presence or absence of the effect. According to this the prevention of a reaction can be concluded only if in an ion-free solution the reaction does not take place at all.

In those cells which excitatorily react to 5HT the excitation takes place in Cl^--free solution, too *(Fig. 5b)*. Though A5 cell is an exception on which the Cl^--free solution prevented the 5HT effect, so much so, that it even reversed it in a single case.

In the majority of the cells the Na^+-free solution completely blocks the excitatory effect of 5HT, however, there were some preparations on which the effects were maintained even in such conditions, for example, on the cell A5 in three of seven measurements *(Fig. 5d)*.

On P1 cell the Na^+-free solution hardly influences the excitatory reaction *(Fig. 6)*.

The behaviour of the neurones in ion-free solutions excited by 5HT is demonstrated in *Table I*.

TABLE I

Changes in the excitatory effect of 5HT in ion-free solutions

Cell	5HT effect in normal solution	5HT effect in Na-free solution	5HT effect in Cl-free solution
A6	+	0	+
A14	+	0	+
A11	+	0	+
A10	+	0	+
P13	+	v	+
P1	+	+	+
A5	+	♀	0

+ excitation; — inhibition; v variable reaction; ♀ and 0 excitation and inhibition, respectively, occurring only in some of the preparations

The table shows that two special cells can be found in this group: P1 is not sensitive to the removal of either the ions, and A5 is sensitive to the lack of both Cl^- and Na^+.

In three cells responding to 5HT with inhibitory or equivocal reactions (P12, P11 and A2), the absence of neither of the ions prevents the reaction

(Fig. 3b, c). On the latter two cells, for which the 5HT reversal potential is near to the resting membrane potential, in certain cases the type of effect changes from excitation to inhibition and vice versa.

The analysis of the behaviour of A9 neurone is incomplete since its activity suffers heavy losses in Na-free solution, and under such a condition the effect of 5HT cannot be examined. On the other hand, Cl^--free solution prevents the reaction.

TABLE II

Changes in the inhibitory and variable effect of 5HT in ion-free solutions

Cell	5HT effect in normal solution	5HT effect in Na-free solution	5HT effect in Cl-free solution
P11	—	v	v
P12	—	—	—
A2	v	v	v
A9	v		0

+ excitation; — inhibition; v variable reaction

III. *Effect of dopamine in normal solution*

Dopamine is effective on a less number of cells as compared to 5HT, and even among these only 3 cells show unequivocal excitatory reaction and one shows inhibition from preparation to preparation. Excitatory effect of dopamine results in a marked increase in the firing rate of the spontaneous activity or in activation of silent cells respectively, the latency of reaction ranges from 1.6 to 11.2 sec. The desensitization of dopamine-sensitive receptors exhibits only small differences and generally is of small degree.

On the basis of the type of dopamine effect the neurones can be classified into the following groups:

1. Dopamine stimulates the eactivity of A9, A11 and P13 cells in all of the preparations.

2. Dopamine stimulates A5 and A14 cells *(Fig. 7)* in 64 per cent of the preparations, in others (36 per cent) exerts no effect at all.

3. On P11 cell the effect of dopamine varies from time to time. The reversal potential of the reaction is -36.7 ± 6.4 mV *(Fig. 8)*. Its average resting potential is -41.7 ± 3.7 mV.

4. Dopamine inhibits A10 cell in some of the preparations, in others exerts no effect. The inhibition results in a slight decrease of the firing rate (28.8%), which occurs with a long latency (average 8.3 ± 3.6 sec). The reversal potential of the effect is -45.2 ± 4.9 mV *(Fig. 9a, b)*.

IV. *Effect of dopamine in Na^+- and Cl^--free solutions*

In the case of A11, P13, A5 and A14 neurones reacting to dopamine with excitation, the Na^+-free solution does not prevent the effect *(Fig. 7b, e)* although on A11 cell inhibits it in 50 per cent of the preparations.

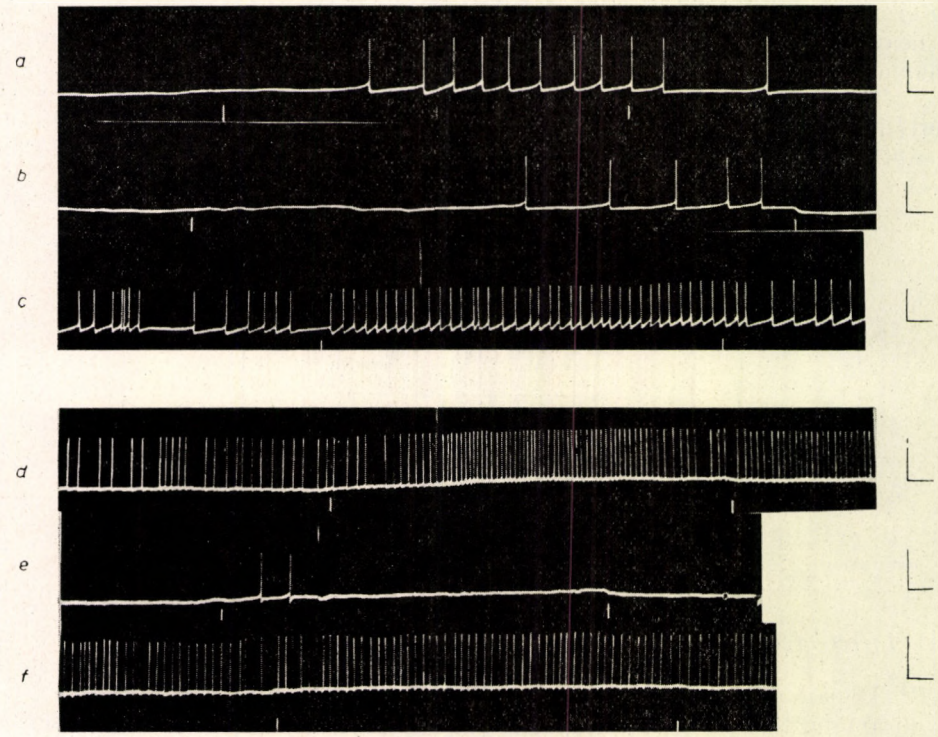

Fig. 7 Excitation caused by dopamine and its ionic dependence on A5 cell *(a; b; c)* and on A14 cell *(d; e; f)*. A5 neurone is silent in normal solution and is activated by dopamine *(a)*, while on cell A14 dopamine increases the firing rate of the spontaneous activity *(d)*. In Fig. *b* and *e* it can be seen that the Na^+-free solution does not block the reactoin of either neurone. .The Cl^--free solution does not block the excitatory effect of dopamine in the case of A5 neurone *(c)*, while on A14 cell its development is inhibited by Cl^--free solution *(f)*. Calibration: 50 mV; 2 sec

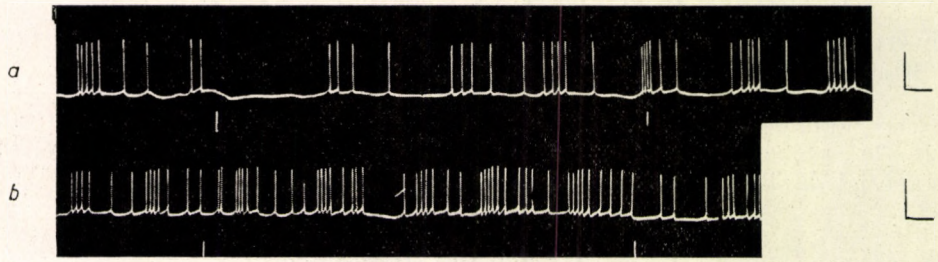

Fig. 8. On P11 neurone the type of the dopamine effect is a function of the actual membrane potential. *a)* At the membrane potential level of −30 mV dopamine inhibits the spontaneous activity. *b)* At the membrane potential level of −40 mV dopamine causes a slight excitation. Calibration: 50 mV; 2 sec

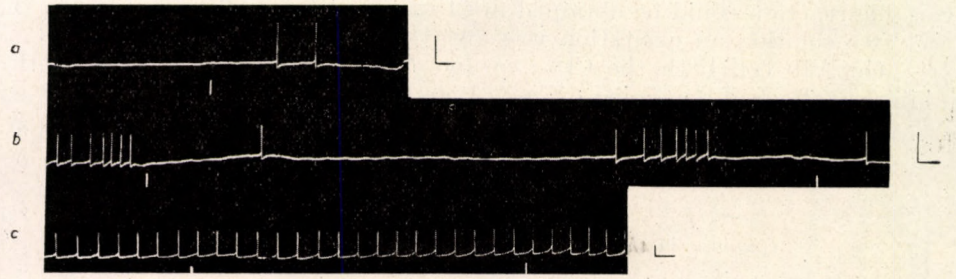

Fig. 9. a. and *b.* On A10 cell dopamine generally causes an inhibition but this effect can be reversed by changing the membrane potential. *a)* Effect of dopamine at a membrane potential level of −48 mV. *b)* Effect of dopamine at a membrane potential level of −20 mV. Calibration: 50 mV; 2 sec. *c)* On A10 cell localized in another preparation, the spontaneous activity persists in Na^+-free solution, but dopamine does not cause any inhibition

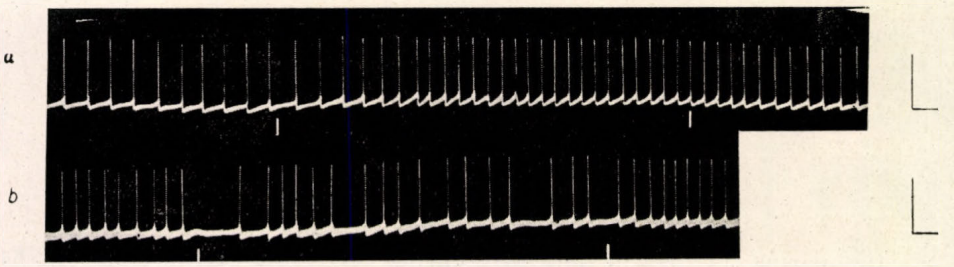

Fig. 10. On P11 cell the effect of dopamine reverses in Cl^--free solution in some cases. *a)* Control activity and effect of dopamine. *b)* In Cl^--free solution dopamine slightly inhibits the activity. Calibration: 50 mV; 2 sec

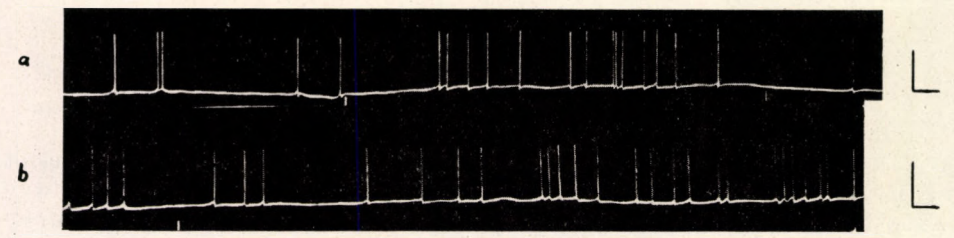

Fig. 11. On A9 neurone the Cl^--free solution reverses the effect of dopamine. *a)* Control activity and effect of dopamine. *b)* Effect of dopamine in Cl^--free solution. Calibration: 50 mV; 2 sec

Cl^--free solution influences the effect of dopamine in each case except in A5 cell *(Fig. 7c)*. This effect is rather complicated and varies from cell to cell. It blocks the effect unequivocally on A14 cell *(Fig. 7f)* and on A11 cell only in 50 per cent of the preparations. But on A9 and P13 cells in one of the cases it reverses the effect from excitation to inhibition *(Fig. 11)*. On P11 neurone, on which the effect of dopamine varies from time to time, similarly

variable reaction could be obtained in Cl^--free solution *(Fig. 10)*, but in the Na^+-free solution an excitation was always observed.

On A10 cell both the Cl^--free and Na^+-free solutions prevented the inhibitory effect of dopamine *(Fig. 9c)*, and in 50 per cent of the cases, even a slight excitation was obtained. The changes of the neuronal reactions in ion-free solutions to dopamine are demonstrated in *Table III*.

TABLE III

Changes in the effect of dopamine in ion-free solutions

Cell	Dopamine effect in normal solution	Dopamine effect in Na^+-free solution	Dopamine effect in Cl^--free solution
A11	+	±	±
P13	+	+	v
A9	+		v
A5	±	+	+
A14	±	+	0
P11	v	+	v
A10	—	±	0

+ excitation; — inhibition; ± excitation only in some of the preparations; v variable reaction.

Discussion

Comparing the present data with our earlier results (Kiss and Salánki, 1971) obtained by adding serotonin and dopamine to the bathing solution, it can be established that concerning the classification of the neurones in the case of 5HT the general picture obtained with either method is the same. The great majority of the neurones are sensitive to serotonin and can be classified into three categories:

1. Cells reacting unequivocally with excitation.
2. Cells reacting unequivocally with inhibition.
3. Cells showing variable reaction.

We observed also some differences between the previous data and the results of the present work in the case of three neurones. A12 and A11 cells always reacted with excitation upon 5HT applied to the bathing solution. By contrast, cell A12 is not sensitive to the iontophoretically applied 5HT at all, and A11 cell is sensitive to 5HT in some of the preparations only. In the case of these neurones 5HT obviously exerts the effect mainly at synaptic level and not on the somatic receptors. On A14 cell excitation was always obtained by iontophoresis, while using the previous method this neurone used to show variable reaction. Presumably this variable reaction was of a mixed effect, in which both the somatic receptors and the synaptic region might have taken part.

By our earlier method 6 neurones were found to be sensitive to dopamine. Some of these proved to be sensitive also to the iontophoretically applied dopamine. On A11 cell excitation, while on A10 cell inhibition was observed similarly to the earlier results. Previously on P11 neurone an inhibition was

always registered, however, dopamine applied iontophoretically caused a reaction varying from time to time, but of excitatory type in most instances. To account for this difference, it must be supposed that dopamine applied to the bathing solution influences the inhibitory postsynaptic membrane, while that applied iontophoretically affects the somatic receptors. On A6 and A2 neurones no effect was obtained by using iontophoresis in contrast with the previous data, accordingly, the somatic receptors may be considered to be insensitive to dopamine.

A5, A14 and A10 neurones responded to dopamine in some of the preparations only. To these cases ASCHER's (1972) assumption may be applied according to which the receptors may be located at small distanse of the soma, the effect depending on the position of the electrode used for application.

In the case of both 5HT and dopamine there is a striking difference between the inhibitory reactions obtained by the two different methods. When the substances added to the bathing solution caused an inhibition in a given neurone, it realized in a temporary blockade of the potential generation almost in all of the cases. Upon the iontophoretic application only a decrease in the firing rate of the spontaneous activity can be observed in most instances. To explain this phenomenon we should consider that the concentrations of substances applied to the bathing solution or by iontophoresis are not identical. It was approximately calculated by an equation used by GERSCHENFELD and STEFANI (1966) that the peak concentration attainable at 5HT receptors is 7.5×10^{-7} M in our case, which is less by at least one-two orders of magnitude than the concentration estimated in our earlier work. Even this fact cannot explain the occasionally too long latency of the effect found for example on A10 neurone in the case of both the excitatory effect of 5HT and the inhibitory effect of dopamine. KERKUT et al. (1975) also found such neurone in *Helix aspersa*, where the excitatory effect of dopamine was realized after a long latency.

As regards the neurones showing variable reactions occasionally our previously proposed assumption concerning ACh (KISS et al., 1972) can be extended to 5HT and dopamine, too. The type of the effect also in the latter cases may be determined by the relation between the actual membrane potential and the reversal potential of 5HT or dopamine, respectively. In the case of 5HT, the reversal potential characterizing cells A9 and A2 is extremely near to the average value of their membrane potential, In the case of dopamine on P11 cell the agreement is not of such a degree, however, here the membrane potential varies over a quite wide range (30–68 mV).

In the Na^+-free solution the stimulation caused by 5HT does not realize, or do realize in some of the cases only, consequently, it can be established that this effect is Na-dependent. Though P1 neurone is an exception, in which the generation of the spontaneous activity appeared to be also independent of Na^+ (KISS and SALÁNKI, 1973). At the same time the lack of Cl^- does not prevent the excitation excepting A5 cell, which needs also Cl^- beside Na^+ for an undisturbed reaction.

The inhibitory and variable effects of 5HT in the case of P11, P12 and A2 cells realize on such receptors that are not blocked by the absence of either Na^+ or Cl^-. Among these it is theoretically conceivable on P12 cell that the realization of the inhibition is due to a change of K^+conductance of the membrane, since when increasing the membrane potential up to -50 mV no

reversal can be recorded. The role of the electrogenic Na-pump (THOMAS, 1972) may be taken into consideration too, however, its verification requires further experiments. However, the −44.7 and −38.1 mV reversal potential characterizing the P11 and A2 cells, respectively, does not permit to postulate a change in K^+conductance. In these cases the ionic mechanism of the effect is not clear like in the case of neurones which are not classifiable in either D or H type on the basis of their reactions given to ACh (KISS and SALÁNKI, 1971). We suppose that in the development of this type of effect not only one ion takes part, but Cl^- and Na^+ alike. The value of the reversal potential is determined by the ratio g_{Na}/g_{Cl} This supposition was described by MACHNE et al. (1973) for ACh on the cells of *Helix pomatia*, where the E_{ACh} value characterizing the different neurones was found to range between very wide limits (−40−+16 mV).

The reaction of A9 cell to 5HT appeared to be Cl^--dependent on the basis of both the reversal potential of −34,1 mV and its susceptibility to be blocked by Cl^--free solution.

Summarizing the ionic dependence of 5HT effect the excitatory, reaction is Na^+-dependent excepting P1 neurone, it is conceivable that it is realized at the "A" type receptors described by GERSCHENFELD (1971). Nevertheless, to finally settle this question extensive pharmacological investigations are required. On the other hand, the receptors localized on the neurones showing inhibitory and variable reaction surely cannot be identical with the "B" and "C" receptors also described by GERSCHENFELD (1971) and GERSCHENFELD and PAUPARDIN-TRITSCH (1974), since they exhibit another type of ionic dependence.

In those cases, when the results are not elucidated unequivocally the ionic dependence of 5HT effect the possible role of Ca^{2+} must also be considered. During the present work no experiment was carried out in order to control the Ca^{2+}-dependence of the effects, because in the literature concerning the neurones of Gastropods only a limited role is attributed to Ca^{2+} in the realization of the effect of 5HT and dopamine (GERSCHENFELD, 1973). Nevertheless, it cannot be disregarded that on the examined cells of *Lymnaea* Ca^{2+} has a greater importance as a charge-carrier during the mediator effects. To answer this question further examinations are in progress.

In contrast with serotonin the effect of dopamine is emphasized at those receptors which are slightly sensitive to the removal of external Na^+, at the same time, the reaction of most cells is very sensitive to the Cl^--free solution. In one part of the cases the development of the excitation and inhibition may be explained by Cl^-current flowing through the membrane. When the neurones stimulated by dopamine in normal solution give no reaction or variable reaction in the Cl^-free solution, this indicates the Cl^-dependence of the effect. Namely, the Cl^--free solution displaces the value of E_{Cl} and via this may influence the reversal potential of the dopamine effect. This explanation is not good for A5, A9 and P11 cells because of the ineffectiveness of the Cl^--free solution. It is possible however, that on these cells, a combined role of Na^+ and Cl^- ions similarly to the 5HT effect is manifested.

Our results contradict ASCHER's (1972) conclusion referring to the excitatory effect explained exclusively with a Na^+influx.

The reversal potential of −45.2 mV measured on A10 cell is higher than the average resting potential. Accordingly, the inhibitory effect of dopamine

may realize via increasing the conductance either of K^+ or of Cl^-. The latter is suggested by the fact that the Cl^--free solution blocks the effect of dopamine.

Unfortunately, in *Lymnaea* the intracellular concentration of either ion is yet almost unknown — the only estimation for K^+ was described by SATTELLE (1974) — but one may reach a further quantitative conclusion only with this knowledge. The following example supports that considering our present work the discussion cannot be based on data obtained previously on different Gastropods. According to the measurements of KERKUT and MEECH (1966) on D-cells of *Helix* E_{ACh} is —39.4 mV, while CHIARANDINI et al. (1967) determined an E_{ACh} value of —25.1 mV for D-cells in *Chryptomphallus aspersa*. The basis of this difference is the difference in the intracellular Cl^- concentrations measured 27.5 mM in the first case and 43 mM in the latter case. It follows from the foregoing that there may be very large differences in the intracellular concentrations of some ions depending on the species, and the reversal potential characterizing certain cells develops according to this.

Summary

On some identified giant neurones of the central nervous system of *Lymnaea stagnalis* the effect of 5HT and dopamine applied iontophoretically was investigated in normal as well as Na^+- and Cl^--free solutions.

Some of the neurones were stimulated, others inhibited by 5HT and dopamine applied to the soma. In addition several cells were found which exhibited excitatory and inhibitory reaction alternately in the different preparations.

In the majority of the neurones, the effect is similar to that obtained previously by perfusion, but on some of the cells it differs from the results of earlier findings.

The excitatory reaction caused by 5HT is Na^+-dependent in all of the neurones except one. On the neurones showing inhibitory and variable from time to time reactions the effect of 5HT realizes at such type of receptors, which are not blocked by the absence either of Na^+ or of Cl^-. It may be suggested that in the development of this type of effect not only one ion participates but Cl^- and Na^+ alike. It is supported also by measuring the reversal potential on these cells.

The effect manifested at the dopamine receptors appeared to be Cl^--dependent in the majority of the neurones or it may be accounted for by a combined ionic mechanism similarly to the 5HT effect.

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IONTOFORETIKUSAN ALKALMAZOTT SZEROTONIN ÉS DOPAMIN ANALIZISE *LYMNAEA STAGNALIS* L. KÖZPONTI IDEGRENDSZERÉNEK IDENTIFIKÁLT NEURONJAIN

Kiss István

Összefoglalás

Lymnaea stagnalis központi idegrendszerének identifikált óriás neuronjain iontoforetikusan applikált 5HT és dopamin hatását vizsgáltuk normál, valamint Na^+- és Cl^--mentes oldatokban. A neuronok egy része serkentő, más része gátló reakcióval válaszolt a szómájukra adott 5HT-ra és dopaminra. Ezenkívül találtunk néhány olyan sejtet, amelyek a különböző preparátumokon váltakozva, hol serkentő, hol gátló reakciót adtak. A hatás a neuronok nagy részén megegyezik a perfúzióval korábban kapott adatokkal egyes sejteken azonban eltér attól.

Az 5HT által okozott serkentő reakció egy kivételével valamennyi neuronon Na^+-függő. A gátló és esetenként változó reakciót mutató neuronokon az 5HT hatása olyan receptorokon valósul meg, amelyeket sem a Na^+ — sem a Cl^--hiány nem blokkol. Valószínűsíthető, hogy az ilyen hatás kialakulásában nem egyetlen ion játszik szerepet, hanem a Cl^- és a Na^+ egyaránt. Erre engednek következtetni az e sejteken végzett reversal potenciál mérések is.

A dopamin receptorokon megvalósuló hatás a neuronok többségén Cl^--függőnek mutatkozott, vagy az 5HT hatáshoz hasonló kombinált ionmechanizmussal magyarázható.

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MEMBRANE EFFECTS OF CYCLIC NUCLEOTIDES AND THEIR ROLE IN THE REALIZATION OF TRANSMITTER ACTIONS ON THE HEART MUSCLE CELLS OF *HELIX POMATIA* L.

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In recent years the effect of cyclic nucleotides was extensively studied on the electrical and contractile activity of the heart. The cyclic nucleotides had the positive inotrope effect on the mammalian, molluscan and insect hearts alike (SKELTON et al., 1970; BERTELLI et al., 1972; MEINERTZ et al., 1973; S.-RÓZSA, 1968; 1974), furthermore, they affected the pacemaker and plateau phases of the spontaneous action potentials in the electrical activity (TSIEN et al., 1972; TSIEN, 1973; REUTER, 1974; S.-RÓZSA, 1974). In vertebrates the effect of catecholamines and cyclic nucleotides proved to be similar, on the Purkinje fibers and blood vessels the participation of the second messenger system together with the catecholamine effect was also verified (SOMLYO et al., 1970; SIGGINS et al., 1971; TSIEN, 1973). Direct connection was also found between the content of the cyclic adenine monophosphate (cAMP) and the periodicity of the heart (BROOKER, 1973).

Earlier, the positive inotrope effect of 3',5'-AMP and the modification of the transmitter effect have been described on the contractions of *Helix* heart (S.-RÓZSA, 1968). Also the presence of adenylate cyclase and its activation for different periods in different concentrations of dopamine (DA) and 5-hydroxytryptamine (5HT) were proved (WOLLEMANN and S.-RÓZSA, 1975) in the molluscan hearts. The membrane effect of cyclic nucleotides has not yet been studied, though it might surrender information on the sites of action. Therefore, the aim of the present investigations was to describe the membrane effects of cyclic nucleotides and the substances influencing the enzymes taking part in their destruction, furthermore, the role of the second messenger system in the realization of transmitter effects.

Material and methods

The experiments were carried out on the spontaneously beating or electrically driven isolated ventricle of snail, *Helix pomatia* L. The isolated ventricle was kept at room temperature under permanent perfusion. In the chamber containing the preparation the volume of the solution was 0.5 cm^3, which was changed once during the substance-testing experiment. The physio-

logical solution used was described earlier (KISS and S.-RÓZSA, 1972). The substances studied were solved in physiological solution.

The following substances were used: cAMP-adenosine-3',5'-cyclic phosphate (Calbiochem); DB-cAMP-N,O-dibutyryl-adenosine-3',5'-cyclic phosphate (Calbiochem); theophylline (Reanal); imidazole (Sandoz); 5HT-5—hydroxytryptamine creatinine sulphate (Reanal); ACh — acetylcholine chloride (Sigma).

For stimulation a suction electrode with a tip diameter of 0.2—0.3 mm was used. For an indifferent electrode a silver plate of about 0.5 cm^2 was employed. Since most of the current crossed the extracellular space the absolute value of the effective stimulatory current could not be detected. The approximate value of the current was 10^{-8} A with a duration of 1 sec. In the figures the relative intensity of the current is seen on the scale 1—5 at constant voltage.

For the registration of the membrane (MP) and action potentials (AP) glass microelectrodes filled with 3 M KCl of 5—15 MOhm resistance were used. Simultaneously with the microelectrode for registration another microelectrode was immersed into the physiological solution. The same Ag-$AgCl_2$ wire served as the indifferent electrode for both microelectrodes. The output of two preamplyfiers was connected with the inputs of the differing amplyfier of the oscilloscope. Using this method, the capacitive artifact arising from the resistance of the microelectrodes or the stimuli can be compensated if the properties of two microelectrodes were the same. The experimental arrangement can be seen in *Fig. 1.* To compensate the artifact, similar experimental conditions were described by BONKE (1973).

The characteristics of the voltage-current was designed on the basis of responses arising as an average to five stimuli of different magnitude. The

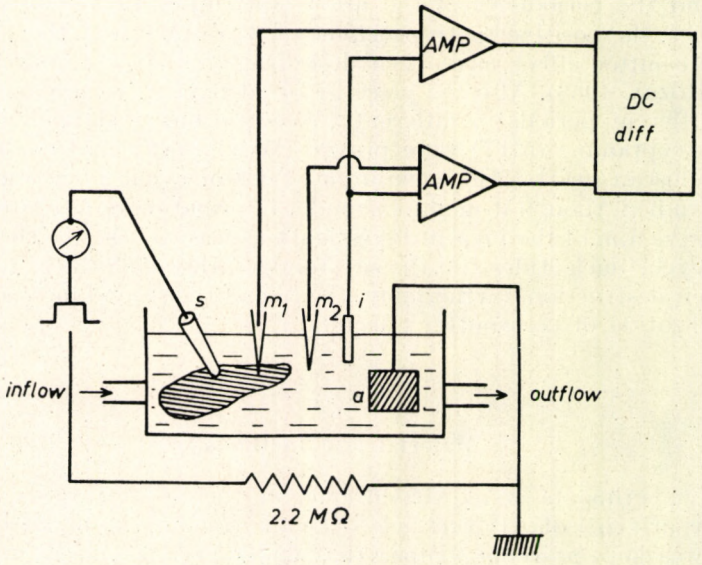

Fig. 1. Scheme for registering and stimulating. m_1, m_2 — microelectrodes; i, a — indifferent electrodes; s — suction electrode

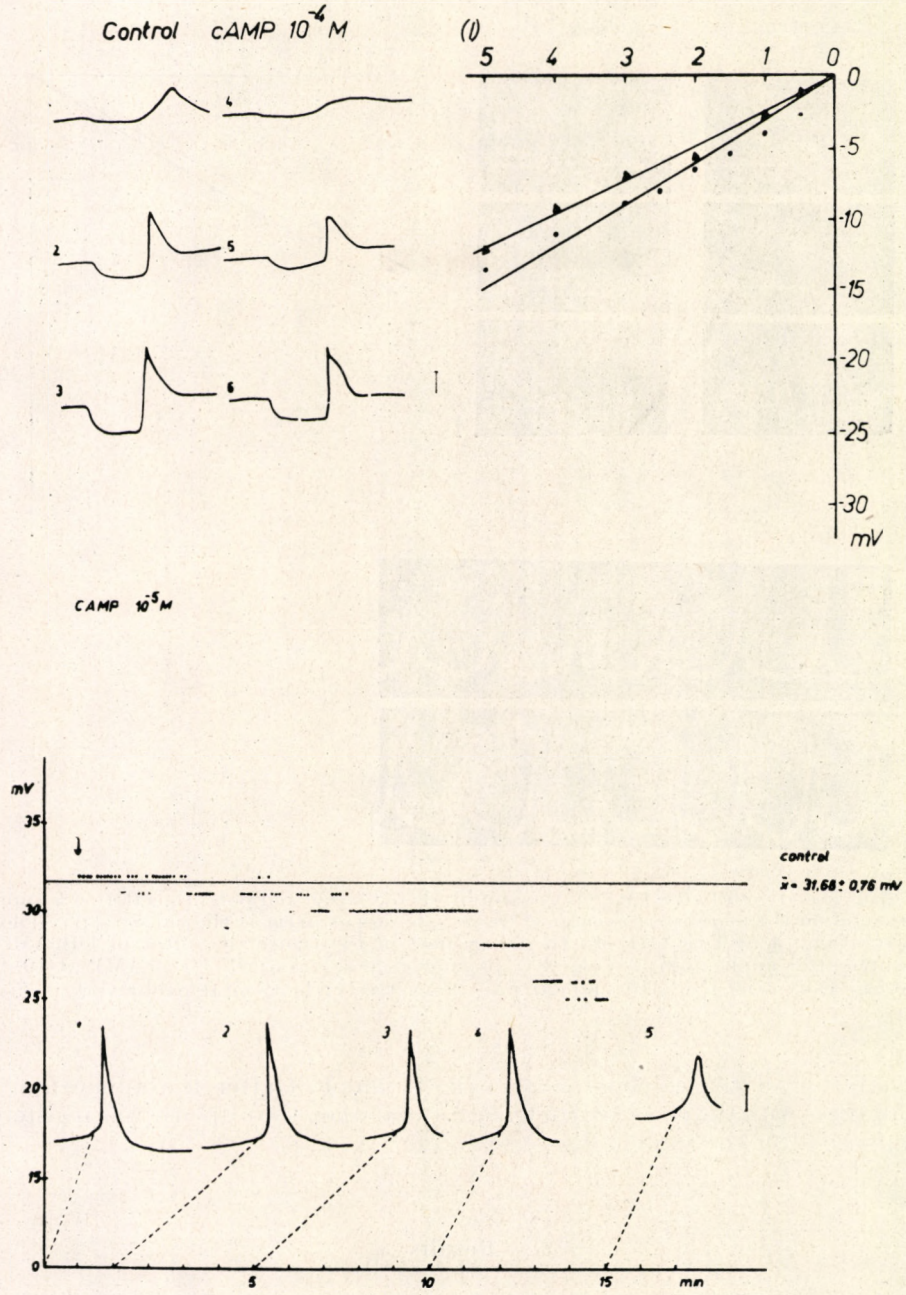

Fig. 2. The effect of cAMP on the electrotonic potentials, amplitude of action potentials (left side, upper part), and the resistance of the membrane (right side). On the graph the results obtained for physiological solution (•) and for cAMP (▲) are seen. In the lower part of the figure the time dependence of the amplitude of the action potentials is shown, marked with the dots. The vertical calibration is 10 mV

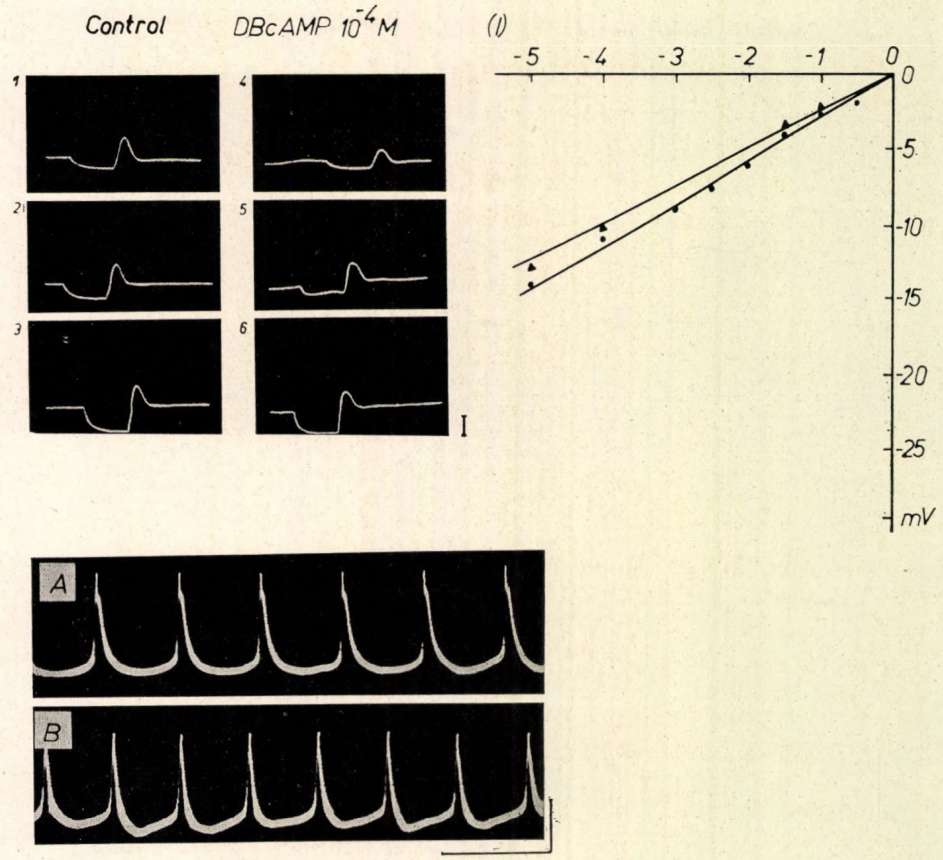

Fig. 3. The effect of DBcAMP on the amplitude of the evoked potentials (left side, upper part) and on the membrane resistance (right side, upper part) is shown. • = physiological solution, ▲ = DBcAMP. In the lower part of the figure the effect of DBcAMP 8 minutes after application is seen. *A* — physiological solution; *B* — DBcAMP at 10^{-4}M. Vertical calibrations are 10 and 20 mV, respectively; horizontal calibration is 5 sec

registration of the response was made 10 minutes after the application of substances with the exception of transmitters, when the response was registered 1 minute after their application.

Results

1. *The membrane effect of cAMP, DBcAMP, theophylline and imidazole*

In the experiments the effect of substances was studied at concentrations 10^{-8}—10^{-3} M. cAMP, DBcAMP and theophylline were to be effective only in high doses. No dose-dependent effect was found.

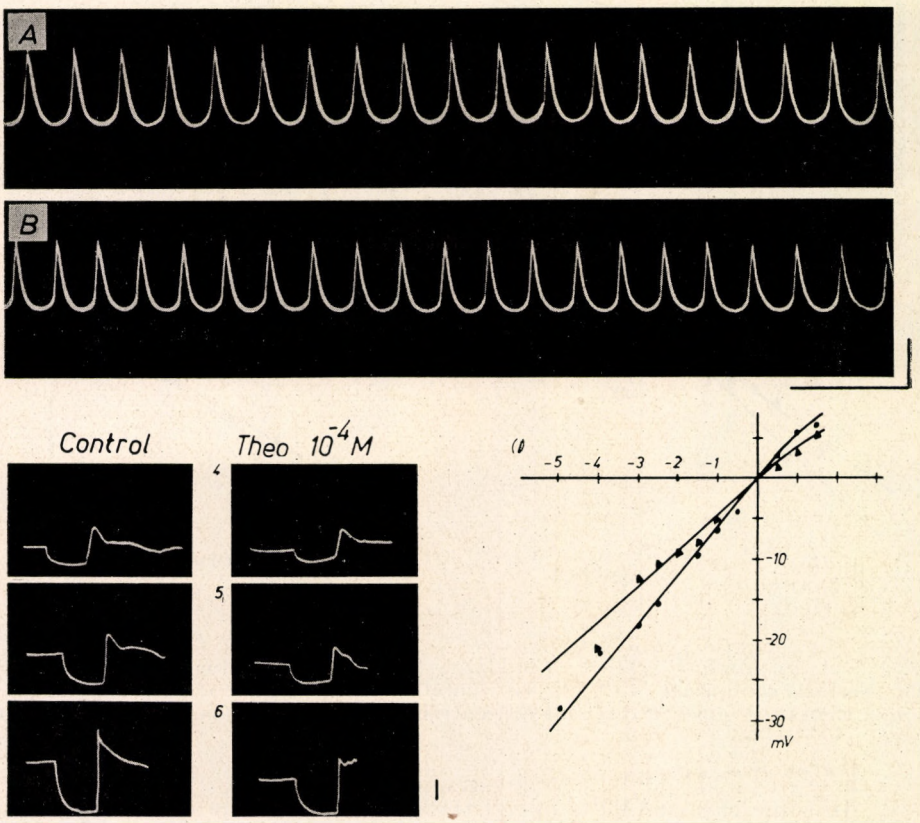

Fig. 4. The effect of theophylline (10^{-4}M) upon spontaneous activity (upper part) is seen. *A* — control; *B* — theophylline. Vertical calibration is 20 mV; horizontal calibration 5 sec. At the left side (below) the changes in the amplitude of the evoked potentials in the presence of theophylline, at the right side (below) the decrease in membrane resistance are shown. Vertical calibration is 10 mV. • physiological solution, ▲ theophylline

cAMP (10^{-4} M) increased the frequency of spontaneous activity at an average of 13 per cent, while the amplitude of the action potentials was significantly lowered. The maximum effect was observed at 5–20 minutes following the application of cAMP. The effect of cAMP appeared consistently. Comparing the registrations 1 (control) and 4 (cAMP) in *Fig. 2* (below) under the influence of cAMP, the increase in diastolic depolarization, the prolongation of the repolarization phase and the decrease in amplitude of the action potentials are readily seen. The same changes were observed on the action potentials accompanying the electrotonic potentials (*Fig. 2*, left side, upper part). In *Fig. 2* registrations 1–3 demonstrate active and passive responses of the membrane of the heart muscle cells to increasingly strong stimuli in normal physiological solution. Registrations 4—6 were made on the heart pretreated by cAMP. Comparing registrations 3 and 6 the slowing of repolarization and as a result the prolongation of the plateau phase and a decrease in the amplitude of the action potentials can be seen under the influence of cAMP.

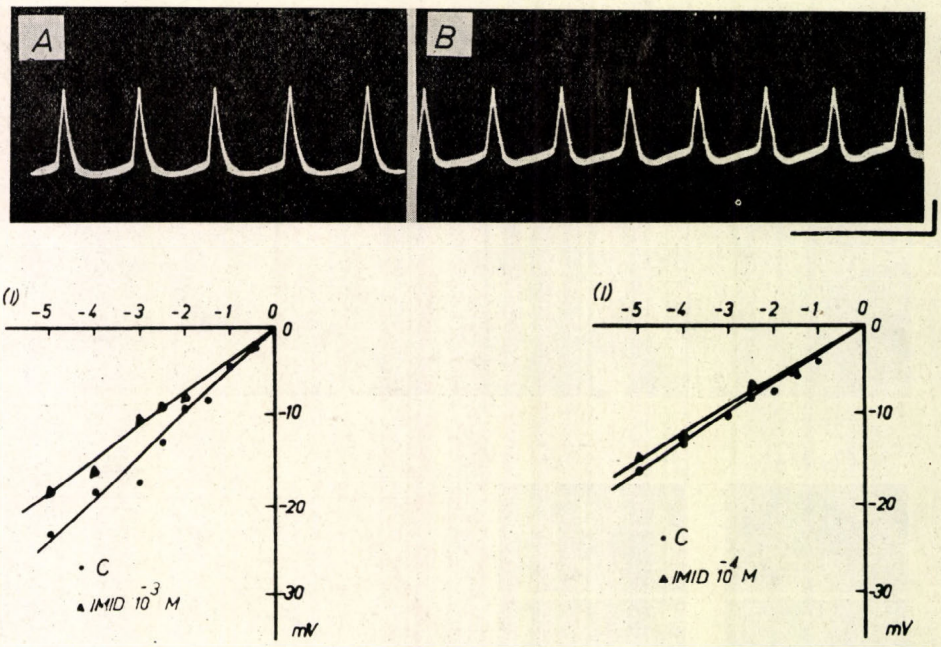

Fig. 5. A — control; *B* — effect of imidazole (10^{-4}M) upon spontaneous electrical activity. Vertical calibration is 10 mV; horizontal calibration is 5 sec. In the lower part the graph shows the effect of different concentrations of imidazole on membrane resistance

In some cases cAMP stopped the generation of the spontaneous action potentials but, even at this stage stimuli could evoke action potentials. The polarity of the membrane was changed by cAMP, the values in both directions of the membrane potential were almost the same *(Table I)*. Out of 12 preparations in 9 cases cAMP decreased the input resistance (R_{eff}) of the membrane by 5–20 per cent, in 2 cases it had no effect, and in one case it caused an opposite effect. In *Fig. 2* (right side, upper part) the effect of cAMP on the membrane resistance is shown by the characteristics of the voltage-current. The decrease in the direction-tangent of the curve corresponds to the decrease in the resistance of the membrane.

The effect of DBcAMP was variable on the frequency of spontaneous activity because in some cases it increased but in others decreased the frequency. The decrease in amplitude was also smaller than when cAMP was used. The repolarization phase was also influenced to a less degree in comparison with cAMP. DBcAMP frequently caused arrhythmia in the heart beats and in some cases stopped the spontaneous electrical activity. However, using electrical stimulation action potentials were evoked again. The membrane was hyperpolarized in the average with several millivolt *(Table I)*. In *Fig. 3* (upper part) the effect of DBcAMP on the spontaneous electrical activity is demonstrated. In *Fig. 3B* the increase in the diastolic depolarization and the slight decrease in the amplitude of the action potentials can be seen. The same decrease in the amplitude of the action potentials was registered also at the

evoked potentials (*Fig. 3* left side, upper part). The input resistance of the surface membrane was lowered in 6 cases and raised in one case under the influence of DBcAMP. The maximum effect appeared 5—10 minutes after application. In *Fig. 3* (upper part) the decrease in the electrotonic potentials produced by DBcAMP can be seen.

TABLE I

	Membrane potential in mV					
No,	Control	cAMP	Changes in per cent	Control	DBcAMP	Changes in per cent
1	41.6	55.7	33.8+	56.0	60.0	7.1+
2	52.8	50.0	5.3—	50.0	58.0	16.0+
3	44.2	50.0	13.1+	40.3	43.7	8.4+
4	42.2	46.0	9.5+	63.3	57.6	9.1—
5	51.5	55.0	6.8+	60.0	70.0	16.6+
6	50.0	50.0	— o	62.5	62.5	— o
7	50.0	55.0	10+	—	—	—
8	57.1	54.1	5.3—	—	—	—
9	61.0	65.2	6.9+	—	—	—
10	51.6	56.6	9.7+	—	—	—

No,	Control	Imidazole	Changes in per cent	Control	Theophylline	Changes in per cent
1	55.7	52.5	5.8—	41.0	43.0	2.4+
2	50.0	46.8	6.4—	50 0	55.9	11.8+
3	50.0	60.0	20 +	52.5	47.5	9.5—
4	40.6	49.6	22.2+	52.0	58.0	11.5+
5	40.0	40.0	—	64.0	56.3	12.0—
6	48.0	55.0	14.6+	42.0	43.0	2.4+
7	41.6	40.0	3.9—	50.0	55.9	11.8+
8	—	—	—	—	—	—
9	—	—	—	—	—	—
10	—	—	—	—	—	—

Note: Each value corresponds to the average of membrane potentials registered from 10—15 heart muscle fibers. + = hyperpolarization. — = depolarization. o = MP remained unchanged

The theophylline (10^{-4} M) and imidazole (10^{-3}–10^{-6} M) increased the frequency and decreased the amplitude of the spontaneous action potentials (*Figs 4* and *5*). Their maximum effect was observed 1—2 minutes following application. Their effect on the membrane potential is summarized in *Table I*. In most of the cases cyclic nucleotides, theophylline and imidazole caused both depolarization or hyperpolarization, however, their effect was not convincing and remained within the limits of error. In *Fig. 4* (upper part)the increase in frequency and the decrease in amplitude can be seen when using theophylline. A drop in amplitude observed on the action potentials following the electrotonic potentials (4–6 registrations) is shown during theophylline application compared to the control (1–3 registrations). In this figure (right

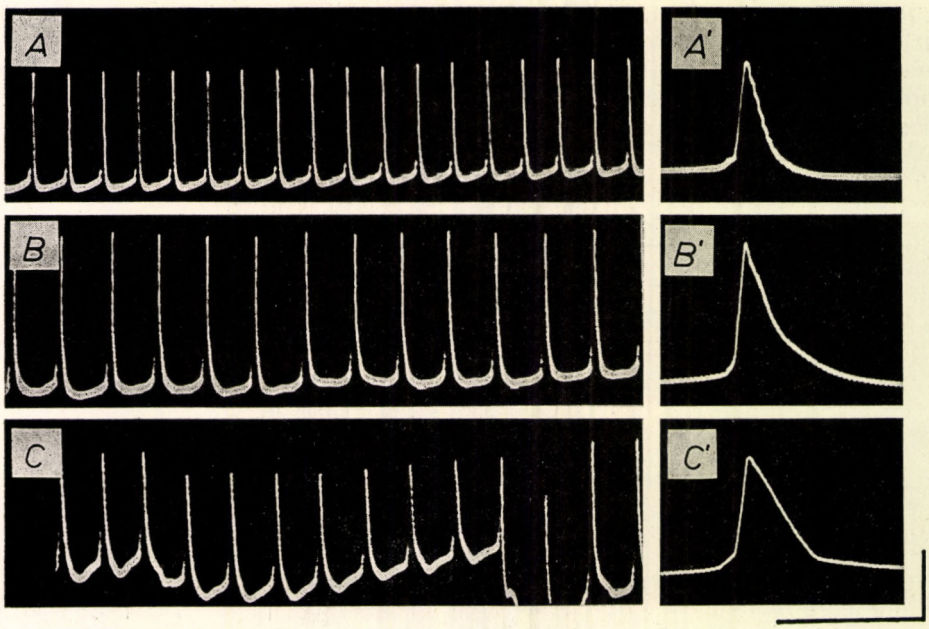

Fig. 6. *A* — control; *B* — cAMP 10^{-4}M; *C* — effect of 5HT ($5 \cdot 10^{-6}$M) on the heart pretreated with cAMP. *A'*, *B'* and *C'* correspond to the above three effects but the action potential was registered with hight speed of the beam. Vertical calibration is 20 mV; horizontal calibrations are 5 and 0.5 sec, respectively

side, below) also the characteristics of voltage and current demonstrate this relationship.

In *Fig. 5A, B* the effect of imidazole (10^{-4} M) exerted on the spontaneous activity is seen. Imidazole increased the frequency and decreased the amplitude of the spontaneous action potentials with the synchronous raising of diastolic depolarization.

On 5 preparations the input resistance of the membrane was decreased by 10—25 per cent with theophylline and by 5 per cent with imidazole. This effect was consequent in all cases.

Taking into account the decrease in membrane resistance (R_{eff}), the order of the investigated substances was: theophylline > cAMP > DBcAMP> > imidazole.

The effect of cyclic nucleotides was observed also in the hearts pretreated with theophylline for blocking the activity of phosphodiesterase. As a result, it would be expected that the potentiation of the effect of cyclic nucleotides were stronger. On the contrary, theophylline eliminated the decrease in amplitude caused by cAMP. After pretreatment with theophylline DBcAMP decreased the diastolic depolarization rising under the influence of theophylline and the amplitude of the action potentials, while the spontaneous activity becomes more frequent. The decrease in the amplitude of the action potentials was greater than that when using DBcAMP alone.

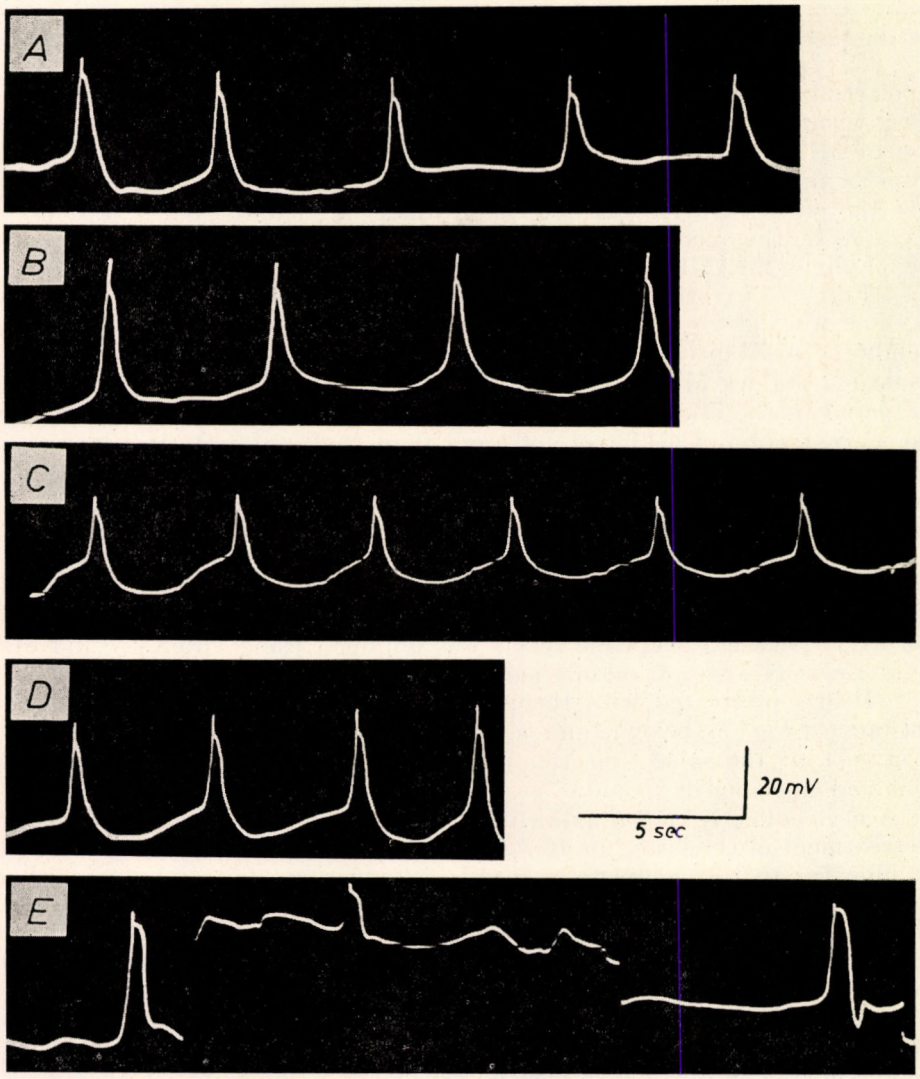

Fig. 7. *A* — control; *B* — and *C* — effect of DBcAMP (10^{-4}M) at 1 and 6 minutes after application. *D* — and *E* — effect of 5HT (10^{-4}M) on the heart, pretreated with DBcAMP. On register *E* a peak in the membrane potential is seen which is the result of dislocation of microelectrode after strong contractions

2. *Influence of the cyclic nucleotides and theophylline on the effects of acetylcholine and 5-hydroxytryptamine*

On the *Helix* heart under the influence of 5HT the frequency of spontaneous electrical activity is increased, the rate of rise of the action potentials also increased and the repolarization phase is prolonged. This characteristic effect

cannot be realized if the heart was pretreated with cyclic nucleotides or theophylline *(Figs 6, 7)*. The effect of cAMP and 5HT applied together is seen in *Fig. 6C, C'*. Some rising in the diastolic depolarization and prolongation of the repolarization phase still are present but in a reduced form compared to the effect when 5HT was applied alone. DBcAMP was less effective on the modulation of 5HT effect. In *Fig. 7B, C* the typical DBcAMP effect is seen, i.e. lowering the amplitude and raising the diastolic depolarization, it can be seen that the effect of 5HT was not significantly influenced by DBcAMP *(Fig. 7D, E)*. In the modulation of 5HT effect the following order was found: theophylline > cAMP > DBcAMP.

Further investigations were carried out: cAMP, DBcAMP and theophylline were given together with 5HT in order to describe their effect on membrane resistance and evoked action potentials. After pretreatment with cyclic nucleotides 5HT decreased the input resistance of the membrane as compared to the effect of cyclic nucleotides. This effect was observed when the concentration of 5HT was not lower than $10^{-6}-10^{-7}$ M. At a lower concentration an opposite effect occurred, i.e. an increase in membrane resistance. In *Fig. 8* (upper part) the effect of cAMP alone, then together with 5HT is shown on membrane resistance. In the same figure, on the right side, the electrotonic and action potentials are demonstrated. Compared the to control *(Fig. 8a)* the amplitude of the action potentials was decreased and the duration of the repolarization phase was prolonged *(Fig. 8b)* when applying cAMP and 5HT together. The same result was obtained using DBcAMP and 5HT simultaneously *(Fig. 8*, middle part).

Hearts pretreated with theophylline showed an increase in membrane resistance *(Fig. 8*, below), and a prolongation in the repolarization phase *(Fig. 8b)*, at the same time the amplitude of the evoked action potentials remained unchanged.

Acetylcholine is known as an inhibitory transmitter on the *Helix* heart. Pretreatment of the heart for 10—20 minutes with cyclic nucleotides or theophylline led to the complete or partial elimination of its inhibitory effect at 10^{-4} M *(Figs 9* and *10)*. As a results of pretreatment not only the inhibitory effect of acetylcholine was eliminated but also some increase in frequency was achieved *(Fig. 9E* and *Fig. 10C)*. Nevertheless, in all three cases in the amplitude of the action potentials considerable decrease was observed.

In *Fig. 11* the effect of acetylcholine is demonstrated on the electrotonic and evoked action potentials applied together with cAMP, DBcAMP or theophylline. The effect of acetylcholine in the presence of cAMP is shown on the upper part of *Fig. 11*. It can be seen from the characteristics of voltage-current that the membrane resistance was not significantly changed when ACh was added to cAMP .In *Fig. 11* (right side) a slight decrease in the amplitude of the evoked action potentials can be seen and a prolongation in the repolarization phase.

The membrane resistance decreased compared to the effect of DBcAMP when the DBcAMP and ACh were added simultaneously *(Fig. 11*, middle part). Similar decrease can be obtained on the electrotonic potentials, too *(Fig. 11*, right side). The amplitude of the evoked action potentials also decreased significantly, but the excitability of the heart muscle cells was unaffected.

No differences were found in the degree of the decrease in the input membrane resistance whether theophylline was used alone or jointly with ACh.

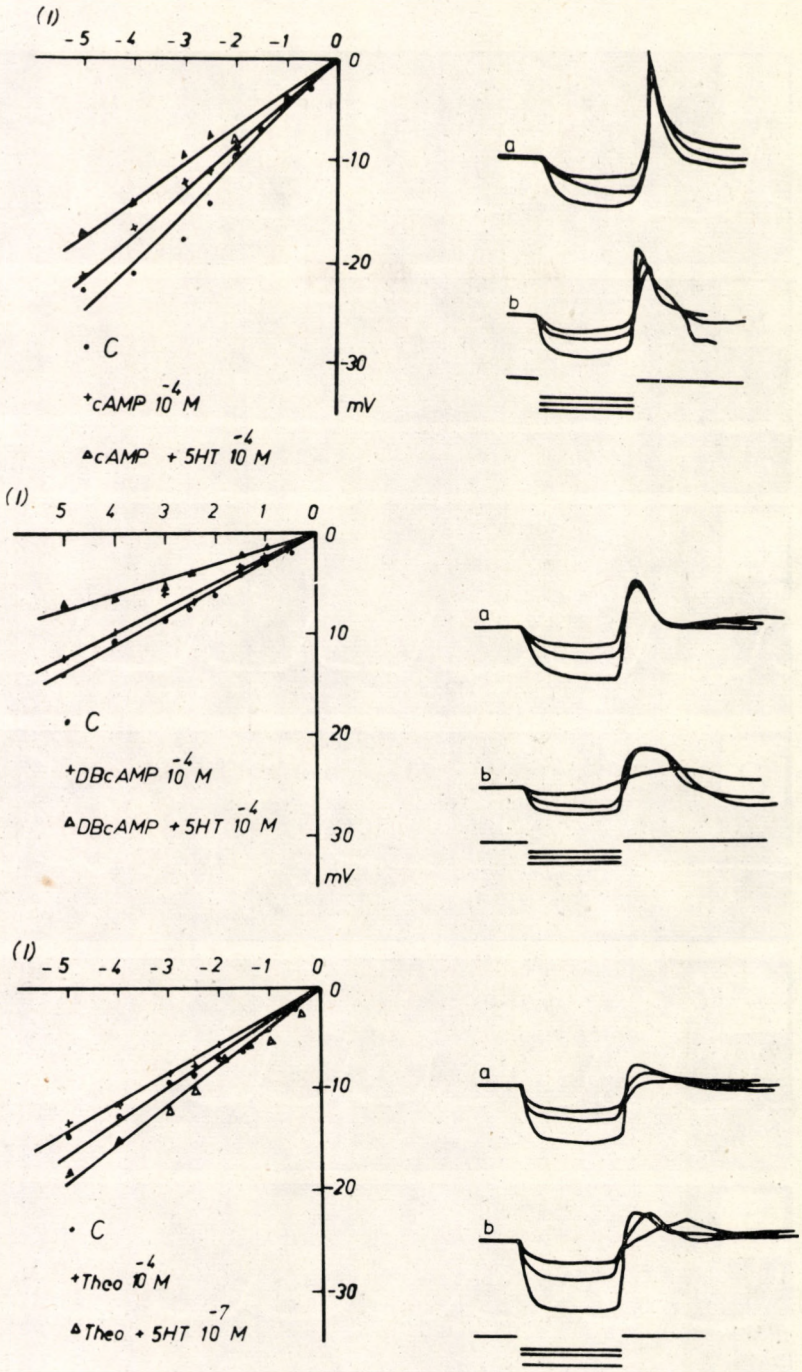

Fig. 8. Changes in electrotonic potentials using hyperpolarizing stimuli and that of membrane resistance during 5HT application, then the same on pretreated heart by cyclic nucleotides or theophylline. *a* — control; *b* — simultaneous effect of 5HT and cyclic nucleotides

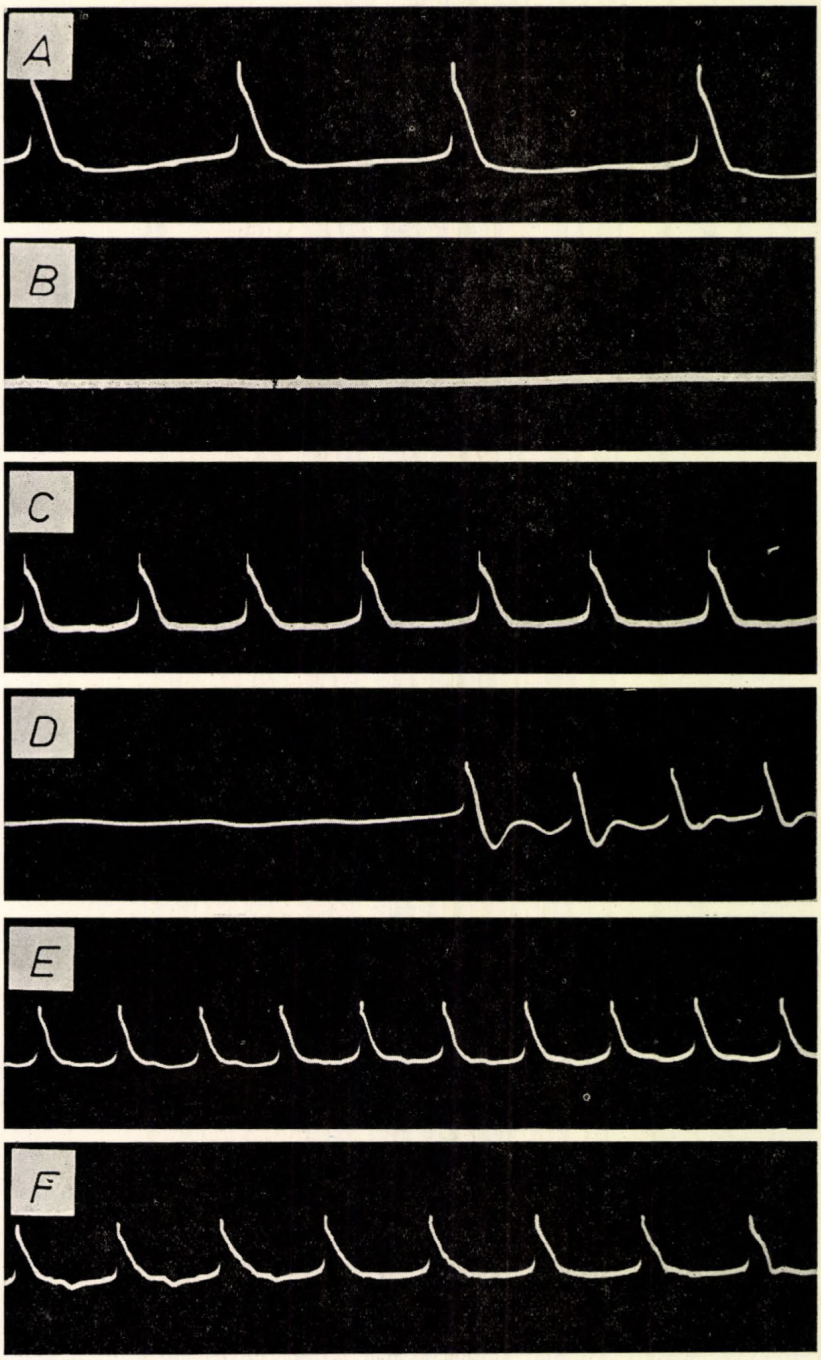

Fig. 9. *A* — control; *B* — effect of ACh (10^{-4}M). *C* — control; *D* and *E* — simultaneous effect of theophylline and ACh (10^{-4}M) immediately after their appliation. *F* — control.

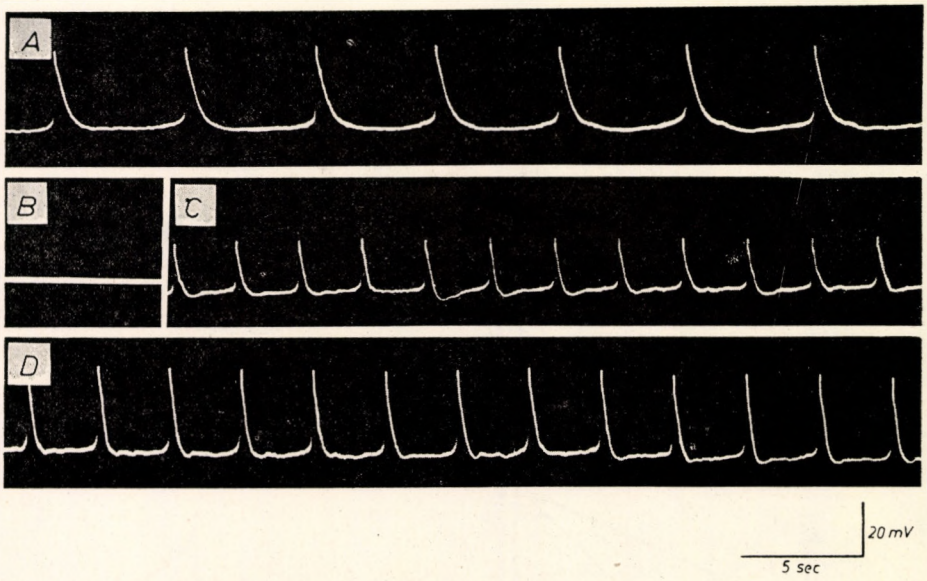

Fig. 10. *A* — control; *B* — effect of ACh ($5 \cdot 10^{-6}$M). *C* and *D* — effect of ACh on the heart pretreated with DBcAMP (10^{-4}M), 2 and 10 minutes after application

However, the excitability of the membrane was significantly lowered. This is demonstrated in *Fig. 11* (below, at right side) but by increasing the strength of the stimulus (broken line) the action potentials were generated again.

Discussion

On the *Helix* heart, according to our data cAMP at the beginning of its action increased the frequency of the spontaneous action potentials, diastolic depolarization and prolonged the phase of repolarization. In the second phase of its action cAMP decreased the amplitude of the action potentials, then the spontaneous beating of the heart cells ceased. DBcAMP had no constant effect on the frequency of the spontaneous action potentials but decreased their amplitude and the duration of repolarization. Theophylline and imidazole showed similar effects to cyclic nucleotides. The maximum effect of cyclic nucleotides occurred 10—20 minutes after their application, on the other hand, the same occurred 1—2 minutes after the application of theophylline and imidazole. Similar to the vertebrate hearts (Tsien, 1973; Reuter, 1974; Yamasaki et al., 1974) these data also refer to the intracellular site of actions of the above substances.

In our experiments on the heart muscle cell membrane, cyclic nucleotides and inhibitors of phosphodiesterase caused slight hyperpolarization or depolarization but this two were not significant. Cyclic nucleotides hyperpolarized the membrane of the smooth muscle (Somlyo et al., 1972) and depolarized the membrane of heart muscle cells (Reuter, 1974). Their hyperpolarizing

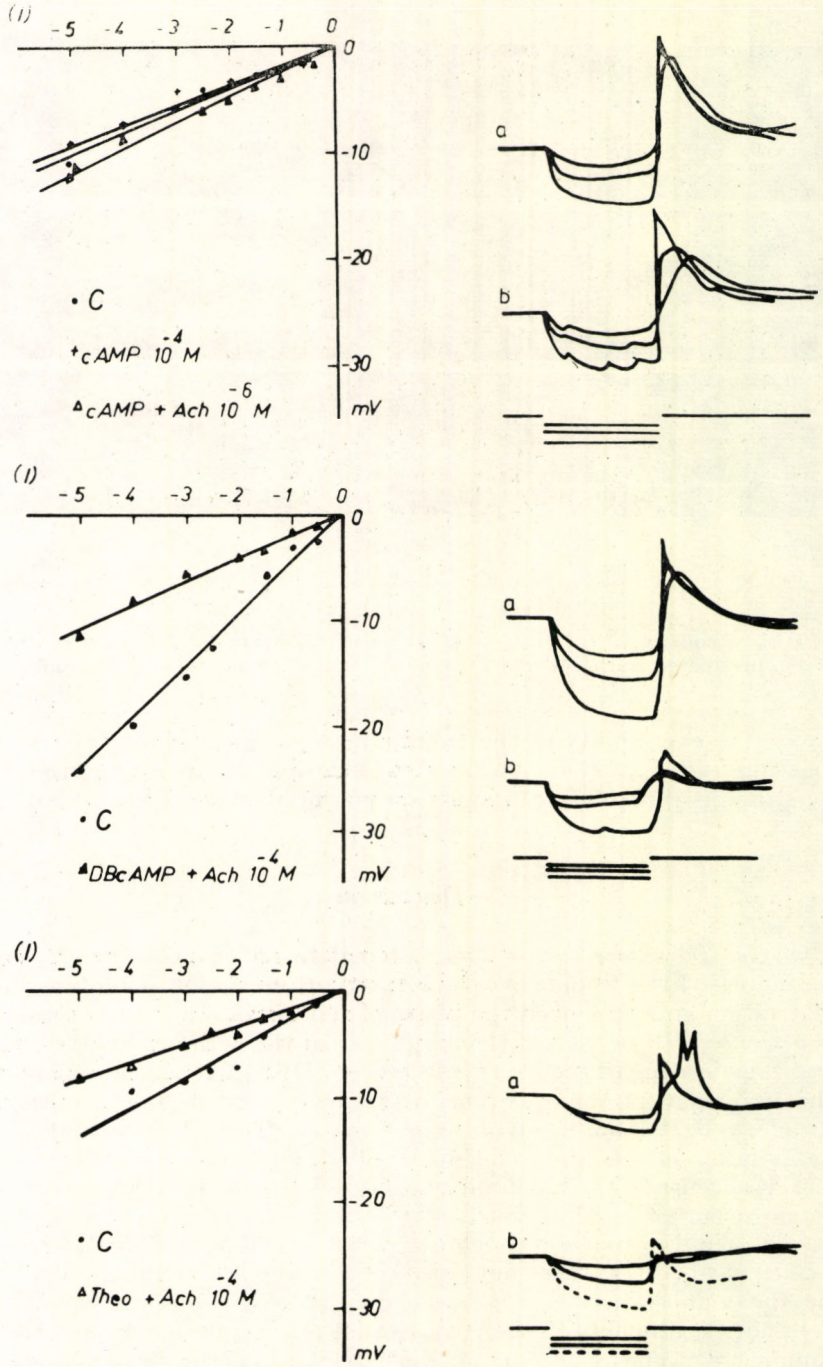

Fig. 11. Changes in electrotonic potentials, using hyperpolarizing stimuli, and that of membrane resistance during ACh application, then the same on pretreated by cyclic nucleotides or theophylline heart. *a* — control; *b* — simultaneous effect of ACh and cyclic nucleotides

effect was found to be dependent on the extracellular concentrations of K-ions (SOMLYO et al., 1972).

Influencing the initial depolarization and the repolarization phase of the spontaneous action potentials the cyclic nucleotides, theophylline and imidazole, similarly to the mammalian hearts, on the *Helix* heart also effected the K current, responsible for the pacemaker component, and the Ca-current, connected with the plateau phase (VASSORT et al., 1969; TSIEN et al., 1972; TSIEN, 1974; REUTER, 1974). On the membrane of the vertebrate heart muscle cell both adrenaline and DBcAMP forced the K current, responsible for the initial depolarization, in the same direction, assuring faster inactivation, thereby an increase in the frequency of action potentials of the spontaneously beating hearts could occur (TSIEN, 1974; REUTER, 1974). It can be stated that the increase in the initial depolarization caused by cyclic nucleotides on the *Helix* heart is also in close connection with the decrease of K conductivity similarly to other pacemaker tissues.

On the vertebrate heart the prolongation of the plateau phase caused by cyclic nucleotides was connected with the increased Ca-permeability of the membrane (TSIEN, 1973; REUTER, 1974) and the same conclusion was drawn on the insect heart (S.-RÓZSA, 1974). The relation between cyclic nucleotides and Ca-ions in the contractile and biochemical events was proved in a number of cases (JOST and RICKENBERG, 1971; KUKOVETZ and PÖCH, 1972; ANDERSSON, 1972; PRINCE and BERRIDGE, 1973; TORDA, 1974; etc).

Through a feed-back mechanism cAMP and Ca-ions can mutually control each other's concentration. Intracellular increase in the cAMP concentration led to the decrease in the concentration of free Ca-ions which resulted in the lowering of the intracellular level of the free Ca-ions, but the low level of Ca-ions induced the elevation of the activity of phosphodiesterase and this led to the decrease of the cAMP concentration (ANDERSSON, 1972) again leading to the activation of adenylate cyclase.

The regulatory role of cyclic nucleotides on the permeability of the membrane can be realized by activating protein kinases, since the phosphorylation of the different proteins and lipids of the membrane can change the penetrating conditions of the ion channels, too (RASMUSSEN and TENENHOUSE, 1968; KUKOVETZ and PÖCH, 1972). Supposedly on the *Helix* heart the investigated phenomenon that in high concentrations of cAMP the transmitter effect failed to occur in the usual way can also be related to the phosphorylation of the membrane components. According to our data the effect of cyclic nucleotides was negligible on the surface membrane and it changed mainly the resistance of the membrane, nevertheless they significantly influenced the transmitter effect or even turned it to the opposite in sign. For this reason the importance of secondary changes in conformations of the membrane protein should be stressed during membrane application of cyclic nucleotides preventing the realization of transmitter actions while their influence on the generation of potentials is negligible (GREENGARD and KEBABIAN, 1974). The changes are connected with the prolonged alterations in permeability and during this time the adenylate cyclase localized in the membrane can only be activated to a small degree so the conditions for the realization of the transmitter effects are absent.

The decrease in membrane resistance was studied on the salivary gland of insects during cyclic nucleotide application (BERRIDGE and PATEL, 1968;

HAX et al., 1974) and it was established that a high cAMP level improves cell connections owing to a decrease in membrane resistance. The same role can be attributed to the decrease in membrane resistance on *Helix* heart. However, in order to elucidate the relations between changes in membrane resistance and transmitter effects, further experiments are needed.

Summary

Studying the membrane effect of the substances relating to the second messenger system on the *Helix* heart, it was stated, that:

1. At the beginning of their application cAMP, DBcAMP, theophylline and imidazole increase diastolic depolarization, the duration of the plateau phase and the frequency of the spontaneous action potentials. At the second period of their action, the amplitude of the action potentials is lowered then the potential generation ceased. The intracellular site of the actions was prevailing on their effect.

2. The above substances depolarize or hyperpolarize the membrane only slightly, synchronously lowering membrane resistance.

3. The changes in the different phases of the action potentials are attributed to the decrease in K-permeability and increase in Ca-permeability. During the application of cyclic nucleotides the alterations in permeability were only secondarily appearing as a consequence of phosphorylation of the membrane components.

4. After pretreatment with cyclic nucleotides or theophylline the effect of 5HT and ACh failed to occur on the membrane of *Helix* heart. The common application of 5HT with cyclic nucleotides decreased while theophylline increased the resistance of the surface membrane, but in the presence of ACh both the cyclic nucleotides and theophylline caused a decrease in membrane resistance. The changes in membrane effects of the transmitter can be explained by prolonged alterations of permeability caused by cyclic nucleotides.

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CIKLIKUS NUKLEOTIDOK MEMBRÁNHATÁSA, VALAMINT SZEREPÜK TRANSZMITTEREK HATÁSÁNAK REALIZÁLÁSÁBAN *HELIX POMATIA* SZIVIZOMSEJTJEIN

Kiss Tibor és S.-Rózsa Katalin

Összefoglalás

A második messenger rendszer membránhatásának vizsgálata alapján megállapítottuk, hogy *Helix* sziven

1. a cAMP, DBcAMP, theophylline, és imidazole, kezdetben növelik a spontán AP-k diasztolés depolarizációját, a plátó fázis idejét, valamint a frekvenciát.

Hatásuk második fázisánan az AP amplitúdóját csökkentik, majd felfüggesztik a potenciálgenerálást. Hatásukban intracelluláris támadáspont dominál.

2. A fenti anyagok a membránt nem szignifikánsan hiperpolarizálják vagy depolarizálják, a membrán ellenállását csökkentik.

3. A spontán AP komponenseinek változása a K-permeabilitás csökkenésére, és a Ca-permeabilitás növekedésére vezethető vissza. A permeabilitás változások ciklikus nukleotidok hatására másodlagosan, a membrán komponensek foszforilálásának eredményeként lépnek fel.

4. Ciklikus nukleotidok és theophylline inkubálás után az 5HT és ACh hatása *Helix* szív membránján nem realizálódik. 5HT-vel együtt alkalmazva a ciklikus nukleotidok csökkentik, theophylline növeli a felszíni membrán ellenállását, míg ACh-val együtt adva a ciklikus nukleotidok és theophylline egyaránt a membrán ellenállás csökkenését hozzák létre. A transzmitterek membránhatásának megváltozását ciklikus nukleotidok által létrehozott tartós permeabilitás változásokkal magyarázzuk.

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SITE OF ACTION OF 5-HYDROXYTRYPTAMINE ON THE MEMBRANE OF HEART MUSCLE CELLS IN *HELIX POMATIA* L.

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In the molluscan hearts the transmitter role of 5-hydroxytryptamine (5HT) was verified by morphological, physiological and biochemical data (WELSH, 1957; WELSH and MOORHEAD, 1960; DAHL et al., 1962; KERKUT and COTTRELL, 1963; S.-RÓZSA and GRAUL, 1964). In Gastropods the liberation and reaccumulation of 5HT in the heart nerve (S.-RÓZSA and PERÉNYI, 1966; TAXI and GAUTRON, 1969) were also proved.

Investigations concerning the membrane effect of 5HT were started only in recent years (KISS and S.-RÓZSA, 1972; S.-RÓZSA et al., 1973; WILKENS and GREENBERG, 1973; HILL, 1974), and the data refer to varying effects in the heart of the different species of Molluscs. In the central nervous system of Gastropoda no less than six sites of action are known for 5HT (GERSCHENFELD and PAUPARDIN-TRITSCH, 1974 a, b), connected with the regulation of permeability for different ions. On the heart membrane of *Helix pomatia* our earlier results proved the biphasic action of 5HT (KISS and S.-RÓZSA, 1972) and called attention to the possibility of different ion-dependence of this effect (S.-RÓZSA et al., 1973). The present investigations aimed at further analyzing the ion-dependence of the membrane effect of 5HT and the pharmacological description of the sites of its action. The investigations also included the analysis of the evoked potentials besides the previously studied spontaneous action potentials.

Material and method

The experiments were carried out on the isolated ventricle of *Helix pomatia* L. The spontaneous electrical activity was registered by using microelectrodes filled with 3 M KCl, their resistance being 5—15 MOhm.

The electrotonic potentials were produced by extracellular stimulation of the heart by using suction electrodes and applying square wave impulses of 10^{-8} A amplitude and 1 sec duration. The way of stimulation was described in detail elsewhere (KISS and S.-RÓZSA, 1975).

The isolated heart was kept in a chamber of 0.5 cm^3 under permanent perfusion with physiological solution. The perfusion was stopped when the investigated substances were applied. The drugs were dissolved in physiological solutions or in the required ion-free medium. The components of the

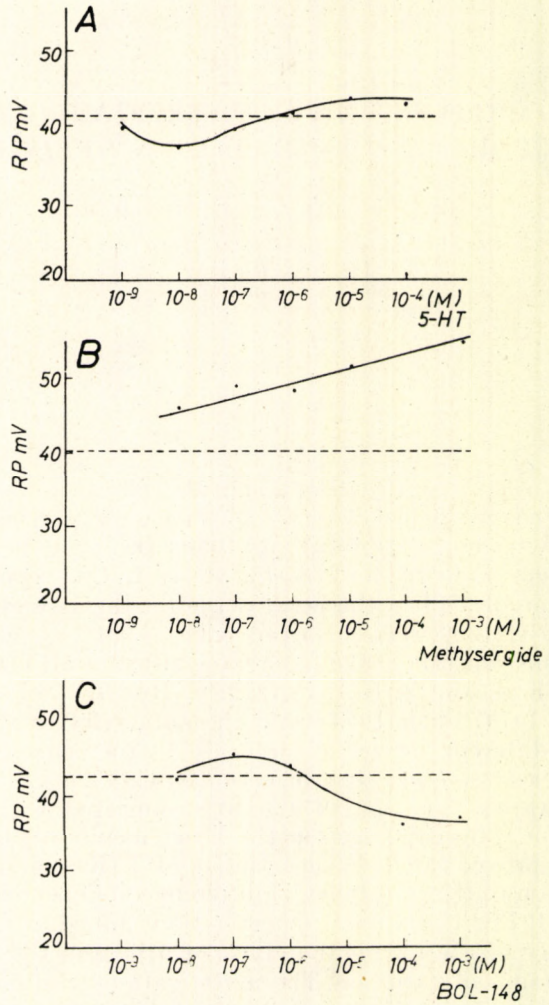

Fig. 1. Dependence of the membrane potential (MP) on the logarithmic concentrations of 5HT (*A*), methysergide (*B*) and BOL-148. Each point corresponds to 10—10 measurements on 22, 28 and 17 heart preparations

used ion-free solution are listed in *Table I*. The experiments were performed at room temperature (22—24°C).

The following substances were used: 5-hydroxytryptamine creatinine sulphate (5HT, Reanal); 2-Bromo-D-lysergic acid diethylamide (BOL-148, Koch-Light Labs); methysergide bimaleate (Sandoz).

Results

1. *The membrane effect of 5HT*

According to our previous data (KISS and S.-RÓZSA, 1972) the threshold concentration of 5HT was 10^{-10} M on the heart membrane of *Helix*. 5HT

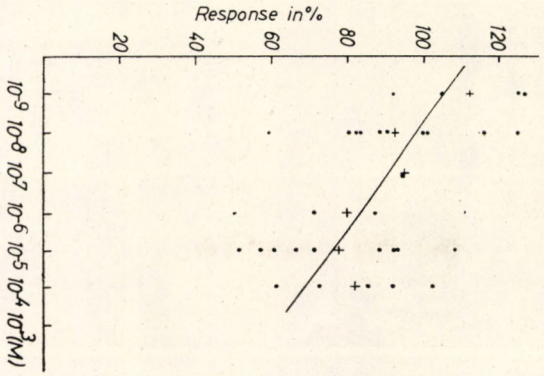

Fig. 2. Changes in the electrotonic potentials depending on the logarithmic concentrations of 5HT at per cent compared to the control value. The designed line is approximative

TABLE I

The compounds of solutions in mM

Substance	Cl-free	Na-free	Ca-free	Physiological solution
NaCl	—	—	112.0	111.0
KCl	—	2.0	2.0	2.0
$NaHCO_3$	2.4	—	2.4	2.4
$CaCl_2$	—	1.8	—	1.8
Choline Cl	—	118.6	—	—
Na-propionate	114.5	—	—	—
K-propionate	1.8	—	—	—
Ca-propionate	1.3	—	—	—

increases the amplitude of the spontaneous action potentials while decreases their frequency. The most typical effects of 5HT were the sharp rise of the spontaneous action potentials and the duration of the phase of retarded repolarization. On quiescent hearts 5HT evoked both AP generation and contractions.

The effect of 5HT on the membrane potentials (MP) was not described earlier, these results are summarized here. In *Fig. 1* the effect of different concentrations of 5HT is shown. On the membrane of *Helix* heart the effect of 5HT was biphasic, since in low concentrations (10^{-9}—10^{-7} M) it depolarized the surface membrane, while in high concentrations (10^{-5}—10^{-4} M) it caused hyperpolarization. At a concentration of 10^{-6} M 5HT failed to change the membrane potential, or its effect was negligible.

In *Fig. 2* the relation between the amplitude of electrotonic potentials and the concentrations of 5HT is demonstrated. As it can be seen in *Fig. 2*, when increasing the concentrations of 5HT, the amplitude of the electrotonic potentials decreased, i.e. the conductivity of the membrane was elevated. However, at low concentrations (10^{-9}—10^{-8} M) 5HT increased the amplitude

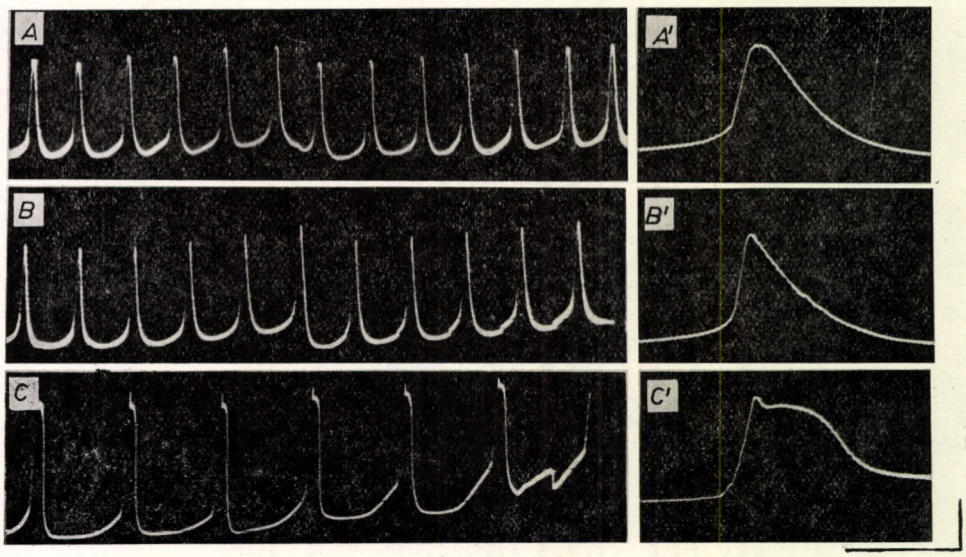

Fig. 3. *A* and *A'* — spontaneous electrical activity in normal physiological solution. *B* and *B'* — effect of Cl-free solution 10 min after application. *C* and *C'* — effect of 10^{-5}M 5HT at Cl-free solution. Horizontal mark is 5 sec and 0.5 sec, vertical mark is 20 mV in each case

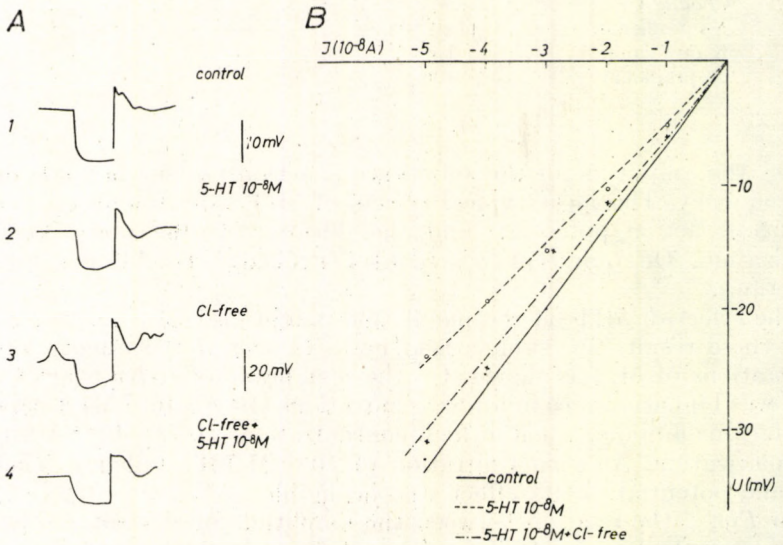

Fig. 4. *A* — changes in the electrotonic potentials and evoked action potentials in the case of Cl deprivation in the presence of 5HT. *B* — changes in the conductivity of the membrane

of the electrotonic potentials by 5—10 per cent, so that 5HT caused biphasic effect on the electrotonic potentials, too. The demonstrated relation was dose-dependent.

2. *The ion-dependence of the effect of 5HT*

Both Ca^{2+} and Na^{+} dependences were found in the effect of 5HT in our earlier experiments (S.-Rózsa et al., 1973). This ion-dependence was studied in detail and the participation of Cl^{-} ions in the effect of 5HT was ascertained.

Cl$^-$-free solution: The amplitude and frequency of the spontaneous activity was not influenced in Cl-free medium substituted by propionate, however, the duration of the action potentials was slightly shortened *(Fig. 3B, B')*. The spontaneous activity was not eliminated in Cl-free solution but the amplitude of the electrotonic potentials rose indicating a decrease in membrane conductance. The membrane was hyperpolarized by several millivolts. In Cl-free solution 5HT increased the amplitude and the duration of the retarded repolarization, while the frequency of the spontaneous action potentials was decreased. However, the decrease in frequency *(Fig. 3C)* under the influence of 5HT in Cl-solution was lower than the same in the control medium. In Cl-free solution the rate of rise of the action potentials remained unchanged compared to the control *(Fig. 3C')*, nevertheless, the positive afterpotential disappeared. In Cl-free solution 5HT increased the amplitude of the electrotonic potentials to a greater degree than the same in the normal physiological solution *(Fig. 4B)*. The average value of decrease in the conductivity of the surface membrane was 64 per cent.

Comparing the second and fourth photographs in *Fig. 4A*, it becomes evident that the positive afterpotential on electrotonic potentials is eliminated in the Cl-free solution. It means that this hyperpolarization wave is directly connected with the Cl-ions.

Na-free solution: It has been published previously that in choline chloride substituted Na-free solution the spontaneous activity was inhibited a few minutes after the exchange of the physiological solution (S.-Rózsa et al., 1973). The membrane is hyperpolarized by approximately 20 per cent in Na-free medium. In Na-deprivation 5HT renewed the generation of the potential. In Na-free solution 5HT failed to form the characteristic retarded repolarization but the positive afterpotential was present. The amplitude and the frequency of the action potentials were lower than the control value.

In Na-free solution the conductivity of the membrane lowered simultaneously with the stopping of the spontaneous activity, but upon stimulation AP was elicited, though its amplitude was lower than the control *(Fig. 5A*, 2nd photo). In Na-free solution 5HT reduced by 25 per cent the conductivity of the membrane. In this case the 5HT effect in normal physiological solution was taken as control. In *Fig. 5B* one of the typical experiments can be seen where at control conditions 5HT (10^{-6} M) increased, while at Na-free solution decreased the conductivity of the membrane. This effect emphasizes the necessity of Na-ions for 5HT action on the surface membrane of heart muscle cells. In Na-deprivation only a gradual rise in the action potentials was observed and it was not compensated by 5HT *(Fig. 5A)*. On the evoked action potentials it can also be seen that in Na-free medium the characteristic retarded

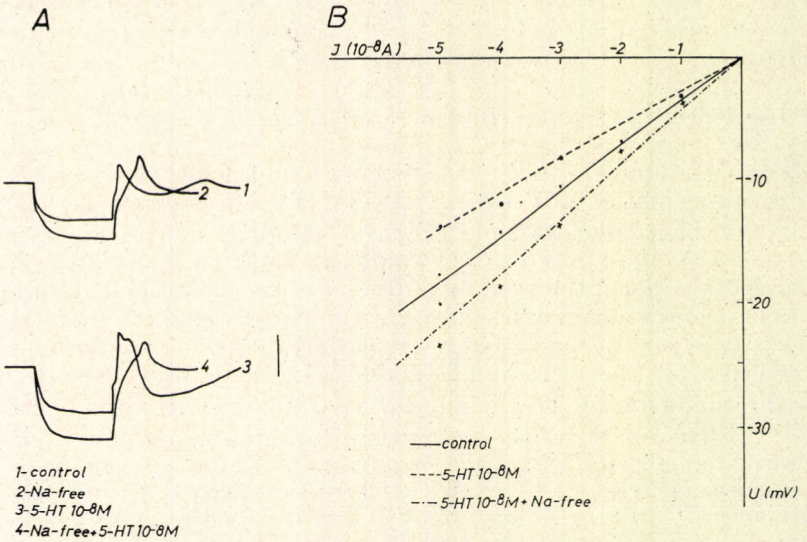

Fig. 5. A — changes in the electrotonic potentials and evoked action potentials in Na-free solution containing 5HT. *B* — changes in the conductivity of the membrane

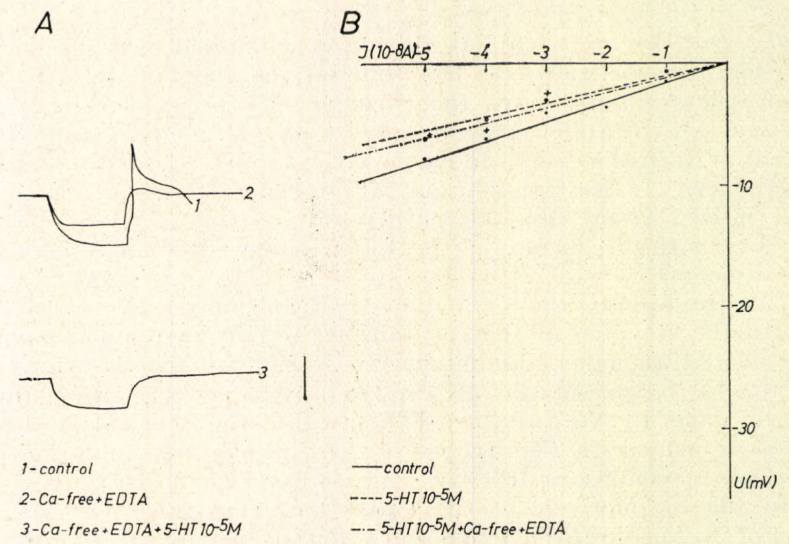

Fig. 6. A — changes in the electrotonic potentials and evoked action potentials in Ca-free medium containing 5HT. *B* — changes in the conductivity of the membranes

repolarization produced by 5HT is submitted to changes compared to the control *(Fig. 5A, 4)*.

Ca-free solution: According to our earlier data (KISS and S.-RÓZSA, 1972), the generation of the spontaneous action potentials was stopped at 30–100 min in Ca-free solution, the process was accelerated by EDTA. In Ca-free medium 5HT caused temporal potential generation (S.-RÓZSA et al., 1973). Detailed analysis of this phenomenon showed that in Ca-deprivation, in the presence of EDTA, the potentials were only local without a steeply rising phase. The retarded oscillatory fluctuation may be regarded as a repolarization phase. In Ca-free solution the stimulation led, though not in all the cases, to the potential generation and the same is true when adding 5HT *(Fig. 6*, photographs 2 and 3). In Ca-free solution with 3mM EDTA the inward resistance of the membrane was decreased then after adding 5HT it increased compared to the control. In this case also the effect of 5HT was taken in normal physiological solution as control *(Fig. 6B)*.

3. *The membrane effect of BOL-148 and methysergide and their influences on the action of 5HT*

BOL-148 from the beginning of concentration 10^{-8} M influenced the surface membrane of the heart. At low concentrations it increased the frequency, amplitude and rate of rise of the spontaneous action potentials, but from 10^{-6} M it began to inhibit these parameters *(Fig. 7D, F)*. At a high concentration (10^{-4} M) it mostly stopped the generation of the spontaneous action potentials.

The membrane potential was affected by BOL-148 similarly to 5HT but with an oposite sign *(Fig. 1C)*. At low concentrations (10^{-8}–10^{-7} M) BOL-148 caused hyperpolarization, while in high concentrations (10^{-5}–10^{-3} M) it depolarized the membrane. The turning-point was around 10^{-6} M.

The effect of 5HT was well antagonized by BOL-148. In *Fig. 9* the control (photograph D) and the simultaneous effect of 5HT and BOL-148 at a concentration of 10^{-6} M (E and F photographs) are demonstrated. The mutual effect of 5HT and BOL-148 is shown by two *(Fig. 9E)* and five *(Fig. 9F)* minutes after their application. In comparison with the control neither the amplitude nor the frequency of the spontaneous action potentials were changed. The shape of the action potentials has altered to a certain degree. During a simultaneous application of 5HT and BOL-148 (1.5×10^{-6} M) the membrane was hyperpolarized by 6–8 mV.

Methysergide from 10^{-8} M increased the frequency of spontaneous activity. From 10^{-6} M it altered the shape of the spontaneous action potentials without influencing the amplitude, then at high concentrations it evoked the postsynaptic potentials *(Fig. 8G)*. It hyperpolarized the membrane in a dose-dependent manner *(Fig. 1B)*. Methysergide did not stop the spontaneous activity, on the contrary, from time to time, it made beat the quiescent hearts.

The simultaneous effect of 5HT and methysergide at 10^{-6} M is shown in *Fig. 9A, B, C*. Disregarding the slight increase in frequency no significant changes were observed as compared to the control. The 5HT effect was eliminated by methysergide. In this case too the postsynaptic potentials appeared upon the influence of methysergide *(Fig. 9B)*. The simultaneous effect of

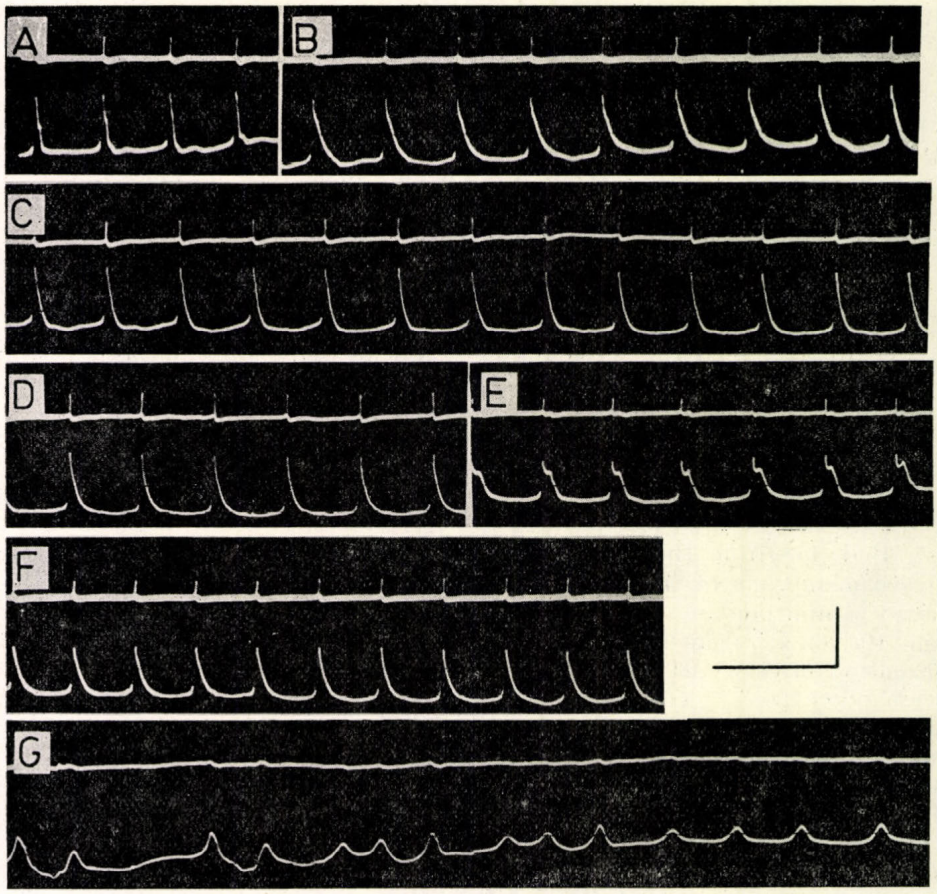

Fig. 7. A — control. *B* — and *C* — effect of BOL-148 (10^{-8}M) by 8 and 12 min after application. *D* — and *E* — effect of BOL-148 (10^{-6}M) at the 5HT and 7th min of its application. *F* — and *G* — effect of BOL-148 (10^{-4}M) at the 3rd and 8th min of its application. Horizontal mark is 5 sec, vertical mark is 20 mV

5HT and methysergide is demonstrated by two and five minutes following their application *(Fig. 9B, C)*.

The membrane was hyperpolarized by 6—8 mV upon the joint effect of 5HT and methysergide (5×10^{-6} M).

Discussion

According to our data on the heart muscle membrane of *Helix pomatia* 5HT has several sites of action similarly to the neurones (Gerschenfeld and Paupardin-Tritsch, 1974 a, b) and its membrane effect was manifested as a hyperpolarization or depolarization.

As on the hearts of the other species of Molluscs (Wilkens and Greenberg, 1973; Irisawa et al., 1973; Hill, 1974) the 5HT effect showed Na-

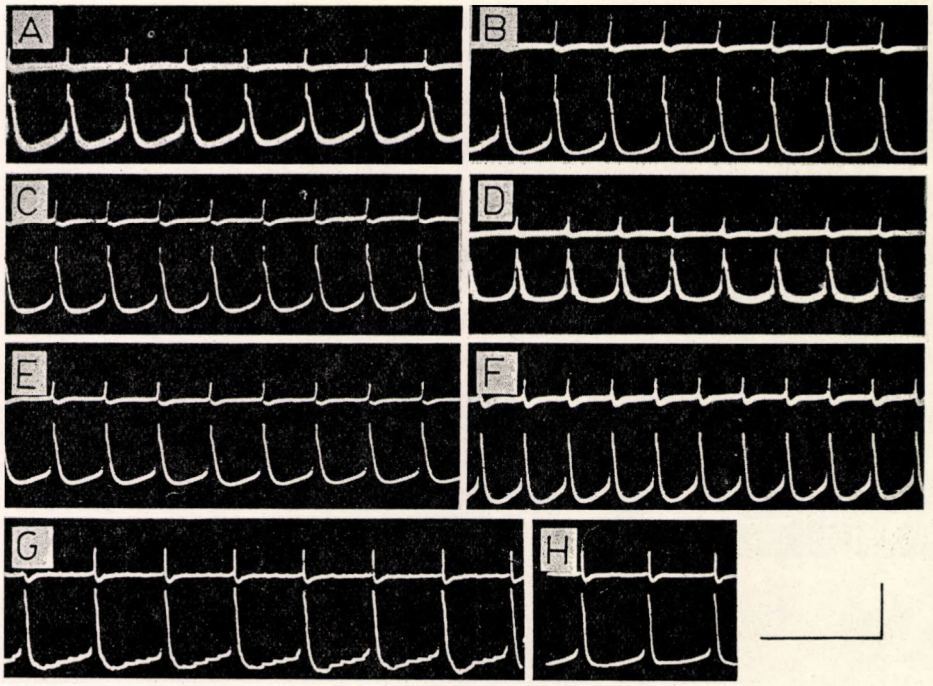

Fig. 8. *A*—control. *B* — and *C* — effect of methylsergide (10^{-8}M) at 8th and 14th min of its application. *D*, *E* and *F* — effect of methylsergide at 4th, 10th and 21st min of its application. *G* and *H* — effect of methylsergide (10^{-4} M) at 9th and 15th min of its application. Horizontal mark is 5 sec, vertical mark is 20 mV

and Ca-dependence on the *Helix* heart, too. Studying the 5HT effect on electrotonic potentials, its definite influence on the membrane resistance was proved which appeared as an increase or a decrease in the conductivity for one or several ions. The decrease in membrane resistance under the influence of 5HT was also found on the retractor and heart muscles of *Modiolus* (HIDAKA et al., 1967; IRISAWA et al., 1973), while on the *Mytilus* heart, the membrane resistance was not affected by 5HT. Using ion-free solutions, the role of Na-, Ca- and Cl-ions was verified in the realization of 5HT effect. The positive afterpotentials appearing under 5HT application proved to be Cl-dependent. The membrane hyperpolarization elicited by 5HT can also be regarded as a result of the increase in Cl-conductivity similarly to one of the 5HT effects found in Gastropod neurones (GERSCHENFELD, 1971). On the hearts of different *Mytilus* species in the 5HT effect a K-dependent phase was also found participating in the hyperpolarization (IRISAWA et al., 1973).

The depolarization caused by 5HT may be explained with an increased conductivity for the Na-ions in the membrane like in the A-response of 5HT at the neurones (GERSCHENFELD, 1973), but the role of Ca- and K-permeability changes should not be excluded either.

In the plateau phase formed by 5HT, both Na- and Ca-ions may participate. In Na-deprivation the shortening of the retarded repolarization period by 5HT may be related not only to the retarded inactivation of Na-permeability

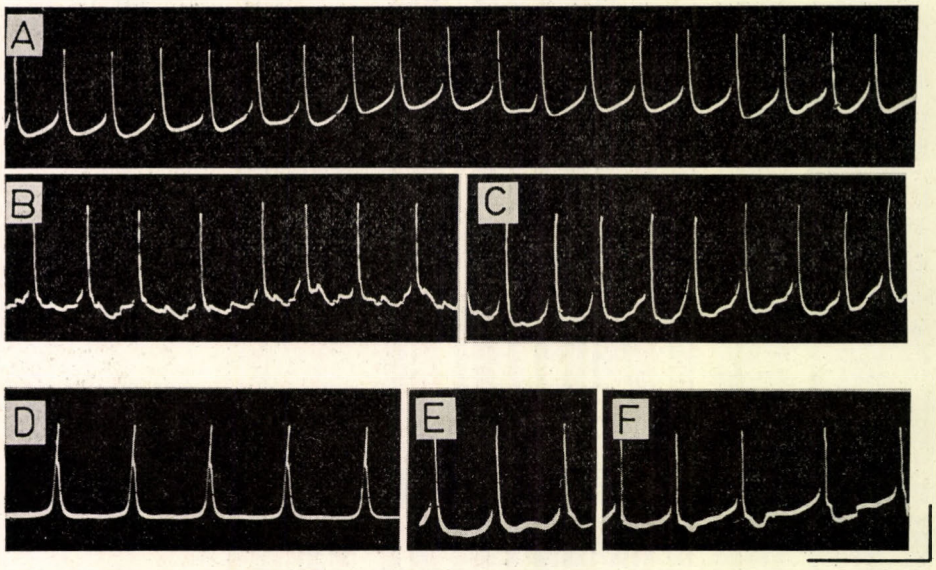

Fig. 9. A — control. *B and C* — simultaneous effect of methylsergide and 5HT (10^{-6}M) on the spontaneous electrical activity at the 5th and 7th min following exchange of solution. *D* — control. *E* and *F* — simultaneous effect of BOL-148 and 5HT (10^{-6}M) on the spontaneous electrical activity at the 4th and 6th min after the application of the above substances. Horizontal mark is 5 sec, vertical mark is 10 mV

but also to changes of the permeability for other (primarily Ca and K) ions evoked by Na-deprivation.

The complex nature of 5HT effect was emphasized with a great variety of ions playing a role in it. Different ions are responsible for the dose-dependent depolarization and hyperpolarization caused by 5HT. It was not unexpected that for the realization of 5HT effect both Na- and Ca-ions are needed, since the generation of the action potentials was found to depend also on the same two ions (Kiss and S.-Rózsa, 1973; S.-Rózsa et al., 1973).

The used antagonists (BOL-148 and methysergide) eliminated the effect of 5HT on the spontaneous electrical activity. Both inhibitors adding together with 5HT hyperpolarized the membrane by 6–8 mM at such concentration (10^{-6} M) which had no effect alone on the membrane potential (with the exception of methysergide). Both antagonists exerted effect owing to their properties as a general 5HT receptor inhibitors and not as specific agent acting on ion permeability. In order to understand the different effects of 5HT it is necessary to use more specific antagonists to be able to separate the receptors.

On the *Helix* heart, 5HT influenced also the intracellular events beside the changes of the electrical properties of the surface membrane. The intracellular effect of 5HT involves the action of adenylate cyclase (Kiss and S.-Rózsa, 1975) causing long-lasting changes in permeability. The connection between the 5HT effect and the second messenger system was proved on the heart of Molluscs, too (S.-Rózsa, 1968; Higgins, 1974; Higgins and Green-

BERG, 1974; WOLLEMANN and S.-RÓZSA, 1975) it is realized by the movement, liberation and reaccumulation of Ca-ions.

Accordingly, in *Helix* heart at the realization of the 5HT effect the Ca-ions play an important and complex role in spite of the fact that the passive electrical properties of the membrane during the 5HT effect was influenced in a higher degree by Na- and Cl-ions.

Summary

Studying the membrane effect of 5HT on the isolated ventricle of *Helix pomatia* L. it was found that:

1. On the heart muscle membrane of *Helix* 5HT had a double effect, at low concentrations (10^{-9}—10^{-7} M) it depolarized, at high concentrations (10^{-5}—10^{-4} M) it hyperpolarized, at 10^{-6} M had no effect, or had only a negligible effect on the membrane.

2. On the electrotonic potentials 5HT had also a double effect. 5HT at high concentrations decreased, while at low concentrations increased the amplitude of the electrotonic potentials. The effects of 5HT are connected with the increase (10^{-9}—10^{-8} M) or decrease (10^{-5}–10^{-4} M) in the conductivity of the membrane.

3. The effect of 5HT on the spontaneous action potentials and on the electrotonic potentials was complex depending on the presence of Na-, Ca- and Cl-ions alike. The positive afterpotentials formed by 5HT were Cl-ion dependent, while Na- and Ca-ions participate in the rising and plateau phases of the action potentials.

4. BOL-148 and methysergide eliminated the effect of 5HT on the membrane and action potentials as well as on the electrotonic potentials. 5HT has, at least, two sites of action on the membrane of *Helix* heart, and, in addition, its intracellular action is proved.

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5-HYDROXYTRYPTAMINE HATÓHELYÉNEK VIZSGÁLATA *HELIX POMATIA* L. SZIVIZOMSEJTJEINEK MEMBRÁNJÁN

Kiss Tibor és S.-Rózsa Katalin

Összefoglalás

Vizsgálva az 5HT membránhatását *Helix pomatia* L. izolált szívkamráján, megállapítást nyert, hogy:

1. Az 5HT *Helix* szív membránján kettős hatású, alacsony koncentrációkban (10^{-9} — 10^{-7}M) depolarizál, magas koncentrációkban (10^{-5}—10^{-4}M) hiperpolarizál, 10^{-6}M koncentrációban membránhatása jelentéktelen, vagy nincs.

2. Az 5HT elektrontónusos potenciálok nagyságát is kettősen befolyásolja. Nagy koncentrációkban az 5HT csökkenti, alacsony koncentrációkban növeli az elektrotónusos potenciálok amplitúdóját. Az 5HT hatás a membrán vezetőképességének növekedésével (10^{-9} — 10^{-8} M), vagy csökkenésével (10^{-5} — 10^{-4} M) kapcsolatos

3. A spontán aktív AP-on és elektrotónusos potenciálon az 5HT hatása összetett volt, Na, Ca és Cl-ionok jelenlététől egyaránt függött. A Cl-ionok az 5HT hatására megjelenő pozitív utópotenciálok megjelenéséért felelősek, a Na és Ca ionok az AP felszálló szárának és a plató fázis kialakításában vesznek részt.

4. A BOL-148 és methysergide az 5HT membrán és akciós, valamint elektrotónusos potenciálokra kifejtett hatását egyaránt megszünteti. Az 5HT-nak legalább két támadáspontja van *Helix* szív membránján, s emellett intracelluláris hatással is rendelkezik.

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SEASONAL ALTERATIONS OF Ca-, K-, Na-, AND Cl-IONS IN THE HAEMOLYMPH OF *ANODONTA CYGNEA* L. A SIMPLE, RAPID DETERMINATION OF Ca- AND Cl-IONS

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Data concerning the ionic composition of the haemolymph of freshwater mussel are quite heterogeneous (HAYES and PELLUET, 1947; FLORKIN and DUCHATEAU, 1950; POTTS, 1953). In our Institute when physiological and biochemical investigations were carried out on *Anodonta* we generally use MARCZINSKY's physiological saline solution (1959). However, the question arose, whether the ionic composition of the haemolymph of the species available over here corresponds to the composition of MARCZINSKY's solution. On the other hand, it is known that the behaviour of the mussel shows a considerable seasonal variety (SALÁNKI and VÉRÓ, 1969; SALÁNKI et al., 1974), furthermore, the osmolarity of the plasma changes depending on temperature (UMMINGER, 1971), therefore, we aimed to estimate the concentration of the most important inorganic ions: Ca^{2+}, Na^{+}, K^{+} and Cl^{-} in the lymph of *Anodonta cygnea* and to clear up the possible seasonal alterations.

Material and method

For our measurements *Anodonta cygnea* specimens of 200 g body weight were used, which were kept in continuously flowing Balaton water at a temperature similar to the natural condition. The lymph of mussels was extracted directly from the heart. For a series of measurement generally the total amount of lymph extracted from five mussels was used. The measurements were performed about on every 25th day of a 14-month period. All of the data presented here are the average of 5–8 measurements. For estimating Na- and K-ions flame-photometric method, for estimating Ca- and Cl-ions spectrophotometry were used. The flame-photometric measurement was performed by a Spectromom 381 L and a direct-pulverizing burner working with hydrogen–oxygen gas mixture was used. The pressure of the hydrogen gas was 0.08—0.10 atm, that of oxygen gas 0.5—0.6 atm, the velocity of pulverization was 2 ml distilled water/min.

For the determination of Ca-ion glyoxal-bis(2-hydroxianil) (GBHA) — a reagent used only lately — was applied. According to the literature (KERR, 1960; UMLAND and MECKENSTOCK, 1960; WILLIAMS and WILSON, 1961;

LAPID and PICKHOLTZ, 1969), the reagent is highly specific to calcium-ion, the determination can be carried out rapidly and with ease.

The spectrophotometric assay was carried out by UNICAM SP 700 spectrophotometer at 520 nm (19.23) wavelength in cuvettes of 1 cm wide. The reagent-solution is yellow and has a slight self light-absorption at 520 nm, which is to be considered by using blind test. For the estimation a calibrating curve was plotted. The accuracy of measurement at 0.01 mM Ca^{2+}/l is ± 5 per cent relative error. A hundredfold amount of Mg-ions, twentyfold amount of Fe(III)-ions and two-threefold amount of phosphate-ions did not influence the results.

The standard solutions are prepared from a stock-solution of 8 mg/l Ca^{2+} concentration as follows: 0.5; 1.0; 2.0; 3.0; 4.0; 5.0 ml of the stock-solution, respectively, is filled up with ion-exchanged water to 10 ml, then 2—2 ml borate-buffer of pH 12,9 and 10—10 ml ethanol are added to the solution. After shaking it up the mixture is rapidly cooled under water-tap and after the addition of 0.5—0.5 ml methanol containing 0.05 per cent GBHA reagent, it is filled up with ethanol to 25 ml. The blank is made in a similar way, the reagents are diluted by 10 ml ion-exchanged water. From the test solution 1 ml sample is transferred to a 25 ml volumetric flask, then the reagents are added. Having prepared the solutions they are allowed to stand for 20 min, light absorption is measured at 520 nm wavelength in a cuvette of 1 cm width. Estimation of the data is done by graphic method, the result is given in mg/l considering the dilution and the primary measuring out of the lymph.

For the determination of chloride a mixture containing mercuric rodanide and Fe(III) reagents (ZALL et al., 1956; IWASAKI et al., 1956; YAMAMOTO et al., 1970) was used. The light sbsorption of the coloured complex is proportional to the quantity of chloride-ions over the range from 1 to 10 mg Cl^-/l concentration.

For the determination of chloride-ions a previously prepared sodium chloride solution of 100 mg Cl^-/l concentration is stored, which are diluted to a concentration of 10 mg Cl^-/l on every occasion. Ferrous ammonium sulphate diluted in nitric acid of 8 per cent and 0.1 per cent of mercuric rodanide diluted in a mixture containing 2 : 1 dioxane and absolute alcohol are prepared as reagents. For preparing the standard solutions 2.0; 4.0; 6.0; 8.0; 10.0 ml of 10 mg Cl^-/l solution, respectively, are measured out and complemented to 10 ml with distilled water. 2 ml of ferrous (III) ammonium sulphate and 3 ml of mercuric (II) rhodanide solution are added and the solution is filled up with distilled water to 25 ml. The blank is made in the same way, the reagents are diluted with 10 ml distilled water. 1 ml of the sample solution is transferred to a 25 ml volumetric flask, then the reagents are added. The extinctions are measured against the blank at 460 nm wavelength in glass cuvettes of 2 cm width after 15—20 min waiting period. The estimation is made by graphic method, the result is given in mg/l considering the dilution and the measuring out.

Preparation of the test solution: 5 ml lymph is diluted with ion-exchanged water to 50 ml. The solution is used for determining the potassium-ion and a given volume of this solution is taken for the determination of Na^+, Ca^{2+} and Cl^-.

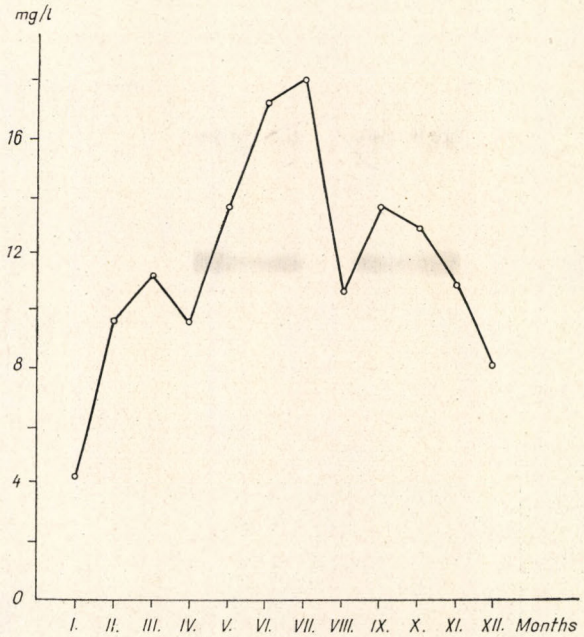

Fig. 1. Seasonal alteration of K-ions in the haemolymph of *Anodonta cygnea* L.

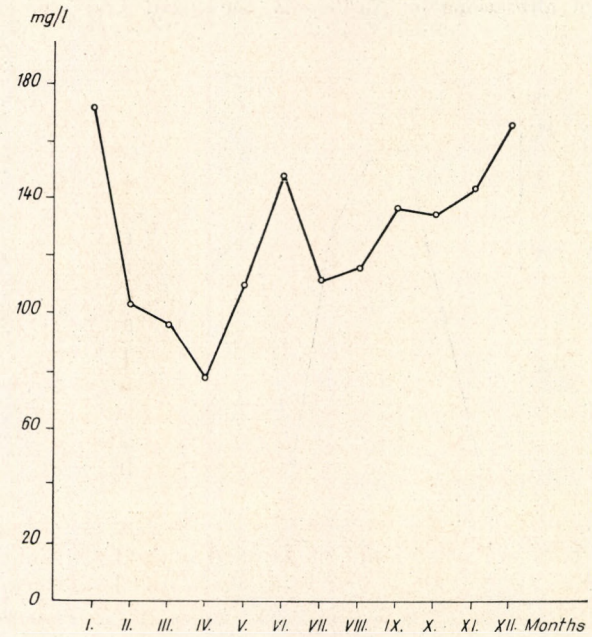

Fig. 2. Seasonal alteration of Ca-ions in the haemolymph of *Anodonta cygnea* L.

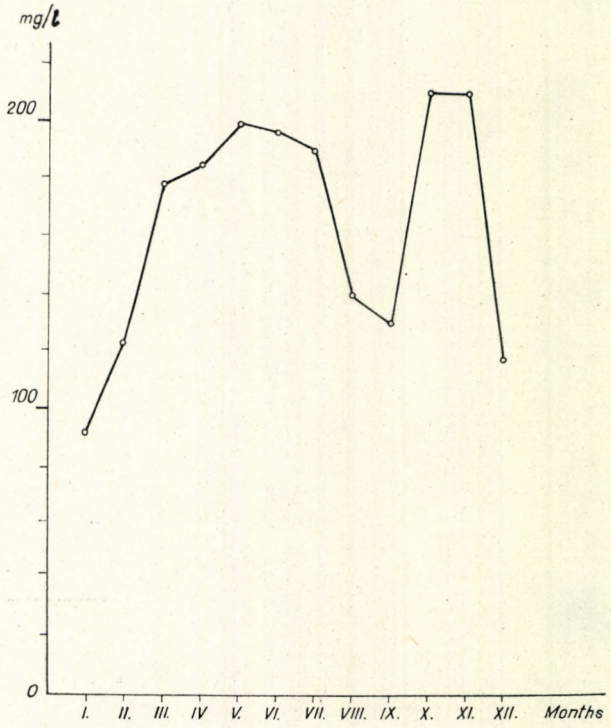

Fig. 3. Seasonal alterations or Na-ions in the haemolymph of *Anodonta cygnea* L.

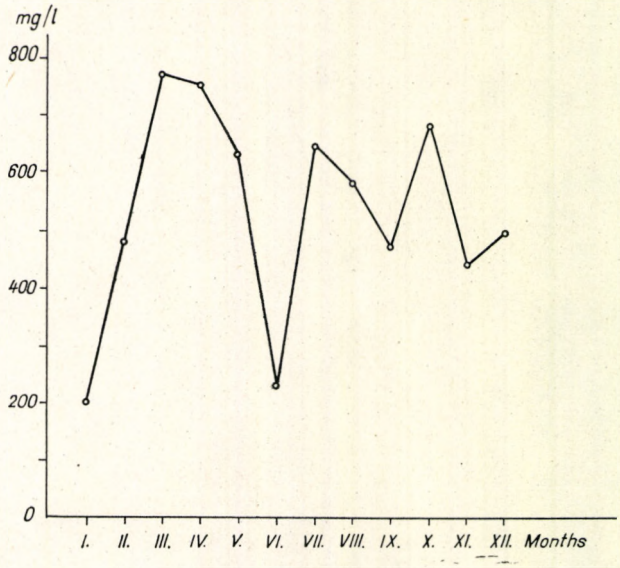

Fig. 4. Seasonal alteration of Cl-ions in the haemolymph of *Anodonta cygnea* L.

Results

According to the results, obtained the concentration of all investigated ions exhibit seasonal alterations in the lymph of the mussel. It is especially apparent, that the seasonal changes in the concentration of K- and Ca-ions are directed oppositely. K-ions are present in the lowest of concentration in winter and highest in summer *(Fig. 1)*. For winter the lowest value (4.2 mg/l) was measured in January. From this time on the concentration of K-ions increases continuously until July. The maximal value was found in July (18 mg/l). Beginning with the next month the concentration of K-ions continually decreases and the value measured in December approaches the minimum recorded in January.

The change in Ca-ions is opposite comparing to that of potassium *(Fig. 2)*, namely, the highest Ca-level was observed in autumn and winter, while the lowest in spring and summer months. The minimum value was 78 mg/l measured in April. From May the Ca-level gradually increases until reaching a maximum — a value of 165—175 mg/l — in December and January. From February till April a decrease can be observed again.

In the case of Na- and Cl-ions the seasonal alteration is of other nature as compared to those observed in the case of K- and Ca-ions. In the seasonal change of Na-ions 2 maximum and 2 minimum values can be seen *(Fig. 3)*. The lowest concentration is measured in January (92 mg/l). Increasing uniformly from February till May the concentration of Na reaches one of the highest peaks (199 mg/l) during the seasonal alteration. From July to September a significant decrease is recorded (130 mg/l), then in October and November another considerable increase in concentration occurs again. The values measured in these two months give the highest — 210 mg/l — value during the seasonal change of Na-ions. The December value approaches again the January minimum.

The seasonal alterations of Cl-ions *(Fig. 4)* appeared to be the most irregular ones. Also in this case the minimum value (200 mg/l) was measured in January, however, a similar minimum of Cl^--concentration (230 mg/l) can be observed in June, too. The highest values: 775 mg/l and 755 mg/l were measured during the spring, in March and April. The values found in summer and in autumn are heterogeneous. Since the most considerable portion of the total ionic concentration of the haemolymph is constituted by the ions measured by us, on the basis of this also osmotic pressure was calculated considering the concentration of the above four ions, which also showed seasonal alteration *(Fig. 5)*. The osmotic pressure of the lymph exhibits the lowest value — 23.4 mOsmol $\pm$ 2.77 (S.E.M.) — in winter, while in the other season it is 30.9 $\pm$ 1.44 (S.E.M.) — 33.4 mOsmol $\pm$ 0.75 (S.E.M.) showing no significant fluctuation.

Discussion

The character of the alteration of Ca-ions is in good agreement with the data of A. Tilgner-Peter (1958) obtained for snails. These results demonstrated that in the haemolymph of the snail the Ca^{2+}-concentration measured in winter was much higher than that measured in summer. Barfurth (1881) and Burton (1972) also observed that the quantity of the Ca-rich cells, the

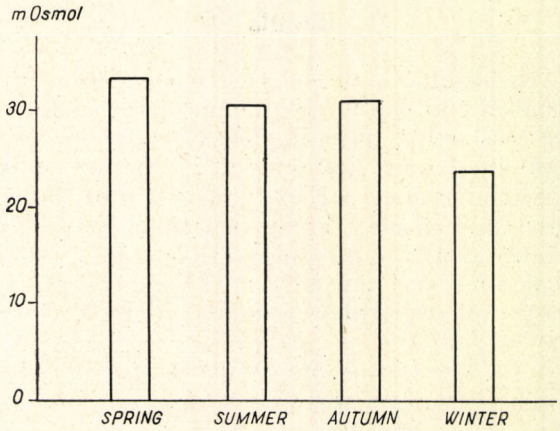

Fig. 5. Seasonal alteration of the osmotic pressure in the haemolymph of *Anodonta cygnea* L.

so-called Ca-sphaerules of *Helix pomatia* showed a marked decrease during winter, which may be a reason of the higher Ca-concentration of the lymph, since Ca-accumulation taking place in summer is eliminated in winter.

Among the estimated four ions Cl-ions were present in highest concentration.

Calculating the average values of the observed ionic concentrations and comparing them with the data of other authors referring to the concentrations of the same ions, the following conclusions can be drawn *(Table I)*: The ionic

TABLE I

Ionic concentrations measured by different authors in the haemolymph of Anodonta cygnea L.

	concentration mg/l			
	Na	K	Ca	Cl
POTTS (1953)	358	19	337	415
MARCZINSKY (1959)	444	30.4	17.7	803.6
Present results	164	12	126	538

concentrations measured and described by different authors differ from each other quite substantially. The annual average value of the concentration of Na-ion measured by us is only half of the value given by POTTS and one-third of that given by MARCZINSKY. In the case of K-ions a deviation of approximately similar proportions is obtained considering the data of the above two authors, as POTTS gave 19 mg/l, while MARCZINSKY 30.4 mg/l for the concentration of K-ions of *Anodonta* lymph, contrarily to the value of 12 mg/l measured by us.

The most considerable difference can be found in the case of Ca-ions. The Ca^{2+}-level is only about two-fifths of the value (337 mg/l) given by POTTS and 7 times greater than that given by MARCZINSKY (17.7 mg/l).

In the case of Cl-ions, the Cl^--concentration measured in our work (438 mg/l) is lower than that described by MARCZINSKY (804 mg/l) and higher than that published by POTTS (415 mg/l). The differences in comparing the ionic concentrations may partly result from the fact that the determination of the ionic composition was not carried out in the same season by the above authors, on the other hand, it may happen even in the same species, that the composition of the lymph depends on the external environment. For example, it is known, that the same species of mussels or snails living in different type of water forming their environment is characterized by different Ca-metabolism. thus, the Ca^{2+}-level of the lymph may likewise be different. One of the outward forms of this, that the snails and mussels living under different environmental conditions secrete thinner or thicker shells depending on the Ca^{2+}-level of water.

The fact is also known, that in the case of the fresh-water species the osmotic pressure of the plasma decreases in the course of cold-adaptation. The osmotic pressure calculated from the ionic concentrations measured by us is also is in good agreement with the above establishment.

Summary

Investigating the seasonal alterations of Ca-, K-, Na-, and Cl-ions in the haemolymph of *Anodonta*, the following results were obtained:

1. All the examined ions exhibit seasonal changes.

2. The seasonal alterations of Ca- and K-ions are directed oppositely: the highest concentration of Ca-ions was measured in winter, the lowest in spring and summer, while in the case of K-ions the maximum value can be determined in the summer months and the minimum in the winter months.

3. The seasonal alterations of Na- and Cl-ions are about the same.

4. The method for determining Ca and Cl can be carried out rapidly and with ease, therefore, it is suitable to estimate the concentration of Ca- and Cl-ion in other biological objects, too.

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Ca-, K-, Na- és Cl-IONOK SZEZONÁLIS VÁLTOZÁSA *ANODONTA CYGNEA* L. HAEMOLYMPHÁJÁBAN. Ca- és Cl-IONOK EGYSZERŰ, GYORS MEGHATÁROZÁSA

Nemcsók János és D. J. Szász Ágnes

Összefoglalás

Vizsgálva *Anodonta* haemolymphájában a Ca-, K-, Na- és Cl-ionok szezonális változását a következőket kaptuk:

1. Valamennyi vizsgált ion szezonális változást mutat.
2. A Ca- és K-ionok szezonális változása ellentétes irányú: a Ca-ionok koncentrációja télen a legmagasabb, tavasszal és nyáron a legalacsonyabb, míg a K-ionok esetében a maximum a nyári, a minimum pedig a téli hónapokban mérhető.
3. A Na- és Cl-ionok szezonális változása nagyjából megegyező.
4. Az általunk használt Ca- és Cl-meghatározás gyorsan és könnyen elvégezhető, ezért alkalmas más biológiai minták Ca- és Cl-ion koncentrációinak meghatározására is.

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MONOAMINES IN THE CENTRAL NERVOUS SYSTEM OF *LYMNAEA STAGNALIS* (GASTROPODA) AND THE EFFECT OF PHARMACONS ON THE MONOAMINE LEVEL

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The significant concentration of biogenic monoamines, the existence of the synthetizing and decomposing enzymes (OSBORNE and COTTRELL, 1970 MARSDEN, 1972; HIRIPI 1970) as well as the data obtained by examination of their functional role (GERSCHENFELD, 1973) refer to a transmiitter function of monoamines in the organism of Gastropods. Serotonin and dopamine have been demonstrated in a number of Gastropods, while the identification of noradrenaline in the central nervous system of some species was successful only lately (OSBORNE and COTTRELL, 1970; HIRIPI, 1972; JOURIO and KILLICK, 1972).

In our previous examinations a significant concentration of serotonin was measured in the central nervous system of *Lymnaea stagnalis* (HIRIPI, 1968), at the same time the quantity of dopamine and noradrenaline in this species is unknown. According to our earlier examinations in the nervous system of *Lymnaea* MAO takes part in the enzimatic elimination of 5HT (HIRIPI, 1970) but the same enzyme is also implicated in the break-down of catecholamines. Therefore, the inhibition of MAO can be expected to result in the increasing of 5HT, DA and NA. Our investigations performed on a Pelecypod *(Anodonta cygnea)* have shown that the effect of MAO-inhibitors affecting the monoamine level changes the behaviour of the animals (HIRIPI, 1973; HIRIPI et al., 1974) and a similar phenomenon may occur also in *Lymnaea*.

Our examination was aimed at determining concentration of dopamine and noradrenaline in the central nervous system of *Lymnaea*. In addition, the effect of iproniazid, nialamid and tranylcypromine inhibiting MAO, pCPA inhibiting the synthesis of 5HT as well as the effect of reserpin on the membrane of vesicles participating in the storage of monoamines upon the level of serotonin, noradrenaline and dopamine and on the behaviour of the animals were investigated.

Material and method

For the experiments adult specimens of *Lymnaea stagnalis* were used. Catecholamines were estimated by the method of ANTON and SAYRE (1962; 1964) while serotonin by the method of SNYDER et al. (1965).

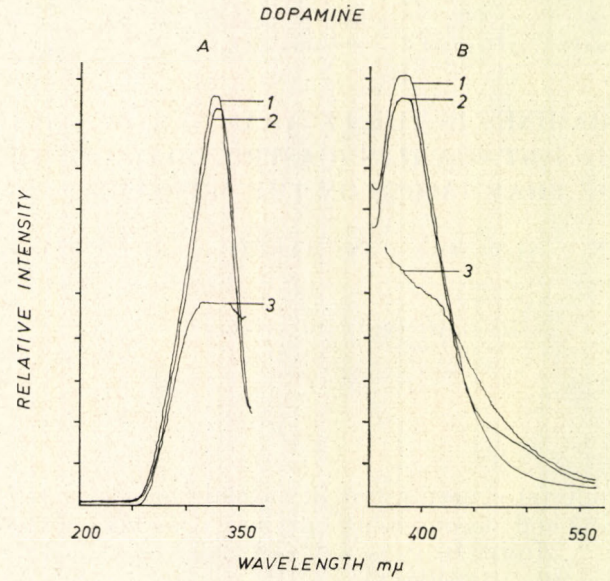
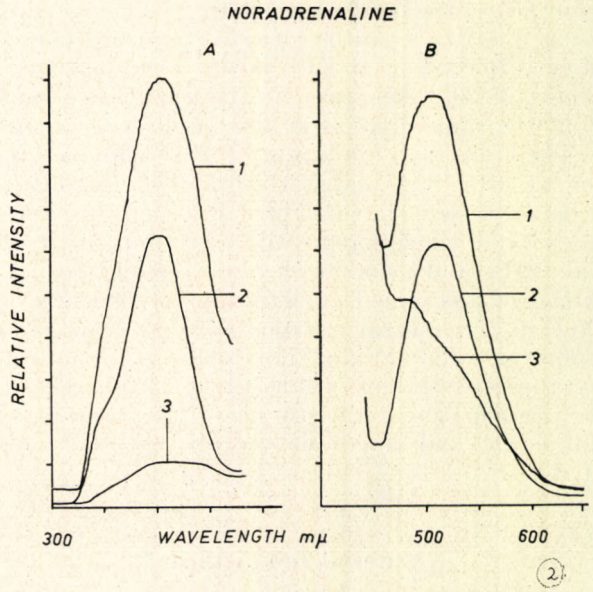


Fig. 1. a) Excitation *(A)* and emission *(B)* spectrum of dopamine. The maximum of excitation spectrum is 325 mμ The maximum of emission spectrum is 385 mμ. 1 — tissue homogenizate, 2 — authentic DA, 3 — blank



b) Excitation *(A)* and emission *(B)* spectrum of noradrenaline. The maximum of excitation spectrum is 415 mμ. The maximum of emission spectrum is 519 mμ. 1 — tissue homogenizate, 2 — authentic NA, 3 — blank

The pharmacological treatment was carried out as follows: the pharmacon was dissolved in filtered Balaton-water, then 15 snails were placed in 1 litre of solution and the animals were kept at room temperature for 24 hours. At the end of the first day the animals were transferred back to Balaton-water. The control animals were kept in Balaton-water for 24 hours at room temperature. The experiments were carried out during summer. The results mean the average of 3—5 measurements.

The name of the pharmacons and their concentrations are listed below: p-chlorophenylalanine-(pCPA)-methylaester-HCl 100 mg/l (SERVA Finebiochemia); Reserpin (Rausedyl inj.) 1 mg/l (Kőbányai Gyógyszerárugyár); Nialamid (SIGMA Chemical Company) 5×10^{-4} M; Iproniazid (SIGMA Chemical Company) 5×10^{-4} M; trans-2-phenylcyclopropylamine (Tranylcypromine) 10^{-4} M (Aldrich Europe).

The concentration of serotonin, dopamine and noradrenaline was measured on the 1st, 2nd, 3rd and 6th day after the application of the pharmacons. During the entire experimental period the behaviour of the animals was watched with attention.

Results

a) *5HT, DA, NA in the ganglion of Lymnaea*

The results of the fluorimetric examinations indicate the existence of dopamine and noradrenaline besides serotonin in the nervous system of *Lymnaea*. The spectra of dopamine and noradrenaline isolated from the nervous system were identical with that of authentic DA and NA *(Fig. 1A, B)*. Namely, the maximum of the excitation spectra was at 325 and 415 mμ, while the maximum of emission spectra was at 385 and 519 mμ, respectively. The concentration of serotonin was found to be 20.35 $\pm$ 1.06 μg/g (S.E.M.), that of noradrenaline 0.63 $\pm$ 0.08 μg/g (S.E.M.), while that of dopamine 12.98 $\pm$ $\pm$ 0.46 μg/g (S.E.M.).

b) *Effect of pharmacons on the serotonin level*

The most significant alteration in the serotonin level was caused by reserpin *(Fig. 2)*. At the end of the first day ,following the application of the pharmacon, the serotonin level shows a 50 per cent decrease and on the third day the degree of decrease is approximately 80 per cent. On the basis of the observations the effect of pharmacon on the activity of the animals can be divided into two phases: on the first day, the activity of the animals increases then markedly decreases on the 2nd and 3rd day following the application of the pharmacon. At this time animals sink to the bottom of the vessel, they stop moving and the majority of the animals lie in upturned position (foot up) withdrawn into the shell. The death of the animals occurs on the 2nd day, and on the 3rd day 60—65% of them are perished. On the 4th day, after the application of the pharmacon, no live animal was found.

pCPA also decreased the serotonin level significantly *(Fig. 2)*. The decrease reached a maximum between the 3rd and 6th day following the application of the pharmacon, namely, at this time, the serotonin level was

about 40 per cent compared to that of the control animals. Following the treatment the motility of the animals slightly decreased.

Among the MAO inhibitors nialamid and iproniazid *(Fig. 3)* decreased the serotonin level gradually by 25—30 per cent until the 3rd day, then on the 6th day a restoration of serotonin level ensued. The effect of tranylcypromine is somewhat different *(Fig. 3)*: the level of 5HT measured on the first day hardly differs from that of the control, the level of 5HT measured on the 6th day exhibits a 50 per cent decrease.

c) *Effect of pharmacons on the level of CA*

Reserpin did not significantly alter the level of dopamine and noradrenaline on the first day following the treatment. However, on the 2nd day already a notable decrease was recorded both in dopamine and in noradrenaline level, i.e. the level of noradrenaline fell to 40 per cent, while that of dopamine to 60 per cent *(Fig. 3)*.

Tranylcypromine affected the level of dopamine and noradrenaline diversely *(Fig. 4)*: it did not significantly alter noradrenaline level on the first day, while on the 2nd day noradrenaline level decreased to 65 per cent, then restored on the 3rd day and a 20 per cent increase was measured. Dopamine level was hardly influenced by tranylcypromine.

Discussion

The concentration of serotonin approximately corresponds to previous data (Hiripi, 1968). The concentration of dopamine is in good agreement with the data described in other snails, while in the case of noradrenaline the concentration measured in the present work is the highest one, which has ever been found in Gastropods (Welsh, 1972). It is possible that the quantitative differences are connected with the significance of a given monoamine in the regulation of seasonal changes, but there may also be differences in the storing system.

The behavioural change caused by reserpin is in agreement with that found in other Gastropod species (Mirolli and Welsh, 1965; Kerkut et al., 1966), as well as with that observed in the fresh-water mussel (Hiripi, 1973). The initial increase in the activity probably is caused by a part of monoamines released from the storing site by reserpin and depleted rapidly toward the periphery exerting an excitatory effect there. The considerable decrease caused by reserpin can be accounted for that reserpin — as it is known — not only depletes the neurotransmitters via affecting the membrane of the storing vesicles but also inhibits their rebuilding.

pCPA caused a considerable decrease in the 5HT level, which obviously can be explained by decreasing the serotonin level to a high degree via the inhibition of the activity of tryptophane-hydrolaze by pCPA (Koe and Weissmann, 1968).

The MAO inhibitors did not increase but rather decreased the 5HT level in the central nervous system of *Lymnaea* similarly to the results obtained in *Anodonta* (Hiripi et al., 1974), although in the central nervous system of *Lymnaea* MAO activity was previously demonstrated (Hiripi, 1970). It has

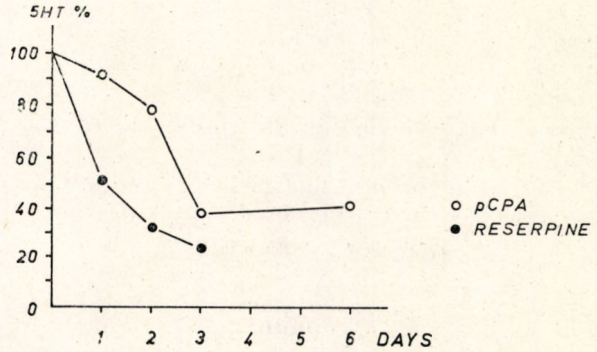


Fig. 2. Effect of pCA and reserpin on 5HT level

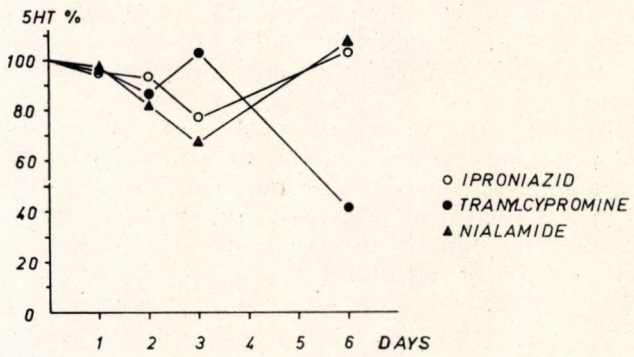


Fig. 3. Effect of MAO inhibitors on 5HT level

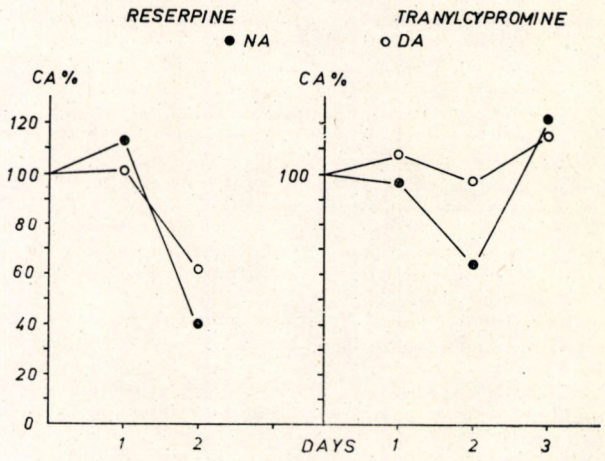


Fig. 4. Effect of tranylcypromine and reserpin on CA level

been known that the mechanism of the effect of tranylcypromine, a MAO inhibitor, considerably inhibits monoamine uptake (KNOLL and MAGYAR, 1972). Consequently, the decrease in the serotonin level caused by tranylcypromine presumably is a result of this process, while the decrease in the serotonin level resulted by nialamid and iproniazid cannot be accounted for at this moment.

The effect of tranylcypromine on the level of noradrenaline and dopamine probably is also associated with the depletion of catecholamines by the pharmacon from the storing vesicles to the periphery.

Summary

1. The excitation and emission spectra recorded from the tissue sample correspond to the excitation and emission spectra of DA and NA.

2. Besides serotonin the central nervous system of *Lymnaea stagnalis* contains dopamine (12.98 μg/g) and noradrenaline (0.63 μg/g) to a high degree. DA and NA present in a significant concentration besides serotonin may suggest that DA and NA also play a significant role in the neurotransmission in *Lymnaea stagnalis*, too.

3. Reserpin and pCPA decreased the serotonin level to a high degree. Nialamid, iproniazid and tranylcypromine known as MAO inhibitors contrary to the expectation, did not increase but rather decreased serotonin level.

4. The reserpin treatment decreased considerably also the level of DA and NA, while tranylcypromine decreased only the NA level noticeably, and hardly altered the level of dopamine.

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MONOAMINOK A *LYMNAEA STAGNALIS* (GASTROPODA) KÖZPONTI IDEGRENDSZERÉBEN ÉS FARMAKONOK HATÁSA A MONOAMINSZINTRE

Nemcsók János, L. N. Markova és Hiripi László

Összefoglalás

1. A szövetmintából felvett gerjesztési és emissziós spektrumok megegyeznek a DA és NA gerjesztési és emissziós spektrumaival.

2. *Lymnaea stagnalis* központi idegrendszere a szerotonin mellett jelentős mértékben tartalmaz dopamint (12,98 μg/g) és noradrenalint (0,63 μg/g). A szerotonin mellett jelentős koncentrációban előforduló DA és NA alapjául szolgálhat annak a nézetnek, hogy a DA és NA is jelentős szerepet játszik a neurotranszmissziós folyamatokban *Lymnaea stagnalis* esetében is.

3. A reseprin és pCPA jelentős mértékben csökkentette a szerotoninszintet. A MAO-gátlóként ismert nialamid, iproniazid és tranilcipromin a várakozástól eltérően nem emelte, hanem inkább csökkentette a szerotoninszintet.

4. A reserpinkezelés a DA és NA szintjét is jelentősen lecsökkentette, míg a tranilcipromin csak a NA szintjét csökkentette figyelemre méltóan, a dopamin szintjét azonban alig változtatta meg.

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EFFECT OF VERATRINE ON THE HEART RECEPTORS AND CENTRAL NEURONES REGULATING HEART BEATS IN *HELIX POMATIA* L. (GASTROPODA, MOLLUSCA)

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In vertebrates veratrine is known as a vagus excitator giving rise to the BEZOLD-JARISCH's reflex when infused intracardially and causes in this way bradycardia and a decrease in blood-pressure (JARISCH and RICHTER, 1939). Although veratrine has some effect also on the presso- and chemoreceptors of sinus caroticus, caroticus communis and lung of vertebrates, it is taken as a specific agent for the excitation of heart mechanoreceptors (NEIL et al., 1949; KILLIP, 1963). The isolated hearts are influenced in the same manner by veratrine and heart glycosides increasing Na-inward current and changing K-permeability resulting in the depolarization of the heart cell membranes following arrhythmia when the application is prolonged (BARTELS and ROSENBERRY, 1973; HORACKOVA and VASSORT, 1974; SPERELAKIS and PAPPANO, 1969).

The presence of mechano-, and chemoreceptors in the *Helix* heart as well as their representation in the central nervous system was proved by our earlier results (S.-RÓZSA and SALÁNKI, 1973 a, b; 1974). Since a considerable overlap was found in the central representation of the two receptors, an attempt was made to separate them by using veratrine. In the present paper the reaction of the central neurones is described giving answer to the stimulation of heart mechano- and chemoreceptors during intracardial perfusion by veratrine.

Material and method

The experiments were carried out on active *Helix pomatia* L. at room temperature (20—22°C) in autumn and winter. Snails kept in hibernation during winter were waken and activated. In the experiments the brain-heart preparation of *Helix pomatia* described earlier was used (S.-RÓZSA and SALÁNKI, 1973 a). For keeping the tissues wet and for solving substances Meng-solution (MENG, 1958) was used. Mechanoreceptors were stimulated on the heart surface by using a fine brush.

The electrical activity of the intestinal nerve was recorded extracellularly by bipolar Ag-AgCl electrodes, while the membrane and action potentials of the central neurones were recorded with glass microelectrodes filled with 2.5 M KCl, with resistance from 5 to 15 MOhm. In the experiments a high

input impedance amplifier (VÉRÓ, 1971) was used, and the polarization of central neurones could be performed with an appropriate bridge circuit. In one part of the experiments, in order to amplify and record the potentials, ALVAR instruments were used, while when besides the nerve and neurone activities, the heart contractions were also registered, the equipment BIOCOMB-5 (Orion, EMG-4581) was used, assuring simultaneous recording for the three events.

The identification and mapping of the investigated central neurones were described earlier (S.-RÓZSA and SALÁNKI, 1973 a, b). Veratrine sulphate (Merck) was used intracardially.

Results

1. *The effect of intracardially perfused veratrine on the activity of heart nerve and central neurones*

During intracardial perfusion of veratrine intensive afferent signalization has started from the heart to the CNS which could be traced at the level of central neurones, too. Veratrine from 10^{-6} M increased the frequency of the potentials of the heart nerve characterizing the excitation of mechano- and chemoreceptors *(Figs 1, 2* and *3)*. According to our earlier results (S.-RÓZSA, 1972) the excitation of chemoreceptors caused changes in the heart nerve activity with 200—300 μV amplitude while the excitation of mechanoreceptors influenced the potentials with 300—500 μV amplitude.

The effect of intracardially given veratrine is shown on cell V13 *(Fig. 1)*. This cell according to our earlier results (S.-RÓZSA and SALÁNKI, 1973 b) was responding to tactile stimulation of the heart with a decrease in frequency, while the excitation of the heart chemoreceptors caused an increase in its firing. In *Fig. 1* the incomplete inhibition of the firing of cell V13 can be seen during tactile stimulation of the heart *(Fig. 1B)*, then the effect of veratrine follows *(Fig. 1C)*. It is obvious from these figures that the two effects cannot be regarded identical, and the veratrine effect is not confined only to the mechanoreceptors of the heart. This suggestion is supported by the fact that a decrease in the firing rate of cell V13 during veratrine perfusion is lower than that during the tactile stimulation of the heart *(Fig. 1B, C)*, so the effect of veratrine also reflected the influences on the chemoreceptors of the heart. In prolonged intracardial perfusion of veratrine the heart nerve activity becomes periodic, then the firing pattern of various intensity and frequency began to run towards the central nervous system *(Fig. 1C, D)*. The intensity of this firing decreased in a time-dependent manner. Comparing the intensity of the heart nerve activity with the 3rd and 6th min of veratrine perfusion, a 60% decrease in the firing rate is found *(Fig. 1C, D)*. Periodicity in the heart nerve activity was not observed during the stimulation of heart mechanoreceptors.

There were some central neurones showing different responses to the tactile stimulation of the heart and veratrine perfusion although in both cases the increased electrical activity run from the heart nerve to the central neurones *(Fig. 2B ,C)*. In the case showen in *Fig. 2B* the activity of the central neurone was inhibited by means of postsynaptic potentials arising upon the

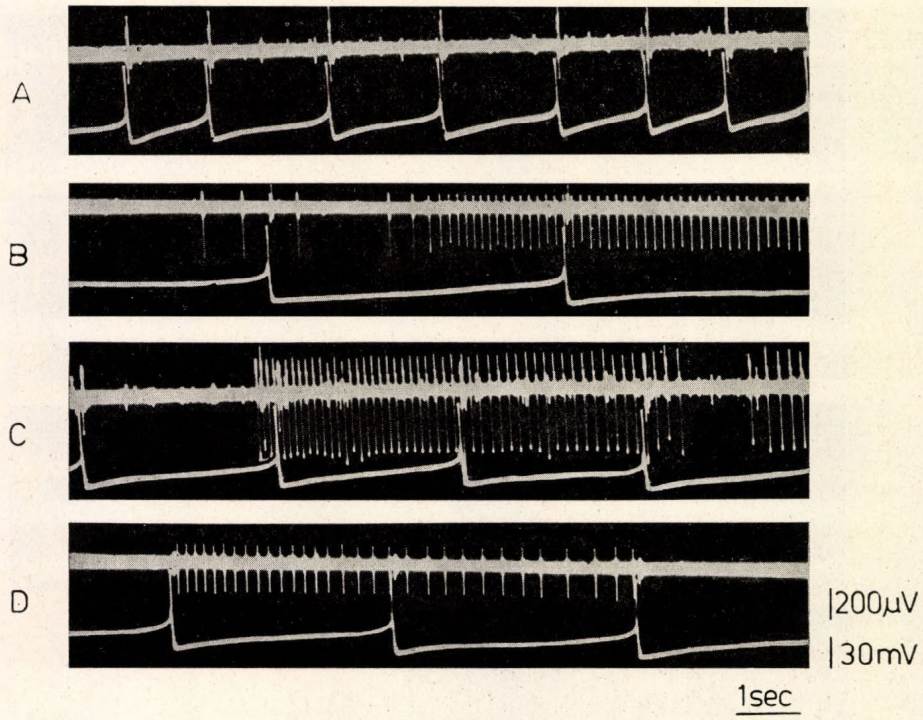

Fig. 1. The effect of intracardial perfusion of veratrine and that of the tactile stimulation of the heart on the heart nerve and cell V13. Here and in *Figs 2* and *3:* upper — extracellular recording from the heart nerve; lower — intracellular recording from the soma of the neurone. *A* — control; *B* — tactile stimulation of the heart; *C* — intracardial perfusion of veratrine at concentration 10^{-6}M, the recording was made 3 min after from the beginning of the perfusion; *D* — the effect of veratrine at the 6th min of its perfusion

tactile stimulation of the heart. After intracardially administered veratrine the inhibition of the same neurone was observed only for a short time (*Fig. 2C*, first part), then in comparison with the control a 60% increase in frequency was recorded *(Fig. 2C*, second part). Washing out veratrine from the heart the control value of the activity was restored *(Fig. 2D)*.

Some further central cells had no definite response to the tactile stimulation of the heart *(Fig. 3B)*, but to the intracardial perfusion of the veratrine their activity changed significantly for a long time *(Fig. 3C, D)*. In this case the double reaction was observed on the central neurone since at the beginning of veratrine perfusion (10^{-4} M) the firing of the neurone was inhibited, then the neurone was synaptically activated *(Fig. 3C, D)*. The synaptic potentials appearing on the neurone were synchronous with the potentials running from the periphery to the heart nerve *(Fig. 3D)*. At the third minute of intracardial perfusion of veratrine the central effect took also periodical character *(Fig. 3C)*.

No central neurone was found to give the same response to the stimulation of mechanoreceptor and that of the intracardial perfusion of veratrine.

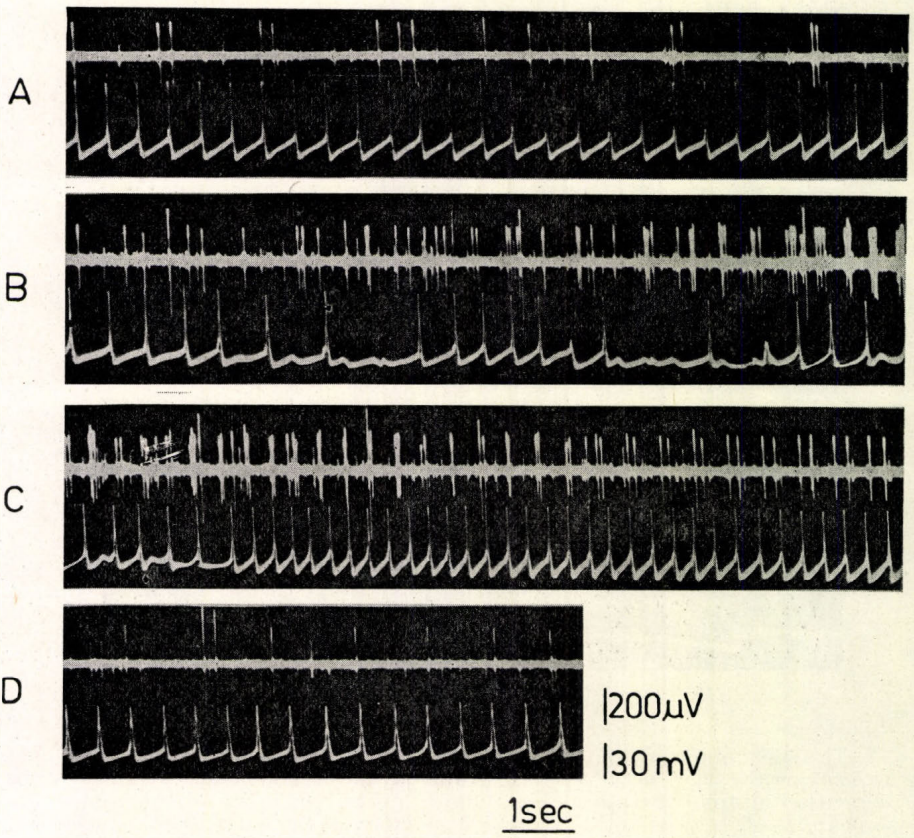

Fig. 2. The effect of veratrine and tactile stimulation of the heart. *A* — control; *B* — tactile stimulation of the heart; *C* — effect of intracardial perfusion of veratrine at concentration 10^{-6}M; *D* — after washing out veratrine

2. *The direct and reflex influences of veratrine on the heart muscle*

In order to study the local and the reflex changes in the heart beats during intracardial perfusion of veratrine, the heart contractions were registered simultaneously with the heart nerve and central neurone activities. This method allowed us to study the effect of central regulatory unit in the modification of heart rate.

These investigations were carried out on the motoneurones (V12, V13) identified earlier and interneurones (V20, V21). From the two motoneurones the firing rate of V12 was increased under the stimulation of heart mechanoreceptors, while the chemoreceptor excitation resulted in the biphasic answer (S.-Rózsa and Salánki, 1973 b). During intracardial perfusion of veratrine (10^{-5} M) cell V12 also showed biphasic response; depending in direction from the type of the initial cell activity. During intracardial perfusion of veratrine the changes in the activity of cell V12 reflected the activation of mechano- and chemoreceptors too *(Fig. 4A, B)*. During veratrine perfusion the increase

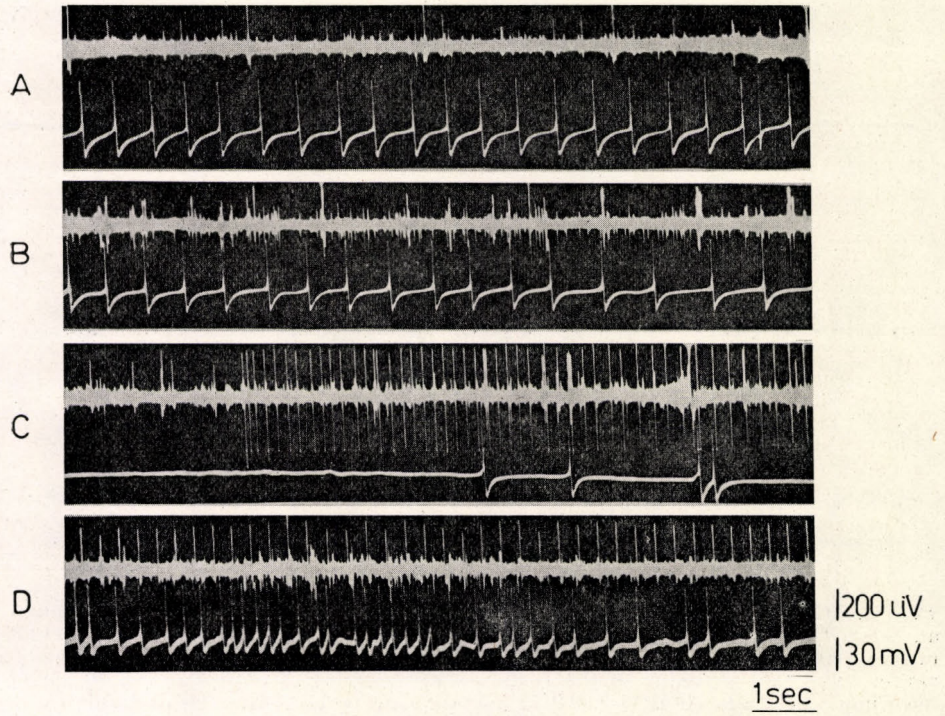

Fig. 3. The effect of veratrine and tactile stimulation of the heart. *A* — control; *B* — the tactile stimulation of the heart; *C* — effect of veratrine at concentration 10^{-4}M; *D* — the effect of veratrine at the 3rd min of its perfusion

in heart nerve activity is observed. In the first case the heart nerve activity was showing the activation of chemoreceptors *(Fig. 4A)*, while in the second case the activation of mechanoreceptors also took place especially at the beginning of the veratrine effect *(Fig. 4B)*. In the case when the activity of cell V12 was increased during veratrine perfusion it was the result of synaptic activation, as the pre-potential of action potentials on cell V12 increased significantly *(Fig. 4B)*. On cell V13 also the activation of chemoreceptors prevailed over the mechanoreceptors during veratrine perfusion and as a result the firing of cell V13 was increased *(Fig. 4C)*.

Veratrine did not increase heart contractile activity as a result of its direct effect on the heart muscle. On the contrary, the heart beats stopped for several seconds after giving veratrine (*Fig. 4A, B, C*, middle lines) then after 30—60 sec the heart beats were restored and some increase in frequency was observed (*Fig. 4*, second part), however, it can be assigned to the reflex influences of veratrine and not to its direct effect on the heart muscle.

From the interneurones taking part in the regulation of heart beats the reaction of cells V20 and V21 was studied during intracardial perfusion of veratrine. Cell V20 is a vasoconstrictor and receives inputs from many receptor areas of the cardio-vascular system (S.-Rózsa, 1975). In the first phase of veratrine perfusion it reacted with decrease in frequency and amplitude, then

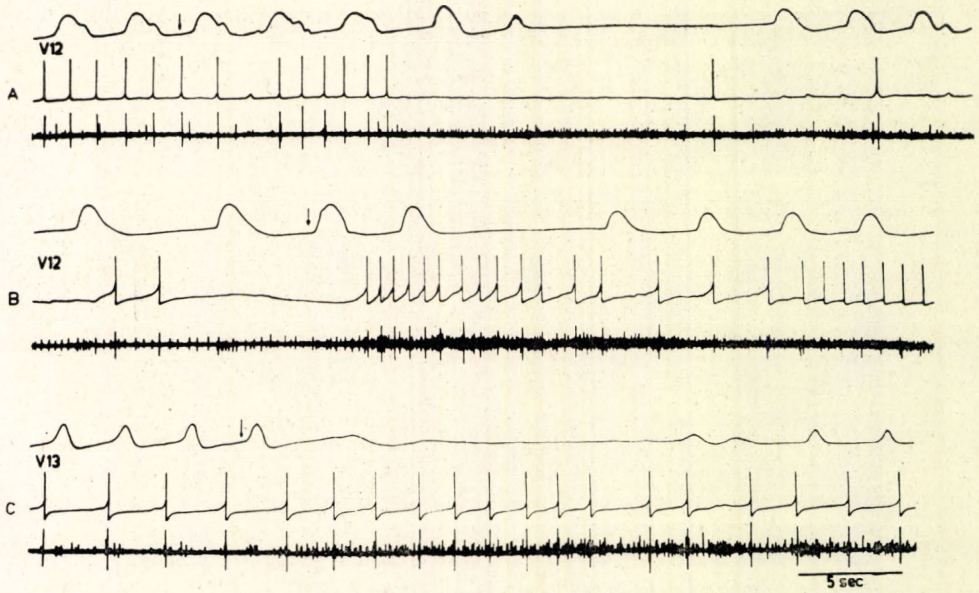

Fig. 4. Effect of veratrine on the heart, heart nerve and activity of motoneurones V12 and V13. Here and in *Fig. 5:* upper — the contractile activity of the heart; middle — intracellular activity of the central neurone; lower — extracellular activity of the heart nerve. ↑ — the beginning of the perfusion of veratrine at concentration 10^{-5}M. This recording was made on BIOCOMB-5, not allowing to ascertain the absolute value of potentials but the two extracellular potentials different in amplitude on the heart nerve correspond also to the excitation of chemo- or mechanoreceptors of the heart

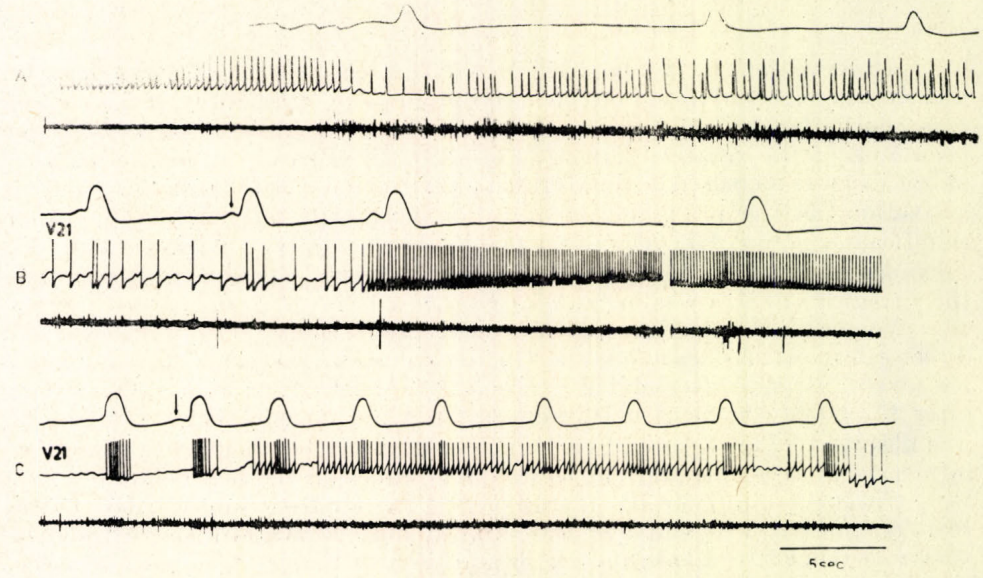

Fig. 5. Effect of veratrine on the heart, heart nerve and activity of interneurones V20 and V21. The registrations as in *Fig. 4* the perfusion of veratrine at concentration 10^{-5}M

it produced for a long time doubled potentials differing in amplitude. The effect was evidently connected with synaptic activation appearing inevitably upon veratrine perfusion.

Interneurone V21 having tonic or phasic types of activity (S.-Rózsa, 1975) showed variable reaction to the intracardial perfusion of veratrine. In some cases the activity of cell V21 turned to phasic remaining such even after the cessation of veratrine perfusion *(Fig. 5B)*, while in other cases neither the burst-type activity nor the heart beats were influenced by veratrine perfusion *(Fig. 5C)*, nevertheless, in this later case the firing rate of cell V21 was again increased. In all but one experiments *(Fig. 5C)* veratrine caused short-term inhibition in the contractile activity of the heart.

The effect of veratrine was easily eliminated by washing out, but during application it is able to keep the heart receptors active for a long period of time. The periodicity in the response was mainly observed when using high concentrations (10^{-4}—10^{-3} M) of veratrine.

Discussion

Apart from mammalian hearts where veratrine has a specific effect on the mechanoreceptors (Jarisch and Richter, 1939; Neil et al., 1949) in *Helix* heart the chemo- and mechanoreceptors are alike sensitive to veratrine. The representation of baro- and chemoreceptors is separated at the cellular level in the cardio-vascular centers of the brain stem of mammals (Miura and Reis, 1972), but in the central nerve system of *Helix* the same neurone responds to the stimulation of heart mechano- and chemoreceptors (S.-Rózsa and Salánki, 1973 b). The same conclusion can be drawn from the veratrine treatment of the heart as the excitation of both mechano- and chemoreceptors can be traced on the motoneurones and interneurones (*Figs 1, 4* and *5*).

In *Helix* heart the veratrine effect took place mainly on the heart receptors because the contractions were influenced only for a short time. Its specific effect is supported also by the fact that on the central neurones the changes arose as a result of synaptic activation (*Figs 3* and *5*). In most cases the changes in the firing of central neurones during intracardial perfusion of veratrine was elicited by excitatory postsynaptic potentials. Neither on the motoneurones nor on the interneurones were these effects monosynaptic. This suggestion is supported by the fact, that during veratrine perfusion the reaction opposite in sign can be observed at some neurones (V13, V12) emphasizing dependence of the central effect of veratrine besides the activation of peripheral receptors also from the properties of other information occurring in the neuronal network. Taking into account this information, too the potentials started from the periphery under the influence of veratrine can produce excitation or inhibition at the given central neurone. These results supporting our earlier findings proved the coupled character of central regulation of heart beats and of the type of regulation based on the functioning of interneurones with double actions. In *Helix* neither on the periphery nor in the central neurones an effect, specific for mechanoreceptors, of veratrine was found concerning mammals (Korner, 1971; Miura and Reis, 1972).

In CNS of Molluscs the neurones firing synchronously with heart beats are as rare as in mammals (Seller and Illert, 1969; Miura and Reis, 1972),

where the neurones with burst activity are regarded as target cells of the heart baroreceptors. However, at the central nervous system of *Helix* cell V21 showing burst activity synchronously with the heart beat was target cell not only for information coming from heart receptors, but this is the cell with changing activity under the activation of several inputs from the phasic type to tonic one (S.-Rózsa, 1975), and the tonic firing above a certain frequency always stopped the heart. In one of the shown experiments the frequency appearing during veratrine perfusion was under the critical level *(Fig. 5C)* so that changes in the heart beat failed to appear.

The periodicity of the heart nerve activity observed by using high concentrations of veratrine is connected with the periodical events at the heart receptors being related to the membrane oscillations as in other systems (Frank, 1958; Danko et al., 1974). This oscillation can be explained with incomplete repolarization of the membrane as well as with changes in the K^+ and Na^+permeabilities (Ulbricht, 1972; Horackova and Vassort, 1974) leading to the temporary insensitivity of the receptor membrane.

Summary

Studying the peripheral and central effects of the veratrine in *Helix* heart it was found that:

1. Both the mechano- and chemoreceptors of the heart were excited during intracardial perfusion of veratrine from 10^{-6} M concentration. In *Helix* heart the veratrine cannot be regarded as a specific stimulator of mechanoreceptors.

2. The intensity of potentials registered from heart nerve was decreased as a function of time. Using high concentrations of veratrine (10^{-4}—10^{-3} M) the response of the receptors became periodic, which can be explained by the oscillation of the receptor membrane.

3. Veratrine had no significant effect on the heart muscle, the obtained effect was the result of reflex influences.

4. The central effect of veratrine was realized on the double-action cells of neuronal network. The veratrine effect at the cellular level depends also on the activity of other inputs in the system. The effects caused by veratrine on the central neurones were always the result of synaptic activation.

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VERATRIN HATÁSA *HELIX POMATIA* L. (GASTROPODA, MOLLUSCA) SZIV RECEPTORAIN, VALAMINT A SZIVMŰKÖDÉST SZABÁLYOZÓ KÖZPONTI NEURONOKON

S.-Rózsa Katalin

Összefoglalás

Vizsgálva *Helix pomatia* L. szivén a veratrin perifériás és központi hatását megállapítottuk, hogy

1. Intracardiálisan perfuzálva a veratrin 10^{-6}M koncentrációtól kezdődően a szív mechano- és kemoreceptorait egyaránt ingerületbe hozza. A veratrin *Helix* szíven nem specifikusan a mechanoreceptorok serkentője.

2. Veratrin hatására a szívidegről regisztrált jelek intenzitása az idő függvényében csökken. Magas veratrin koncentrációk (10^{-4}M, 10^{-3}M) alkalmazásával a receptorok válasza periodikussá válik, ami a receptor membrán-oszcillációs jelenségeivel kapcsolatos.

3. A veratrin hatása s szívizomsejteken nem kifejezett, a létrejött változások reflexműködésre épülnek.

4. A veratrin központi hatása kapcsolt neuronhálózat többhatású sejtjein realizálódik. Az egyes sejtek szintjén jelentkező veratrin effektus a rendszer más bemeneteinek aktivitásától is függ. A veratrin által indukált változások a központi neuronokon mindig szinaptikus aktiválás eredményei.

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PHARMACOLOGICAL PROPERTIES OF THE RECEPTORS AT THE HEART MUSCLE MEMBRANE OF *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. F. (INSECTA)

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In recent years while studying the sites of actions of different substances the analysis of their effect on the membrane prevailed both on the nerve and muscle cells, in order to discover the pharmacological properties as well as the ion-mechanisms of the receptors (GERSCHENFELD, 1973; TRAUTWEIN, 1973; MILLER, 1973). Recent results proved the membrane effect of transmitters on the heart of numerous invertebrate and vertebrate species and in some cases even their sites of action and ion-dependence were described (MILLER, 1973; HOLLEY and DELALEU, 1972; WILKENS and GREENBERG, 1973; IRISAWA et al., 1973). In *Locusta* heart our previous results verified the transmitter role of acetylcholine (Ach), 5-hydroxytryptamine (5HT) and gamma-aminobutyric acid (GABA), as well as the Na- and Ca-ion dependence of the effect of Ach and 5HT (S.-RÓZSA and V.-SZŐKE, 1973; S.-RÓZSA et al. 1973). To pursue these studies the investigations of the site of action of transmitters were emphasized for clearing up whether on the heart muscle membrane of insects the receptors of Ach, 5HT and GABA can be compared to the structures known for other classes of animals or whether they had some distinguishable properties. The present paper summarizes the results of these experiments.

Material and method

Experiments were carried out on the adults of 2—7 weeks old *Locusta migratoria migratorioides* R. F., both male and female specimens were used. The animals were obtained from breeding, kept at a temperature of 28—32°C with 12 hours photoperiodism. The experiments were made at room temperature (22—26°C).

The experiments were performed on the half-isolated heart, it was described earlier in detail (S.-Rózsa and V.-SZŐKE, 1970). The recording of action potentials was made by conventional glass microelectrodes filled with 2.5 M KCl using the equipment described earlier (S.-RÓZSA and V.-SZŐKE, 1972).

In the experiments the physiological saline prepared for insect hearts (LUDWIG et al., 1975) was used and the investigated substance was dissolved in the same solution immediately before application. The substances were studied at concentrations 10^{-10}—10^{-3} M observing their effect over a period

of 10 minutes. The pretreatment with inhibitors took 5—15 minutes, then the transmitter was added together with the blocking substance. In case of biphasic effect of the transmitter the blocking of both effects was studied.

The following substances were used: acetylcholine chloride (Sigma); 5-hydroxytryptamine creatinine sulphate (Reanal); gamma-amino-butyric acid (Reanal); nicotine hydrogen (t)-tartrate (BDH); d-tubocurarine dichloride (Schuchardt); atropine sulphate (BDH); benzoquinonium chloride, mytolon (St. W. Res. Inst); bromo-d-lysergic acid diethylamide, BOL-148 (Sandoz); methysergide bimaleate (Sandoz); ergometrine (Fluka); picrotoxin (Fluka); bicuculline (Pierce chemical Co).

Results

1. *Effect of antagonists on the membrane of Locusta heart*

According to our data, the antagonists possess own effects on the heart varying from an insignificant decrease or increase in frequency and sometimes in amplitude to the stopping of heart beats.

TABLE I

Effects of receptor antagonists on the spontaneous action potentials of Locusta heart

Antagonists and concentration used (M)		Changes in spontaneous action potentials	
		frequency	amplitude
Antagonists of Ach receptors			
d-Tubocurarine	10^{-6}—10^{-4}	decrease, increase	slight decrease
	10^{-3}	decrease, stopping	ceased
Atropine	10^{-8}—10^{-6}	decrease, increase	unchanged
	10^{-5}—10^{-3}	decrease	unchanged
Nicotine	10^{-8}—10^{-6}	increase, decrease	unchanged
	10^{-5}—10^{-3}	increase, decrease	unchanged
Mytolon	10^{-7}—10^{-6}	increase, decrease	unchanged
	10^{-5}—10^{-3}	decrease	unchanged
Antagonists of 5HT receptors			
BOL-148	10^{-5}	increase, decrease	decrease
	10^{-4}—10^{-3}	increase, decrease stopping	ceased
Methysergide	10^{-6}—10^{-4}	decrease, stopping	decrease, ceased
Ergometrine	10^{-9}—10^{-4}	increase, decrease	decrease
Antagonists of GABA receptors			
Picrotoxin	10^{-9}—10^{-7}	decrease	unchanged
	10^{-6}—10^{-4}	increase, decrease unchanged	unchanged
Bicuculline	10^{-9}—10^{-7}	increase, decrease stopping	unchanged
	10^{-6}—10^{-5}	decrease	unchanged
	10^{-4}	decrease, increase stopping	unchanged

Note: The lowest concentration seen in the *Table* reflects the threshold concentration of the pharmacons. The effect demonstrated at the first place shows the effect of drugs on the action potentials at the first minute of application, then the change of effect is seen if occurred few minutes after the application of the drugs

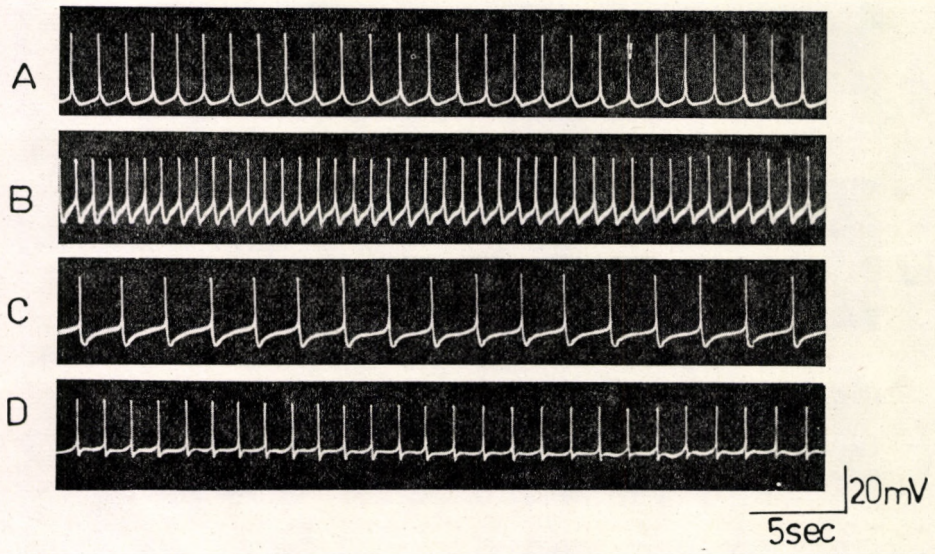

Fig. 1. Effect of nicotine on the generation of action potentials on *Locusta* heart. *A* — control; *B* — effect of nicotine (10^{-3}M) 1 minute after application; *C* — same as *B* but 5 minutes after application of drug; *D* — activity after washing out nicotine

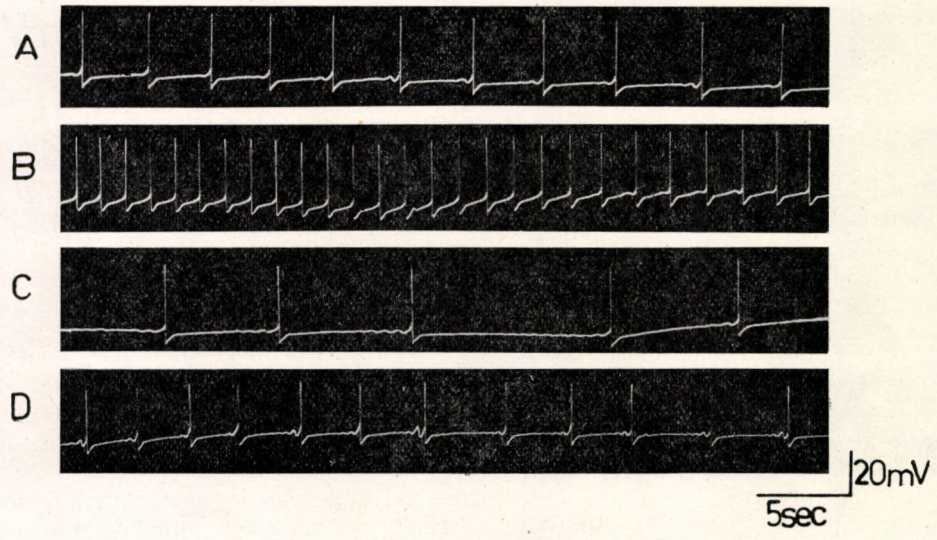

Fig. 2. Effect of mytolon on spontaneous action potentials. *A* — control; *B* — applying 10^{-3} M mytolon; *C* — effect of mytolon 2.5 minutes after application; *D* — activity after washing out the drug

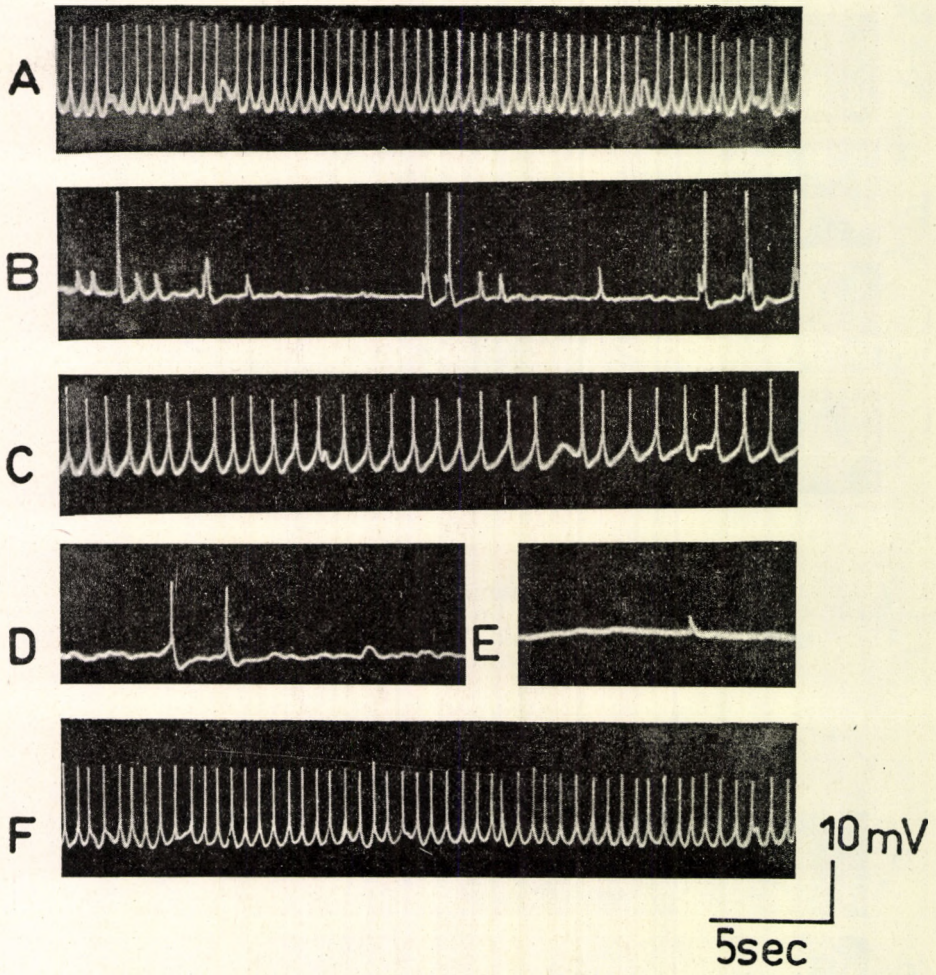

Fig. 3. Effect of d-tubocurarine on the membrane of *Locusta* heart. *A* —ontrol; *B* — beginning of the action of d-tubocurarine (10^{-4}M); *C* — same as *B* after 3.5 minutes; *D* — same as *B* after 5 and 6 minutes of application, respectively; *E* — activity after washing out d-tubocurarine

Among anticholinergic substances d-tubocurarine, atropine, nicotine and mytolon, among antagonists of the 5HT, BOL-148, methysergide and ergometrine, while among antagonists of GABA picrotoxin and bicuculline were studied. The results can be seen in *Table I* regarding the spontaneous action potentials of the *Locusta* heart.

The investigated substances influenced more often the frequency than the amplitude fo the action potentials. The effect on the amplitude was negligible with the exception of the antagonists of 5HT *(Table I)*. Using antagonists the biphasic effect can often be observed indicating the time-dependent appearance of the inhibitory or excitatory influences in the same concentration

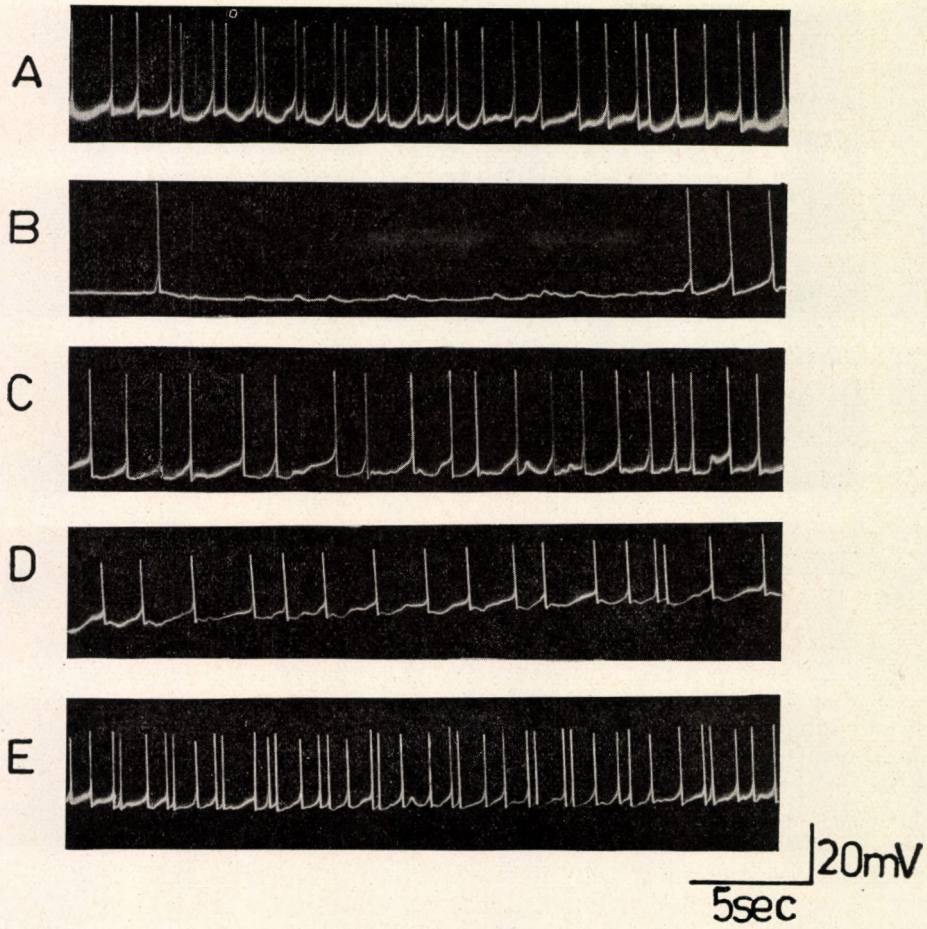

Fig. 4. Effect of atropine on the sponatneous action potentials of *Locusta* heart. *A* — control; *B* — applying atropine at 10^{-6}M; *C* — effect of atropine 1 minute after application; *D* — effect of atropine 2 minutes after application; *E* — activity after removing atropine

of the drugs. In *Table I* both the first and secondary effects of the pharmacons are shown.

Using cholinergic antagonists the changes in frequency were fairly small. Thus, nicotine at 10^{-3} M at the beginning of its application increased frequency by 50 per cent then decreased it by 30 per cent *(Fig. 1)*. At the same concentrations mytolon, at the beginning, doubled the initial frequency but after 2.5 minutes it failed to achieve even half of the control value *(Fig. 2)*. The time-dependent changes were the same in sign using nicotine or mytolon, but nicotine influenced mainly the diastolic depolarization of action potentials which appeared even during the decrease of frequency *(Fig. 1C)*, while mytolon caused oscillation in the membrane potential *(Fig. 2B, C)*. During its application d-tubocurarine influenced the synaptic potentials *(Fig.3)* and

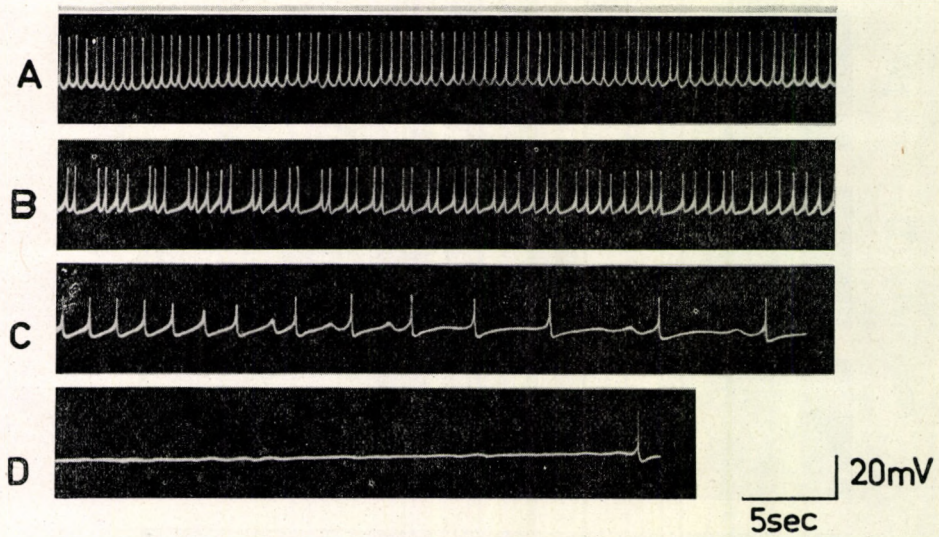

Fig. 5. Effect of BOL-148 on the membrane of *Locutsa* heart; *A* — control; *B* — application of 10^{-3}M BOL-148; *C* — effect of BOL 148 5 minutes after application; *D* — same as *C* after 8 minutes

periodically abolished potential generation *(Fig. 3)*. The inhibition of the spontaneous activity appeared at the beginning and even after 5 and 6 minutes interrupted with the periods of firing *(Fig. 3C)*. At the beginning of its application atropine caused strong inhibition, then step by step the generation of action potentials was restored *(Fig. 4)*. Here also during diastolic depolarization the significant increase in synaptic potentials was observed prevailing even after the stopping of spike generation *(Fig. 4B)*. High concentrations (10^{-4}—10^{-3} M) of atropine sometimes caused slight temporary increase in frequency but it was followed by a decrease. The effect of cholinergic antagonists was eliminated easily by washing out.

Among receptor inhibitors of 5HT, BOL-148 exerted effect from a concentration of 10^{-5} M. At this concentration BOL-148 caused slight increase in frequency but at 10^{-4}—10^{-3} M it gradually slowed the firing and ceased the geneartion of the action potentials at 8—10 minutes after application *(Fig. 5D)*, this latter was slow or irreversible. Methysergide eliminated the generation of action potentials without excitatory phase in lower than 5HT threshold concentration *(Fig. 6)*, but this inhibition ceases after washing out. The effect of ergometrine was variable, beginning with 10^{-9}M after 4—5 minutes of application it increased frrequency by 8—10 per cent by simultaneously lowering the amplitude to 50 per cent of the control. However, in other cases ergometrine caused only inhibition. The effect of ergometrine was hardly reversible.

Among the receptor antagonists of GABA the effect of picrotoxin was very variable, it caused inhibition and excitation alike or was even ineffective. In this variation no concentration dependence was ascertained, although at low concentrations the increase in frequency was more usual *(Table I)*.

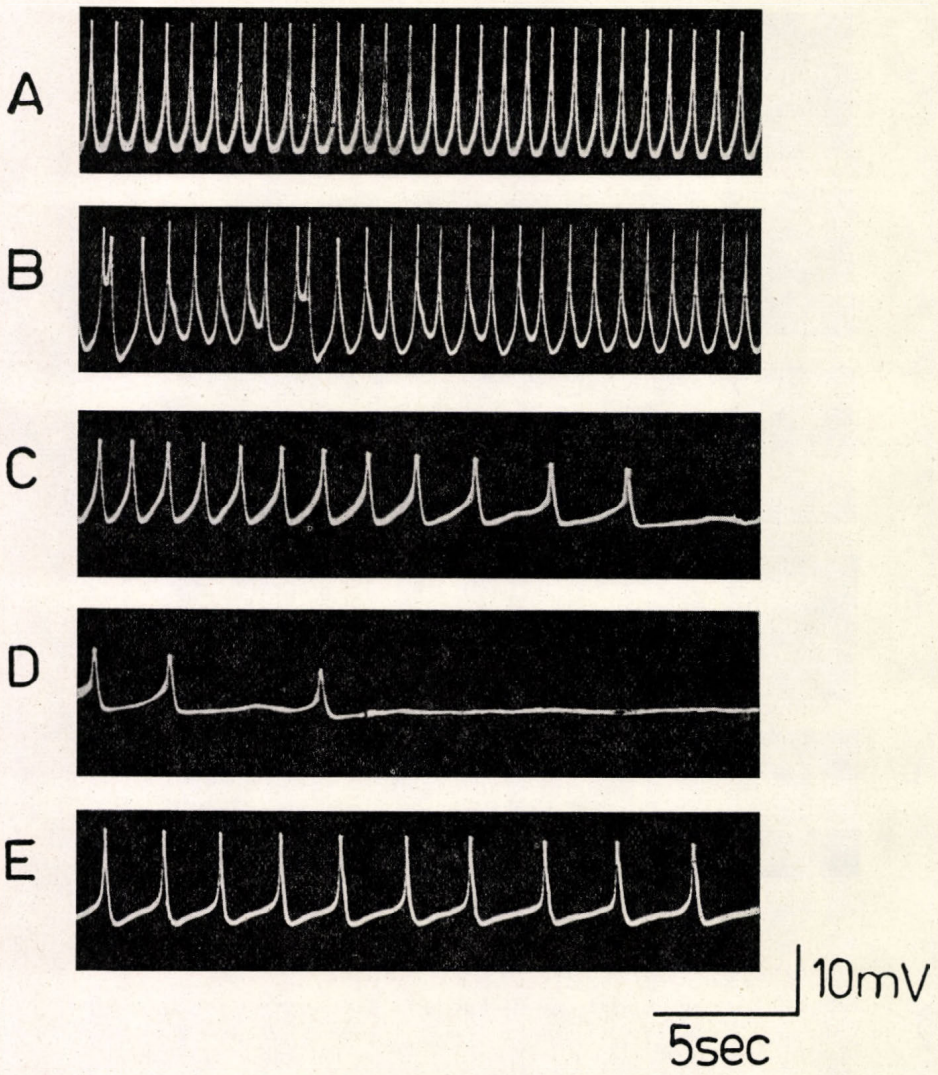

Fig. 6. Effect of methysergide on the membrane of *Locusta* heart. *A* — control; *B* — effect of methysergide (10^{-6}M) immediately after application; *C* — effect of methysergide 2 minutes after appliation; *D* — same as *C* after 3 minutes; *E* — after methysergide removal

This effect was reversible. Bicuculline at concentrations 10^{-9}—10^{-7} M first increased the frequency of spontaneous firing then inhibited it, while at 10^{-6}—10^{-5} M only decreased the frequency of action potentials, but at 10^{-4} M, similarly to the d-tubocurarine, inhibition was interrupted from time to time by firing *(Fig. 7)*. Inhibition took place within 5 minutes of application. Using bicuculline, the secondary stopping of the generation of action potentials was not regular. The synaptic potentials become more frequent, prevailing

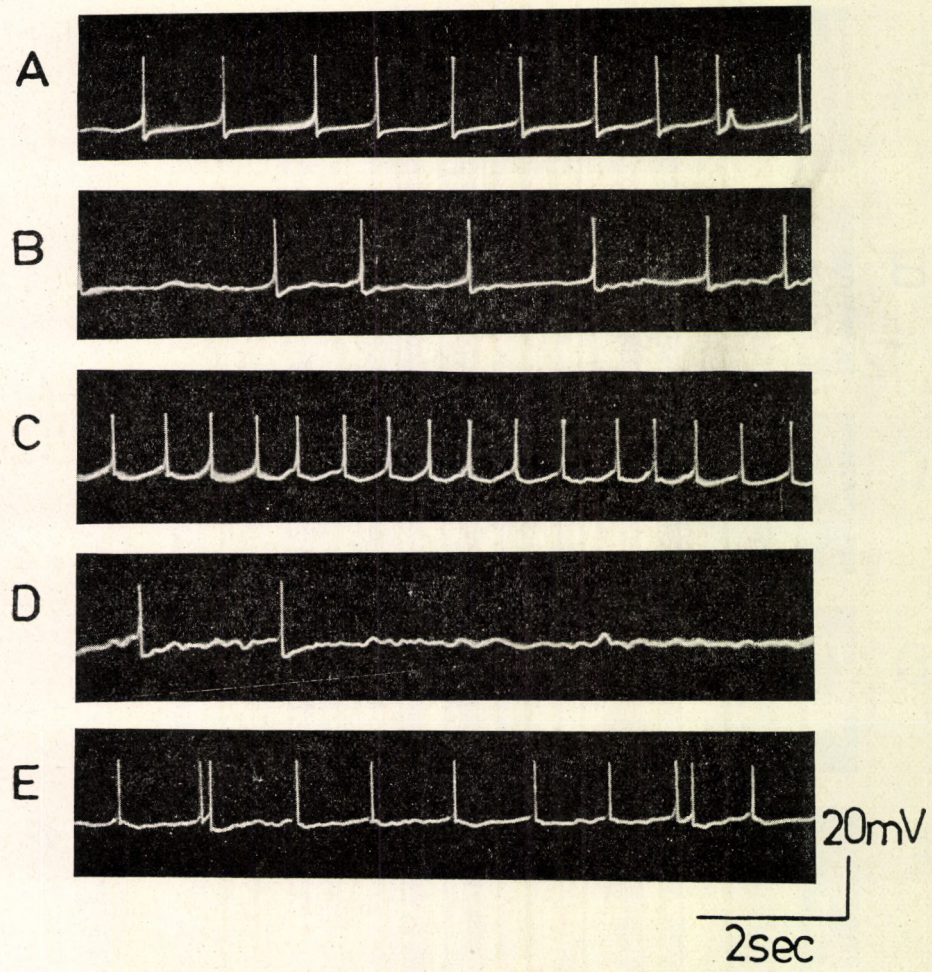

Fig. 7. Effect of bicuculline on the membrane of *Locusta* heart. *A* — control; *B* — effect of bicuculline (10^{-4}M) 30 se after application; *C* — same as *B* after 2.5 minutes; *D* — same as *B* after 3.5 minutes; *E* — after bicuculline removal

also during the stopping of the heart by bicuculline *(Fig. 7D)*. The effect of bicuculline was slowly reversible even after repeated washing out. The amplitude of the spontaneous action potentials was not influenced by the two antagonists of GABA receptors.

2. The sites of action of Ach, 5HT and GABA

According the our previous results in the spontaneous action potentials of *Locusta* heart Ach and 5HT caused both the inhibitory and excitatory effects, while GABA produced partial or complete inhibition (*S.*-Rózsa and

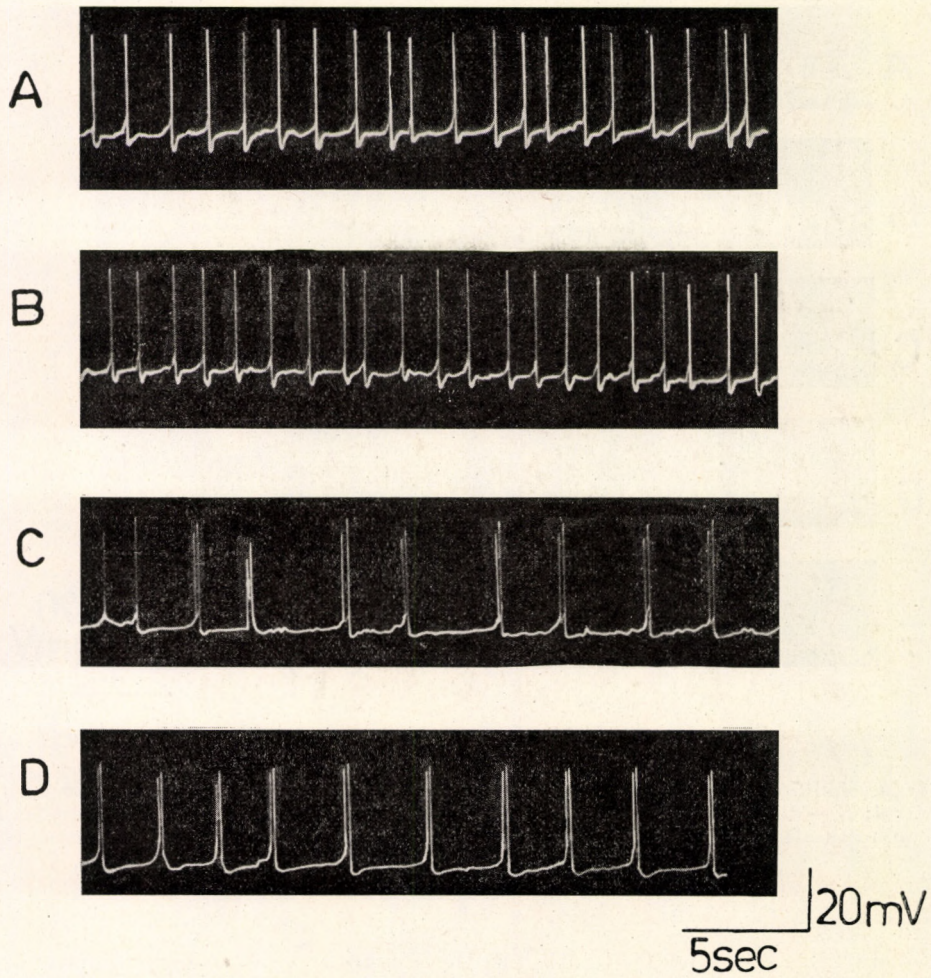

Fig. 8. Modification of Ach effect after pretreatment of *Locusta* heart by atropine. *A* — control; *B* — first minute of the atropine (10^{-5} M) treatment; *C* — effect of simultaneous application of atropine (10^{-5} M) and Ach (10^{-3} M); *D* — activity after washing out atropine and Ach

V.-Szőke, 1972; S.-Rózsa et al, 1973). The abolishment of these effects was studied here by using the above pharmacons.

The results are summarized in *Table II*, referring to the wide limits of antagonists used, since most of them eliminated or turned the effect of transmitter applied to the opposite. The antagonistic properties of pharmacons were more obvious in the blocking effect at low concentrations of transmitters. On the Ach receptors of the heart membrane of *Locusta* d-tubocurarine possessed somehow low activity, because it failed to block the inhibitory effect of Ach, and also its excitatory effect was turned into the opposite in some of the cases, mainly in old animals. The inhibitory effect of Ach (10^{-9} M) normally was

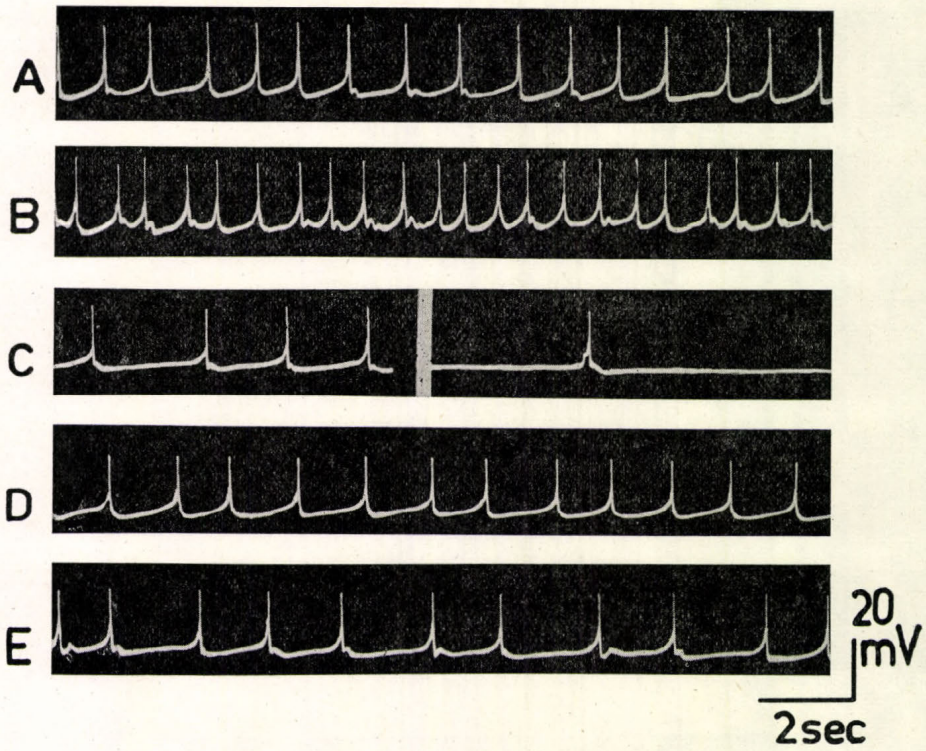

Fig. 9. Modification of 5HT effect by pretreating *Locusta* heart with BOL-148. *A* — control; *B* — beginning of the pretreatment of the heart with BOL-148; *C* — same as *B* 15 minutes after pretreatment; *D* — effect of simultaneous application of BOL-148 (10^{-4} M) and 5HT (10^{-4} M)

abolished by atropine (10^{-4} M) but the synaptic potentials appeared more frequently and the heart beats became arrhythmic. After pretreatment with atropine the excitatory influences, of Ach was eliminated but the arrhythmic heart beats were characteristic too *(Fig. 8)*. In some cases following the pretreatments with atropine the high concentration of Ach caused an inhibition *(Table II)*. Both the inhibitory and excitatory effects of Ach were abolished by nicotine on young animals, while on older ones the inhibitory effects prevailed or instead of the usual excitatory response at 10^{-6} M Ach the inhibition occurred. Mytolon ($10^{-6}-10^{-3}$ M) failed to influence the effect of Ach.

Among the antagonists of 5HT both BOL-148 and methysergide abolished the excitatory influences of 5HT. At most of the cases BOL-148 ($10^{-4}-10^{-5}$ M) not only eliminated the excitation caused by 5HT but stopped the heart beats *(Table II)*. BOL-148 applied alone caused also a decrease in the frequency of action potentials which could not be completely compensated by adding 5HT *(Fig. 9)*. The effect of ergometrine was variable on the receptors of 5HT, sometimes at $10^{-4}-10^{-5}$ M it abolished the excitation caused by 5HT but in other cases it remained ineffective *(Table II)*.

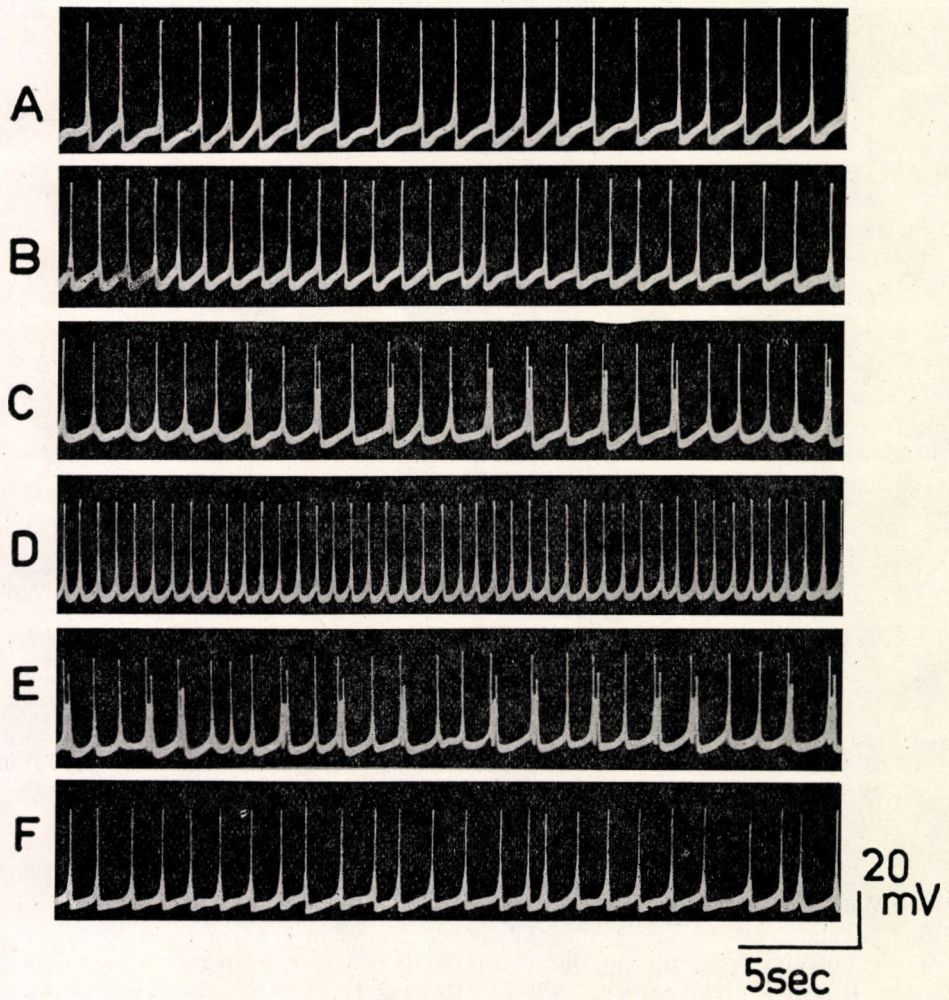

Fig. 10. Modification of GABA effect by treating the heart with bicuculline. *A* — control; *B* — 7 minutes after pretreating the heart with bicuculline (10^{-7} M); *C* — same as *B* after 20 minutes; *D* — effect of simultaneous adding of bicuculline (10^{-7} M) and GABA (10^{-5} M); *E* — same as *D* after 5.5 minutes; *F* — after removing the drugs

From the receptor antagonists of GABA picrotoxin abolished the inhibition caused in low concentrations (10^{-7}, 10^{-6} M), while in high concentrations (10^{-5}—10^{-4} M) decreased in degree but without a complete elimination *(Table II)*. The bicuculline proved to be a more effective antagonist on the GABA receptors because, beginning with 10^{-8} M, it completely or partially abolished the inhibitory effect of GABA *(Table II)*. In some of the cases, after treatment with the bicuculline, the inhibition caused ba GABA turned to excitation which persisted throughout application *(Fig. 10 D, E)*. During GABA application the heart beats became arrhythmic but they were quickly restored after the drug was washed out *(Fig. 10F)*.

TABLE II

Modification of the effects of Ach, 5HT and GABA applying specific inhibitors to the receptors of the heart of Locusta

Transmitter (M)	The effect of the transmitters on AP (control)	Antagonist	Effect of transmitter after pretreatment of the heart with antagonists
Acetylcholine			
10^{-9}	inhibition	d-TC	ineffective or inhibition
	inhibition	atropine	ineffective or inhibition
	inhibition	nicotine	ineffective or inhibition
10^{-6}	excitation	atropine	inhibition
	excitation	mytolon	excitation
10^{-4}	excitation	d-TC	excitation, stopping
	excitation	atropine	ineffective, inhibition
	excitation	nicotine	excitation
	excitation	mytolon	excitation
5-hydroxytryptamine			
10^{-6}	excitation	BOL-148	inhibition (to stopping)
10^{-5}	excitation	BOL-148	inhibition (to stopping)
	excitation	methysergide	inhibition
	excitation	ergometrine	excitation, ineffective
GABA			
10^{-6}	inhibition	bicuculline	excitation, inhibition
	inhibition	picrotoxin	ineffective, slight inhibition
10^{-5}	inhibition	picrotoxin	inhibition
10^{-4}	inhibition	bicuculline	excitation

Note: The concentrations of antagonists: d-TC 10^{-5} M, atropine 10^{-6}—10^{-4} M, nicotine 10^{-9} M, 10^{-4} M; mytolon 10^{-6} M, 10^{-4} M; BOL-148 10^{-6} M, 10^{-5} M, methysergide 10^{-5} M, 10^{-4} M, ergometrine 10^{-5}, 10^{-4} M, bicuculline 10^{-8}, 10^{-5} M, picrotoxin 10^{-6}, 10^{-4} M

Discussion

We have shown that the antagonists of different receptors exert also an effect on the generation of the action potentials of insect hearts as on the contractile activity, although we have reported already on specific dependence, too (S.-Rózsa and V.-Szőke, 1970; S.-Rózsa, 1974). On other insect hearts the effect of the antagnoists of Ach have been described earlier, according to this nicotine caused excitation on the heart of several species (Jaeger and Gahan, 1937; Davenport, 1949; Naidu, 1955), while atropine and curare abolished the Ach effect (Hamilton, 1939; Metcalf et al., 1964; Jones, 1964). According to our earlier data regarding the contractile activity of *Locusta* heart, mytolon, nicotine and methysergide exerted biphasic effect (excitation, inhibition), while BOL-148 caused only inhibition (S.-Rózsa, 1974). The action potentials of the heart were affected with these pharmacons in a similar way exepting BOL-148 which also causes excitation *(Table I)*. The other pharmacons used here have not been studied, as far as contractile activity of the heart is concerned. The changes in the action potentials of *Locusta* heart showed the temporary character of the excitation arising during the first minutes of the application of pharmacons reversing soon to inhibition which often stopped potential generation. This latter phenomenon is connected with the adaptation and desensitization of receptors. The significant increase in the amplitude of the

action potentials was found only under the influence of antgonists of 5HT receptors *(Table I)*.

All the antagonists used, exept mytolon, abolished the effect of certain transmitters in different degrees or turned it to a response opposite in sign. In abolishing the generation of action potentials on *Locusta* heart among cholinergic antagonists atropine and nicotine, among the inhibitors of 5HT receptors BOL-148 and methysergide, while among GABA antagonists bicuculline were the most effective agents. The above correspond to the data obtained on the contractile activity of the heart exepting the efficiency of methysergide (S.-Rózsa, 1974). Our results emphasized the presence of similar structures in *Locusta* heart to the vertebrate receptors. The specificity of the receptors cannot be discussed here since the antagonists were tested only on one kind of receptors, when our earlier data showed the mixedtype of the sites of action of the transmitter in insect hearts (S.-Rózsa and V.-Szőke, 1970, S.-Rózsa, 1974).

Although systematic studies have not been carried out to prove the dependence of the effects of receptor antagonists on the age of the animals, still some decrease was found in the efficiency of these drug in older *Locusts*. Age-dependence in Ach and 5HT effects was not obvious in our earlier studies although it was ascertained by several authors (McFarlane, 1967; McFarlane and Ting-Ya Fong, 1971; Roussel, 1974). The changes in receptor sensitivity observed during the application of the pharmacons also directed attention to the importance of the age of th animals.

On the heart membrane of arthropods and especially insects only limited amount of data is available regarding the receptor properties. The effect of GABA was analyzed only on *Periplaneta* and *Porcellio* hearts being abolished by picrotoxin (Holley and Delaleu 1973; Miller, 1973; Richter, 1973). Although our present data added some further information to the sites of the action of transmitters on insect heart the exact localization may be found only on completely isolated hearts. On semi-isolated hearts both the tranmitter and pharmacons can act on the heart and nerve tissues alike, and the separation of the two sites involves some difficulties.

Summary

The effect of receptor antagonists of Ach, 5HT and GABA were studied on the membrane of the heart muscle cells of *Locusta* and their interactions with the transmitters. The used antagonists of cholinergic (d-tubocurarine, atropine, nicotine), serotoninergic (BOL-148, methysergide, ergometrine) and GABA-ergic (picrotoxine, bicuculline) receptors had effect on the membrane of *Locusta* heart. In the majority of the cases the initial excitatory or inhibitory effects turned to opposite in sign at the 5th minute of the application. Among cholinergic antagonists d-tubocurarine, atropine and nicotine abolished both the excitatory and inhibitory effects of Ach partially or completely, while mytolon was ineffective. Among the inhibitors of 5 HT receptors BOL-148 and methysergide eliminated the excitatory effect of 5HT moreover, BOL-148 turned it to inhibition but the ergometrine only partially blocked 5HT effect. Picrotoxin abolished the effect of low concentrations of GABA, however, the effect of high concentrations of GABA (10^{-4} M) was antagonized only by bicuculline.

According to these results the receptors of the heart membrane of *Locusta* may be characterized with the same phycological properties as known for vertebrates, so that they may be regarded similar to each other.

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TRANSMITTEREK RECEPTORAINAK FARMAKOLÓGIAI TULAJDONSÁGAI *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. F. (INSECTA) SZIVÉNEK MEMBRÁNJÁN

S.-Rózsa Katalin és V.-Szőke Ida

Összefoglalás

Vizsgálták kolinerg, serotoninerg és GABA-erg receptor bénítók hatását *Locusta* szívizomsejtjeinek membránján és azok kölcsönhatását transzmitterekkel. Megállapították, hogy a kolinerg (d-tubocurarine, atropine nicotine), serotoninerg (BOL-148, methysergide, ergometrine) és GABA-erg (picrotoxine, bicuculline) bénítók *Locusta* szív membránján saját hatással is rendelkeznek. Az esetek többségében a kezdeti serkentő vagy gátló hatás ellenkező előjelűvé válik a kezelés 5. perce körül. Kolinerg bénítók közül a d-tubocurarine, atropine és nicotine különböző mértékben antagonizálják az acetylcholine gátló és sekentő hatását, a mytolon hatástalan. A serotoninerg blokkolók közül a BOL-148 és methysergide az 5HT serkentő hatását megszüntetik, a BOL-148 gátlóvá alakítja azt, míg az ergometrin részleges 5HT-hatás kivédést eredményez. A picrotoxin GABA hatását alacsony koncentrációk alkalmazásakor kivédi, de magasabb (10^{-4}M) BAGA koncentrációk hatását csak a bicuculline antagonizálja.

Az eredmények szerint rovar szívek membránján a receptorok farmakológiai blokkolása magasabbrendűeken ismert bénítókkal valósítható meg, így azokkal azonos tulajdonságúaknak tekinthetők.

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SENSORY INPUT CHARACTERISTICS AT THE CHEMICAL STIMULATION OF THE LIP IN THE SNAIL *HELIX POMATIA* L.

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The role of contact chemoreceptors in food detection, in escape reaction to predators and also in forming some other behavioural patterns was investigated both in terrestrial and aquatic gastropods (in: CHARLES, 1966). In experiments testing the reaction of the whole animal to various substances it has been shown that the receptors located around the mouth can differentiate among chemicals (KIECKEBUSCH, 1953). This finding is in correlation with earlier morphological data describing various receptor structures in the lip of the snail (SCHULZ, 1938).

Electrophysiological experiments on snail chemoreceptors have been done mainly on the osphradial nerve in order to elucidate the chemoreceptive characteristics of its peripheral organ. Although from the nerve itself no action potentials could be recorded (KOHN, 1961), when stimulating the osphradium with some chemicals the specific response of several central neurones was observed (BAILEY and LAVERACK, 1963; STINNAKRE and TAUC, 1969). Lately some special chemoreceptive ability of the heart and the central representation of the afferent inputes were demonstrated in *Helix* (S.-RÓZSA, 1972; S.-RÓZSA and SALÁNKI, 1973).

In our recent investigations we are dealing with the irritability of the receptive field of the mouth in the snail. We aim to explore the effect of different chemical substances and to clear up the peripheral and central mechanisms of the discrimination between them. In the present paper the methods applied and further, some characteristics of the afferent activity recorded from the lip nerve upon stimulating the chemoreceptors with various substances are described.

Material and method

Experiments were carried out partly on brain-lip preparations partly on isolated lip preparation of *Helix pomatia* L. When making the brain-lip preparation, these structures and the nerve connecting them were isolated, while in the case of the isolated lip only the radula, lips and a part of the lip nerve were included *(Fig. 1)*.

Using free moving animals, the shell of the snail has been removed, the coelom sack was cut and the central nervous system (CNS) with the nerves

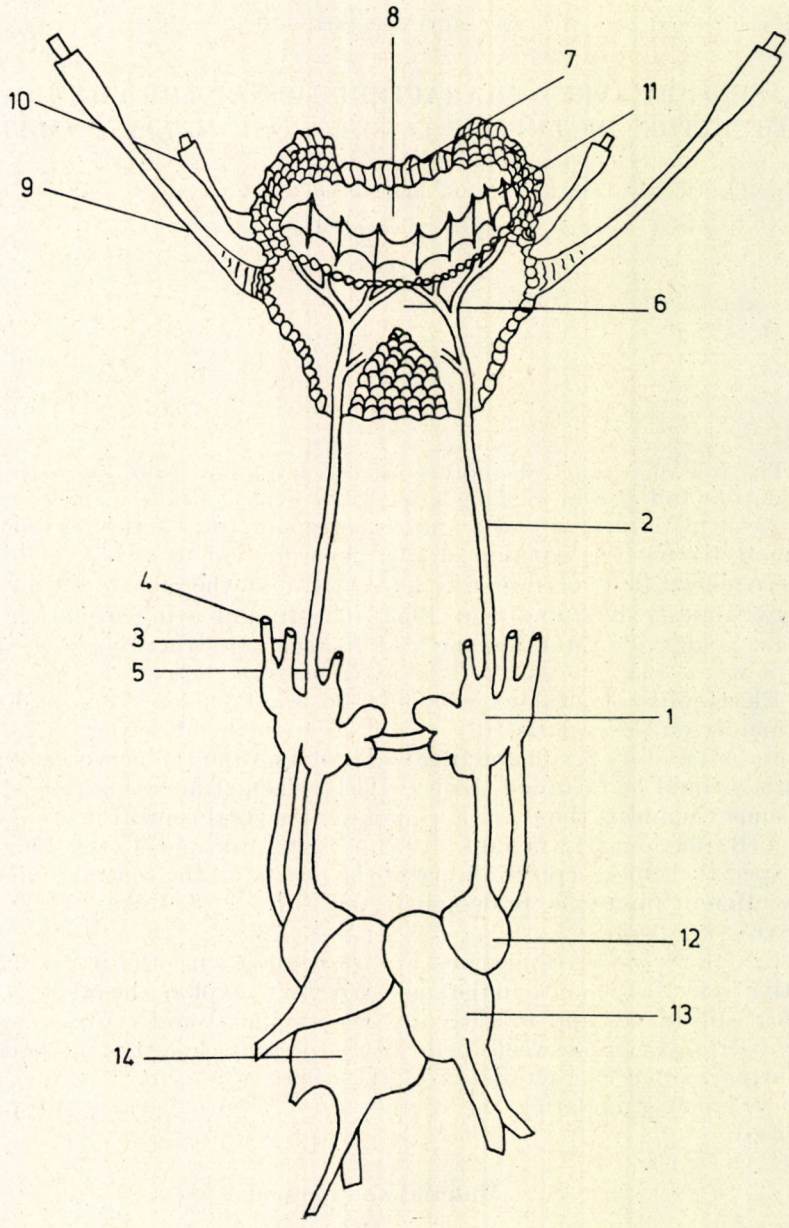

Fig. 1. Gross anatomy of the head and the CNS in *Helix pomatia* L. 1 — right cerebral ganglion; 2 internal lip nerve; 3 — medial lip nerve; 4 — external lip nerve; 5 — cerebro-buccal connective; 6 — upper lip; 7 — lower lip; 8— mouth; 9 — upper tentacle; 10 — lower tentacle; 11 — radula; 12 — right pleural ganglion; 13 — right parietal ganglion; 14 — visceral ganglion

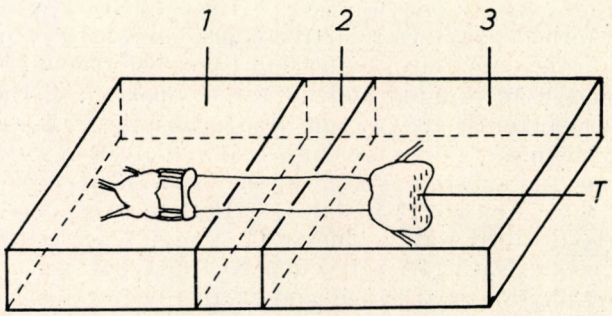

Fig. 2. Preparation placed in the experimental chamber. 1 — circumoesophageal ganglionic ring; 2 — lip nerves; 3 — mouth preparation; T = test zone

running towards the mouth were explored. Then the lip was isolated from the surroundings. The oesophagus was eliminated too, and so only the radula and the lips remained intact together with their nerve connections. Finally the CNS was nearly totally isolated: all its nerves were cut except the pair of lip nerves running to the upper part of the mouth.

The preparation was placed in a three parted plexiglass chamber *(Fig. 2)*. In the first the ganglionic ring, in the third the oral part, while between them, in the second, the lip nerves were located, the latter running through thin cuttings made on the two inner walls dividing the three sections. After placing the preparation in, the middle part was isolated from the others by filling up the cuttings with vaseline. The cut parts of the feeding organ, the nerve and the CNS were in physiological saline, while the lip was above the solution. When electrodes were placed under the nerve, the physiological solution was suctioned from the middle part and was replaced by paraffin oil, rendering recording possible and protecting the nerve from drying at the same time.

The isolated lip preparation was made similarly but the lip nerves were also cut at branching off from the CNS. Accordingly only the activity originating from the periphery was recorded. Both preparations remained in good accondition for hours at room temperature (20—24°C).

The method of testing the activity of the receptors was as follows: the electrical activity was recorded from one of the lip nerves by bipolar platinum electrodes fed into the input of an Alvar AC amplifier and registration took place from the screen of oscilloscope to film. It was cleared up in the preliminary experiments that upon lip stimulation the highest synchronous activity increase can be recorded from the internal lip nerves in isolated preparations. The exact area which gave the highest response on the nerve to tactile stimulation was determined by trials in every case *(Fig. 2,.T)* and this test zone was exposed locally to various chemicals during the investigations.

The application of substances was performed by using 5×5 mm filter paper soaked in solution prepared for testing, then it was put gently onto the test zone. At the first time for each preparation the piece of filter paper applied was soaked in physiological saline. This way the response to the tactile stimulus was tested. During the experiments the preparation was washed with physiological solution after each test, and the tests were carried out at 5—10 min intervals to assure the recovery of the receptor sensitivity.

The change of activity on the nerve at the appliation of test solutions was compared to the spontaneous activity preceding the application. At processing the data the potentials produced in 10 sec before and after the application of the drugs were counted and for the comparison of the values the control activity was taken as 100 per cent. Each substance was used in several preparations and the mean values are compared and given in *Table I.*

The solutions and substances used in the experiments were: physiological saline (NaCl 6.5 g, KCl 0.14 g, $CaCl_2 \cdot 2H_2O$, 0.12 g, $NaHPO_4$ 0.01 g, $NaHCO_3$ 0.02 g/l), distilled water; glucose (1, 2 and 5 per cent); saccharose (1, 2 and 5 per cent); NaCl (5.8 per cent); KCl (7.5 per cent). Sugars were dissolved both in physiological saline and in distilled water. The effect of several plant protecting agents which are used in agricultural practice against weeds, insects and molluscs, were also tested. These are: dazomet (3,5-dimethyl-tetrahydro-1,3,5-tiadiazin-2-tion) (10^{-2} and 10^{-3} M); dipterex (0,0-dimethyl 2,2,2-trichloro-1 hydroxyethyl phosphate) (10^{-2} and 10^{-3} M); malathion (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate) (from 10^{-4} to 10^{-8} g/ml). The first two were dissolved in physiological solution while the third in distilled water.

The experiments were carried out throughout the year.

Results

A. *Brain-lip preparation*

Activity could be recorded from the nerve also in the case when no stimuli were applied to the test zone. This "resting" activity is composed of irregularly distributed fast components of various amplitudes (50—100 μV). The frequency varies in different preparations and changes also in the same preparation in time between 3—8 imp/sec. As a mean of 110 measurements it was found 4.6 spikes/sec *(Fig. 3).* When touching the lip with a fine brush the

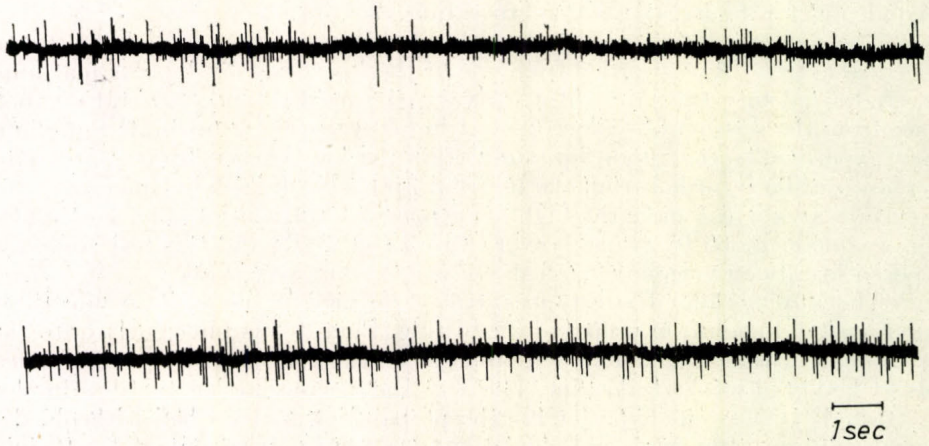

Fig. 3. Spontaneous activity recorded from the lip nerve of two brain-mouth preparations

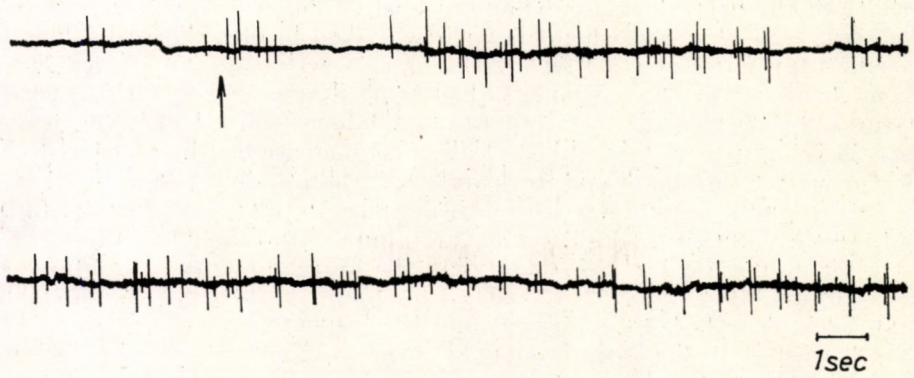

Fig. 4. Effect of filter paper soaked in physiological saline applied onto the lip. The second row is the continuation of the first one (in each further case too)

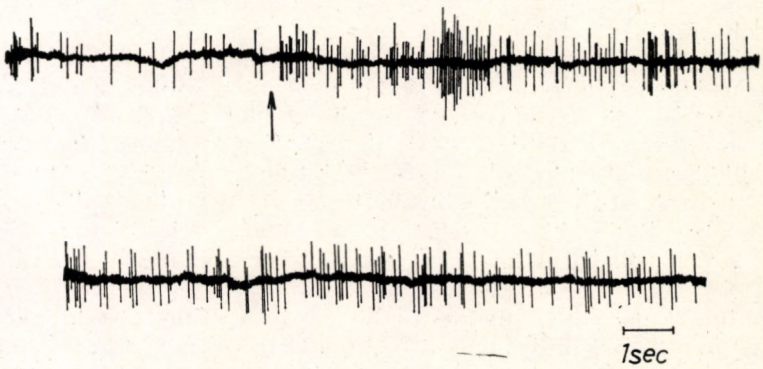

Fig. 5. Effect of distilled water

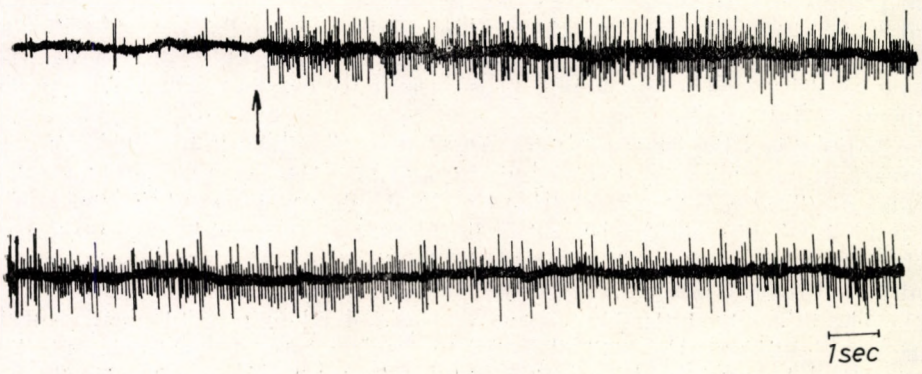

Fig. 6. Effect of 1 mol NaCl

activity of the nerve increased simultaneously. Similarly to this, the increase of activity was observed when filter paper soaked in physiological saline was placed onto the test zone *(Fig. 4)*. The highest activity was recorded just at the moment of placing the paper, then some decrease of activity occurred. Taking into consideration the first 10 sec, the increse of activity was in these cases 28 per cent as a mean of 17 trials. This increase can be ascribed to the effect of the tactile stimuli and to the effect of the physiological saline. Taking this into account, at applying various drugs only the change of activity differing from this value was considered as a repsonse of the chemoreceptors.

When applying distilled water onto the lip the activity of the lip nerve was considerably increased *(Fig. 5)*. The spikes, both of lower and higher amplitudes, became more numerous and the response was long-lasting, tonic in its character. In the mean of 10 experiments the increase of frequency was by 53 per cent above the control.

Applying 1 mol solution of NaCl onto the lip a very high increase in activity was detected *(Fig. 6)*. Both the lower and higher amplitudes became more frequent. The response is most expressed in the first seconds, decreasing somewhat after 6—10 sec but at continuous application it did not disappear within 1 min. In the mean of 8 experiments the increaase of the activity surpassed the control by 119 per cent.

The highest activity increase occurred at the application of KCl in 1 mol concentration. After a transient period of 1—1.5 sec, when the activity increase was only moderate, a very frequent and of high amplitude (150—200 μV) impulsation appeared. It decreased somewhat in 3—4 sec, then, in the case of continuous application of KCl these high frequency spikes reappeared again in the form of burst-like oscillations with somewhat lower amplitudes *(Fig. 7)*. The effect was tonic, mixed with these secondary series of burst-like spikes. Under the effect of KCl the activity was 147 per cent higher than that of the control.

Adding 1 per cent glucose dissolved in distilled water the activity response *(Fig. 8)* was differing in its character from that observed for distilled water. The increase of activity was not significant, it preceded the control only by 54 per cent, however, the newly appearing groups of spikes consisting at the beginning of 8—10, later on of 2—4 potentials of high amplitudes show a great difference both from the control and from the effect of distilled water. The effect of glucose was more expressed when it was solved in physiological saline and when different concentrations were used. 1, 2 and 5 per cent glucose caused 51, 68 and 78 per cent increase in activity, respectively, what is regarded to be significant. The effect of glucose gradually decreased but did not disappear within 1 min.

Applying 1 per cent solution of saccharose dissolved in distilled water an increase of activity was observed again, and also the frequency of potentials of high amplitudes is characteristic *(Fig. 9)*. Under the effect of saccharose the activity was increased by 41 per cent in the first 10 sec, and in the appearance of high potentials some periodicity could be observed. The response was tonic, and lasted over 1 min.

The effect of 1 per cent saccharose dissolved in physiological solution was also significant, the evoked increase of activity reached 89 per cent. The same effect was recorded for 2 per cent saccharose evoking a 89 per cent increase, while a 5 per cent solution of saccharose did not cause any increase,

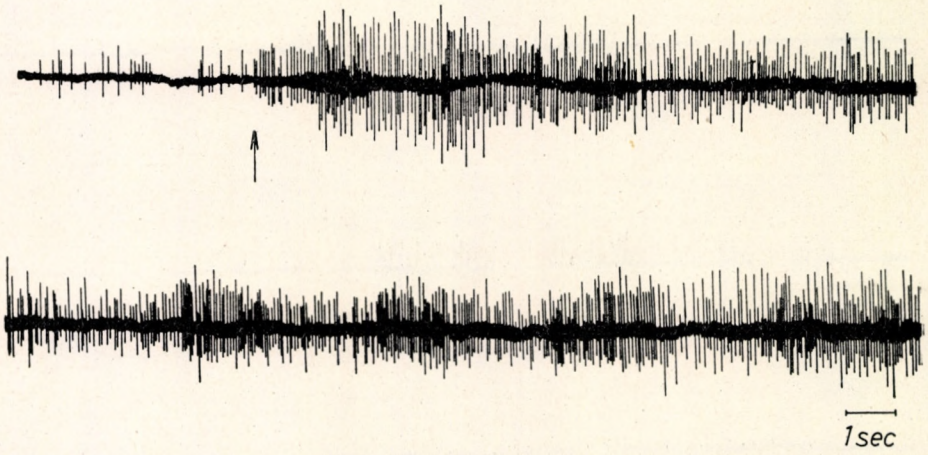

Fig. 7. Effect of 1 mol KCl

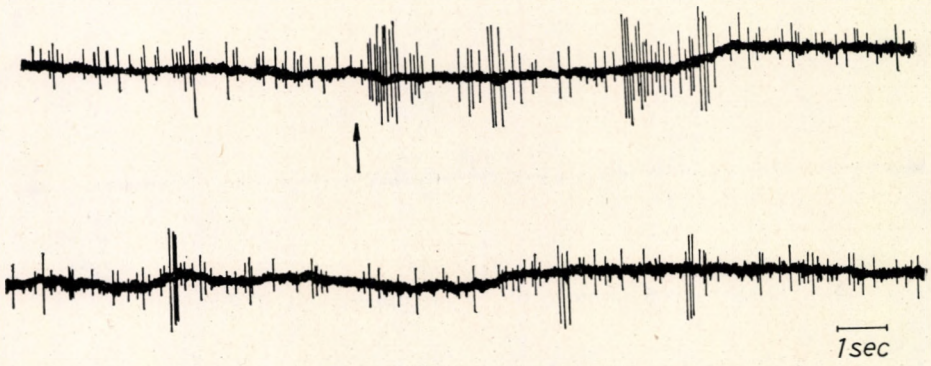

Fig. 8. Effect of 1 per cent glucose dissolved in distilled water

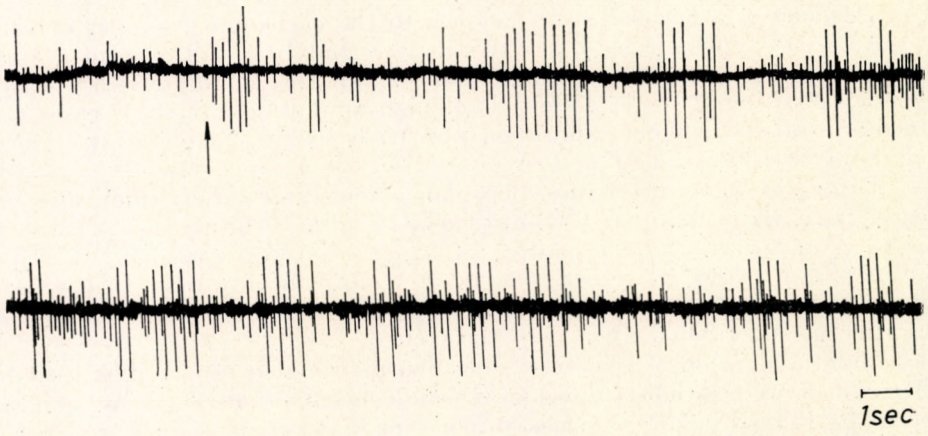

Fig. 9. Effect of 1 per cent saccharose dissolved in distilled water

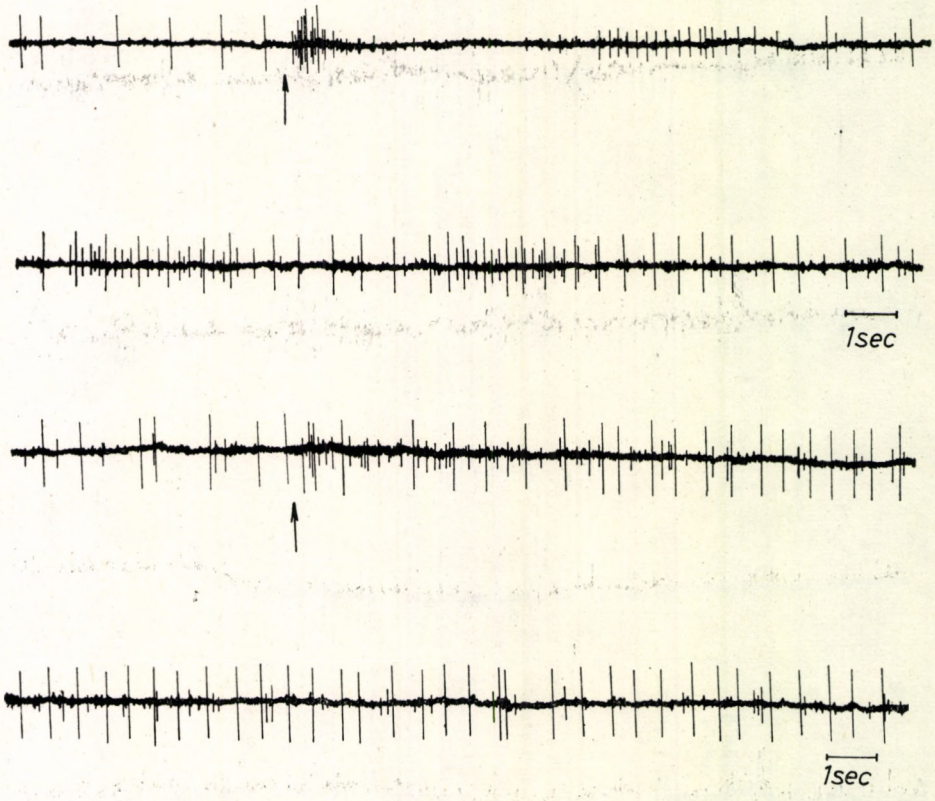

Fig. 10. Effect of dasomet 10^{-3} mol (upper) and 10^{-2} mol (lower)

activity corresponded only to 102 per cent of the control. Considering the effect of tactile stimulation (28 per cent increase), the effect of the 5 per cent saccharose must be interpreted as inhibitory.

Dasomet is a herbicide and belongs to the carbamat group, it evoked 70 and 55 per cent increase of activity in 10^{-2} and 10^{-3} mol concentrations, respectively. The effect was characteristic, dominating in the increase nearly regularly distributed single potentials of high amplitudes. In some cases the mixed appearance of higher and lower potentials, belonging probably to several axons was observed *(Fig. 10)*.

Dipterex, being an organic phosphoric acid ester (insecticide), also increased the activity when applied onto the lip *(Fig. 11)*. The increase of activity was tonic and surpassed the control by 92 per cent when used in 10^{-2} mol concentration, while by 41 per cent in 10^{-3} mol concentration. In 6—8 sec after application there was a decrease in activity, nevertheless, it was also at this time considerably higher than the control.

Malathion belongs also to organic phosphoric acid esters, it is used to kill molluscs. We tested its effect in a wide concentration range. At application of 10^{-4} g/ml the activity was 85 per cent higher than the control, while between 10^{-6}—10^{-8} g/ml it evoked only about 40—60 per cent increase, not

differing much from the effect of the distilled water, used this case as a solvent. The increase both of lower and of higher spikes was observed *(Fig. 12)*, but the variation of amplitudes was less than when adding distilled water alone.

B. *Isolated lip preparation*

When the two lip nerves connecting the feeding organ and the CNS were cut at their origin from the cerebral ganglia, the control activity was suddenly increased. In about 10 min it decreased gradually than kept a constant level being somewhat higher, than before cutting off the ganglia. The activity varied between 2 and 11 imp/sec, yielding a mean of 5.3/sec (from 236 measurements). The amplitudes of the potentials measured 50—100 μV.

It must be noted that spontaneous activity could be recorded from the nerve even if it was cut also at entering the lip, i.e. if an isolated nerve preparation was made. However, this activity ceased entirely within 8—10 min, or very rarely occurring low (20—30 μV) potentials of long duration could be observed over ten minutes.

Applying the above substances onto the lip different activity increases could be observed, however, it was always less than we found on the brain-lip preparation. 15 per cent increase of activity caused by tactile stimulus was not

TABLE I

Electrical activity recorded from the lip nerve. The number of potentials occurring within 10 sec was counted. Values are expressed as percentage of the control

Substance	Brain-mouth preparation	Number of experiments	Isolated mouth preparation	Number of experiments
Physiological saline (control)	100		100	
Physiological saline applied on filter paper (tactile stimulus)	128	17	115	14
Distilled water	153	10	107	9
NaCl 5.8 per cent in dist. w.	219	8	145	12
KCl 7.5 per cent in dist. w.	247	7	166	16
Glucose 1 per cent in dist. w.	154	9	115	18
Glucose 1 per cent in physiol. saline	151	3	102	14
Glucose 2 per cent in physiol. saline	168	3	115	15
Glucose 5 per cent in physiol. saline	178	3	124	14
Saccharose 1 per cent in dist. w.	191	9	132	18
Saccharose 1 per cent in physiol. saline	189	3	132	15
Saccharose 2 per cent in physiol. saline	185	3	127	15
Saccharose 5 per cent in physiol. saline	102	3	141	15
Dasomet 10^{-3} mol in physiol. saline	155	9	112	8
Dasomet 10^{-2} mol in physiol. saline	170	8	123	10
Dipterex 10^{-3} mol in physiol. saline	141	8	122	9
Dipterex 10^{-2} mol in physiol. saline	192	7	122	8
Malathion 10^{-8} g/ml in dist. w.	142	7	145	8
Malathion 10^{-6} g/ml in dist. w.	160	7	140	6
Malathion 10^{-4} g/ml in dist. w.	185	7	111	6

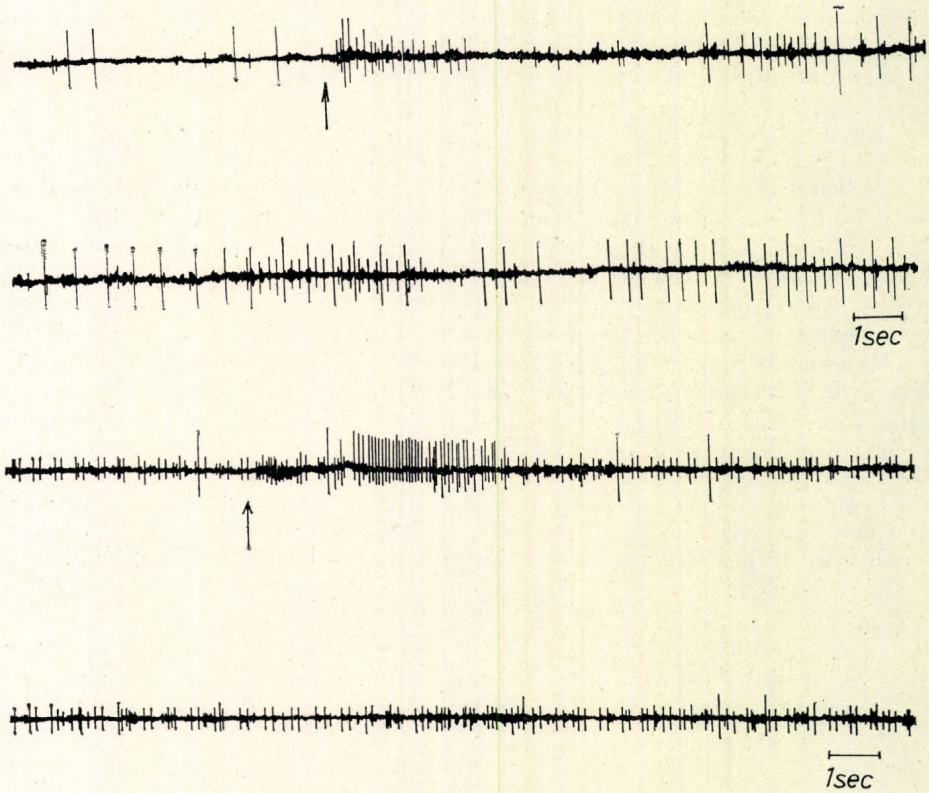

Fig. 11. Effect of dipterex 10^{-3} mol (upper) and 10^{-2} mol (lower)

surpassed upon applying distilled water, 1 and 2 per cent solutions of glucose solved both in distilled water or in physiological solution, dasomet in 10^{-3} mol and malathion in 10^{-4}—10^{-8} g/ml. In the other cases the increase of activity varied between 22 and 66 per cent. The mean values obtained at the application of substances is presented in *Table I*. For comparative purposes the values obtained on the brain-lip preparation are also presented.

Discussion

When investigating the feeding habits and food detection of several gatropods the specificity of receptors localized around the mouth was shown by several authors. KEICKEBUSCH (1953) carried out experiments on the behaviour of *Helix pomatia* offering various sugars and salts in solution. Based on the time of reaction he concluded that the chemoreceptors of the head, foot and mantle margin are different according both of sensitivity and type of the response. CARR (1967a, b) investigated the sensitivity of *Nassarius obsolatus* to the components of crab extract and found that the receptors of the proboscis are especially sensitive to several amino acids, amines and some other substances

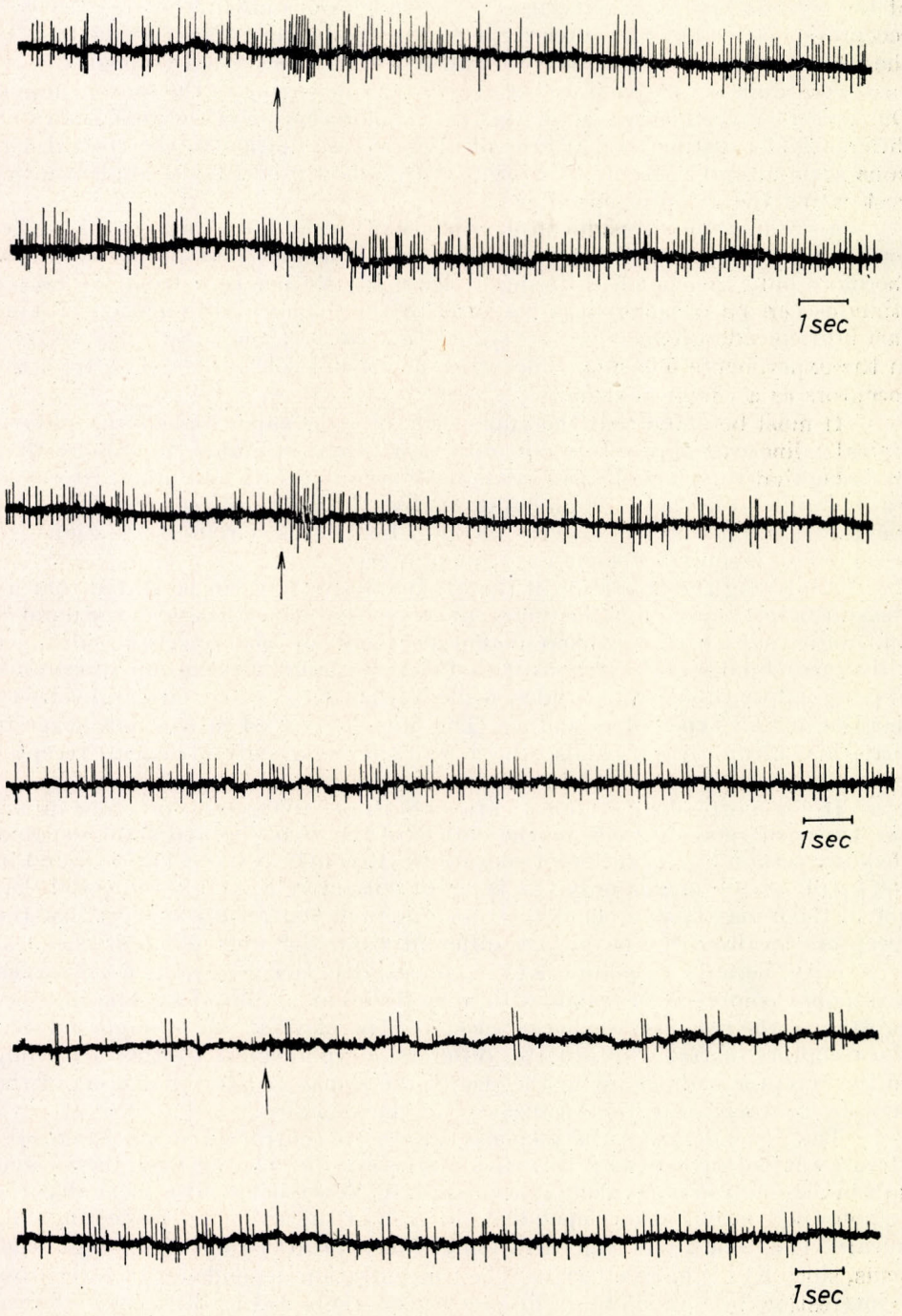

Fig. 12. Effect of malathion. Upper: 10^{-8} g/ml, middle: 10^{-6} g/ml, lower: 10^{-4} g/ml

of the extract. In these experiments the conclusions about the specificity of receptors localized on the outer surface of the body were drawn on the basis of the behavioural reaction of the animal, however, no electrophysiological analyses were done for clearing up the specificities occurring in the sensory input. Our present investigations show that lip chemoreceptors of the snail send well differentiable, distinctive patterns of afferent impulsation to the central nervous system, and it seems to us that the method used can be applied in investigating the receptor specificity.

According to our results on the surface of the lip both chemo- and tactile-receptors are present. The test method used includes also tactile stimulus, therefore only the values differing at least 15—20 per cent from the tactile stimulus can be considered as response to the chemical stimulation. Taking this into consideration, it can be stated that each of the substances we used in the experiments has such concentration which evokes a response from the receptors as a chemical stimulus.

It must be noted that the application of filter paper soaked into physiological saline can play a role not only as a tactile stimulus. It is more than probable that the physiological saline itself stimulates the receptors. However, for making and keeping the preparation alive the use of physiological saline is inevitable and it contacts also the receptor field. These circumstances must be accepted as a source of some experimental error.

Analyzing the character of the evoked activity it can be stated that all the substances caused long-lasting, tonic responses, nevertheless, in the number and amplitudes of the evoked potentials there are characteristic substance differences. So it was characteristic that sugars stimulate the group appearance of potentials with high amplitudes, while NaCl or KCl caused a general activity increase for all types of potentials. The effect observed in dasomet suggests that this substance causes also the activity increase only of several receptor types.

In his morphological investigations SCHULZ (1938) described four different types of receptor cells in the cuticle of the *Helix* lip and supposed that they are responding to different sensations. Recently NAVONI (1973) found in the lip of opistobranchia only two types of sensory cells, and he supposed that one of them may have a chemoreceptor function. Our results suggest that the receptors localized in the lip are different from the wiev point of chemical sensitivity, because on adding KCl, a general depolarizing agent, we observed a response composed of spikes with very different amplitudes while in other substances the response was more selective. Therefore, it can be supposed that the receptors of *Helix* lip are also different morphologically either according to the receptor endings, or in the size of the somas or axon diameters of the sensory neurone, or in the localization of the somas.

The dependence of the response on the concentration of the same substance was not investigated this time systematically, neither were the various substances used in equivalent concentrations. Nevertheless, it is clear that the response is concentration-dependent (in case of sugars, plant-protecting chemicals). The same 1 mol concentration of NaCl and KCl caused different reactions, since KCl is more effective. The concentration-dependency of saccharose is interesting, because on brain-lip preparation there was no difference between the effect of 1 and 2 per cent solution, while 5 per cent saccharose was inhibitory in action. This may parallel the observation of KIECKEBUSCH (1953)

who found that *Helix* responds less to 5 per cent saccharose than to a 2 per cent solution of the same.

The activity increase observed on brain-lip preparation and on isolated lip preparation are differing mainly quantitatively, nevertheless, in several cases also qualitative differences occurred. So, in isolated lip preparation the control activity proved to be higher by about 15—20 per cent, but the increase of activity on the application of various substances was nearly always lower on this preparation. There seem to be two possible explanations for the latter. One possibility is that because of the higher control level the same activity increase results in a lower value if expressed in the percentage of the control. The other possibility is that the cutting of the nerve between the CNS and the lip causes the switch out of some receptors. The latter is more probable, since the sensory fibres having their soma inside the ganglion got eliminated by cutting their axons and they do not show spike activity any more.

The fact that the nerve of the isolated lip preparation gives a higher spontaneous activity than that of the brain-lip preparation refers to the well-known phenomenon that CNS has a regulatory role on the sensitivity and activity of the receptors. It seems that in sensory neurones relieved from central control there is a higher resting activity, but at the same time their sensitivity drops, too. Especially notable is the low sensitivity of receptors to distilled water and sugars in the isolated lip preparation, what can be explained by one of the above reasons.

The spontaneous activity observable in isolated nerve preparation for a short period of time can be ascribed to the firing of neurone somas located in this nerve, elicited by the injury of the axons. The presence of neuronal bodies in the intestinal nerve of *Helix* was shown by SCHLOTE (1955).

Summary

1. The chemical sensitivity of the lip receptors to various chemicals is different what can be detected by recording the electrical activity from the lip nerve both in brain-lip preparation and in isolated lip preparation.

Applying the general depolarizing agent KCl to the lip, the variation of spike amplitudes is very wide in the increased activity, the selectivity of the responding receptors is well expressed in the case of glucose and saccharose.

3. Distilled water seems to be a stimulus increasing the activity of the receptors differently than does physiological saline. The effects of NaCl, glucose, saccharose and of drugs used in plant-protecting chemistry (dasomet, dipterex and malathion) were also significant in some concentrations.

4. The basic activity of the lip nerve was higher on the isolated lip preparation than on the brain-lip preparation. This refers to the role of the CNS in the control of the activity of sensory neurones. Our electrophysiological records prove that there are neurones in the isolated lip nerve which function even after the isolation of the nerve.

5. Isolated lip preparations gave a somewhat lower response to the same chemical substance than did brain-lip preparations. This can be explained with the presence of a part of the sensory neuronal bodies in the ganglion itself.

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SZENZOROS BEMENET ELEKTROFIZIOLÓGIAI VIZSGÁLATA A SZÁJ KÖRÜLI RECEPTOROK KÉMIAI INGERLÉSE ESETÉN *HELIX POMATIA*-N

Salánki János és Truong Van Bay

Összefoglalás

1. Száj körüli receptorok kémiai érzékenysége különböző anyagokra eltérő, s ez az elektromos aktivitás ajakidegről történő elvezetése során agy-szájszerv preparátumon és izolált szájszerv preparátumon egyaránt jól detektálható.

2. Az általános depolarizáló hatású KCl ajakra való applikálásakor regisztrált aktivitásban az amplitúdóvariáció nagy, a glukóz, szacharóz esetén a szelektivitás jól kitűnik.

3. A desztillált víz a fiziológiás oldattól eltérő aktivitásfokozó ingert jelent az ajak receptoraira. Ugyancsak jelentős a NaCl, a glukóz, szacharóz, valamint a növényvédelemben használatos dasomet, dipterex és malathion hatása is.

4. Izolált szájszerv preparátum esetén az ajakideg alapaktivitása magasabb, mint agy-szájszerv preparátumnál. Ez a központi idegrendszer szenzoros sejtaktivitást szabályozó szerepére utal. Elektrofiziológiai adataink azt bizonyítják, hogy neuronok az izolált idegben is előfordulnak, melyek egyideig az ideg izolálása után is működnek.

5. Az izolált szájszerv preparátum ugyanazon kémiai ingerre kisebb aktivitással reagál, mint ami agy-szájszerv preparátum esetében megfigyelhető. Ez a központi idegrendszer receptorérzékenységet befolyásoló hatásával lehet összefüggésben ill. azzal, hogy a szenzoros neuronok egy része a központi idegrendszerben helyezkedik el.

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ION CURRENT TEMPERATURE DEPENDENCE OF Br-TYPE NEURON OF *HELIX POMATIA* L.

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The temperature dependence of neuron activity patterns and of neuron activity parameters is known both for vertebrate animals (BARKER and CARPENTER, 1970) and for invertebrate animals (KERKUT and TAYLOR, 1956). The change of ambient temperature will change the neuron resting potential (HODGKIN and KATZ, 1949), and a similar significant change will also be introduced in the repetition frequency of the cells (CARPENTER, 1967). Investigation of certain giant neurons showing burst activity proved the temperature dependence of the activity pattern: the characteristic activity pattern of the control disappears at low temperatures below 12°C and at high temperatures above 33°C (WACHTEL and WILSON, 1973; SALÁNKI et al., 1973).

Previous investigations carried out in our Institute (SALÁNKI et al., 1975) and also the work of other authors showed that probably several mechanisms were responsible for the slow periodical membrane potential change resulting in the burst activity pattern, and for the generation of the action potential.

Investigations of different ion content solutions also showed that the generation of slow waves and action potentials is dependent on the ions involved (JUNGE and STEPHENS, 1973; SALÁNKI et al., 1975). It is known that the ion current during voltage clamp measurements has a marked temperature dependence (HODGKIN et al., 1952). Recognizing the decisive role of temperature in the activity pattern, the purpose of our present investigations has been the determination of the temperature dependence in the Br-type RPal cell of *Helix pomatia* L.

Material and method

The isolated ganglion of *Helix pomatia L.* has been placed in a perfusion chamber having a volume of 3 cm^3. The temperature of the physiological solution and the temperature of the ganglion have been adjusted to the values of 7, 22 and 33 degrees Centigrade, resp. The temperature adjustment has been performed by PELTIER batteries driven by a special circuit (VÉRÓ, 1974a).

Glass microelectrodes filled with 2.5 M KCl having a resistance in the range of 4 to 7 Mohm have been used for recording the membrane and action potentials. Perfect compensation of the electrode potential was possible by the

use of a high input impedance negative capacitance amplifier. The voltage clamp measurement method (VÉRÓ, 1974b) was used for the determination of ion currents.

The components of the physiologicl solutions used in our experiments are given in the following *Table*

		Normal solution	Na^+ free solution	Ca^{2+} solution
NaCl	(mM)	51	—	51
KCl	(mM)	4.6	4.6	4.6
$MgCl_2 \cdot 6H_2O$	(mM)	12.0	12.0	12.0
$CaCl_2 \cdot 2H_2O$	(mM)	10.0	10.0	—
$NaHCO_3$	(mM)	2.3	—	2.3
Tris-HCl	(mM)	—	53.3	22.0

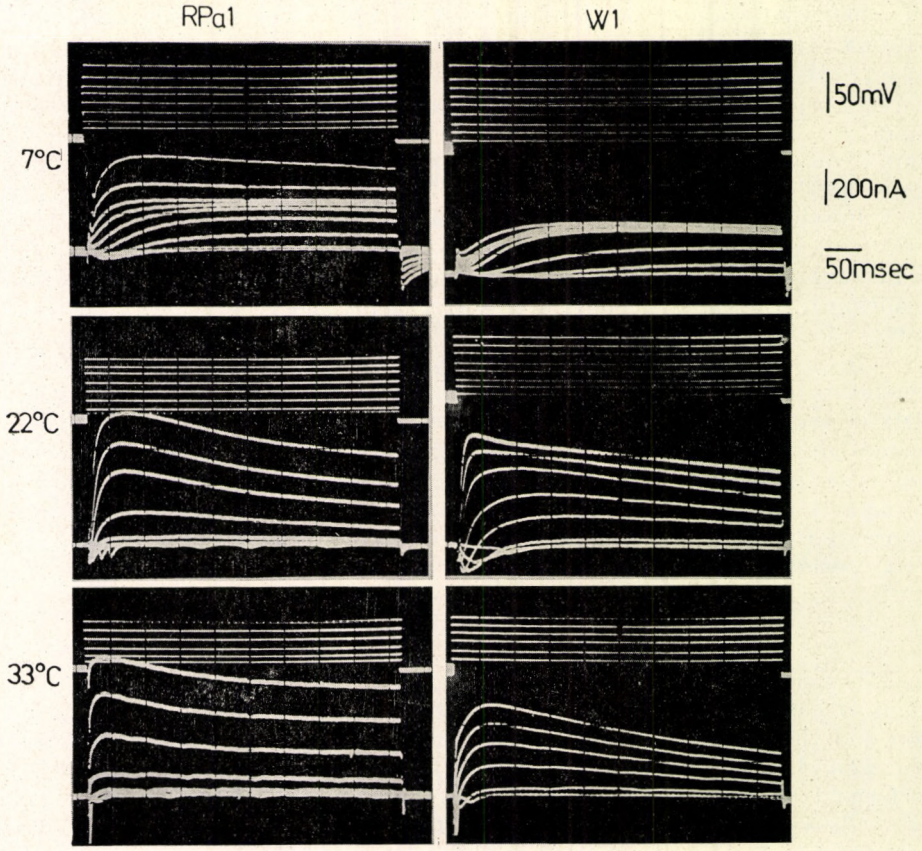

Fig. 1. Temperature effect on ion currents of PRa1 and W1 giant neurons

Results

1. Temperature effect on ion currents of RPal and Wl giant neurons

The ion current change of the RPa1 cell has been compared with the current change of the monomodal pacemaker neuron in the visceral ganglion, denoted by W1. *Fig. 1* shows the ion currents of cell RPa1 and W1 at different temperatures. It is seen that there is only a quantitative difference between the temperature dependences. The current changes due to temperature are adequately reflected by the votage-current characteristics plotted during the experiments. Results show that the highest inward current component of neuron RPa1 is 160 $\pm$ 10 nA at 22°C, 50 $\pm$ 11 nA at 7°C and 200 $\pm$ 20 nA at 33°C (see *Fig. 2*, left side). The outward current is first higher at the low temperature of 7°C than at 22°C and 33°C, but the derivative of the current-voltage characteristic shows a marked decrease for more positive voltages. For the W1 neuron, the highest value of the inward current is 190 $\pm$ 30 nA at 22°C, 33 $\pm$ 7 nA at 7°C and 220 $\pm$ 30 nA at 33°C. The outward current values are similar to those of the RPa1 neuron (*Fig. 2*, right side). *Fig. 3* shows the relative conductance change of the RPa1 neuron as a function of the membrane potential. It can be seen that the initial conductance for the outward current is high at 7°C, but the conductance is lower in the positive voltage range. The temperature dependence of the conductance for inward currents is not so marked.

The temperature change had not only effects on the value of the ion current but also on it's time dependence. *Fig. 1* shows clearly the inward current decay time increase at 7°C as compared with that at 22°C. At 33°C,

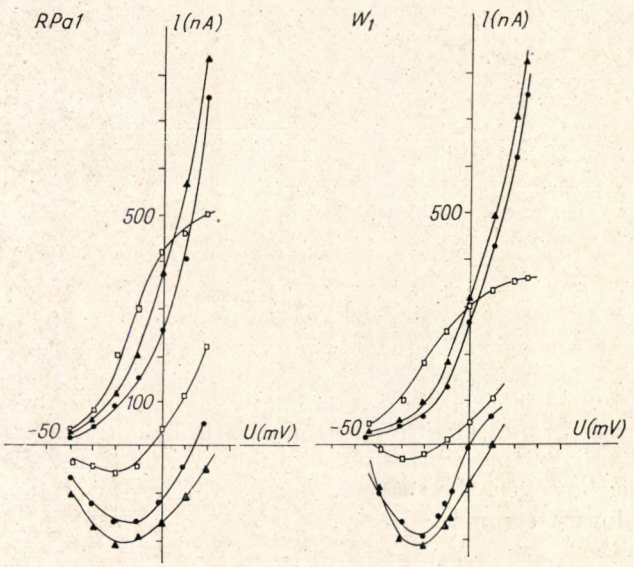

Fig. 2. Current-voltage characteristics of RPa1 and W1 neurons. □: 7°C, ○: 22°C, ▲: 33°C

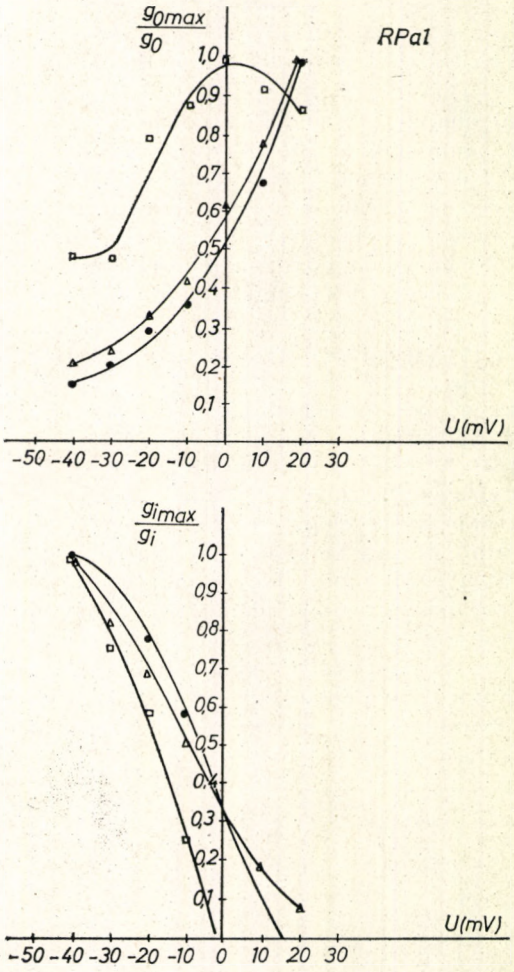

Fig. 3. Voltage dependence of RPa1 neuron relative conductances (g_i: Na^+ conductance, $g_{i\,max}$: highest Na^+ conductance, g_0: K^+ conductance, $g_{0\,max}$: highest K^+ conductance. □: 7°C, 0: 22°C, ▲: 33°C

the inward current decay time is substantionally lower than the decay time at 22°C. *Fig. 4* shows the inward current decay time as a function of the membrane potential for different temperatures. It can be seen that for the lowest command pulse of 10 mV there is an approximately 50 msec decay time difference between values measured at different temperatures, corresponding to a 50% change. For the highest command pulse of 90 mV, the time difference is only 2.5 . . . 3 msec; however, the percentage time difference is equal to that obtained for the lowest command pulse.

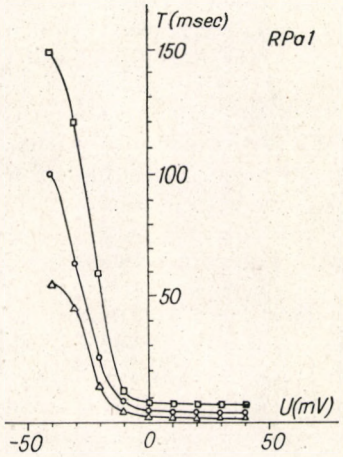

Fig. 4. RPa1 neuron inward current time durations as a function of membrane potential at different temperatures. □: 7°C, ○: 22°C, ▲: 33°C

2. Temperature effect on RPa1 neuron ion currents in Na^+-free and Ca^{2+}-free solutions

RPa1 neuron iron current data for physiological solutions having different ion contents have been reported in our previous investigations (SALÁNKI et al., 1975). Thus at 22°C, the inward current practically disappears in Na^+-free solutions, and shows a 30% decrease in Ca^{2+}-free solutions. The membrane current changes are identical to those obtained in normal physiological solutions; in this case, the control values have been those obtained with an ion-free solution at 22°C. Thus the inward current in the Na^+-free solution appears not even at 7°C. The inward current in the Ca^{2+}-free solution is decreased for lower temperatures and increased for higher temperatures by the same percentage as in normal physiological solution. In an ion-free solution, the temperature effect on the current time dependence will be the same as in normal solutions.

3. The effect of conditioning hyperpolarization on RPa1 neuron ion currents in normal physiological solutions

It is known that the outward current of some giant neurons has two components; a low delayed component which, though possessing some inactivation, is present during the whole period of the command pulse, and a fast component which precedes even the inward current and is characterized by complete inactivation; this fast component is produced by conditioning from a voltage level below the resting membrane potential (NEHER, 1971). This conditioning hyperpolarization has been investigated for the RPa1 neuron, and it was found that in the temperature range covered, the fast component is not present.

Further experiments have been carried out to investigate the slow outward current at different temperatures. A given constant voltage level

has been approached from different holding levels (conditioning hyperpolarizations). The initial conditioning value was equal to the membrane potential of −50 mV, the highest value was −150 mV, and the time duration was 250 msec. The outward current at 7°C showed an increase during the whole period of the command step, both with −50 mV and with −150 mV conditioning. At 22°C and with −50 mV hyperpolarization, the outward current attained it's steady state value in 50 msec. With higher hyperpolarization levels, a maximum appeared on the current-time plot. At 33°C, a maximum — though not so pronounced — was present even with −50 mV. This maximum tends to be more pronounced with higher conditioning, and the highest maximum value occurs with −150 mV hyperpolarization at t = 25 msec.

Fig. 5. shows the outward current maximum values and the approximately steady-state current values at the end of the command-step duration as a function of the holding level at different temperature. It is seen that at 7°C, the current value at the end of the command step duration represents simultaneously the highest value and the steady-state value. At 22°C, the highest deviation between the maximum and steady state current values (67%) is present with a holding level of −150 mV, but with −50 mV conditioning, the difference practically disappears. At 33°C, the highest deviation occurs at −150 mV conditioning (63%), while −50 mV will result in a current difference of less than 5%.

Discussion

It is known that the resting potential as determined from the equation of NERNST is temperature dependent. The potential difference effected by the Na^+ and K^+ pumps, which is also responsible for the generation of the resting potential, is temperature dependent too (GORMAN and MARMOR, 1970a; GORMAN and MARMOR, 1970b). The resting potential is further dependent on on the resting permeability of Na^+ and K^+ which are themselves temperature dependent parameters (MARCHIAFAVA, 1970; LIVENGOOD and KUSANO, 1972).

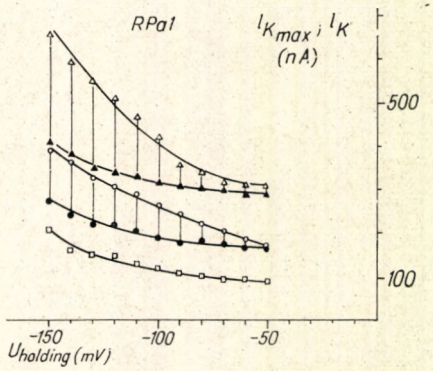

Fig. 5. PRA1 neuron outward current highest and steady state values at different temperatures as a function of the conditioning hyperpolarization.
□: 7°C, U_h = − 50 and − 150 mV; ●: 22°C, U_h = − 50 mV; ○: 22°C, U_h = −150 mV; ▲: 33°C, U_h = −50 mV; △: 33°C, U_h = −150 mV.

Thus the temperature effect on the membrane potential can also be verified indirectly. The membrane conductance in the case of different ions is proportional to the permeability (FRANKENHAUSER, 1963), so it can easily be concluded that the temperature dependence of ion currents is due to the change of conductance. Thus in the cases of RPa1 and W1 neurons too, the current decrease with decreasing temperature is caused by the decrease of Na^+ and K^+ conductance.

The temperature changes produced definite effects on the current function time parameters: at lower temperatures, the duration and the rise/fall times of the inward currents decreased. This implies that not only the Na^+ and K^+ conductance values are decreased but their time function is also changed. While the temperature effect on the conductance value is primarily determined by the highest time-independent conductance constant (HODGKIN and HUXLEY 1952), the temperature effect on the conductance time dependence is mainly effected by the membrane time constant change. The application of different conditioning hyperpolarizations showed that the activation and inactivation potential of the membrane is temperature dependent (MAGURA et al., personal communication). Our investigations showed that at 7°C, both the Na^+ and the K^+ activation is slow and nearly simultaneous. We believe that the current increase in time is primarily caused by the slow Na^+ activation. At higher temperatures, the Na^+ activation is substantially accelerated, and a significant increase of K^+ activity results in complete inactivation; thus the inactivation of the outward current is shown by the over-all current function. This proves that the temperature has different effects on the conductances of Na^+ and K^+; according to our investigations, Na^+ has a higher temperature sensitivity.

Our investigations also showed that the temperature dependence in case of an Na^+- and Ca^{2+}-free solution cannot be proved in this way. However, taking into account the role of these ions, especially that of Ca^{2+}, in the seasonal changes (BARKER and GAINER, 1973), the membrane properties are probably effected by the temperature dependent Ca^{2+} concentration in a manner which has not yet been cleared.

No specific feature of the RPa1 neuron which would distinguish this neuron showing a characteristic actvity pattern from other pacemaker neurons could be discovered. The RPa1 neuron parameters have a temperature dependence which is practically the same as that of other neurons with non-periodic activity patterns. The mechanism responsible for the Br-type activity pattern, though temperature dependent (SALÁNKI et al., 1973), cannot be determined by the methods outlined in this paper.

Summary

1. The magnitude of the inward current decreases, the duration of the inward current increases with temperature.

2. The outward current steady state value in the case of low command step values is highest at 7°C, and in the case of 50 mV and higher command steps, it is lowest at 7°C.

3. The K^+ conductance is decreased at low temperaturs in the case of command steps higher than 50 mV.

4. The conditioning hyperpolarization effect is temperature dependent; a temperature increase results in higher activation of both Na^+ and K^+, but they have a different decrease in their time durations. Thus at higher temperatures an increasing inactivation of the outward current can be ascertained.

5. The temperature dependence of currents measured with Na^+ and Ca^{2+} free solutions is not different from the temperature dependence of currents from the temperature dependence of currents measured with normal physiological solutions.

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HELIX POMATIA L. BR-TÍPUSÚ SEJTJE IONÁRAMAINAK HŐMÉRSÉKLETFÜGGÉSE

Vadász István és *Véró Mihály*

Összefoglalás

1. Hőmérséklet csökkenésekor a befelé irányuló áram nagysága csökken, időtartama nő.

2. A kifelé irányuló áram állandósult értéke kis feszültség ugrások esetén 7°C-on a legmagasabb, 50 mV-os és nagyobb kommand impulzusok esetén 7°C-on a legkisebb.

3. A K^+ vezetőképesség alacsony hőmérsékleten, 50 mV-nál nagyobb kommand impulzus esetén csökken.

4. Az előkondicionáló hiperpolarizáció hatása hőmérsékletfüggő; a hőmérséklet növelésekor mind a Na^+, mind a K^+ aktiváció mértéke nő, de itőtartamuk különböző mértékben csökken, így magasabb hőmérsékleten a kifelé irányuló áram növekvő inaktivációja figyelhető meg.

5. A Na^+ és Ca^{2+} ionok hiányában mért áramok hőmérsékletfüggése nem különbözik a normál fiziológiás oldatban tapasztalt hőmérsékletfüggéstől.

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THE GROWTH OF BLEAK (*ALBURNUS ALBURNUS* L.) (PISCES, CYPRINIDAE) IN LAKE BALATON AND THE ASSESSMENT OF MORTALITY AND PRODUCTION RATE

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The food, growth, mortality and production of pike-perch population inhabiting Lake Balaton have been studied both in larval and mature stages in details within the period 1965—73 (BIRÓ and ELEK, 1969; BIRÓ, 1969; 1970; 1972; 1973; 1975a, b). From the results obtained it could be established that in the food of pike-perch (*Stizostedio lucioperca* L.), the main predatory fish of Lake Balaton, the bleak played the most important role. In earlier papers (ENTZ, 1949—50; 1951; ENTZ and LUKACSOVICS, 1957; WOYNÁROVICH, 1959), however, the priority of pope (*Gymnocephalus cernua* L.) was noticed, and the bleak was mentioned as secondary prey-fish. The significance of bleak as food fish of pike-perch has increased due to its greater frequency parallel to the strong decrease or even disappearance of several small-sized fish species (BIRÓ, 1971; 1974; PONYI et al., 1972).

ENTZ studied the growth of bleak on material collected in the years 1947—49, and reported a fairly fast growth rate (ENTZ, 1949—50). Changes in quality of food and rate of growth of pike-perch and those of different prey fishes were observed (BIRÓ, 1971; BIRÓ and GARÁDI, 1974). The necessity came up to repeat studies concerning the growth and food of bleak in order to reveal the food-chain of pike-perch. In addition we have obtained data on mortality and production of bleak population, too, because no such data have been published till now as yet.

Material and method

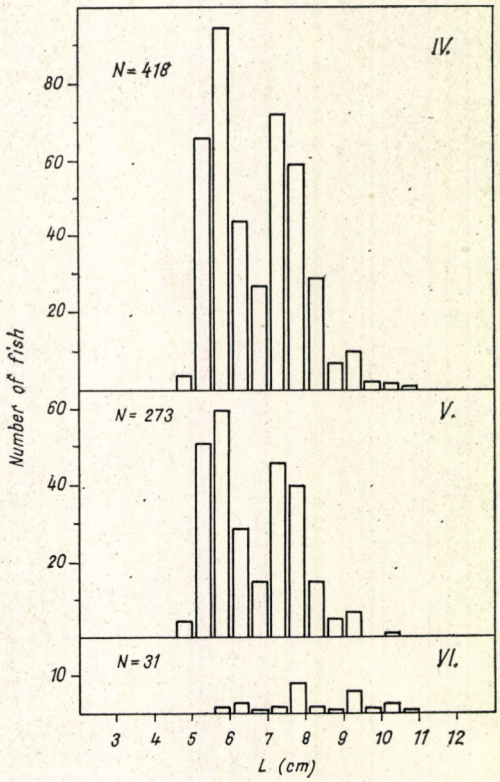
Collections were made in Lake Balaton using a 5 m long and 3 m wide otter-trawl of 5 mm mesh size altogether 47-times during the years 1968—70. For this study 1112 bleaks of different measures have been worked up collected from April to October 1968 *(Table I)*. The specimens caught by net were preserved in 4—5 per cent formaldehyde solution, and their standard and total lengths, as well as their weights were measured. 10—15 scales were detached from the area above the lateral line behind the posterior margin of the left pectoral fin *(Fig. 4)*. After cleaning, the wet scales were placed between slides, and studied with profile projector at a 50-times magnification. The total length of caual radii and the annual ring distances from the focus were measured

TABLE I

Time of samplings, the number of bleaks caught and the limits of measured lengths and weights (1968)

Date of collection	Number of fish	Standard length (cm)	Weight (g)
April	418	4.6—11.0	1.0—21.0
May	273	4.8—10.2	1.5—13.5
June	31	5.5—10.7	2.0—15.0
July	298	5.1—10.0	1.5—14.0
August	12	6.5— 9.9	3.0—13.5
September	71	3.5—11.1	0.5—20.0
October	9	6.7— 7.9	4.5— 7.5
Total	1112		

(Fig. 4). More detailed scale investigations and age-determinations were carried out on 294 specimens. In order to determine the seasonal variances of length-weight relationship (viz. BEVERTON and HOLT, 1957) data of body meas-



Figs 1—2. Histograms of standard length distribution of bleaks collected during April-October, 1968. N = number of fish investigated, L = standard length

urements of 1112 specimens were utilized. To define the length structure of the stock, length-data of 1112 bleaks were studied. The relationship between the standard lengths and total caudal radii of scales was determined by the least square method. The intercept of this line on the abscissa was taken into consideration as a correction factor in the back-calculations of fish lengths (FRASER, 1916). The growth of bleak was graphically represented by FORD–WALFORD's method (WALFORD, 1946) with use of the back-calculated standard lengths. For the description of growth in length, BERTALANFFY's (1938; 1957) growth-model was applied. Mortality and production of bleaks belonging to different age-groups were assessed according to RICKER and FOERSTER (1948) and RICKER (1958). The instantaneous coefficients of growth in weight necessary to the assessment of production were calculated after CHAPMAN (1968) and TESCH (1968).

Results

1. Lenght-distribution in different months

The standard lengths of bleaks caught in Lake Balaton varied between 3.5—11.0 cm *(Table I)*. By standard length the population showed a bimoda

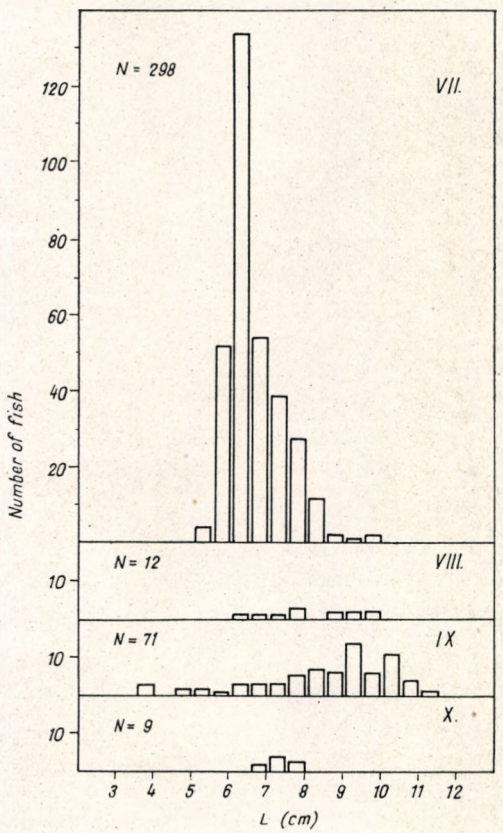


Fig. 2. Text see at *Fig. 1.*

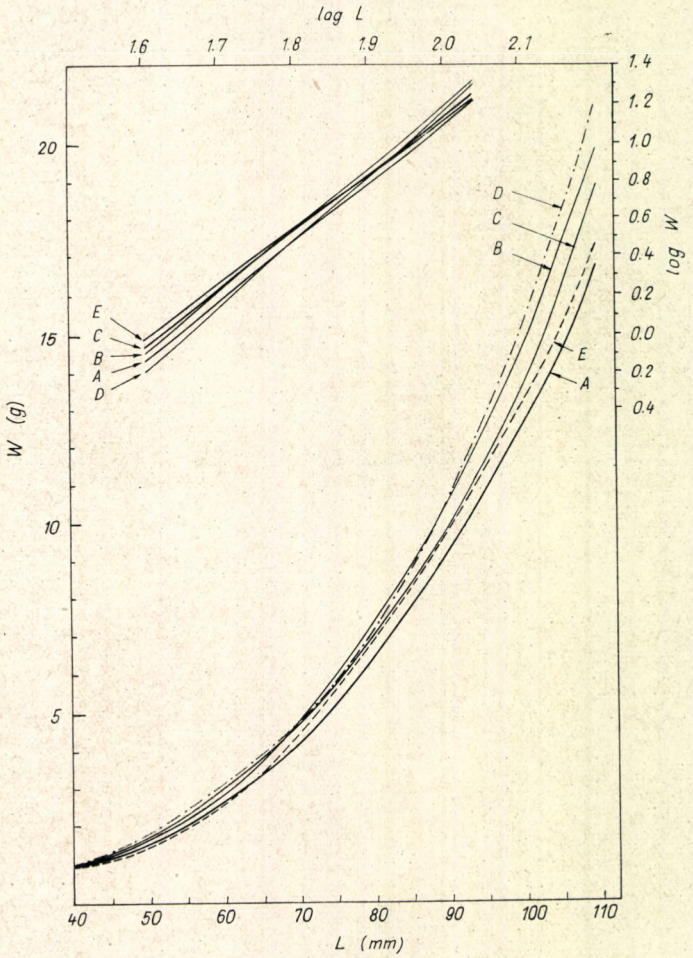


Fig. 3. Seasonal variation in length–weight relationship of bleak of Lake Balaton during consecutive months of 1968. L = standard length, W = weight, A = April, B = May, C = June, D = July, E = August

distribution during spring time (April-May). During summer and autumn (July-September) its distribution was as well asymmetrical, considering the fish material collected in different months *(Figs 1—2)*. In spring months the sample is divided into two dominant leng-groups due to the numerous occurrence of second (1+), third (2+), as well as four-summer-old (3+) specimens. During summer, it was compensated but the asymmetric length distribution remained unchanged. This phenomenon should have been only in a smaller degree the result of growth compensation.

2. Seasonal variation in length–weight relationship

The coefficient expressing the growth rate of linear dimensions in proportion to the weight, varied between 2.27—3.43 during the period of studies.

Their deviations experienced during successive months are significant ($P < < 0.01$). The relationships were as follows *(Fig. 3, curves A—F):*

April	$\log W = -4.9919 + 3.0447 \times \log L$		(A)
May	$\log W = -5.1927 + 3.1788 \times \log L$		(B)
June	$\log W = -4.8398 + 2.9840 \times \log L$		(C)
July	$\log W = -5.6741 + 3.4262 \times \log L$		(D)
August	$\log W = -5.5925 + 3.3749 \times \log L$		(E)
September	$\log W = -4.6139 + 2.8662 \times \log L$		(F)

The average of six months:

$$\log W = -5.1508 + 3.1458 \times \log L$$

where L = standard length in mm; W = weight in grams. As can be seen in *Fig. 3,* there are no great variations in body weights as compared to standard lengths. The seasonal fluctuations are rather significant among specimens of 9—11 cm sizes. The shape of curves calculated for August (*E*) and September (*F*) are uniform covering up each other.

3. Relationship between standard body length and total scale radius

The relationship between average total caudal radii of "key-scales" and standard body lengths was found linear on 294 bleaks. For this the next equations have been calculated *(Fig. 4):*

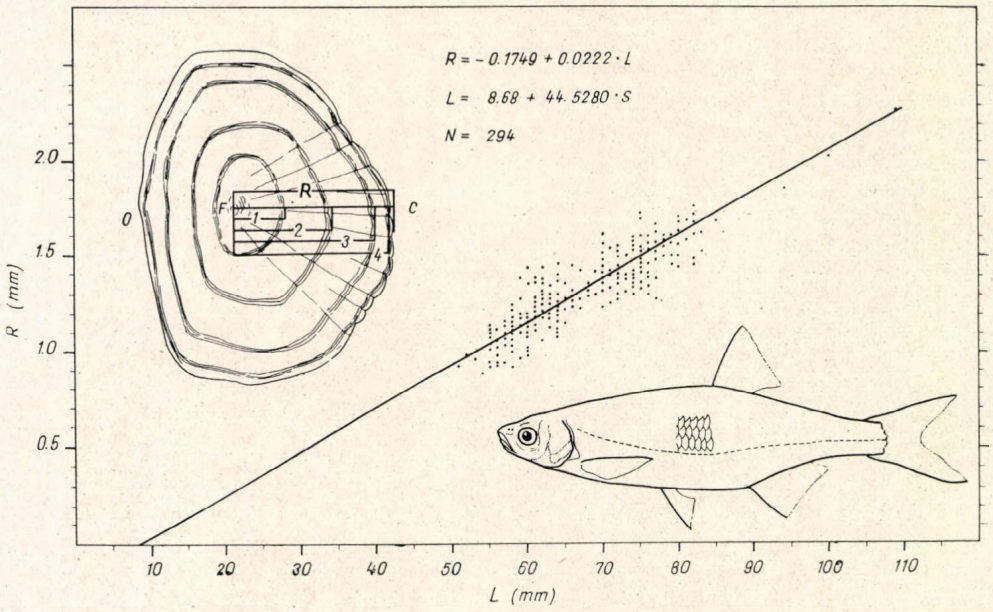


Fig. 4. Linear regression of average caudal radii of scales (R) in the function of standard lengths (L) established for 294 bleaks caught in Lake Balaton. Its intercept on the abscissa 8.7 mm. Points of measurement indicated on the sketch of scale are:
F = focus, R = total caudal radius, O = oral edge of scale, C = caudal edge of scale

$$R = -0.1749 + 0.0222 \times L$$
$$L = 8.68 + 44.528 \times R$$

where L = standard length in mm; R = total caudal radius of scale in mm.

Average scale radius and standard length calculated by both equations show only small deviations as compared to the measured averages *(Table II)*.

TABLE II

Relationship between the standard lengths (L = mm) and the total caudal radii of scales (R = mm) of bleak

Measured		Calculated	
standard length	caudal radius of scale	standard length*	caudal radius of scale**
55	1.02	54.1	1.05
60	1.17	60.8	1.16
65	1.26	64.8	1.27
70	1.44	72.8	1.38
75	1.51	75.9	1.49
80	1.57	78.6	1.60
85	1.64	81.7	1.71
100	2.03	99.1	2.04
109	2.28	110.2	2.27

* calculated according to the equation of $L = 8.68 + 44.528 \times R$
** calculated according to the equation of $R = -0.1749 + 0.0222 \times L$

About 30—40 per cent of the scales examined showed spots of regeneration after different mechanical damages and irregularly developed marks due to parasitic effects. The year rings on normal scales, developed symmetrically, were usually formed regularly, their radii being just the same.

The larval annuli usually do not separate definitely on the scales. Number of sclerits developing within the first year ring varied between 15—25. A stepped, gently S-shaped relationship seems to exist between the standard lengths and the average year-ring distances established for different age-groups, which reflects the exponential pattern of growth.

4. Growth in standard length

Average standard lengths back-calculated from the distances of the year rings are shown in *Table III*. No positive Lee-phenomenon was observed on the basis of minimal and average standard lengths back-calculated for the five age-groups, but it was found to be positive in the case of maximal values. The growth of bleak in Lake Balaton based on the back-calculated average values *(Table III, C)* seems to be slow, but even smoothed *(Fig. 5)*. Analysing the growth of five different age-groups by year-class strengths, the linearity of average growth in length has changed and preferably a stepped growth could be observed primarily in age-groups 4+ and 5+ *(Fig. 6)*. Based on differences in mean length of age-groups, the annual growth is small. The

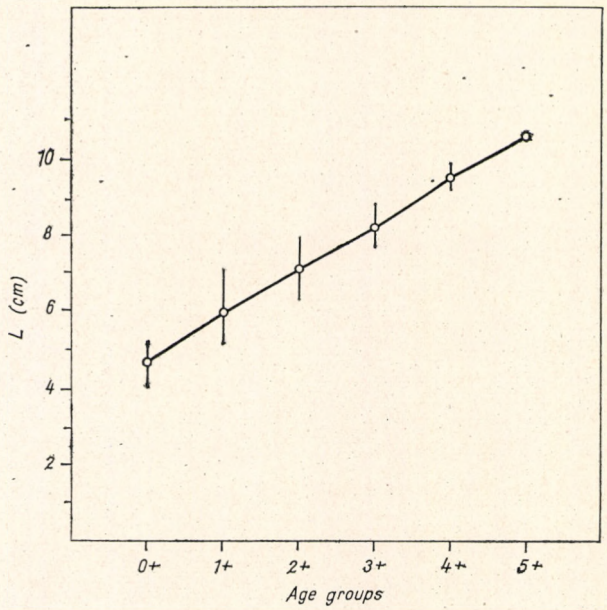


Fig. 5. Average annual growth in standard length of bleak in Lake Balaton from the one-summer (0+) to six-summer-old (5+) age

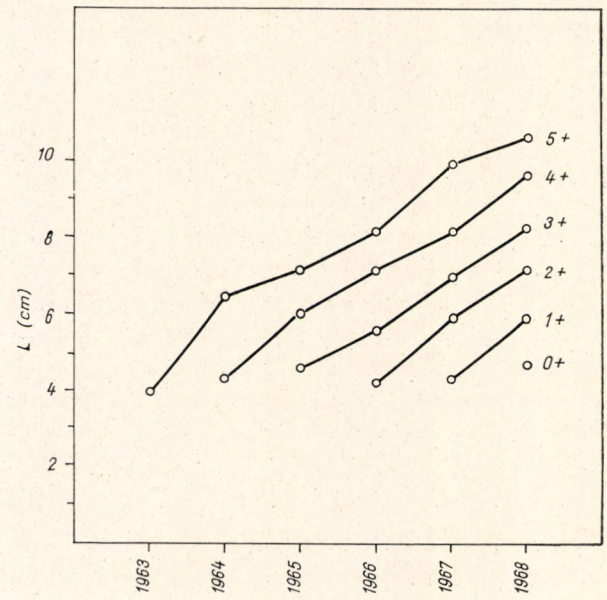


Fig. 6. Growth by year-class strength of age-groups 0+ to 5+ in Lake Balaton

TABLE III

Back-calculated standard lengths (cm) of bleak in Lake Balaton

Standard length		Age-groups						Average	Increase	W* (g)
		0+	1+	2+	3+	4+	5+			
L_0	A	4.1	3.7	3.4	3.9	4.2	—			
	B	5.2	5.2	5.1	5.2	4.5	—			
	C	4.7	4.3	4.2	4.6	4.3	3.9	4.3	4.3	1.0
L_1	A		5.2	4.9	5.1	5.8	—			
	B		7.1	7.0	5.9	6.3	—			
	C		5.9	5.9	5.6	6.0	6.4	6.0	1.7	2.8
L_2	A			6.3	6.7	7.0	—			
	B			8.0	7.4	7.3	—			
	C			7.1	6.9	7.1	7.1	7.1	1.1	4.7
L_3	A				7.7	8.0	—			
	B				8.9	8.2	—			
	C				8.2	8.1	8.1	8.1	1.0	7.1
L_4	A					9.3	—			
	B					9.9	—			
	C					9.6	9.9	9.8	1.7	13.0
L_5	C						10.6	10.6	0.8	16.6

A = minimum; B = maximum; C = average value

* calculated weight according to the average length-weight relationship:
$\log W = -5.1508 + 3.1458 \times \log L$

slowest growth period of age-group 5+ coincides in time with the mass fish kill (1965). The back-calculated standard lengths were graphically represented according to FORD-WALFORD'S method. Plotting the lengths in t-time (L_t) in the function of one year later values (L_{t+1}), the dots determine a straight line of which the intercept by the diagonal line passing through the origin at an angle of 45 degrees, gives a theoretically attainable, maximum length, i.e. $L_\infty = 18.8$ cm *(Fig. 7)*. Except the L_0 value, the dots are placed closely along the straight line. From our data the other parameters of BERTALANFFY'S growth-model were also determined, as well as the start point of the exponential curve ($t_0 = -1.25$ year) and the growth coefficient ($K = 0.1142$) *(Fig. 8)*.

TABLE IV

Back-calculated standard lengths of bleak in Lake Balaton

Age groups	Back-calculated standard length (cm)		
	From scales	Ford-Walford's plot	Bertalanffy's model
0+	4.3	3.8	4.3
1+	6.0	5.9	5.8
2+	7.1	7.4	7.3
3+	8.1	8.4	8.5
4+	9.8	9.2	9.6
5+	10.6	10.8	10.6

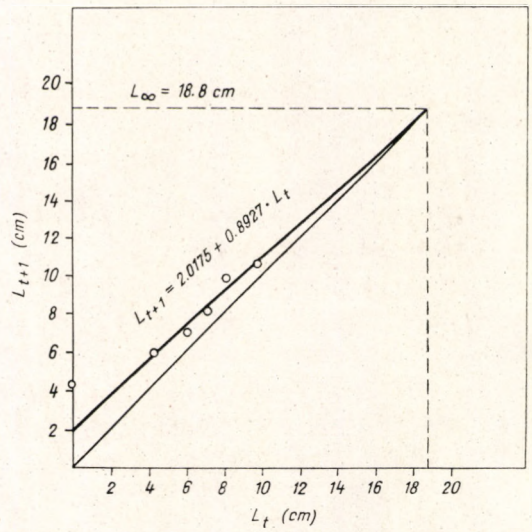


Fig. 7. FORD-WALFORD's plot. L_t = standard length in every t-period of time, if t = 1 year; L_{t+1} = the same one year later; L_∞ = maximum attainable standard length

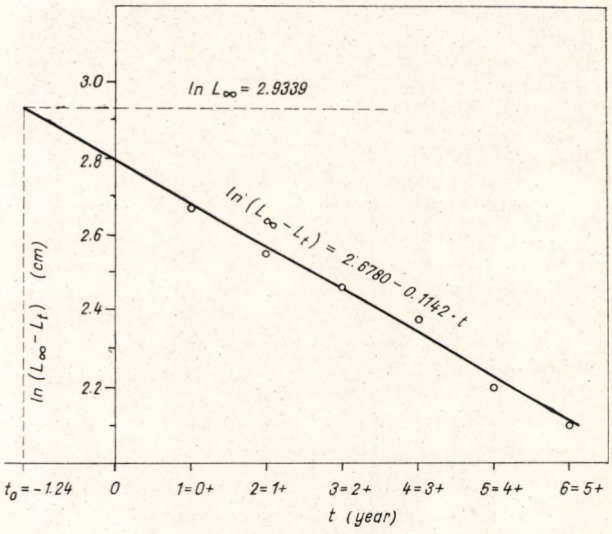


Fig. 8. Estimation of t_0 and K, the parameters of BERTALANFFY's growth model (For explanation see text)

Representing the exponential growth by the parameters obtained, we got a flat-like curve *(Fig. 9)*, the numerical equation being as follows:

$$L_t = 18.8\ (1 - \exp - 0.1142/t + 1.25/)$$

where L_t = standard length given in cm in every t-period of time, if t = 1 year.

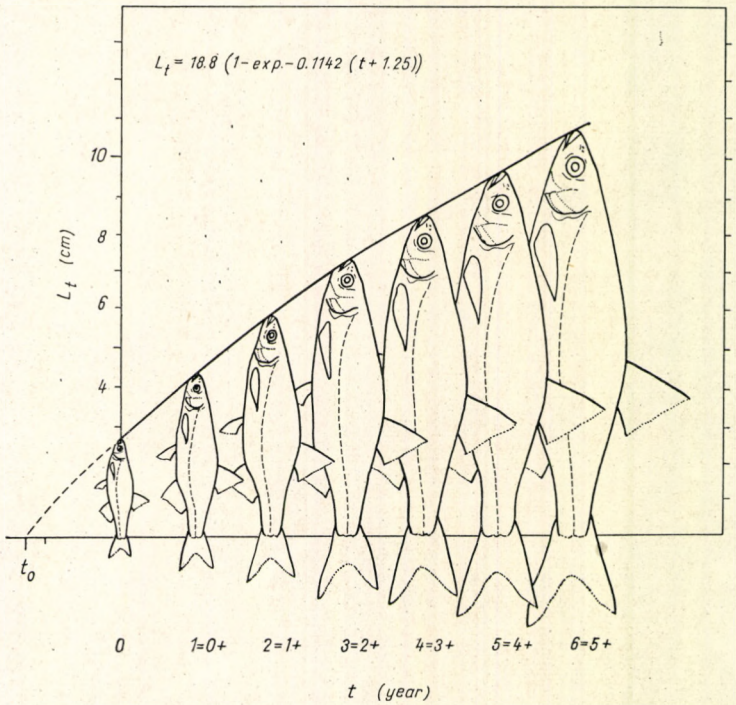


Fig. 9. Growth in length of bleak in Lake Balaton by BERTALANFFY's growth model (For explanation see text).

Comparing the values back-calculated from scales and those represented according to FORD-WALFORD and by BERTALANFFY's model, there is a deviation of 2 to 5 mm in different age-groups *(Table IV)*. This insignificant difference proves the suitability of the model for correct description of growth.

5. *Age-distribution and mortality*

From 1112 bleaks studied, altogether 291 specimens were aged on the basis of the number of completely developed annuli. For the age-distribution we got a typical curve *(Fig. 10)*, where the overwhelming majority consisted of age-groups 1+ and 2+ (46 and 34.7 per cent respectively), but the number of older ones decreased significantly (0.3—4.1 per cent). One-summer-old specimens belonging to age-group 0+ were also represented in a restricted number (13.7 per cent). This fact is evidently in connection with sampling techniques applied. The age-distribution of bleaks examined is asymmetrical. Representing the number of specimens in different age-groups, using their logarithms of natural base when the decrease in number from age-groups 1+ to 5+ was taken to be linear, the instantaneous total mortality coefficient (Z) proved to be 1.33. Survival rate calculated from it was S = 26 per cent and that of the annual mortality was A = 74 per cent *(Fig. 11)*. These rates in different age-groups are greatly variable: between age-groups 1+ and 2+

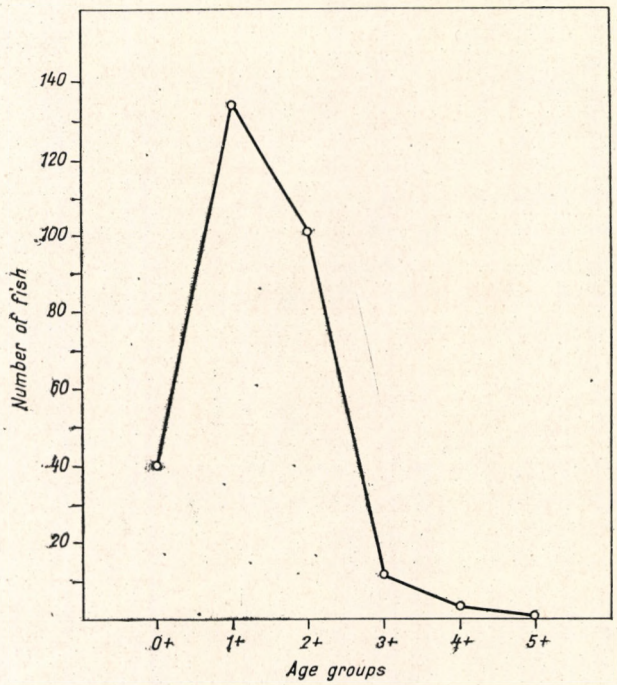


Fig. 10. Age-structure of bleak population in Lake Balaton presented by the sample studied

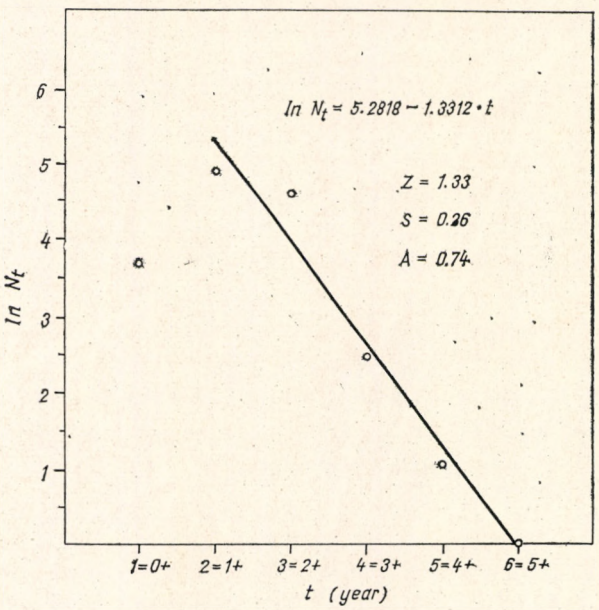


Fig. 11. Mortality of bleak in age-groups 1+ to 5+: the logarithmic decrease of individual number in different age-groups. Z = instantaneous total mortality coefficient; S = survival rate; A = rate of annual mortality

Z = 0.28; S = 76 per cent, A = 24 per cent; in 2+ — 3+ year-old ones Z = 2.13; S = 25 per cent, A = 75 per cent; and finally in 4+ — 5+ year-old specimens Z = 1.10; S = 33 per cent and A = 67 per cent.

6. *Production*

Knowing the initial number of specimens (N_0) and the average weight (W_0) of fish in every age-group and those of the coefficients of mortality (Z) and growth in weight (G), the average biomass ($\bar{B}$) of age-groups 1+ to 5+ have been assessed according to the exponential pattern of growth *(Table V)*. The average biomass proved to be 737 g and its annual increase was 529 g

TABLE V

Average biomass and its annual production in age-groups 1+ to 5+ of bleak in Lake Balaton

Age-groups	N_0 (pc)	W_0 (g)	$N_0W_0 = B_0$ (g)	Z	G	Z—G	$\bar{B}$ (g)	P (g)	$P/\bar{B} \cdot 100 =$ = A. P. (%)
1+	134	2.8	372.5	1.3312	0.9929	0.3383	317.4	315.1	99.3
2+	101	4.7	475.7	1.3312	0.5272	0.8040	325.8	171.8	52.7
3+	12	7.1	85.6	1.3312	0.4146	0.9166	56.2	23.3	41.4
4+	3	13.0	38.9	1.3312	0.6007	0.7305	27.6	16.7	60.5
5+	1	16.6	16.6	1.3312	0.2444	1.0868	10.2	2.5	24.5
Total	251						737.2	529.4	

$$\bar{B} = \frac{B_0(1\text{-exp-/Z—G/})}{Z-G} \quad \text{if } Z > G \quad \Sigma P/\Sigma\bar{B} \times 100 = \text{A. P.} = 71.8 \text{ per cent}$$

in our sample. The ratio of increased biomass ($\bar{B}G$) per average biomass ($\bar{B}$) gives the annual production (P), which on an average proved to be 71.8 per cent for the given age-groups. The annual production of average biomass was the highest in age-group 1+ (99.3 per cent), while in others it ranged from 24 to 60 per cent.

Discussion

The bimodal or asymmetrical length-distribution of bleak population in Lake Balaton has been previously observed by ENTZ (1949—50) during his studies carried out on winter shoals of bleak. Such pattern of length-distribution was also experienced during our investigations. The seasonal character of the age-structure unanimously shows that in spring the bleak population of Lake Balaton is divided into two dominant age-groups. Thereafter, during the summer months, one predominating size-class is formed. This compensation is evidently due to the relatively high number of two-summer-old fish *(Figs 1—2)*. The same could be observed when analysing the length-distribution of bleak remains found in pike-perch stomachs (BIRÓ, 1973). The length-distribution of bleaks proved to be extremely similar in the pike-

perch stomachs and during our present investigations. The spring bimodal and the summer asymmetrical structure have been observed in both cases. It could be concluded that pike-perch selects the most abundant 1—2 year-old specimens of 5.5—7.0 cm being present any time. Since the predatory pike-perch population consumes annually 31—32 kg/ha fish (BIRÓ, 1973; 1975a, b), and its food consists mainly of bleak, it is evident that bleak has a decisive importance in the food-chain as an energy-mediator. To the quantitative estimation of this phenomenon the correct knowledge of real biomass of bleak population expressed in unit of area is wanted. This should be determined by further investigations. In the littoral zone of Lake Balaton the biomass of bleak population present seasonally varies very much because of emigration and immigration. This fluctuation is multiplied by bleak-consumption of different predatory fish (pike-perch, eel, etc.). Bleak can be found along the littoral zone in significant number during the spawning period, when shoals consisting mainly of 1—4 year-old specimens can be observed *(Fig. 12)*.

From the seasonal variation of length-weight relationship it seems to be that the value of its coefficient changes parallel with the periodical spawning and takes place during spring and summer months. More remarkable differences in weight attributed to spawning activities releasing a relatively greater amount of eggs only appeared above 8—9 cm standard length. According to PAPADOPOL (1968), the range of egg number of 1—4 year-old, and of 6.5—11.5 cm sized bleaks originated from Somova (River Danube) was between 592—5700, on an average 1970. The number of ovocytes in different stages of development

Fig. 12. Periodically spawning shoal of bleak consisted of 1—4 year-old specimens at stony littoral zone of Lake Balaton

varied between 898—5814, on an average it was 2437. In the ovary he found in 44.7 per cent ovules and in 55.3 per cent ovocytes. We have no data referring to fecundity of bleaks widespread in Lake Balaton, but the number of their eggs possibly shows the same variation. In seasonal variation of length–weight relationship CHITRAVADIVELU (1974) described very great differences of the coefficients of bleaks caught in ŽOFIN-complex (River Danube) between July and October.

Studies on cycloid scales of characteristic shape showed a linear regression between standard lengths and caudal radii of scales. By this relationship 8.7 cm was calculated for standard length, which gives the size measurable in time of "key-scale" formation *(Fig. 4)*. This is about a half of that CHITRAVADIVELU (1971) has observed on bleaks (16 mm) inhabiting the Labe river system. This difference may be due to the measuring of diagonal radius applied by him. The size of 8.7 mm obtained during our investigations seems to be smaller than the real one, and equals with the length of one-two week-old fry.

Standard lengths back-calculated from annual radii of scales showed a slow growth of bleak in Lake Balaton. Its primary reason might be due to qualitative and quantitative features of food. This is supported by analysis of the gut content of a number of specimens, the food mainly consisting of diatoms (G. TAMÁS, oral information) and in smaller amount of animal food (crustacean-plankton, insects) (ENTZ and LUKACSOVICS, 1957). The monotony of gut contents can reflect the insufficiency of available food for bleak in the littoral zone, but on the other hand, it can be the results of its competition with eel (*Anguilla anguilla* L.) for food (BIRÓ, 1974). Based on these assumptions, the difference experienced in growth rate of bleak as compared to data published by ENTZ (1949—50) can be explained. From this fact, however, we drew the conclusion that the slow and uneven growth of pike-perch (BIRÓ, 1970; 1972) must be in causal connection with the slow rate of growth of bleak recently observed. Decrease recorded in the growth rate of bleak was also described in other prey fishes of pike-perch (BIRÓ, 1971; 1972; PONYI et al., 1972). These signs should be considered if the food chains of different fish species, e.g. pike-perch should be explained quantitatively as related to environmental changes of the lake. Environmental change, toxic effect, etc. concerning the growth by year-class strengths mostly seems from the length increase of bleaks of age-group 5+, where the fish kill taken place in 1965 coincides with the slower growth-period of this age-group. The causal connection of this coincidence in time, however, can not be demonstrated unanimously.

The maximal standard length in the model describing the growth of bleak in Lake Balaton, $L_\infty = 188$ mm, hardly differs from the theoretical size of 192 mm described by CHITRAVADIVELU (1974). According to his findings, this is attained by 13—15 year-old bleaks. In Lake Balaton such old specimens could not be detected. The age of the oldest ones supposed to be present may reach probably 8—9 years. The number of such old specimens, however, may be very small in the lake, because not a single such old specimen was caught during our 47 collections. Mortality of the population is strongly affected by death immediately after spawning, when primarily males die (ENTZ, oral information). The instantaneous mortality coefficient $Z = 1.33$ is higher than that observed in the Danube, which was $Z = 0.82$ (CHITRAVADIVELU, 1974). The assessed value of mortality in Lake Balaton is strongly influenced by significant loss during spawning, too. Besides mature bleaks a great amoun

of eggs laid down dies before hatching of larvae; in consequence of that the eggs become entirely covered by muddy sediment and colloids floated by rough water. This observation refers to other fish species, as well as to bream (*Abramis brama* L.), which spawns on the same stony grounds (Biró and Garádi, 1974).

Because of insufficient knowledge of population number and biomass expressed for unit area, the production could be given only in unit of average biomass. Despite of low survival rate, the production of average biomass is high, it may be estimated about 72 per cent in which the portion of one-summer-old fish is excluded. It is highly probable that because of the great

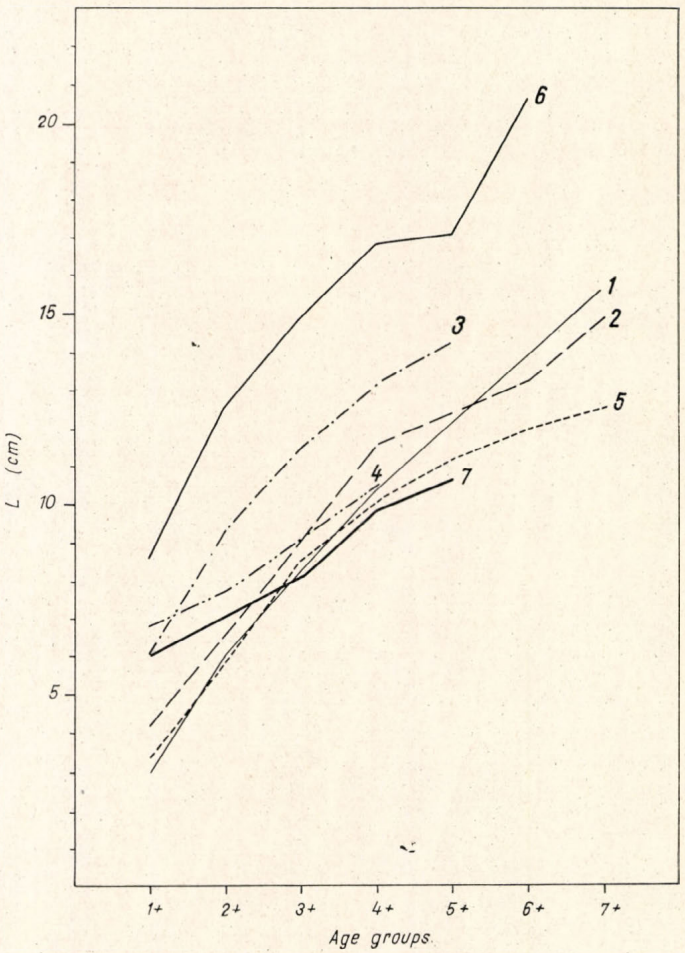


Fig. 13. Growth in length of bleak in different European waters: 1. Lake Langelmaresi (Finland) (Broefeldt, 1917, cit. Berg, 1933), 2. Average of 20 N-German lakes (Bauch, 1955), 3. Lake Ilmen (Soviet Union) (Domracev, 1926, cit. Berg, 1933), 4. Somova (Danube Delta, Roumania) (Papadopol, 1970), 5. Thames (England) (Williams, 1963), 6. Average of Slapy and Lipno Reservoirs (Czechoslovakia) (Vostradovský, 1963), 7. Lake Balaton (Hungary), present investigations

number of one-summer-old fish the ratio of P/B is higher. During his three-year studies CHITRAVADIVELU (1974) found the population density of bleak inhabiting the ŽOFIN-complex (River Danube) to be varying between 158—9675, their biomass ranging from 11 to 170 kg/ha and with a gross production from 5.2 to 91.0 kg/ha. Presumably we have to reckon with similarly significant, but more balanced variability in bleaks of Lake Balaton, because its shallow water gives more stabilized living possibilities as compared to the habitat in lotic environment.

Comparing the data published on growth of bleak in different European waters (BERG, 1933; BAUCH, 1955; OLIVA and FRANK, 1959; ČIHAR, 1961; VOSTRADOVSKÝ, 1963; WILLIAMS, 1963; MANN, 1964; BALON, 1967; KIECKHÄFER, 1967; PAPADOPOL, 1970; CHITRAVADIVELU, 1971; 1974) we found their growth in Lake Balaton to be relatively slow in spite of the fact tha the mean-size of one-year-old specimens is comparatively large *(Fig. 13)*

Summary

Length- and age-distribution of 1112 bleaks, as well as the growth of 294 specimens collected during consecutive months of 1968 have been studied. The seasonal variations in length-weight relationship, the mortality, average biomass and production of the population were studied. It was established that:

1. The population showed in spring a bimodal distribution by standard length, which was transformed to asymmetrical.

2. The length-weight relationship showed significant seasonal variation, which is evidently in connection with ripening cycle of gonads, with spawning as well as changes in condition.

3. Regression between the total caudal radii of scales and standard lengths was linear. The straight line cuts 8.7 mm from the abscissa. On the basis of standard lengths calculated from the annuli of scales, the growth of bleak is usually slow in Lake Balaton, its annual increase in length is small. Exponential growth in standard length could be well represented by BERTALANFFY's model. The observed stunted growth of bleak as compared to previous data may be probably the consequence of food scarcity of the littoral zone and the result of food competition with other fish species.

4. Age-structure of the stock is asymmetrical and its overwhelming majority consisted of age-groups 1+ and 2+ in 46 and 34.7 per cent, respectively. One-summer-old (0+) fishes were present only in 13.7 per cent, and the ratios of older ones, belonging to age-groups 3+ — 5+, were between 0.3—4.1 per cent. Based on decrease in logarithmic number of specimens, the instantaneous total mortality coefficient proved to be $Z = 1.33$, the rate of survival was $S = 26$ per cent and that of annual mortality $A = 74$ per cent. These values have changed in different age-groups. The ratio of average biomass and production on an average was $\bar{P}/\bar{B} = 71.8$ per cent. It was found to be highest in age-group 1+ (99.3 per cent), while in others (2+ to 5+) it varied between 24—60 per cent.

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A KÜSZ (*ALBURNUS ALBURNUS* L.) NÖVEKEDÉSE A BALATONBAN, MORTALITÁSÁNAK ÉS PRODUKCIÓJANAK BECSLÉSE

Biró Péter

Összefoglalás

Vizsgáltuk 1968 különböző hónapjai során gyűjtött 1112 db küsz méret- és kormegoszlását és scalimetrikus mérések alapján összesen 294 példány növekedését. Tanulmányoztuk a testhossz-testsúly viszonyának szezonális variációját, az állomány mortalitását, átlagos biomasszájának produkcióját. Megállapítható volt:

1. Tavasszal az állomány a törzshosszak alapján bimodális megoszlást mutatott, amely a nyári kompenzálódás után aszimmetrikussá vált.

2. A testhossz-testsúly viszonya szezonálisan szignifikáns különbségeket mutatott, ami nyilvánvalóan kapcsolatos a gonádok fejlődési ciklusával, az ívással, valamint a kondicióbeli változásokkal.

3. A pikkelyek teljes kaudális rádiusza és a törzshossz regressziója gyakorlatilag lineáris volt. A „kulcs"-pikkelyek képződésekor mérhető törzshosszra 8,7 mm-t kaptunk. A pikkelygyűrűkből visszaszámított törzshosszak alapján a küsz növekedése a Balatonban általánosan lassú, évenkénti növekedése kismértékű. A törzshossz exponenciális növekedése a BERTALANFFY-féle modellel pontosan leírható. A küsz állományra vonatkozó korábbi adatokhoz képest lassúbb növekedése valószínűleg a tó parti öve táplálékbeli elszegényedésének következménye, illetve más halfajokkal szembeni kompetíció eredménye lehet.

4. Az állomány kor szerinti struktúrája aszimmetrikus, a döntő többséget 1+ és 2+ korcsoportok alkották 46 illetve 34,7%-ban. Csak 13,7%-ban szerepeltek egynyaras (0+) halak, s az idősebb, 3+ — 5+ korcsoportúak részaránya 0,3—4,1% volt. Az egyedszámok logaritmikus csökkenése alapján a totális mortalitás pillanatnyi együtthatója $Z = 1{,}33$ volt, a túlélés rátája $S = 26\%$-nak, az éves mortalitás $A = 74\%$-nak adódott. Ezek az értékek a különböző korcsoportokban változtak. Az átlagos biomassza és produció aránya $P/\overline{B} = 71{,}8\%$ volt, legmagasabbnak az 1+ korúaknál találtuk (99,3%), míg a többi korcsoportban 24—60% között változott (2+ — 5+ korúaknál).

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HORIZONTAL DISTRIBUTION OF ORGANIC CARBON CONTENT IN THE UPPER LAYER OF THE BOTTOM DEPOSIT IN LAKE BALATON

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Informations on the organic matter content of the bottom deposits of Lake Balaton were presented the first time by EMSZT (1911) on the basis of six mud-cores. This study was followed by that of CSAJÁGHY and TOLNAI (1955) publishing data on this topic on the basis of two samples taken in the Kereked Bay. ENTZ et al. (1963) carried out studies on the bottom deposits of the Keszthely Bay.

PONYI et al. (1972) and FRANKÓ and PONYI (1973a; b) provide data of a large number of analyses on organic carbon. The first study concerned examined 64 samples taken at nine transversal sections of Lake Balaton, and the latter one clarified the seasonal fluctuation in the quantity of organic carbon content of bottom deposits at five stations of the Keszthely Bay.

Owing to the current conditions a significant quantity of the sediment and of the particulate organic substances migrate towards the calmest water areas (dead drift spaces) and accumulate there (LIGETI, 1974). Based on previous works on the bottom sediment of the Keszthely Bay it was suggested that the organic matter occurs in spots on the bottom.

The aim of our work was to draw a possibly clear picture on the distribution of the organic matter of bottom deposits on the basis of samples taken from the whole lake.

Localities, dates and methods of collection

Samples were taken with Ekman-Birge bottom sampler from 7 points each of the 24 transversal sections of the lake *(Fig. 1)* in the period of August—September 1971. Detailed description of the sections worked up is given in the study of MÜLLER (1969). Aliquot sampler were taken from the homogenizate of the top 5 cm thick sediment layer and dried at 40—50 °C in an aerated desiccator.

The organic carbon content was determined by the method adapted from WALKLEY and BLACK (1934). The end point of the titration of $K_2Cr_2O_7$ was determined in three parallels with RAVEH's and AVNIMELECH's method (1972) using an automatic titrimeter of the type Radelkis OP-506 connected to Ag/AgCl reference electrode of the type Op-8212 and potentiometric graphit

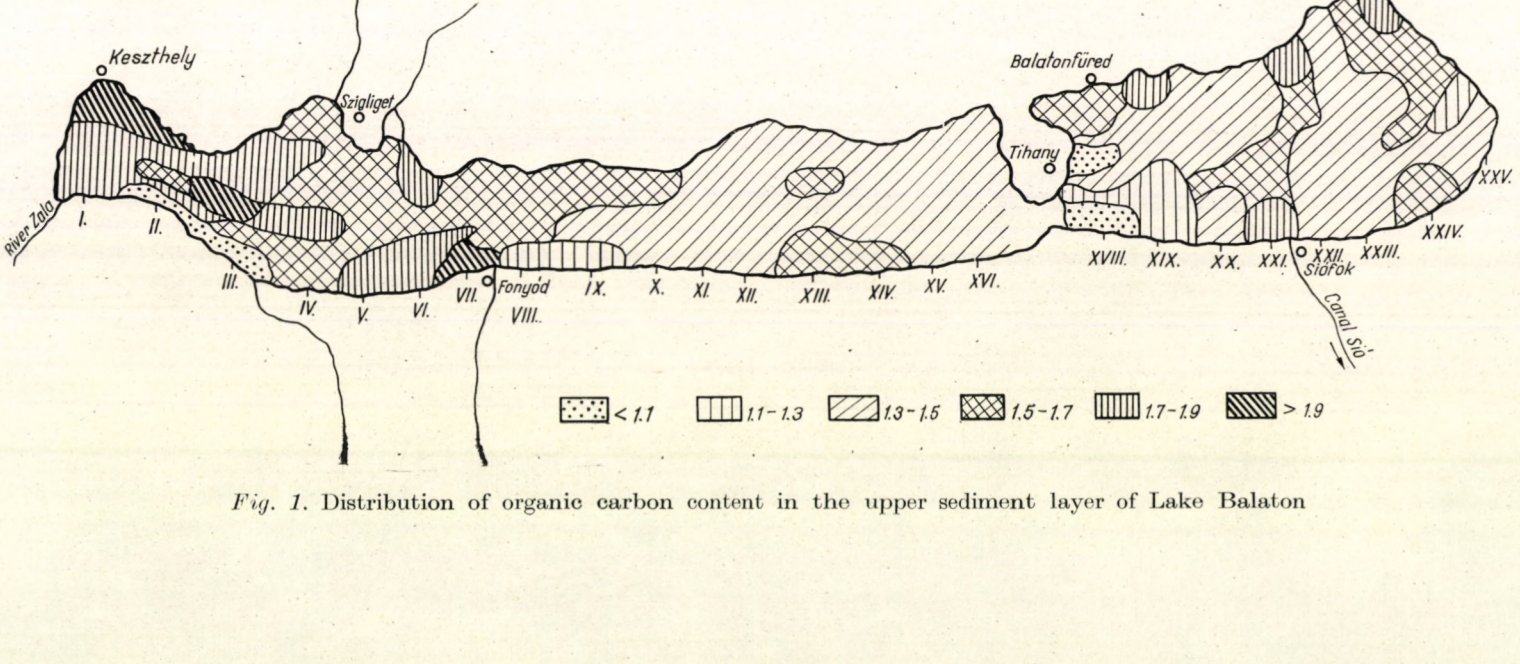

Fig. 1. Distribution of organic carbon content in the upper sediment layer of Lake Balaton

electrode of the type OP-C-7111-D. The determination of organic carbon content (DAVIES, 1974) was not disturbed by the very high $CaCO_3$ content which is characteristic in Lake Balaton (EMSZT, 1911; ENTZ, 1959; FRANKÓ and PONYI, 1973 a; b).

Results and discussion

Compared to other lakes (SCHÖNBORN et al., 1965; HANSEN, 1961; RYBAK, 1969; THOMAS et al., 1973) the results show that the organic carbon content of the open water bottom sediment of Lake Balaton is approximately low. Based on 168 mud-samples analysed *(Fig. 1)* it was found that localities with higher organic carbon content (over 1.9 per cent) are situated in the two south-western basins of the lake. The organic carbon content of these basins (sections I—VII) is distributed in spots. From this point of view the central basin of the lake shows homogenous distribution (sections VII—XVI). The north-eastern basin (sections XVIII—XXV) distribution has a mosaic pattern as well as in the two basins of the south-western region, with the distinction that higher values than 1.9 per cent were absent. On the basis of the average organic carbon content calculated for one section the four basins can well be differentiated from each other *(Fig. 2)*.

The distribution of organic carbon content may be explained by the currents raised by the prevailing winds (MUSZKALAY and STAROSOLSZKY,

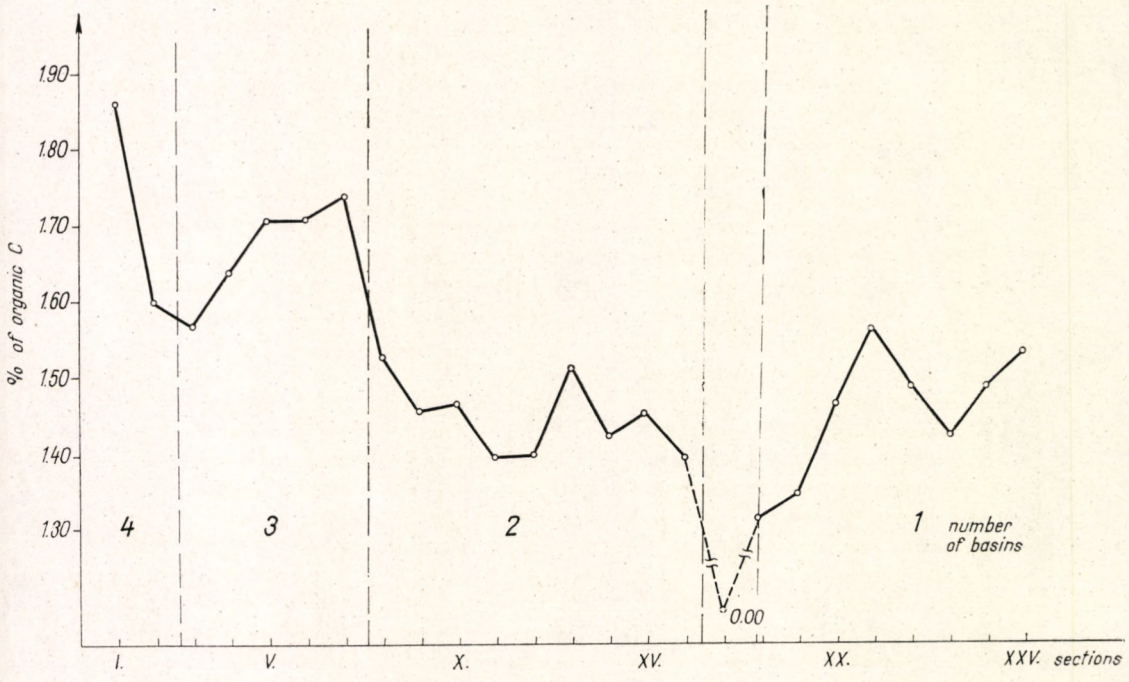

Fig. 2. Variation in the mean organic carbon conent at the sections of the four basins of Lake Balaton

1964; SZESZTAY, 1967; LIGETI, 1974). Since the samples were taken from the top 5 cm thick layer, the map showing the distribution of the organic carbon content based on our investigations, reflects the situation developed in the past 20—30 years (PONYI, 1971).

The open waters under the effects of the most intensive currents (sections II, III, XVIII) have sandy bottoms of low organic carbon content.

Concerning the tributaries, the effect of River Zala on the distribution of organic matter seems to be significant, however, it can be demonstrated only in the Keszthely Bay (PÁSZTÓ, 1963; PONYI et al., 1972; LIGETI, 1974).

In the knowledge of other literary data (OHLE, 1958; 1962; KUSNETZOV, 1968; KAJAK et al., 1970) as the main source of organic carbon content in the bottom deposit the phytoplankton production should be mentioned although there are studies (SEBESTYÉN, 1949; 1964), which underline the role of detritus of macro-vegetation in this aspect. The authors mentioned above have pointed out that not more than 0.8—30.0 per cent of the primary production increases the carbon content of the bottom deposits.

During our investigations carried out monthly in the Keszthely Bay (FRANKÓ and PONYI, 1973a), classified as hyperthrophic (HERODEK and TAMÁS, 1975), the annual increase of organic carbon was found to be about 0.2 per cent. It is suggested that because of its rapid renewal the extremely high alga production (830 gC/m^2/year) (HERODEK and TAMÁS, 1975) remains suspended in the water or can be found only in the uppermost mud layer of some millimetres thickness.

On July 26, 1972 an exploratory study was carried out on the particulate organic matter discharge of River Zala. Water samples of 50—50 litres taken from three points in the mainstream of the river were filtered through a No. 25 net and the filtrates were united. The net became plugged soon and has to be rubbed and patted to filter the total amount of water. It is suggested that organic particles larger than 50 μ in diameters remained in the net.

At the date of the field collection the floating sediment was determined to be 11.68 g/m^3 in River Zala. By using the "dry combustion" method 0.52 g/m^3 organic matter was identified in the sample.

Since the average water output of River Zala is 250—300 million m^3/year (PACHNER, 1972) not less than 131—157 tonnes of particulate organic matter are yearly carried into Lake Balaton by the river. Supposed an uniform spreading of this matter in the Keszthely Bay (surface of 38 km^2/each m^2 would share 3—4 g of it. In practice, however, this value may be several times higher, since the above calculation disregarded the fact that the increase of water output may lead to an increase of 10—20 per cent of the organic matter and that the quantity of particulate organic matter of smaller than 50 μ size may be more than it was measured to be. Despite these facts, the quantity of particulate organic matter carried by River Zala cannot be said to be significant. It becomes obvious when comparing it to the quantity of dissolved organic carbon of the river (FELFÖLDY et al., 1970) found to be as great as 2,215 tons a year in average.

Regarding the rapid water renewal in the Keszthely Bay (14 months; SZESZTAY, 1967) it is seen that each m^2 of the bay shares in an average 60 g dissolved organic carbon a year.

Owing to methodological differences the present data on organic carbon cannot be compared to those obtained previously (FRANKÓ and PONYI, 1973a).

However, the data of "dry combustion" *(Table I)* were to be compared and it was found that in the Keszthely Bay the quantity of organic matter increased to 3-fold during the past 70 years with lower increase at other places. As a whole, the organic matter content of the sediment of the lake is low. It may be attributed to the wave action drifting a part of the organic matter ashore (drifts) and to the currents raised by wind carrying the organic matter towards the wind-protected north shore, the site of reed stands. At this latter area even organic matter content of 40 per cent was found (ENTZ et al., 1963).

TABLE I

Organic matter content of the upper sediment layer of Lake Balaton in different years (at a depth of 3 m, measured by "dry combustion")

	EMSZT 1911 %	ENTZ et al. 1963 %	FRANKÓ and PONYI 1973 %
Keszthely	2.13	3.20	7.95
Akali—Szemes	3.21	—	3.15
Tihany, north shore	1.25	—	2.93

Summary

Having the organic carbon content of 168 mud-samples taken at 24 transversal sections in Lake Balaton analysed with WALKLEY's and BLACK's method, the authors conclude as follows:

1. Compared to other European lakes the organic carbon content of the top 5 cm thick sediment layer of the lake (varying between 0.27 and 2.33 per cent) is very low.

2. In the two basins of the south-western region with most of the waters inflowing here, the organic carbon content is relatively higher (over 1.9 per cent).

3. Apart from the central basin the organic carbon is distributed in spots. This phenomenon is due to the typical hydrodynamic conditions of the lake and partly to the effedt of River Zala (Keszthely Bay).

4. The mean organic carbon content of the sections decreases from the south-west end of the lake (section I) to the beginning of the north-east basin (section XVIII) with a slow increase from here. Probably, owing to the currents the mean organic carbon content decreases between the basins.

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A SZERVESSZÉN HORIZONTÁLIS ELOSZLÁSA A BALATON FELSŐ ISZAPRÉTEGÉBEN

Frankó András és *Ponyi Jenő*

Összefoglalás

A Balaton 24 keresztszelvényből származó 168 iszapminta szervesszén analízisét WALKLEY és BLACK módszerével végezték el a szerzők, melynek alapján a következő megállapításokat tették:

1. A tó felső 5 cm-es iszaprétegének szerves C tartalma más európai tavakhoz viszonyítva igen alacsony és 0,27—2,33% között változik.

2. A tó DNy-i részén fekvő két medencében, ahová a vízbefolyások döntő többsége torkollik, viszonylag magasabbak a szerves C értékek (1,9% felett).

3. A középső medencétől eltekintve a szerves C foltszerűen oszlik el, melyet a tó sajátságos hidrodinamikai viszonyai (ÉK-i medence), és részben a Zala-folyó hatása (Keszthelyi-öböl) magyaráz.

4. A vizsgált keresztszelvények szerves C tartalmának átlagértékei a tó DNy-i végétől (I. szelvény) csökkenő tendenciát mutatnak az ÉK-i medence kezdetéig (XVIII. szelvény), majd újra valamelyest megemelkednek. A medencék között, feltehetően az áramlások miatt, az átlag szervesszén értékek lecsökkennek.

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THE EFFECT OF LIGULOSIS ON THE GROWTH OF BREAM (*ABRAMIS BRAMA* L.) IN LAKE BALATON

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In a previously published paper (BIRÓ and GARÁDI, 1974) the role of ligulosis as a basic effect essentially influencing the growth of this species has been discussed. As well known the 3–5 summer-old bream stock of Lake Balaton is strongly invaded by *Ligula* plerocercoids (MOLNÁR et al., 1968; PÉNZES, 1968). The parasitic effect may be determined directly from the growth as it was earlier published for ruff (*Acerina cernua* L.) of Lake Balaton (PONYI et al., 1972). Since the effect of *Ligula* invasion on the growth of bream has not been studied in details, therefore a scalimetric study of invaded specimens became necessary. The solution of this problem is still important the growth of bream population of Lake Balaton has been found to be fast even in European relation.

Material and methods

The material consisting of 100 *Ligula*-invaded specimens belonging to age-groups 1+ to 7+ was sampled from breams caught at the environs of Fonyód with 1000 m long nets in July, 1974. Their standard length and weight were measured, then 10–20 scales were detached from the area above the latera line of each fish. 4–8 well developed wet scales were placed between slides, and the year-ring distances and the caudal radii of scales were measured at a magnification 30 times with the use of a profile projector. Knowing the relationship between the average caudal radius and the standard length, the body dimensions attained during the previous years were back-calculated after FRASER (1916). The length-weight relationship was determined by HUXLEY (1924) (viz. LAGLER, 1956). The growth in standard length of *Ligula*-invaded breams was described by the methods of WALFORD (1946) and BERTALANFFY (1938; 1957). The findings were compared to the data on the growth of 145 breams of 2+ to 7+ age collected at the environs of Fonyód in 1972 (BIRÓ and GARÁDI, 1974).

Results

Age-distribution of fish in the samples was as follows: four specimens belonged to age-group 1+, 30 were 2+, 36 were 3+, 14 were 4+, 10 were 5+, four breams were 6+ and two were 7+. The length-weight relationship calcu-

lated for *Ligula*-invaded breams could be represented by a parabola fitted in lower position as compared to that of non-invaded ones *(Fig. 1)*. It means that a smaller specific weight belongs to the same standard length, or the relative weight deficiency arising in consequence of the parasitism is partly replaced by the biomass of *Ligula* plerocercoids in the abdominal cavity. The effect of parasitism on the length-weight relationship can be estimated from the value of its coefficient which proved to be 2.7469.

About 50 per cent of the examined scales of *Ligula*-invaded breams were found to be deformed and damaged. The formation of annuli on the proportionally developed scales differs in many respects from that on the sound fish scales. The annuli were generally confused depending on the extent of *Ligula*-invasion even on seemingly undeformed scales. It is especially valid during the first three years of their development. The regression between the caudal radii of scales and the standard lengths was calculated from significantly scattered means. The straight line cuts 1.2 cm from the abscissa *(Fig. 2)*. Average caudal radii of scales from age-group 1+ to 7+ were: 2.0, 2.63, 3.5, 4.26, 4.74, 5.16, 6.33 mm, respectively.

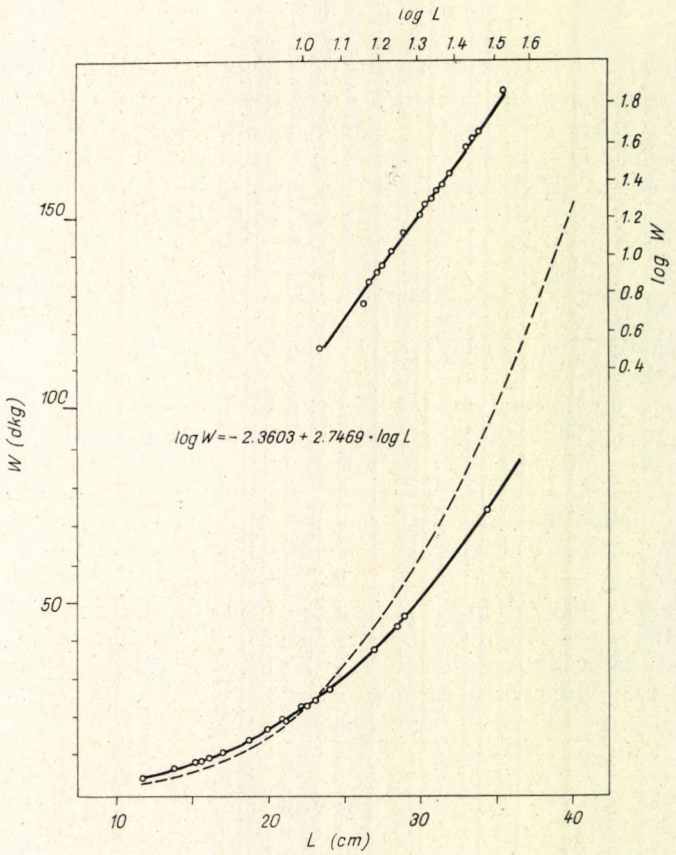

Fig. 1. Length-weight relationship of breams infected with plerocercoids of *Ligula intestinalis* (The dotted line concerns the sound specimens).

Estimating the yearly increments in length with use of standard lengths back-calculated, the growth of specimens invaded by *Ligula* seems to be slower than that of the sound ones. A stepped increase in standard length both in measured and back-calculated values could be observed *(Figs 3—4)*. In

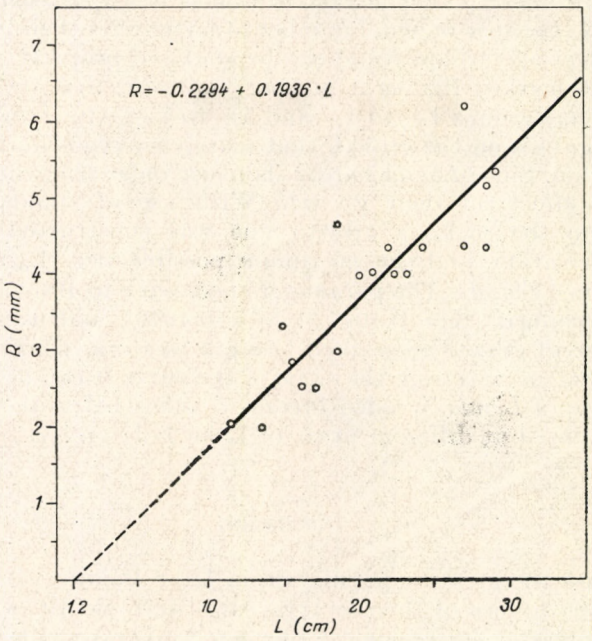

Fig. 2. Linear regression expressing the relationship between the total caudal radii of scales (R) and the standard lengths (L) of the invaded breams

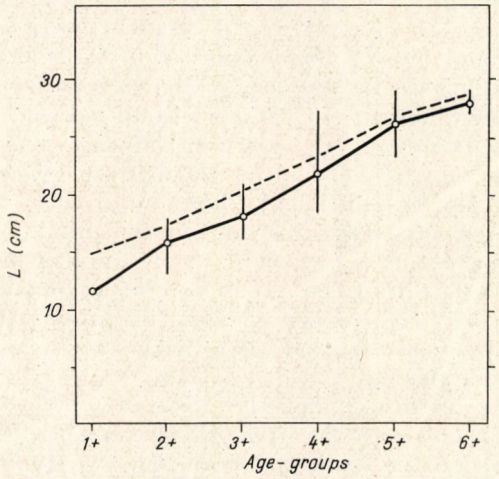

Fig. 3. The measured averages as well as the minimum and maximum values of standard lengths in *Ligula*-infected breams (the dotted line shows the averages for sound individuals)

age-groups 1+ to 6+ the deviations from the mean values were significant, up to $\pm 6-8$ cm.

Average standard lengths of age-groups 1+ to 6+ back-calculated from the scales, were as follows: 7.7, 12.1, 16.3, 19,8, 25.0 and 26.8 cm. Analysing the growth by year-class strengths the growth in age-groups 1+ to 5+ seems to be relatively linear, and contrary to this it is stepwise in age-groups 6+ to 7+ *(Fig. 5)*. Because of small number of latter ones their growth deviating from the younger age-groups could not be analysed properly.

From the marked figures it is clear, however, that there is a different growth rate in age-groups 1+ to 4+ and 5+ to 7+, which reflects the hindering effect of ligulosis on the growth that mainly succeeds during the first five years of their life. Since *Ligula* hardly damages older fish, therefore it cannot have peculiar effect on their growth. With use of standard length back calculated from the scales a growth line was constructed by WALFORD's (1946) transformation, and the maximum possible size (L_∞) was estimated. It was 51.58 cm *(Fig. 6)*. The parameters necessary to BERTALANFFY's model were also determined ($K = 0.1077$, $t_0 = -0.4076$), and using them, the exponential curve of growth in standard length was drawn *(Fig. 7)*. From the parameters obtained and from the slope of the curve it can be established that the growth rate of *Ligula*-invaded breams remains below those of sound fish previously observed at different areas of Lake Balaton.

Discussion

Bream has the greatest population in Lake Balaton, and the fishermen catch 900—1100 tonnes of them every year (BIRÓ and ELEK, 1970). Because of a dense population its ecological role (niche) is especially important from the point of energy turnover of the lake. Strong infection of breams — as the second intermediate host — with *Ligula* plerocercoids in Lake Balaton is well known (MOLNÁR et al., 1968; PÉNZES, 1968). In Hungary a significant *Ligula* infection occurs in natural waters, lakes, and closed arms of the rivers, where the ligulosis decimates the population of bream (*Abramis brama* L.), roach (*Rutilus rutilus* L.) and bleak (*Alburnus alburnus* L.) every year. In the fish ponds significant ligulosis rarely occurs (KOCZYLOWSKI and MIACZYNSKI, 1963; MOLNÁR and SZAKOLCZAI, 1973). The second intermediate host of *Ligula* emerges from the Cyprinids. In the case of a strong invasion, the weight of *Ligula* plerocercoids may reach the one-third of the weight of fish. Consequently, the physiological condition, feeding and growth of the host changes. Their external features may be established from the annuli formed on the scales, or from several deformations and irregular development of them. During the studies it became obvious that the annuli exhibit a compact stock depending on the intensity of the *Ligula* invasion. Their distances have decreased significantly which unanimously showed that the growth rate became slower in relation to the sound fish.

Studying the growth and mortality rate of breams invaded by *Ligula* in the WDZYDZE lake-complex (Poland), BRYLIŃSKI (1969, 1970) found that the annuli developed on the scales of bream one-two years before they have been caught are extremely close to each other. Their distances have been greatly varied related to the number of parasitic *Ligula* plerocercoids being

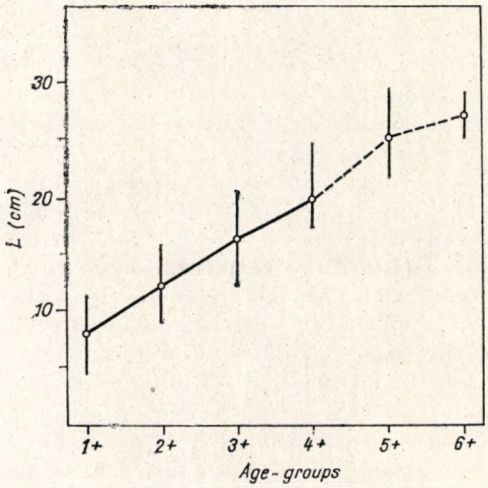

Fig. 4. Average, minimum and maximum standard lengths back-calculated from the scales in different age-groups of the infected breams. Data on the age-groups 4+ to 6+ are represented by the dotted line because of their small individual number.

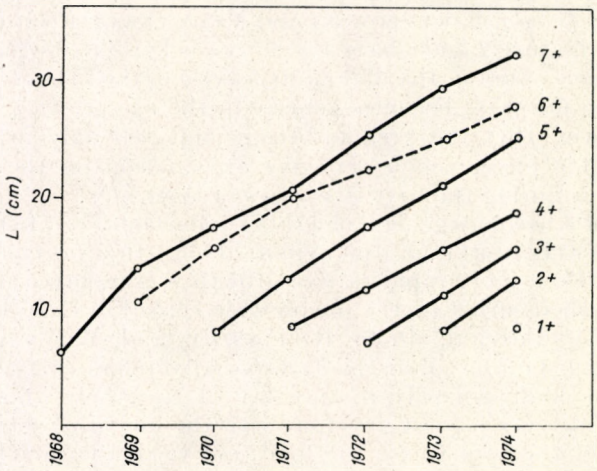

Fig. 5. The growth in length by year-class strengths

present in the abdominal cavity of the fish. Their total amount has reached 15—26 per cent of the body weight of the fish. From the small distances of annuli the parasitic effect clearly follows and manifests itself in the length-weight relationship, too. The growth in length of breams in the age-groups 1+ to 7+ studied by him was slower by 1.5—40.5 per cent as compared to the healthy individuals. The same in weight varied between 20—52 per cent.

The parasitic effect on the growth in length and weight of breams in Lake Balaton may be obviously followed from the length-weight relationship *(Fig. 1)*. At 30 cm standard length the difference in body weights of sound

and invaded fish has reached 10 grams i.e. 17 per cent. From this size the difference in weight rapidly increases.

BRYLIŃSKI (1969) demonstrated that parallel with the increase of invasion in different age-groups the growth rate decreases annually to such an extent that the growth in length stops between 36—38 cm. In the same time there is a significant loss in weight.

The linear regression curve expressing the relationship between the caudal radii of scales and the standard lengths of *Ligula* invaded breams in Lake Balaton cuts 1.2 cm from the abscissa *(Fig. 2)*. The same value for the sound fish proved to be 0.8 cm (BIRÓ and GARÁDI, 1974). The measured standard lengths of breams invaded with *Ligula* were smaller than those of non-invaded ones *(Fig. 3)*, and the deviations from the mean were 6—8 cm in different age-groups. Based on the back-calculated standard lengths *(Fig. 4)* the fact of slow growth is evident from the small differences in mean sizes of the age-groups, and from the slope of the growth curve. Analysing the length increase according to year-class strengths, the relatively slow growth is more striking *(Fig. 5)*. Consequently, the growth in length of bream in Lake Balaton is differently influenced by the invasion of *Ligula* plerocercoids in the first five years (age-groups 1+ to 4+), and further on (age-groups 5+ to 7+). In older individuals, although their small number is forewarning, nevertheless it can be seen that the extent of *Ligula* invasion is smaller, or the breams are less sensible than during the first five years of their life. Concerning the development cycle of *Ligula*, KOCZYLOWSKI and MIACZYNSKI (1963) have described that the life of mature *Ligula* lasts for 2—3 weeks, but its plerocercoid can stay even for three years in the abdominal cavity of the fish. According to our observations the infection becomes general in the age-group 1+. It is highly probable, however, that the breams become infected only in their third or fourth years. This phenomenon can give explanation on the differences in growth of breams during the first five years of their life.

The ligulosis becomes general chiefly in undernourished fish having dense population (KOCZYLOWSKI and MIACZYNSKI, 1963). In a previous paper (BIRÓ and GARÁDI, 1974) a more rapid rate of growth was reported as compared to other data published earlier on the bream wide-spread in Lake Balaton (WUNDER, 1930; WOYNÁROVICH, 1958; RIBIÁNSZKY and WOYNÁROVICH, 1962; PÉNZES, 1966; 1968). This observation especially refers to the SW-ern part of Lake Balaton and primarily to the Bay of Keszthely and waters in its surroundings. This may have connection with the increased eutrophication of different areas of the lake where the food-reserve of the mud has increased. The intensive fishing also contributes to the changes of the population dynamics of the fish. The average mortality of age-groups 3+ to 7+ of bream stock in Lake Balaton has been previously observed as 62 per cent. The estimation of influencing effect of ligulosis on the mortality of bream at different areas of Lake Balaton both from theoretical and practical points of view is wanted.

Summary

On the basis of their scales, the growth of 100 two-to eight-summer-old breams has been studied. This specimens invaded with *Ligula* plerocercoids have been caught by fishermen at the environs of Fonyód in July 1974. It was concluded that

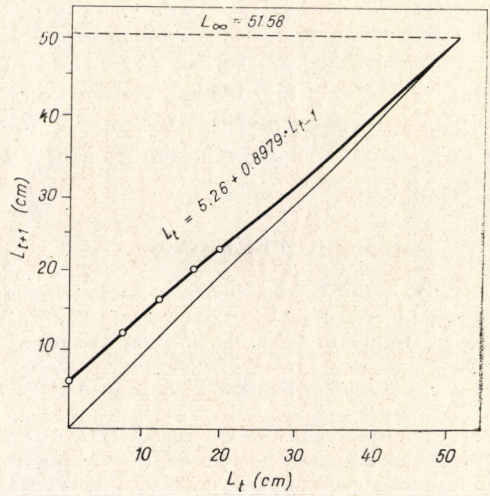

Fig. 6. FORD-WALFORD's plot in *Ligula*-invaded breams. L_t = standard length (cm) in every t-period of time if $t = 1$ year, L_{t+1} = the same one year later, L_∞ = maximum standard length in cm

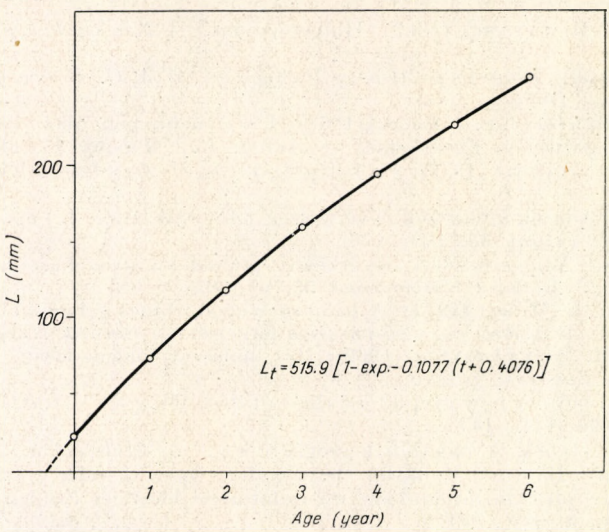

Fig. 7. Exponential growth in standard length of *Ligula*-invaded breams in Lake Balaton represented by BERTALANFFY's model. L_t = standard length in every t-period of time where $t = 1$ year

1. The relationship between the total caudal radii of scales and the standard lengths may be described by a linear regression. It cuts 1.2 cm from the abscissa. The annuli on the scales developed irregularly in connection with the extent of *Ligula* invasion. About a half of the scales studied were deformed and regenerated.

2. Based on the standard lengths back-calculated from the annuli of the scales it could be established that breams invaded with plerocercoids of *Ligula intestinalis* stunt in growth as compared to the sound specimens. Their different pattern of growth in weight has evenly been observed from the length-weight relationship. During the first five years of their development the growth of bream in Lake Balaton has been significantly hindered by ligulosis.

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LIGULÓZIS HATÁSA A BALATONI DÉVÉRKESZEG (*ABRAMIS BRAMA* L.) NÖVEKEDÉSÉRE

Összefoglalás

1974 júliusában Fonyód környékén a halászok által fogott dévérkeszegek közül 100 db, *Ligula intestinalis* plerocerkoidokkal fertőzött másod-nyolcadnyaras példányt választottunk ki és vizsgáltuk pikkelyeik alapján növekedésüket.
Megállapítottuk, hogy:

1. A pikkelyek teljes kaudális rádiusza és a törzshossz összefüggése lineáris regresszióval leírható, a test pikkelyzetének kialakulása 1,2 cm-es törzshosszra tehető. A *Ligula*-invázió mértékétől függően, a pikkelyeken a téli évgyűrűk zavartan s egymásba olvadóan képződtek. A tanulmányozott pikkelyeknek kb. fele deformálódott, vagy regenerálódott volt.

2. Az évgyűrűkből visszaszámított törzshosszak alapján nyilvánvaló, hogy a *Ligula intestinalis* plerocerkoidokkal fertőzött dévérkeszegek évenkénti növekedése elmarad az egészséges példányokétól. A testhossz-testsúly viszonyából a súlynövekedés eltérő volta a fertőzésmentes példányokhoz képest szintén megállapítható volt. A ligulózis a dévérkeszeg növekedését az első öt életévig jelentősen gátolja, majd ettől kezdve a hatás kevésbé érződik.

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THE PRIMARY PRODUCTION OF PHYTOPLANKTON IN THE KESZTHELY BASIN OF LAKE BALATON IN 1973—1974.

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Lake Balaton (surface area of 596 km^2) is composed of four more or less separated basins. The annual cycle of the primary production of the phytoplankton was studied first in the eastern basin in front of the Institute in Tihany (HERODEK and TAMÁS, 1973; 1974). This paper reports on the investigations in the Keszthelybasin. Studies in the Szigligetbasin are now in progress.

The Keszthelybasin, sometimes referred to as Keszthely-bay constitutes the westernmost and smallest section of the lake, with a surface area of 38 km^2 and a maximum depth of 3 m. Its water, exchanged to 99 per cent in 15 months, originates in 90 per cent in the inflows and in 10 per cent in precipitation (BARANYI, 1974). The main inflow empties from the south into the basin. This is the river Zala with a watershed of 2750 km^2, and a water output amounting to nearly the half of all the inflows of the lake. Chemistry of the lake, the basin and the river were studied by ENTZ (1959). On the northwestern shore of the basin is situated the town Keszthely with 17,000 inhabitants. With regard to the river and to the sewage-waters of the town, this part of the lake could be considered as the one most exposed to cultural eutrophication.

Methods

At the deepest point in the center of the basin water was sampled from 25, 100, 200 and 275 cm depths. From known part of the samples the algae were counted by UTERMÖHL's technique, and the mass of the phytoplankton was calculated from the cell volumes. 100 ml of the same samples were transferred into flasks of Pyrex glass and after adding 20 μCi $Na_2{}^{14}CO_3$ they were lowered to their original sites and there exposed for four hours ($10h—14h$). After exposal the flasks were carried to the laboratory in a dark box, cooled to 4—6°C. Here the algae were separated from the water by membrane filters of 0.2μ pore size. Following the samples 50 ml of previously filtered, inactive lake water was passed through the filters, then they were exposed to the fumes of HCl in order to remove all inorganic contamination of ^{14}C. Radioactivity was measured by liquid scintillation detector. Each value was reduced by that of the dark parallel. The total carbonic acid content of the water was deter-

mined, and the weight of carbon fixed by algae calculated. The production per unit of surface area was calculated assuming the samples at 25 and 275 cm depths to represent half metre thick those at 100 and 200 cm depths one metre thick water layers. The daily production was obtained by extrapolating the data from the four hours exposal to the period from sunrise to sunset minus two hours. Total radiation was recorded by the Meteorological Station of Keszthely. Details of the methods have been described earlier (HERODEK and TAMÁS, 1973).

There was a normal warm summer in 1973, followed by a September, much warmer than usual. The winter was exceptionally mild, the lake being covered by ice only for a short period in December. In common winters the lake would be frozen for two months.

Weather conditions during the exposals:

June 4. Overcast, drizzling. Strong wind, strong waves.
June 21. Overcast, drizzling. Light wind, low waves.
July 5. Very hot day. Bright sunshine, few clouds. Light wind, low waves.
July 19. High summer. Bright sunshine, few clouds. Light wind, calm water.
Aug. 2. After a cooler period very hot days again. Sunshine, moderate wind, moderate waves, then flat calm.
Aug. 16. The fortnight before was hot and calm. Sunshine. Flat calm.
Aug. 30. Overcast, at times drizzling. Medium wind, medium waves.
Sept. 13. Overcast. Cloud-drift, at times sunshine. Strong wind, strong waves.
Oct. 18. Overcast, raining. Storm, very strong waves.
Nov. 15. Overcast, drizzling. Moderate wind, weak waves.
Jan. 23. Overcast, then sunshine. Breeze, rippling water.
Febr. 21. Moderate cloud cover, at times sunshine. Medium wind, medium waves.
March 19. Overcast, then sunshine. Strong wind, strong waves, then medium wind, medium waves.
April 17. Sunshine with passing clouds. Storm, very strong waves.
June 5. Moderate cloud cover, then sunshine. Light wind, low waves.

TABLE I

Environmental

Date	4. VI.	21. VI.	5. VII.	19. VII.	2. VIII.	16. VIII.
Water temperature °C	21	21	24	25	22	24
Total radiation during exposal cal · cm^{-2}	142	97	178	236	277	261
Total radiation in the whole day cal · cm^{-2}	360	161	482	504	618	522
Secchi transparency cm	43	44	40	33	68	46
Illumination at the surface Klux	28.5	28.5	63.2	41.4	56.2	56.2
Illumination at the different depths in per cent of the surface illumination						
25 cm	34.1	32.7	48.3	38.7	64.3	53.3
100 cm	4.3	4.6	6.9	4.2	12.5	5.8
200 cm	0.7	0.8	0.9	0.2	2.5	0.5
275 cm	0.1	0.2	0.2	0.0	0.9	0.1

Results

Water temperature, Secchi transparency, total radiation, surface and underwater illumination data are presented in *Table I*. In this basin transparency was very low. Usually at 1 m depth the illumination did not attain the 10 per cent of the surface value, i.e. this depth is not light-saturated. At 2 m depth the illumination never attained the 10 per cent, in most cases not even the 1 per cent, accordingly it was under the compensation point. At 2.75 m there was practically permanent darkness, illumination exceeding 1 per cent was measured only at two occasions. The light attenuation is not only much more expressed here, but is also of a more stable character than in the eastern basin, where by a heavy storm only 2 per cent of the light was found at 1 m depth, but even 20 per cent of the incident light reached the bottom during a long calm period. It also turned out that lower transparencies belong to the same Secchi values in the Keszthely basin than in the eastern one. In the eastern part of the lake water transparency depends basically on the amount of mud, swirled up by the waves, while in the Keszthelybasin it is already determined by the phytoplankton.

In these investigation 122 algal species and 5 forms were found in the samples. Their distribution according to phyla was as follows: Cyanophyta 14, Euglenophyta 10, Pyrrophyta 7, Chrysophyta 49, Chlorophyta 47, Mycophyta 1. To make the survey easier only those species are listed separately in *Table II* whose biomass reached the 200 $\mu g \cdot litre^{-1}$ during the year. The biomass of the other species are summed up for each phylum. In June diatoms prevailed. In July the blue-greens *(Aphanizomenon flos-aquae, Anabaena spiroides)* formed a strong water-bloom. Hereupon the number of *Ceratium hirundinella* (Pyrrophyta) increased very strongly dominating the plankton till September. In the meantime also *Euglena oxyuris* (Euglenophyta) attained significant amounts. Besides the *Ceratium*, two other Pyrrophyta species, *Cryptomonas erosa* and *Peridinium inconspicuum*, appeared also in big masses, the former

factors

30. VIII.	13. IX.	18. X.	15. XI.	23. I.	21. II	19. III.	17. IV.	5. VI.
20	20	13	7	4	7	9	11	20
126	156	21	34	89	78	144	203	241
258	325	61	55	118	167	295	398	510
46	48	36	68	74	42	50	27	51
43.4	30.5	4.8	5.7	22.6	32.5	50.3	60.2	46.3
35.7	39.3	50.0	42.9	62.5	34.7	30.6	40.0	58.4
4.9	4.3	8.3	9.3	18.7	4.4	5.6	2.5	15.6
0.4	0.9	1.6	2.9	7.9	0.6	1.1	0.1	2.8
0.1	0.6	1.0	1.6	5.0	0.3	0.5	0.0	0.8

TABLE II

The biomass of the phytoplankton

	4. VI.	21. VI.	5. VII.	19. VII.	2. VIII.	16. VIII.
Cyanophyta						
Anabaena scheremetievi	—	—	—	80	200	40
Anabaena spiroides	—	14	16	420	200	5
Aphanizomenon flos-aquae	—	45	272	2175	247	262
Other species	87	59	25	58	75	83
Total	87	118	313	2733	722	390
Euglenophyta						
Euglena ehrenbergii	13	17	59	—	25	218
Euglena oxyuris	45	36	18	72	90	1350
Phacus longicauda	—	—	25	517	—	—
Other species	9	12	16	126	21	43
Total	67	65	118	715	136	1611
Pyrrophyta						
Cryptomonas erosa	137	37	125	250	182	1000
Cryptomonas ovata	—	—	—	—	—	—
Ceratium hirundinella	53	397	848	912	8904	2385
Diplopsalis acuta	3	24	231	7	70	346
Gonyaulax apiculata	—	21	31	10	31	333
Peridinium inconspicuum	—	3	—	300	—	1260
Other species	1	1	—	—	1	—
Total	194	483	1235	1479	9188	5324
Chrysophyta						
Cyclotella bodanica	624	244	407	81	22	27
Cyclotella glomerata	4	—	—	4	2	4
Cyclotella ocellata	47	85	85	34	5	20
Diatoma elongatum var. *tenuis*	—	—	—	—	—	—
Melosira granulata	34	30	52	688	13	292
Nitzschia acicularis	47	24	40	47	5	19
Surirella robusta var. *splendida*	344	62	225	50	—	—
Other species	434	265	211	292	151	472
Total	1534	710	1020	1196	198	834
Chlorophyta						
Ankistrodesmus falcatus	31	3	—	3	3	31
Phacotus lenticularis	—	—	3	—	—	255
Other species	163	183	139	123	247	365
Total	194	186	142	126	250	651
Sum total of all algae						
$10^6 \mu m^3 \cdot litre^{-1}$	2076	1562	2828	6249	10494	8810
$g \cdot m^{-2}$	6.2	4.7	8.5	18.7	31.5	26.4

TABLE III

The production of the phytoplankton at

Depth cm	4. VI.	21. VI.	5. VII.	19. VII.	2. VIII.	16. VIII.	30. VIII.
25	131.2	46.0	190.7	1111.2	605.1	583.9	358.4
100	52.1	50.4	147.2	428.1	401.8	267.8	178.4
200	1.6	6.4	9.2	23.0	207.9	16.2	7.3
275	0.7	0.0	4.6	2.1	17.1	0.5	0.6

$10^6\ \mu m^3 \cdot litre^{-1}$

30. VIII.	13. IX.	18. X.	15. XI.	23. I.	21. II.	19. III.	17. IV.	5. VI.
56	32	—	—	—	—	—	—	5
16	32	—	—	—	—	—	—	2
195	45	—	—	—	—	—	—	7
45	79	48	13	70	24	24	50	109
312	188	48	13	70	24	24	50	123
17	34	—	—	—	—	—	—	—
18	54	—	—	—	—	—	—	—
49	25	—	—	—	—	—	—	—
64	43	98	—	—	—	—	10	4
148	156	98	—	—	—	—	10	4
312	175	1937	950	625	1625	37	—	187
262	105	315	665	—	402	—	—	—
10282	5618	265	—	—	—	—	27	238
35	63	—	—	—	—	—	—	—
73	312	—	—	—	—	—	—	—
405	177	—	—	—	—	—	—	—
1	161	—	—	—	—	—	—	46
11370	6611	2517	1615	625	2027	37	27	471
136	81	407	163	814	760	678	2306	950
7	—	46	14	221	265	319	31	6
34	10	373	24	85	1153	220	153	220
—	—	—	—	375	112	13	—	—
82	62	4	—	215	—	—	—	322
4	4	4	1	86	121	252	281	9
25	—	12	—	—	—	—	3750	—
202	304	528	93	747	689	405	440	158
490	461	1374	295	2543	3100	1887	6961	1665
2	10	28	10	367	—	35	24	17
225	75	—	—	—	—	—	—	—
93	326	138	8	179	75	147	91	297
320	411	166	18	546	75	182	115	314
12640	7827	4203	1941	3784	5226	2130	7163	2577
37.9	23.5	12.6	5.8	11.4	15.7	6.4	21.5	7.7

different depths $\mu g\ C \cdot litre^{-1} \cdot hour^{-1}$

13. IX.	18. X.	15. XI.	23. I.	21. II.	19. III.	17. IV.	5. VI.
308.9	16.8	78.2	15.7	90.1	71.2	45.5	46.1
111.3	4.3	45.6	27.5	28.6	58.2	22.8	61.0
8.9	0.4	8.5	18.2	2.8	6.9	0.7	26.3
2.6	0.1	2.4	13.5	0.9	1.8	0.1	8.2

prevailing till February. From January the number of diatoms increased again, and they, mainly *Cyclotella bodanica* and *Cyclotella ocellata* formed the bulk of the phytoplankton until June 1974. The mass of the total phytoplankton increased from the 2 mg · litre^{-1} in June to 6 mg · litre^{-1} at the water-bloom of the blue-greens in July, then by the rapid rise of *Ceratium hirundinella* population it surpassed the 12 mg · litre^{-1} by the end of August. From here on the biomass dropped to 2 mg · litre^{-1} by the middle of November, and varied between 2 and 7 mg · litre^{-1} in the following months.

Fig. 1 shows the mass of algae groupped according to their length. It appears that in summer, in time of the maximal biomass of the total phytoplankton, the amount of those algae shorter than 30 μm is the smallest, not only in relative but even in absolute terms. The mass of algae, shorter than 10 μm showed an inverse relationship with temperature, and reached its maximum in February. This could be effected to some extent by seasonal changes in the number and activity of filterfeeders, grazing on these small algae. For the winter the quantity of cladocerans diminishes, and the feeding intensity of *Eudiaptomus gracilis*, the dominant copepod in this lake drops to a low level (P.-ZÁNKAI and PONYI, 1973). The other factor involved might be the greater specific surface of the smaller forms, forming in the winter by lower nutrient concentration a considerable advantage. In summer, when the water is rich in nutrients these algae are suppressed by the large blue-greens and by *Ceratium hirundinella*. According to a rough estimation the total surface of the phytoplankton was the largest in February. In the eastern basin by a lower nutrient supply, the proportion and in summer even the absolute amount of the algae, shorter than 10 μm exceeded those in the Keszthely basin, and showed less seasonal changes (HERODEK and TAMÁS, 1974).

The primary production was very high *(Table III)*. The highest incorporation was measured on July 19. That day 444 μg carbon has been fixed by the algae in the 100 ml water of the sample exposed at 25 cm depth for four hours. Values as high as this are to be find but exceptionally even in the most polluted waters. It is twenty-three times higher than the maximum in the eastern basin. The vertical profile of the photosynthesis shows the characteristics of hypertrophic waters. The maximum was found at three occasions at 1 m, in all other cases at 25 cm depth. At 1 m depth, where usually the illumination was already suboptimal, the production was about the half of that in the uppermost sample. At 2 m depth, where the illumination remained under 1 per cent, the radioactivity of algae did not reach in most cases the 10 per cent of those in the optimal depth. This means that here the photosynthesis is already less intensive than the respiration, consequently there is no net production. In the deepest layer the photosynthesis was minimal. While in the eastern basin, corresponding to the varying transparency, the vertical profile of the photosynthesis changed from time to time, and in long calms the maximum developed near the bottom, in the Keszthely basin the vertical profile remained stable, with no significant production in the deepest samples.

The production per unit surface *(Fig. 2)* was 1.6 g C · m^{-2} · day^{-1} in early June. From the beginning of July, when the water temperature attained 24°C, by increasing numbers of blue-greens, the production rose rapidly. It reached the maximum on July 19, in the time of the water-bloom caused by *Aphanizomenon flos-aquae* and *Anabaena spiroides*. On this day the production was extremely high, 13.6 g C · m^{-2} · day^{-1}. In early August, by decreased blue-

greens and increased *Ceratium hirundinella* stand, the production remained very high, then diminished gradually till mid-September. The lowest production measured was during a cold storm in October, when the blue-greens disappeared, and *Ceratium* could be detected only in a few specimens. From November to next June the production remained much lower than in summer,

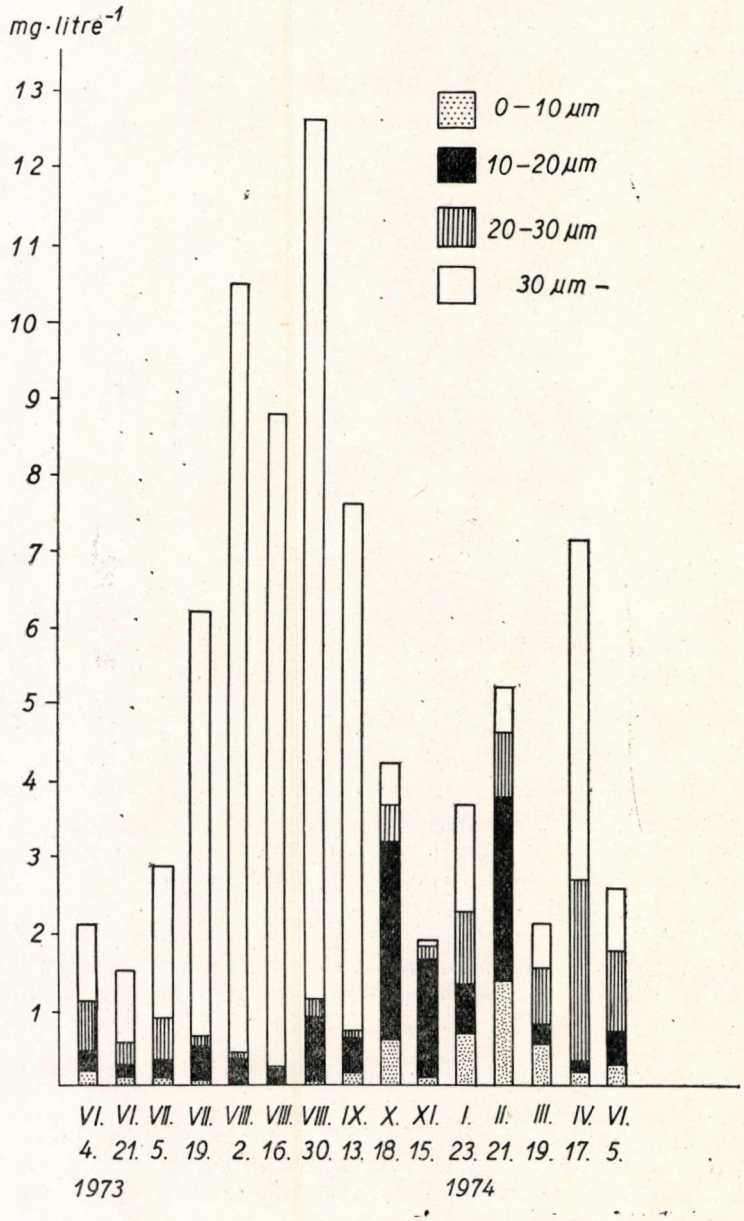

Fig. 1. Annual cycle of the biomass of the total phytoplankton, and that of length groups

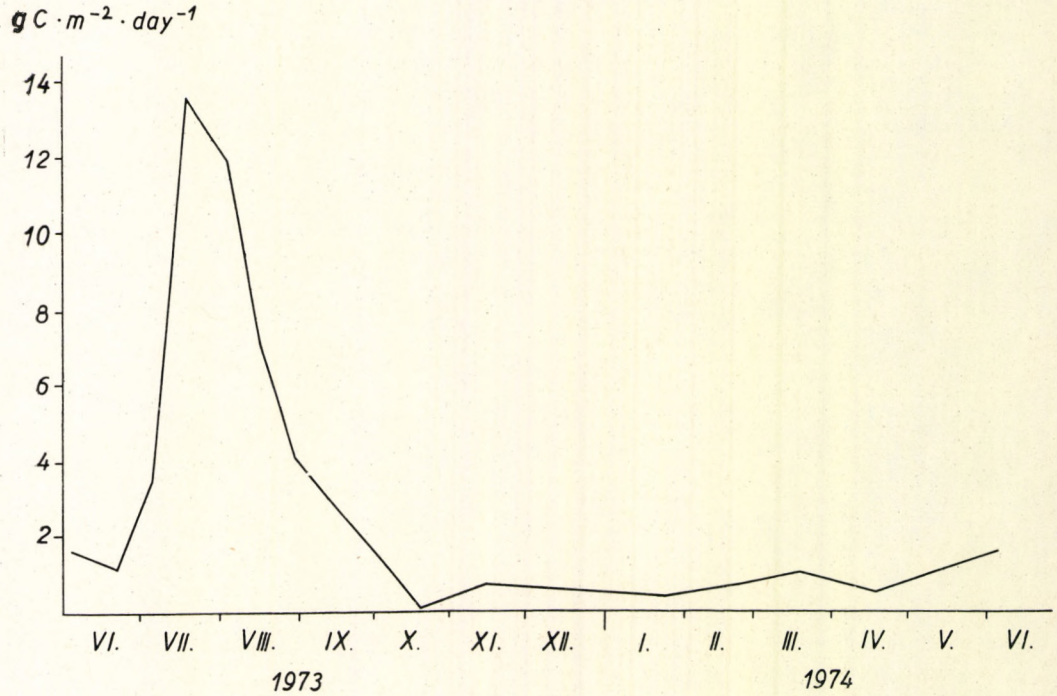

Fig. 2. Annual cycle of the production of phytoplankton

however it exceeded the values obtained during corresponding seasons in the eastern basin. The annual production, calculated from the area under the curve was 831 g C · m^{-2}. This is eight times higher, than that in the eastern basin. The seasonal distribution of production in the two basins differs too. While in the eastern basin the production was well balanced throughout the year in the Keszthely basin a huge summer peak developed, and two thirds of the annual production fell to this period.

Even the chemical composition of the water was modified by the intensive photosynthesis. The total carbonic acid content was 5.13 mM in February, but only 3.11 mM in August due to the incorporation into organic material and to the biogenic lime formation. The seasonal changes of the total carbonic acid in the lake have not been described earlier. In the eastern basin the total carbonic acid content changed much less, it was 4.36 mM in January and 3.94 mM in August.

Discussion

Comparing the standing crop of the phytoplankton, found during these investigations at Tihany and at Keszthely with data of earlier years (Tamás, 1974), it appears that the mass of the phytoplankton increased strongly in both basins and now it is about three times higher at Keszthely, than at Tihany *(Fig. 3)*.

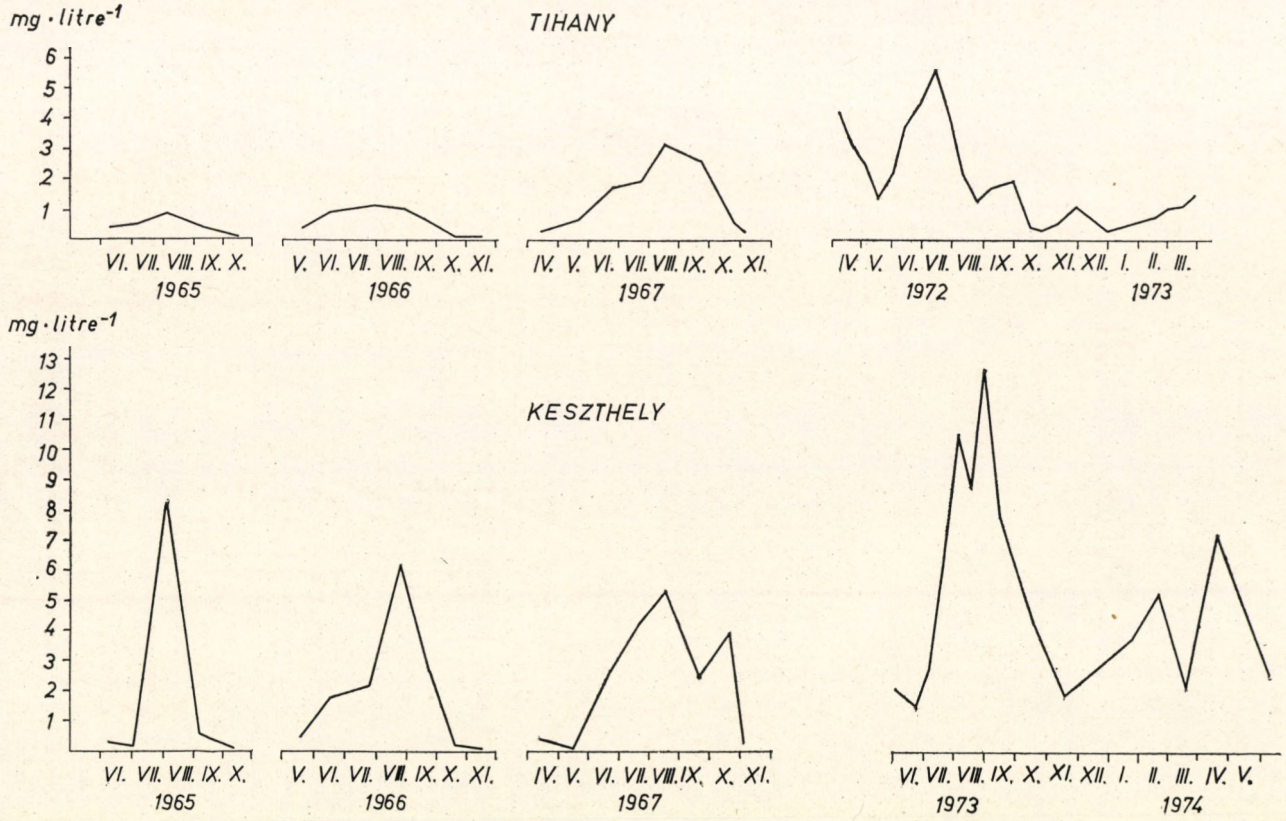

Fig. 3. The rise of the biomass of phytoplankton since 1965 at Tihany and at Keszthely

The increased mass of algae results already in a permanent strong water coloration and causes frequent water-blooms, rendering the water less attractive for swimming. Due to the self-shading effect of the algae the euphotic zone is restricted to the upper two metres, provoking the possibility of O_2 deficiency in long calm periods. The light insufficiency caused by the phytoplankton can be also responsible for the disappearance of submerged macrophytic vegetation from the deeper parts of the basin. In the preceding years the submerged vegetation consisting mainly of *Potamogeton perfoliatus* formed a major nuisance for ships and boats and different ways were suggested to clear the water of them. According to the surveying in 1969 (KÁRPÁTI and VARGA, 1970) of this basin 308 ha were covered by submerged vegetation, and of this 51 ha were the densely covered area. In the course of our measurements in most cases the illumination at 2 m depth was less than 1 per cent of that at the surface, therefore the aquatic weeds should have to grow about 1 m to reach as much light as necessary for a photosynthetic rate, higher than the respiration. The plants are unable to perform this. In the shallow water i.e. where sufficient light reached the bottom, the submerged vegetation persisted, supporting the above explanation. Similar shift of the submerged vegetation towards the shallower waters was observed in the course of eutrophication of Lake Fure (JÓNASSON, 1969).

While in the eastern basin the highest daily production was 0.6 g C $\cdot$ m^{-2}, in the Keszthely basin it was estimated to reach 13,6 g C $\cdot$ m^{-2} on July 19, and 11.9 g C $\cdot$ m^{-2} on August 2. These values are unexpectedly high not only when contrasted to the data obtained in the eastern basin, but even among the numerous lakes worldwide investigated there are but few examples for such a high production. According to RYTHER's (1959) theoretical considerations the maximal primary production by 600 cal $\cdot$ cm^{-2} daily total irradiaton corresponding approximately to the maximal irradiation in Hungary, would be 13.7 g C $\cdot$ m^{-2} $\cdot$ day^{-1}. In outdoor algal cultures 15 – 20 g C $\cdot$ m^{-2} $\cdot$ day^{-1} production could be achieved (VENDLOVÁ, 1969). In the Ethiopean soda lakes, where due to the higher total radiation the theoretical maximum would be 19.2 g C $\cdot$ m^{-2} $\cdot$ day^{-1}, productions corresponding to 15.1 – 20.0 g C $\cdot$ m^{-2} $\cdot$ $\cdot$ day^{-1} were found according to the daily rhythm of the oxigen content of the lakes (TALLING et al., 1973). The maximal production in Indian fish-ponds was 15.6 g C $\cdot$ m^{-2} $\cdot$ day^{-1} (GANAPATI and SREENIVÁSAN, 1972). It appears, that these maximal values actually measured, correspond fairly well with the maxima, calculated on theoretical grounds. Respecting the broader limits of error in our estimations, the very close agreement between the maximal production, found in the Keszthely basin and the theoretical maximum must be regarded in part as a matter of chance. For theoretical ends it seems necessary to determine the summer maximum more precisely, with samples in more depths and more time intervals, embracing the whole day and also with the O_2 techniques. Practically, however, this basin can be regarded as a nutrient-saturated water body, that already attained its maximal productivity.

RODHE (1969) coupling the concept of eutrophication with that of the primary production suggested to use the annual primary production per unit surface as a measure of the trophic state. RODHE (1969) and HÜBEL (1971) following WINBERG (1961) suggested fairly similar productivity ranges for the trophic categories *(Fig. 4)*. When the daily production is calculated by extrapolating from the four hours of the exposal to the whole day-time

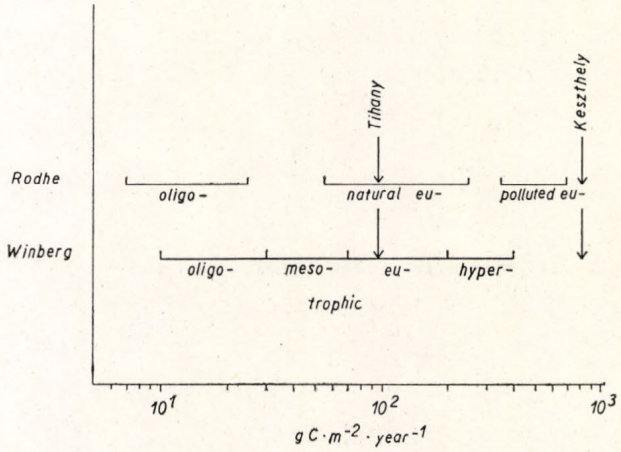

Fig. 4. Position of the two basins on the scale of lakes based on their primary production

(HÜBEL, 1971), the annual production is 114 g C · m^{-2} in the eastern basin and 971 g C · m^{-2} in the Keszthely basin. By extrapolating to the day-time minus two hours gives an annual production of 96 g C · m^{-2} in the eastern and 831 g C · m^{-2} in the Keszthely basin. Indicating these lower values in *Fig. 4.* it appears, that the production at Tihany corresponds to that of the slightly eutrophic lakes, while that at Keszthely to the most hypertrophic waters, this later value actually leaving the scale. Thus while Balaton is often referred to as an oligotrophic lake, according to the primary production measurements the trophic state of its basins ranges from the lower limit of eutrophy to the most developed hypertrophy.

However cultural eutrophication manifests itself as worldwide phenomenon the increase of the primary production was in fact demonstrated but in very few lakes. This is perhaps explained by the relatively recent propagation of investigations of this type. In Lake Balaton the first measurements by the ^{14}C technique were carried out in 1961 (BÖSZÖRMÉNYI et al., 1962). The primary production was measured in front of Tihany at altogether 12 occasions in samples exposed in 1 m depth, and the values obtained at that time are but 30 per cent lower than those found in 1972. It seems therefore, that in the eastern basin the production of the phytoplankton increased only moderately during the last decade. In 1961 when on two occasions water samples were collected in all the basins, no significant differences could be stated in their production. In the samples collected at Keszthely the production was 8.1 μg C · litre^{-1} · hour^{-1} on August 24, 1961 and 4.0 μg C · litre^{-1} · hour^{-1} on September 15, 1961, these values corresponding to 119 and 48 per cents of the production of the simultaneously exposed samples collected at Tihany. The photosynthetic rates at Tihany in 1972 and at Keszthely in 1973 and 1961 are compared in *Fig. 5.* The data of 1961 are extrapolated to a water column of 3 m. Admitting that in 1961 the productivity of the Keszthely basin corresponded to the present one of the eastern basin, it seems that in the last twelve years in the Keszthely-basin the productivity attained an eightfold

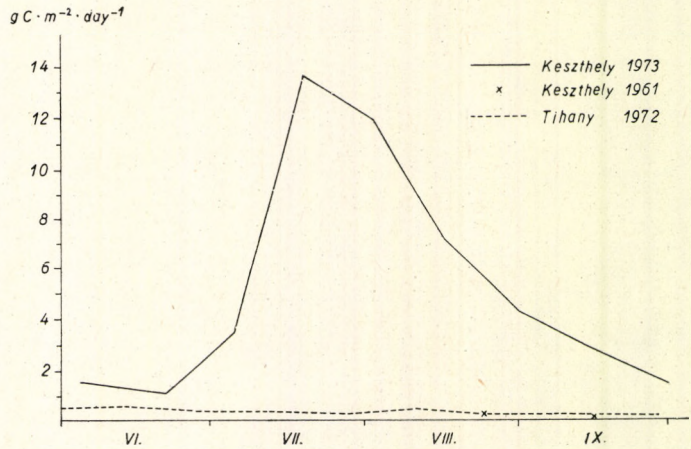

Fig. 5. Comparison of the productivity at Keszthely in 1961 and in 1973 and at Tihany in 1972

increase, the basin getting from the moderately eutrophic to the strongly hypertrophic state.

One of the basic problems of production biology is the relationship between production and biomass. The view, that the production per biomass unit, often referred to as activity coefficient, is inversely related to the population density is supported by phytoplankton studies in many lakes (VERDUIN, 1959; WRIGHT, 1960; FINDENEGG, 1966 a, b; GOLDMAN et al., 1968; HILLBRICHT-ILKOWSKA and SPODNIEWSKA, 1969). The activity coefficient of the phytoplankton used to be lower in eutrophic than in oligotrophic waters, and is usually lower in summer than in autumn or in winter. In the eastern basin, in accordance with this principle, the production per biomass was not in summer the highest, when production attained the maximum, but in autumn, when the biomass was minimal. The principle seems to be valid for the changes that took place through many years. In the eastern basin the production has barely increased since 1961, in turn the biomass in 1965 was only a quarter of that in 1972, suggesting that in 1965 the production per biomass ratio was four times higher than in 1972. In this part of the lake the production seems to be more stable both seasonally and through the years than the mass of

TABLE IV

The relation of primary production

	4. VI.	21. VI.	5. VII.	19. VII.	2. VIII.	16. VIII.
$\frac{\text{g C daily production}}{\text{0.1 · g fresh weight}}$	2.64	2.38	4.15	7.26	3.78	2.67
$\frac{\text{100 · cal production}}{\text{0.46 · cal total radiation}}$	0.73	0.72	1.24	3.71	2.89	1.92

the algae. In the Keszthely basin the situation is just the opposite. Here the highest activity coefficient could be found at the time of the enormous production in summer when also the biomass showed its maximum, and the production changed more seasonally, than the mass of the phytoplankton. The same holds true for the changes that took place through many years. At the present in the Keszthely basin the biomass is three times, the production eight times higher than in the eastern basin. It seems, that the activity coefficient decreases in the first period of eutrophication and suddenly increases in the final stage.

The energy fixation by plants in per cent of the energy of the photosynthetically active part of sunlight is referred to as photosynthetic efficiency. In our latitude 46 per cent of the total radiation falls within the wave-lengths available for the photosynthesis. To avoid the errors arising from the extrapolation the photosynthetic efficiencies are given for the four midday hours *(Table IV)*. Values for the whole days were higher in all probability. The light utilization is very high in this basin as compared to other European lakes (RODHE, 1958), and the efficiency is the highest in summer, when the absolute radiation has its summit.

Lake Balaton, in European terms, is pretty warm in summer, in July-August the water temperature can stay for long periods above 25°C. This is due to Hungary's climate, and to the small thermal capacity of the shallow lake. The analysis within the frame of IBP of the data of many lakes throughout the world showed the productivity to be best correlated with temperature (BRYLINSKY and MANN, 1973). It seems, that at permanently high temperatures communities of high metabolic rates are formed, the intensive synthesizing and decomposing processes resulting in a rapid turnover of the nutrients and in a high photosynthetic efficiency. This explains perhaps the exceptionally high productivity of the Keszthely basin as compared to other strongly polluted but colder lakes.

The increasing biomass of the phytoplankton, the water bloom caused by the blue-greens, the narrowing of the euphotic layer, the restriction of submerged macrophytic vegetation to shallower depths, the enormous peak in summer of the production curve, the unusually high annual primary production all attest the hypertrophic state of the Keszthely basin. The main sources of nutrients are the sewage waters of the town Keszthely and its slaughter-house, and the river Zala. A large-scale project is planned for purification, and in part for diversion of the sewage-waters from the watershed of the lake. With regards to the seriously threatened state of this basin, it

to biomass and to relation

30. VIII.	13. IX.	18. X.	15. XI.	23. I	21. II.	19. III.	17. IV.	5. VI.
1.11	1.26	0.09	1.19	0.38	0.42	1.60	0.25	2.04
2.52	1.54	0.55	2.42	0.59	0.86	0.61	0.20	0.41

would be advisable to solve the sewage-water problems of Keszthely as the first step of the project. The Kis-Balaton, once a basin of the lake, now a marshland belongs with its extended reeds to the most important nature conservation areas in Hungary. Prior to its regulation the river Zala flooded this region before entering the lake. The earlier idea of restoring the original state of the Kis-Balaton seems worth reconsidering because in the reeds the water would drop most of the silt and also the majority of the dissolved nutrients would be removed by the plants. Whether this or another conception would meet the case, the polluting effect of the river Zala must be radically reduced in the very near future too.

Summary

The illumination, composition, biomass and production of the phytoplankton were studied in the basin in 25, 100, 200 and 275 cm depths a whole year through.

In most cases less than 10 per cent of the surface illumination was found at 1 m depth, and less than 1 per cent at 2 m depth. Due to the shading by the algae the rich submerged vegetation of the previous years has been extinguished from the deeper regions of the basin. The biomass was much larger than in the previous years, its maximum attaining 12.6 g fresh weight $\cdot$ m^{-3}. In July strong water bloom was caused by *Aphanizomenon flos-aquae* and *Anabaena spiroides*, in August *Ceratium hirundinella* dominated, while in the colder water diatoms prevailed. The amount of algae shorter than 10 μm correlated inversely with temperature.

Usually the production was the highest in the uppermost sample, it was reduced about to the half for 1 m and to a few per cents for 2 m depth. The production was extremely intensive in summer. In July 1.1 mg C $\cdot$ litre^{-1} $\cdot$ $\cdot$ hour^{-1} production was measured at the optimal depth, and the daily production was estimated to 13.6 g C $\cdot$ m^{-2}. The annual production amounted to 831 g C $\cdot$ m^{-2}. Comparison with earlier data suggests, that it happened in the last twelve years that the basin got from a moderately eutrophic state to the strongly hypertrophic one.

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A FITOPLANKTON ELSŐDLEGES TERMELÉSE A BALATON KESZTHELYI MEDENCÉJÉBEN 1973—74 ÉVEKBEN

Herodek Sándor és *Tamás Gizella*

Összefoglalás

Egy éven keresztül vizsgáltuk a medence közepén 25 cm, 100 cm, 200 cm és 275 cm mélyen a megvilágítást, a fitoplankton összetételét, tömegét és termelését.

Az esetek nagyobbik részében már nem találtuk meg 1 m mélyen a felszíni megvilágítás 10%-át, 2 m mélyen pedig 1%-át sem. Az algák okozta fényhiánnyal magya-

rázzuk, hogy a medence mélyvizéből eltűnt a korábbi évekre jellemző gazdag hínárállomány.

A fitoplankton tömege a korábbi évekhez képest jelentősen emelkedett, és augusztusban elérte a 12,6 g C . m^{-3}-t. Júliusban az *Aphanizomenon* flos-*aquae* és az *Anabaena spiroides* okoztak vízvirágzást. Augusztustól a *Ceratium hirundinella* dominált, a hidegebb vizekben a kovamoszatok törtek előre. A 10 μ-nál rövidebb algák össztömege a hőmérséklettel fordított irányban változott.

A termelés általában a legfelső mintában volt a legnagyobb, 1 m mélyen ennek már csak a fele volt, 2 m mélyen és ez alatt legtöbbször már nem találtunk netto termelést. A legmagasabb termelést a júliusi vízvirágzásnál mértük, 1,11 mgC · $liter^{-1}$ · $óra^{-1}$-t. Az alapterületre számított napi termelés ekkor 13,6 g C · m^{-2} volt.

Az éves termelés 831 g C · m^{-2}, amelynek a fele július—augusztusra esett. Korábbi adatokkal összevetve valószínű, hogy a medence az utóbbi évtizedben jutott a mérsékelt eutrófiától a legsúlyosabb hipertrófia állapotába.

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INVESTIGATIONS BY GAS CHROMATOGRAPH ON THE CHLORINATED HYDROCARBON POLLUTION IN TWO AREAS OF LAKE BALATON

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The first studies on the chlorinated hydrocarbon pollution in Lake Balaton (BARON et al., 1967; PONYI et al., 1968) were based on thin-layer chromatographic measurements summarizing the results of so many analyses that, even today, the great fish kill in 1965 can be ascribed to chlorinated hydrocarbon pollution. The results are supported by the probability of these pesticides being accumulated in the food-chain. The authors mentioned above found γ-HCH, DDT and their decomposition products in fishes, plankton crustaceans and molluscs.

Investigations with gas chromatograph have been carried out at the first by FELFÖLDY and TÓTH (1967), when γ-HCH, aldrin, dieldrin and DDT could be located in fishes. PINKOLA and TÓTH (1971) stated their presence in the water and water weeds. In their exploratory study CZEGLÉDI-JANKÓ et al. (1973) reported on the occurrence of 2.4-D (Dikonirt), in addition to the γ-HCH, DDT and their metabolites, in Lake Balaton.

When collating the literary data the followings become evident:

1. Despite the fact that DDT and its decomposition products have been decreasing since the fish kill in 1965, they are still present in the lake.

2. γ-HCH was also found in significant quantity.

The aim of our studies was to gain further informations on the concentration of γ-HCH, DDT and their decomposition products in the lake and in aquatic organisms. We were going to find explanation to the followings:

1. The degree of chlorinated hydrocarbon pollution in different members of the food-chain (algae, zooplankton, non-predatory and predatory fishes).

2. Whether or not the two water areas of diverging quality differ in the chlorinated hydrocarbon content.

3. The degree of chlorinated hydrocarbon pollution of River Zala and its effect on the lake.

Dates and localities of collecting, materials and methods

Samples were taken along two standard transversal sections of Lake Balaton (A and M), at the inlet of River Zala and 10 km upwards in the river itself *(Fig. 1)*. The selection of these sites was indicated by previous investiga-

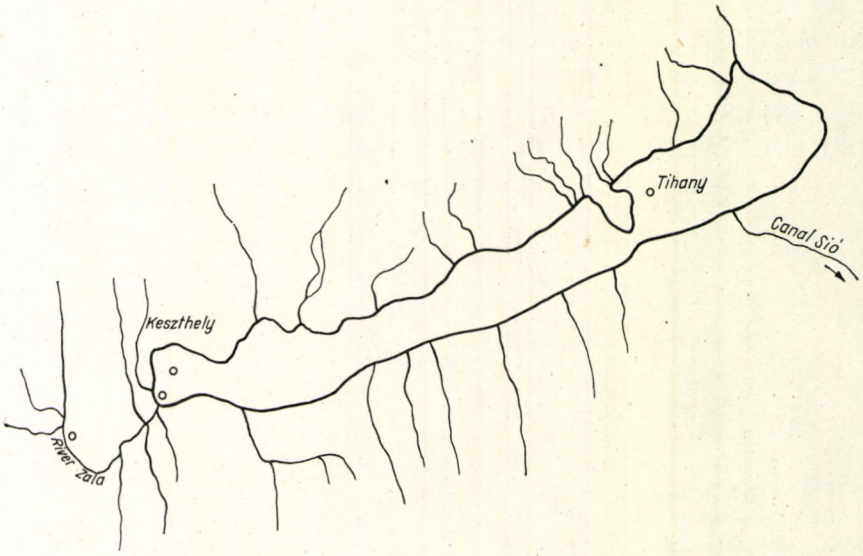

Fig. 1. Sampling places in Lake Balaton

tions (PONYI et al., 1972; PONYI and P.-ZÁNKAI, 1972; PONYI, 1975). Owing to the fact that section M is influenced by the inflowing waters in a remarkable degree and section A only to some extent, the quality of the two water areas differs very much. At the same date of the investigations the two sections were characterized by the following differences:

	section M	section A
Organic carbon content (mg/l)	6.42	5.36
Chemical O_2 consumption (mg/l)	4.94	3.80
Total P (mg/m^3)	86.60	51.50
Bacterium (cells$\times 10^5$/ml)	4.70	3.50
Algae (individual number$\times 10^5$/l)	4.90	1.40
Rotatoria (μg wet weight/l)	24.00	42.00
Planctonic Crustacea (μg dry weight biomass/l)	99.00	69.00

At the sampling places water, phytoplankton, plankton Crustacea, white grass-carp *(Hypophtalamichthys molitrix)*, carp *(Cyprinus carpio)* and pike-perch *(Stizostedion lucioperca)* were collected in the following months:

Date of sampling	water	fish	plankton
April 1973	+	−	−
June	−	+	−
July	−	+	+
October	+	−	+

The phytoplankton and the planktonic crustaceans were filtered with sampling nets No. 25 and 6.

The degree of γ-HCH, DDT, DDE and DDD pollution was examined in all samples.

Water sample of 4000 ml was extracted in petroleum ether of 2×150 ml (Carlo Erba, boiling point of 30—40°C) with shaking. The petroleum etheric phases were brought together and evaporated with anhydrous sodium sulphate of max. 5 g (Merck). After decantation the extracts were condensed in rotary vacuum concentrator of Rotadest type at 40°C. The concentrate was purified on Davidow column in the appliance of CZEGLÉDI-JANKÓ and CIELESZKY (1968). The purification lasted for 1.5 hours. The purified extracts were evaporated almost dry and taken up in hexane of gas chromatographic purity. In the fish samples the degree of pollution was determined in the flesh, brain, liver, eggs and milt. The whole quantity of the brain and liver samples was homogenized with distilled water in the ratio of 1 : 1. 10—10 g flesh of each fish was homogenized with distilled water of the same quantity. The homogenizates of the organs and plankton samples were liofilizated in a final vacuum of 10^{-2} torr, at a sublimation temperature between —25 and —30°C applying an after heating of 35°C.

Since the rest of organs to be determined were compounds diluting easily in lipids, the fat content of the samples was extracted in petroleum ether after liofilization. Of the fat obtained and measured, 0.1—0.15 g was studied on. The analyses resulted three parallel mean values.

The examinations were carried out with an instrument of Packard type. The conditions of the chromatographic experiment were as follows:

Column: made of pyrex glass of 180×0.4 cm; OV 17 and QF-1 partitive liquid of 1.5 per cent layered on solid carrier; temperature: 190°C.
detector: electron capture; tritium foil content (activity of 150 mC); temperature: 196°C
carrier gas: nitrogen of high purity, flow rate 55 ml/minute; vaporizer temperature: 220°C.

The identification and quantitative determination was carried out by means of a stock-solution compounded of reference materials. The solution also contained α-, β-, and δ-HCH. We failed, however, to evaluate the quantity of these substances.

Results

1. *Chlorinated hydrocarbon content of water samples*

The gas-chromatographic investigations showed the γ-HCH pollution of Lake Balaton to be identical at Tihany and in the Keszthely Bay in spring (35 ng/l) *(Table I)*.

In autumn the γ-HCH values decreased at the sampling sites. In the Keszthely Bay and River Zala only 7–14 per cent, while at Tihany 37 per cent of the spring values were found.

In spring the DDT pollution of the water could be denoted in figures only at Tihany. Owing to its low quantity, it could not be worthily measured at other sampling places. In autumn it showed an increase reaching a concentration of 9 ng/l in the Keszthely Bay.

TABLE I

Chlorinated hydrocarbon pollution of Balaton and River Zala, 1973 (μg pesticide/1000 ml water)

Site of sampling	Date of sampling	γ-HCH	DDE	DDD	DDT
Tihany	April	0.035	Ny	0.001	0.002
	Oct.	0.013	Ny	Ny	Ny
Keszthely	April	0.035	Ny	0.002	Ny
	Oct.	0.005	Ny	Ny	0.009
Inlet of River Zala	April	0.037	Ny	0.002	Ny
	Oct.	0.002	Ny	Ny	0.003
10 km upwards in River Zala	April	0.039	Ny	0.003	Ny
	Oct.	0.003	Ny	Ny	0.003

Ny = less than 0001 μg/1,000 ml water

In spring the DDD was of almost identical concentration all over the lake and in the River Zala.

2. *Chlorinated hydrocarbon content of plankton samples*

Phytoplankton could be analysed only once, in October, when the water-bloom was followed by heaps of algae floating on the surface. In this period the alga concentration was as much as 4.2 mg wet weight/litres (HERODEK and TAMÁS, 1974; 1975). In the phytoplankton samples DDT and DDD were found in the largest quantity *(Table II)*. Compared to the values of planktonic Crustacea the quantity of γ-HCH also seemed to be significant.

TABLE II

Pesticide content of the phytoplankton and plankton crustacean samples in two different regions of Lake Balaton, 1973 (mg pesticide/1000 g dry plankton)

Month	Region	γ-HCH	DDE	DDD	DDT	Fat content %
VII.	Tihany, section A Crustacea-plankton	0.129	0.126	0.115	0.025	5.72
VII.	Keszthely, section M Crustacea-plankton	0.171	0.135	0.089	0.042	5.06
X.	Keszthely, section M Crustacea-plankton	0.229	0.165	0.166	0.679	11.69
X.	Keszthely, section M Phyto-plankton	0.635	0.327	3.507	4.086	4.09

The following species of crustaceans were found in the samples: *Eudiaptomus gracilis* (10 individuals/litre), *Cyclops vicinus* (13 individuals/litre), *Mesocyclops leuckarti* (3 individuals/litre), *Daphnia cucullata* (1 individual/litre) and *Cyclops nauplii* (5 individuals/litre).

The γ-HCH and DDT content of planktonic Crustacea collected along the transversal section M in summer and autumn is different *(Table II)*. In the autumn the γ-HCH pollution was 1—2 times, while the DDT pollution 16—17 times as high as it was in the spring.

The comparison of the summer samples taken along sections A and M shows that the degree of pesticide pollution of the two areas differs to some extent. Reducing into water value and regarding the inequality in the plankton density, this difference even grows *(Tables III* and *IV)*.

TABLE III

Estimated quantity of plankton in the period of the investigation (in µg dry weight/litre)
(WINBERG, 1971; HERODEK and TAMÁS, 1973; 1975; PONYI, 1975)

Month	Section	Planktonic Crustacea	Phytoplankton
July	A	122	280
	M	161	1240
October	A	69	420
	M	185	840

TABLE IV

Pesticide pollution of plankton at two sampling areas of Lake Balaton (µg pesticide/m^3 water)

Month	Section	Plankton	γ-HCH	DDE	DDD	DDT
VII.	A	Crustacea-	0.016	0.015	0.014	0.003
VII.	M	Crustacea-	0.028	0.027	0.014	0.007
X.	M	Crustacea-	0.042	0.031	0.031	0.126
X.	M	Phyto-	0.533	0.275	2.946	3.432

3. *Pesticide content of fish samples*

Analyses of the different organs of white grass-carp *(Table V)* showed that chlorinated hydrocarbon is present in larger quantity in the 3 + year-old specimens than in the 2 + year-old ones. The γ-HCH, content of fles was found to be 9 times higher and of brain of 3+ year-old 4 times higher than in case of 2 + old specimens. The difference in the concentration of DDT was 2.5-fold in the flesh. The increased pesticide content of the 3+ year-old specimens is connected with the changed fat-content.

The pesticides found in the different organs of the pike-perch and carp differ in quantity at the two sampling sites *(Table VI)*. The γ-HCH content of the liver, eggs, milt and brain of pike-perches collected in the north-eastern basin is higher than that of the specimens from the Keszthely Bay. The γ-HCH content of these organs of carp is the opposite of these values, found to be higher just in the Keszthely Bay. The distribution of DDT and its decomposition products shows other picture like the γ-HCH did. The degree of pollution of the organs of fish collected at the two areas is nearly the same.

TABLE V

Pesticide content of the organs of white grass-carp (Hypophthalmichthys molitrix) at Keszthely, June 1973 (mg pesticide/1000 g organ, wet weight)

Age	Organ	γ-HCH	DDE	DDD	DDT	Fat content %
3+	Flesh	0.136	0.162	0.179	0.165	24.49
3+	Liver	0.038	0.056	0.051	0.086	13.25
3+	Brain	0.117	0.042	0.026	0.099	6.46
2+	Flesh	0.015	0.012	0.037	0.064	6.87
2+	Liver	0.026	0.060	0.058	0.094	8.52
2+	Brain	0.031	0.061	0.034	0.106	7.20

TABLE VI

Pesticide content of the organs of pike-perch and carp in two regions of Lake Balaton, July 1973 (mg pesticide/1000 g organ, wet weight)

Species	Organ	Site of sampling	γ-HCH	DDE	DDD	DDT	Fat-content %
Pike-perch	Flesh	Tihany	0.0120	0.0074	0.0022	0.0214	0.37
		Keszthely	0.0133	0.0058	0.0029	0.0174	0.35
	Liver	Tihany	0.0266	0.0828	0.0208	0.0232	1.11
		Keszthely	0.0157	0.0672	0.0400	0.0346	1.56
	Milt and eggs	Tihany	0.0131	0.0076	0.0033	0.0260	1.60
		Keszthely	0.0072	0.0104	0.0039	0.0608	1.71
	Brain	Tihany	0.0244	0.1071	0.0137	0.0235	7.87
		Keszthely	0.0078	0.0775	0.0207	0.0292	6.73
Carp	Flesh	Tihany	0.0173	0.0162	0.0073	0.0226	1.10
		Keszthely	0.0129	0.0319	0.0218	0.0213	1.70
	Liver	Tihany	0.0329	0.0360	0.0160	0.0186	3.68
		Keszthely	0.0819	0.0656	0.0440	0.0320	5.63
	Milt and eggs	Tihany	0.0113	0.0101	0.0049	0.0146	1.04
		Keszthely	0.0165	0.0256	0.0192	0.0088	2.34
	Brain	Tihany	0.0057	0.0076	0.0016	0.0078	4.30
		Keszthely	0.0228	0.0196	0.0093	0.0180	5.05

When determining the ratio of DDT and its decomposition products (DDE + +DDD), significant differences were found in three cases *(Table VII)*. A difference in the ratio of DDT/DDE + DDD pollution in the examined organs of pike-perches collected in the two areas was shown only by the eggs and milt. At Tihany it was found to be 2.4 and at Keszthely 4.3. In addition to the eggs and milt, the flesh of carp shows a shift like this in ration, but in an inverse manner. While at Tihany the ratio of DDT/DDE + DDD equals 1, this shifts to the advantage of the decomposition products at Keszthely.

Discussion

Our gas- chromatographic results can be compared to other data on Lake Balaton only restrictedly because, on the one hand, there are only three publications describing similar methods (comp. the introduction); on the other

TABLE VII

Ratio of DDT and its decomposition products found in the organs of pike-perch and carp at the two collecting areas

Collecting station	Pike-perch DDT/ DDE+DDD	Carp DDT/DDE+DDD	Organ
Tihany	2.2	1.0	flesh
Keszthely	2.0	0.4	flesh
Tihany	0.2	0.4	liver
Keszthely	0.3	0.3	liver
Tihany	2.4	1.0	eggs and milt
Keszthely	4.3	0.2	eggs and milt
Tihany	0.2	0.8	brain
Keszthely	0.3	0.6	brain

hand, the comparison is hindered by the way of publishing or the diverging matter of the investigations. Compared to PINKOLA's and TÓTH's (1971) data on 1968—69, now the quantity of γ-HCH found in the water proved to be significantly lower *(Table VIII)*.

TABLE VIII

The quantity of γ-HCH (ng/1000 ml water) measured in Lake Balaton and River Zala in different years

Sampling site	PINKOLA and TÓTH, 1971			PÁSZTOR et al. 1973
	1967	1968	1969	
River Zala	64	130—170	84	3—39
Keszthely Bay	86	30—165	150	5—35
At Tihany	12	50—170	76	13—35

Comparing the data it seems that the quantity of γ-HCH markedly varied from year to year. The values are higher in spring than in autumn. This seasonal fluctuation is strictly connected to the period when γ-HCH is used and is washed by rain into the lake (October 1973 was pretty dry). This is supported by the data of PINKOLA and TÓTH (1971) stating that the high γ-HCH content measured in October 1968 was caused by the rather rainy weather.

DDT and its decomposition products were measured in all samples. Of course, the values were low, varying between 2 and 9 ng/litre. It means that DDT can be still found at present. Owing to the few data at our disposal, its origin cann ot be determined. As a matter of fact in the Keszthely Bay the quantity of DDT was found to be three times highes than in River Zala at the same time in autumn.

Analysing the plankton samples, the same chlorinated hydrocarbons were found similarly in the water. In October the water, phytoplankton and plankton crustaceans were sampled simultaneously. On this basis the distribution of γ-HCH and DDT in the water and in the plankters could be estimated *(Table IX)*. Our results showed that most of the pesticides could be found in the water and the γ-HCH and DDT content of algae was 10 to 28 times higher than that of the plankton crustaceans.

TABLE IX

Percentual distribution of chlorinated hydrocarbon between the water and the plankters in the Keszthely Bay, in October 1973

	γ-HCH	DTD
Water	89.3	70.5
Phytoplankton	9.9	28.5
Planktonic Crustacea	0.8	1.0

Comparing the pesticide content of two plankton Crustacea samples taken in the Keszthely Bay it is visible that it was higher in autumn than in summer *(Tables II* and *IV)*. It may be caused by the phenomenon that in October the macrophytoplankton breaks up into pieces of 10—15 μ size, thus becoming good for serving as food. In this period most of the water weeds of high pesticide content (PINKOLA and TÓTH, 1971) do the same and are fed on by the cladocerans. There was no rain before the samplings giving reason for the increased pesticide level in the crustaceans.

Comparing our data to those on the planktonic Crustacea in 1967 obtained by using the thin-layer chromatographic method (PONYI et al., 1968), it is seen that at the same time and localities place — e.g. along section M —the pesticide content increased with one order of magnitude *(Table X)*.

TABLE X

Comparison of the pesticide content of planktonic Crustacea in Lake Balaton (mg pesticide/1000 g dry plankton)

Month	Site of sampling	γ-HCH		Total DDT	
		* PONYI et al. (1968)	PÁSZTOR et al. 1973	*PONYI at al. (1968)	PÁSZTOR et al. 1973
VII.	A	0.200	0.129	0.730	0.266
VII.	M	0.010	0.171	0.050	0.266
X.	M	0.001	0.229	0.001	1.010

Note: * = Data on wet weight are calculated after WINBERG (1971)

Of the three fish species examined, the greatest pollution of white grass-carp may be ascribed to the relatively high pesticide content of the plankton organisms *(Table XI)*. It is known that the white grass-carp feeds on phyto- and zooplankton, consequently, its pesticide uptake is the highest. Comparing the maximal and minimal quantity of DDT and its decomposition products found in the organs of white grass-carp to earlier literary data *(Table XII)*, it becomes evident that it does not decrease with one order of magnitude as the pike-perch does *(Table XIII)*.

Compared to that in 1966, the γ-HCH pollution level of carp liver increased *(Table XIV)*. On the other hand, the γ-HCH content of pike-perch was lower than it was in 1966 *(Table XV)*. The difference between the degree of pesticide pollution of the carp and pike-perch is explained by food biology. To answer all questions, e.g. what is caused by the different ratio of DDT and

TABLE XI

Comparison of the pesticide level in the fishes collected in the Keszthely Bay in June, 1973 (mg pesticide/1000 g organ, wet weight)

Organ	Species	γ-HCH	Total DDT
Flesh	2+ white grass-carp	0.0150	0.2222
	3+ white grass-carp	0.1360	0.506
	Carp	0.0129	0.1416
	Pike-perch	0.0133	0.1418
Liver	2+ white grass-carp	0.0260	0.2114
	3+ white grass-carp	0.0375	0.1930
	Carp	0.0819	0.1416
	Pike-perch	0.0157	0.1418
Brain	2+ white grass-carp	0.0309	0.2002
	3+ white grass-carp	0.1172	0.1665
	Carp	0.0228	0.0469
	Pike-perch	0.0078	0.1274

TABLE XII

Maximum and minimum value of DDT and its decomposition products found in the organs of white grass-carp and some other species in Lake Balaton (numbers rounded)

Year	Species	Authors	mg/1000 organ wet weight
1965	Pike-perch, carp, bream	DÉNES and CIELESZKY (cit. ap. BARON et al.)	0.1 —8.4
1966	Pike-perch, carp, bream	BARON et al.	0.02—1.88
1967	Volga pike-perch, Mirror carp, Razor fish	FELFÖLDY and TÓTH*	0.01—0.42
1973	White grass-carp	PÁSZTOR et al.	0.17—0.51

Note: * = Unkown whether or not total DDT had been determined

TABLE XIII

Level of DDT and its decomposition products in pike-perch in different years (mg/1000 g organ, wet weight)

Organ	DÉNES and CIELESZKY 1965	BARON et al. 1966	PÁSZTOR et al. 1973
Flesh	0.1—0.2	0.09—1.80	0.026—0.031
Liver	0.4—8.4	0.15—1.85	0.127—0.142

its decomposition products in the eggs and milt of the two species, should be clarified by further investigations.

TABLE XIV

Quantity of γ-HCH in the carp in different years (mg/1000 g organ, wet weight)

Organ	BARON et al. 1966	PÁSZTOR et al. 1973
Flesh	0.01—0.03	0.013—0.017
Liver	0.01—0.03	0.033—0.082

TABLE XV

Quantity of γ-HCH in the organs of pike-perch (mg/1000 g organ, wet weight)

Organ	BARTON et al. 1966	PÁSZTOR et al. 1973
Flesh	0.03—0.07	0.012—0.013
Liver	0.11—0.23	0.016—0.027
Eggs and milt	0.05—0.25	0.007—0.013

Relying the above findings the three questions raised in the introduction can be answered as follows:

1. The organisms playing an important role in the food-chain of Lake Balaton are characterized by different levels of, pesticide pollution. Contrary to the plankton crustaceans the pesticide accumulates in larger quantity in the phytoplankton. Consequently, the pollution level of white grass-carp feeding directly on algae is significantly higher than that of the carp or pike-perch. Since the sixties the pesticide content of carps (γ-HCH) increased to some extent. It is explained by the accumulation of pesticides in the alga-detritus → *Chironomus-Dreissena* → carp food-chain. The lowest pollution level was found in pike-perch.

2. There is a difference in the pollution level between the Keszthely Bay and the open water at Tihany, although this is not significant. This is due to the River Zala directly polluting the water in the Keszthely Bay. On the other hand, even the alga concentration is higher in the bay. The quantity of pesticides fixed in the plankton may increase the chlorinated hydrocarbon pollution of water with its 10—30 per cent.

3. Our data do not point out whether the γ-HCH pollution of the lake originates from River Zala alone or from the other tributaries, as well. This latter suggestion may be supported by the fact that DDT was found in larger quantity in the lake than in River Zala.

Summary

In 1973 the authors studied the chlorinated hydrocarbon pollution in two regions of different water quality of Lake Balaton with gas-chromatographic method. At the collection sites water, samples of phytoplankton and plankton crustaceans were taken, and white grass-carp *(Hypophthalmichthys molitrix)*, carp *(Cyprinus carpio)* and pike-perch *(Stizostedion lucioperca)* were collected. The quantity of γ-HCH, DDT, DDE and DDD chlorinate

hydrocarbons were examined in all samples. After the gas-chromatographic analyses the followings could be established:

1. The γ-HCH pollution of the Keszthely Bay and the open water at Tihany was identical in spring (35 ng/l). At the inlet of River Zala and in the river itself this value was somewhat higher (37 and 39 ng/l). In autumn their quantity decreased at each of the sampling sites.

2. In spring the DDD concentration was distributed evenly in the lake (1.4—1.7 ng/l), however, it was higher in River Zala (3.2 ng/l). In this season, except the surroundings of Tihany (2.1 ng/l), DDT was found only in very low values (less than 1 ng/l) with some increase in autumn and amounting to 8.5 ng/l in the Keszthely Bay.

3. In the Keszthely Bay the autumn phytoplankton samples containing DDT (4.086 mg/kg dry weight) and DDD (3.507 mg/kg dry weight) were in the largest quantity. Regarding the actual alga concentrations at the sampling sites this equals to 7.2 $\mu g/m^3$ pesticide fixed in the plankton.

4. The chlorinated hydrocarbon content of plankton crustaceans shows differences especially in the summer and autumn samples (0.437 and 1.239 mg/kg dry weight). In the autumn samples the value of γ-HCH was found to be 1.3—1.8 times higher than that of DDT being 16—27 times higher than in summer. Regarding the actual concentration of plankton crustaceans at the sampling sites, this equals to 0.05—0.23 $\mu g/m^3$ pesticide fixed in them.

5. In the specimens of the 3+ year-old white grass-carps the chlorinated hydrocarbon was found in significantly larger quantities than in the 2+ year-old ones. Examining the pollution of white grass-carp it was noted that the flesh of the 3+ year-old specimens contained 9 times (0.136 mg/kg), and their brain 4 times (0.117 mg/kg) as much γ-HCH as the 2+ year-old ones. As regards DDT, it was present in 2.5 times larger quantity (0.506 mg/kg) in the flesh of the 3+ year-old specimens.

6. The pesticide pollution (mg/kg organ, wet weight) of the organs of pike-perch and carp is lower than that of the wite grass-carp. In the case of pike-perch collected at Tihany the γ-HCH content of liver (0.027), eggs and milt (0.013) and brain (0.024) was higher than in the Keszthely Bay (0.0161; 0.007; 0.008 respectively). The carps showed higher pollution levels in the Keszthely Bay. As regards DDT and its decomposition products, a significant difference was found between the eggs and milt of pike-perch and that of the carp at the two areas. In the case of carps, even the difference in the flesh pollution is worth mentioning.

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A KLÓROZOTT SZÉNHIDROGÉN SZENNYEZETTSÉG GÁZKROMATOGRÁFIÁS VIZSGÁLATA A BALATON KÉT KÜLÖNBÖZŐ VÍZTERÜLETÉN

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Összefoglalás

A szerzők 1973-ban a klórozott szénhidrogén szennyezettségre vonatkozóan gázkromatográfiás módszerrel vizsgálták a Balaton két, vízminőségben eltérő vízterületét. A vizsgálati helyekről víz, fito- és Crustacea-plankton, fehér busa, *(Hypophthalmichthys molitrix)*, ponty *(Cyprinus carpio)* és süllő *(Lucioperca sandra)* került begyűjtésre. Az összes mintára vonatkozóan vizsgálták a gamma-HCH, DDT, DDE és DDD klórozott szénhidrogének mennyiségét. A gázkromatográfiás elemzések során a következőket állapították meg:

1. A tihanyi és keszthelyi víztérség gamma-HCH szennyezettsége tavasszal azonos (35 ng/lit). A Zala-folyó beömlésénél, valamint magában a folyóban ez az érték magasabb (37 és 39 ng/lit). Ősszel az összes vizsgált helyen mennyiségük lecsökkent.

2. A DDD koncentráció tavasszal a tó vizében közel azonos (1,4—1,7 ng/lit.) csupán a Zala-folyóban magasabb (3,2 ng/lit.). A DDT tavasszal a tihanyi vizeket kivéve (2,1 ng/lit.), csak nyomokban (1 ng/lit. alatt) található. Ősszel valamelyest megemelkedik és a Keszthelyi-öbölben elérte a 8,5 nannogramot literenként.

3. A Keszthelyi-öböl őszi fitoplankton mintájában a legnagyobb mennyiségben a DDT (4,086 mg/kg száraz súly) és a DDD (3,507 mg/kg száraz súly) fordult elő. Figyelembe véve az algák tényleges koncentrációját a gyűjtőhelyeken, ez 7,2 $\mu g/m^3$ planktonhoz kötött peszticidnek felel meg.

4. A Crustacea-plankton klórozott szénhidrogén tartalma különösen a nyári és az őszi minták között mutat eltérést (0,437 ill. 1,239 mg/kg szárított súly). Míg a lindán esetében 1,3—1,8-szor, addig a DDT-re vonatkozóan 16—27-szer nagyobb értékeket figyeltek meg az őszi minták javára. Figyelembevéve a Crustacea-plankton tényleges koncentrációját a gyűjtőhelyeken, ez 0,05—0,23 $\mu g/m^3$ rákplanktonhoz kötött peszticidnek felel meg.

5. A 3 nyaras fehérbusa példányokban számottevően nagyobb mennyiségekben található klórozott szénhidrogén, mint a 2 nyarasokban. A γ-HCH-ra vonatkozóan a 3 nyaras busa húsában kilencszer (0,136 mg/kg), az agyában négyszer (0,117 mg/kg több van mint a 2 nyarasban. A DDT esetében a húsban két és félszeres a különbség (0,506 mg/kg) az idősebb példányok javára.

6. A süllő és a ponty különböző szerveiben található peszticidek mennyisége (mg/kg szerv nedves súly) alacsonyabb, mint a fehér busáé. A Tihany előtti vizekből származó süllőknél a máj (0,027), ivartermék (0,013) és az agy (0,024) γ-HCH tartalma magasabb, mint a keszthelyi példányoké (0,0161; 0,007; 0,008). A ponty esetében ugyanazon szervekre vonatkozóan éppen fordítva igaz, a keszthelyi minták γ-HCH tartalma a magasabb. A DDT és bomlástermékeinek aránya a süllő és ponty esetében a két gyűjtőhelyre vonatkozóan az ivartermékben, a pontynál ezenkívül még a húsban is jelentősen eltér egymástól.

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INVESTIGATIONS ON PLANKTONIC CRUSTACEA IN LAKE BALATON VI. QUANTITATIVE CHANGES IN THE *EUDIAPTOMUS GRACILIS* POPULATION AT VARIOUS REGIONS OF LAKE BALATON

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As regards number of individual and biomass, *Eudiaptomus gracilis* is the most important filter feeder of the planktonic crustaceans in Lake Balaton (SEBESTYÉN, 1960; PONYI, 1968; PONYI and P.-ZÁNKAI, 1972). On the one hand, its decided role has been borne out by those investigations proving that it serves as important food to fry, even fry of pike-perch on the other hand, as the only filtering copepode of the lake, its breeding goes on throughout the year, and all the developmental stages are present in each season. These facts called for detailed studies on this species.

This study presents data on *Eudiaptomus gracilis* on the basis of samples taken during the periods 1965—67, 1972 and partly 1973.

Time table sampling stations and methods

The stations are given in *Fig. 1* and the detailed description of the transversal sections can be found in previous papers (SEBESTYÉN, 1960; P.-ZÁNKAI and PONYI, 1970). The number of sampling sites of the separate sections were different. Samples were taken monthly from 1 point each of the section in 1965, 3–3 points in 1966—67 and 5—5 points in 1972—73. The sampling generally took place in the warm-water period, except some years when the samples were taken in early spring and late autumn.

Dates of sampling:

1965: 9—10 June, 1—2 July, 3—4 August, 7—8 September, 13—14 October.
1966: 17—18 May, 14—15 June, 26—27 July, 23—24 August, 21—22 September, 18—19 October, 15—16 November.
1967: 16—18 May, 20 June, 26 June, 19—20 July, 15—16 August, 19—20 September, 23 October, 26 October.
1972: 19—21 April, 17—19 May, 21—23 June, 24—26 July, 15—17 August, 26—29 September, 16—18 October.
1973: 25 April, 14 May, 4 June, 19 June, 2 July, 18 July, 7 August, 20 September, 2 October, 25 October, 13 November (only at section M).

Samples were taken within a 1—3 days period from the 77 km long Lake Balaton, thus even those of the far-off sections could be compared.

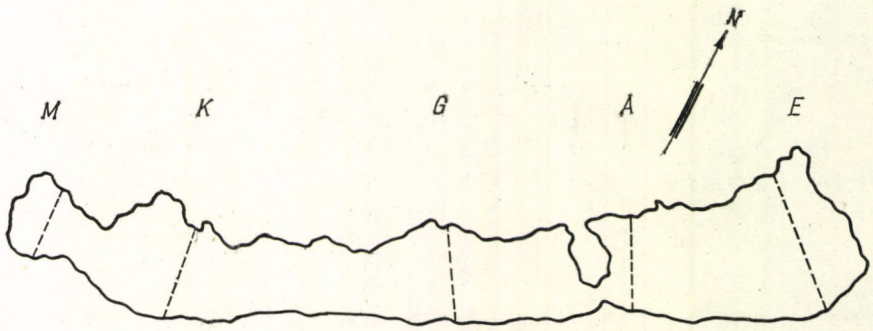

Fig. 1. Sampling places at Lake Balaton

The samples were taken with a water-column-lifting-filtering apparatus (SEBESTYÉN, 1960). For details on this method and the analysis of samples see PONYI and P.-ZÁNKAI, 1972.

Results

Surveying the data of *Eudiaptomus gracilis* samples taken in the periods of 1965—67 and 1972—73, it became evident that the density per litre of each life stage had considerably changed *(Figs 2—6)*. The quantitative fluctuation of the developmental stages was represented with curves. According to the pattern of curves the following eight types could be distinguished:

1. spring peak (abbr. T)
2. summer peak (abbr. N)
3. autumn peak (abbr. Ö)

These three types of curves showed only one peak;

4. peaks in spring and summer (abbr. TN)
5. peaks in spring and autumn (abbr. TÖ)
6. peaks in summer and autumn (abbr. NÖ)

In these types the number of developmental stages increased twice a year. Sometimes, between the two peaks, prior to or after them, a third increase was observed but because of its low value (1 individual/litre) it was disregarded.

7. peaks in spring, summer and autumn (abbr. TNÖ)
8. undefinite peak or peaks (abbr. B)

Of course, in the periods non-investigated or between the investigations further numerical increases or decreases might occur.

When examining the samples of 1 year and the frequency of patterns of the different developmental stages along the neighbouring transversal sections, the followings could be stated:

a) Identical pattern (N) at each of the five sections (from M to E) was shown once by males *(Table I) (Figs 2—6,* 1967).

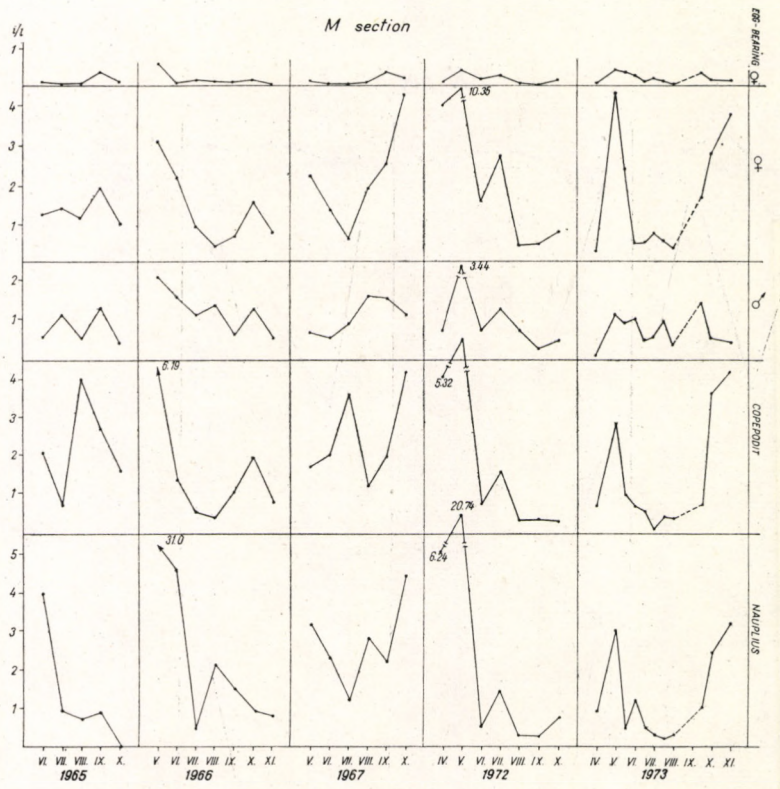

Fig. 2. Variation in the number of developmental stages of *Eudiaptomus gracilis* at transversal section *M* in five years

b) Identical pattern (TÖ) at four neighbouring sections (K-E) was also observed once produced by the copepodite stage *(Figs 3—6,* 1972).

c) Identical pattern at three neighbouring sections was found 5 times. One of them was shown by females (pattern N) in 1965, while other two by males: at sections M-G in 1966 (pattern T) and at sections K-A in 1972 (pattern TN) *(Figs 4—6).* The nauplii showed also identical patterns twice: the first one (T) at sections M-G (1965) and the second one (TN) at sections G-E (1966).

d) Identical patterns of two neighbouring sections were observed 12 times, mostly shown by egg-bearing females (4 times) and females (4 times) *(Table I* and *Figs 2—6).*

Items a—d clarify that the distribution of the developmental stages at the sections diverged very much within the same year. Not more than one-fifth of the so-called patterns showed uniformity.

By summing up the patterns of the different sections, it can be concluded that the seasonal variation of the developmental stages of *Eudiaptomus gracilis* differs section by section. While the spring peak is rather the characteristic of sections M-K (36—40 per cent), the summer peak is of higher frequency at sections A-E (25—40 per cent). The spring-summer development of the

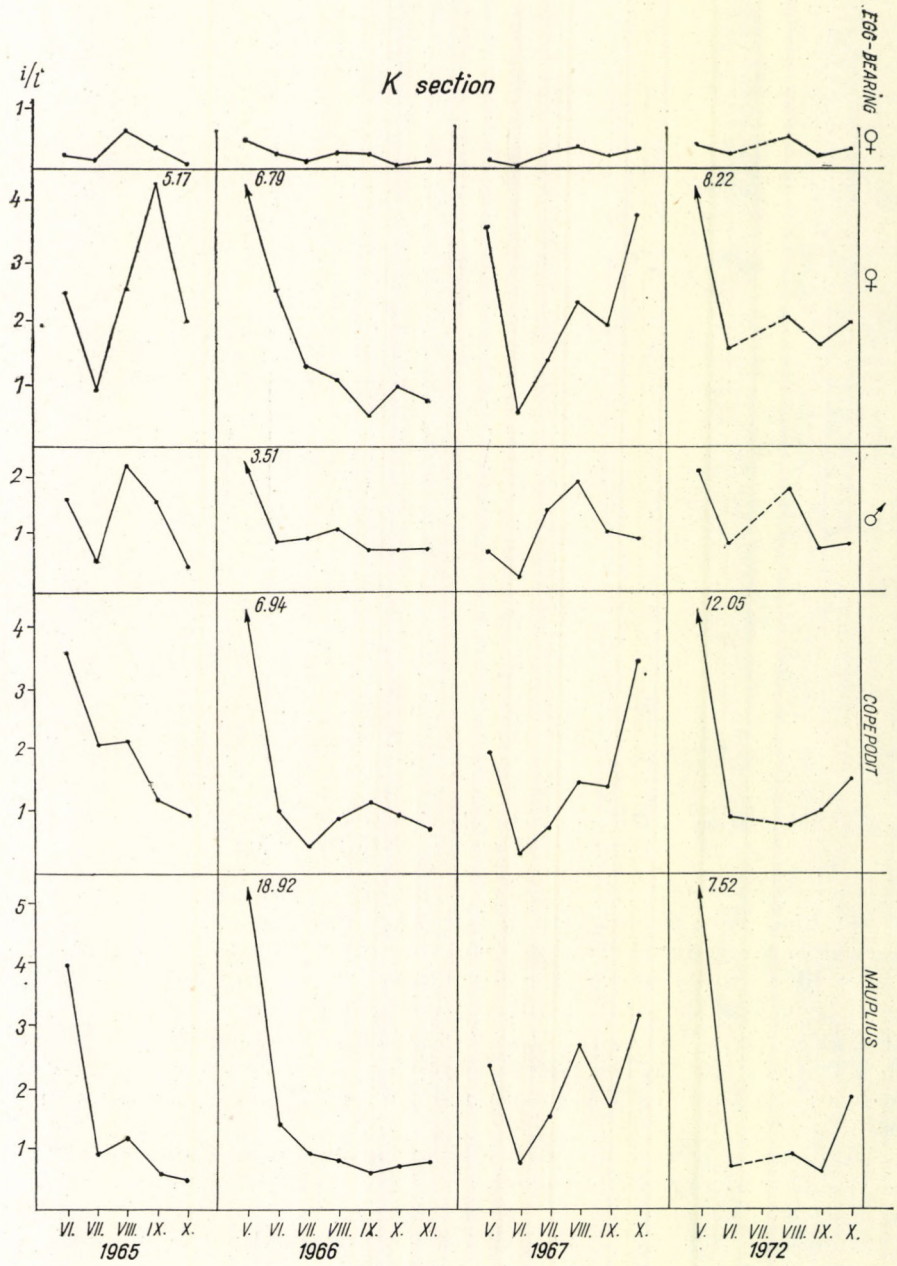

Fig. 3. Variation in the number of the developmental stages of *Eudiaptomus gracilis* at transversal section *K* in Lake Balaton in a period of four years

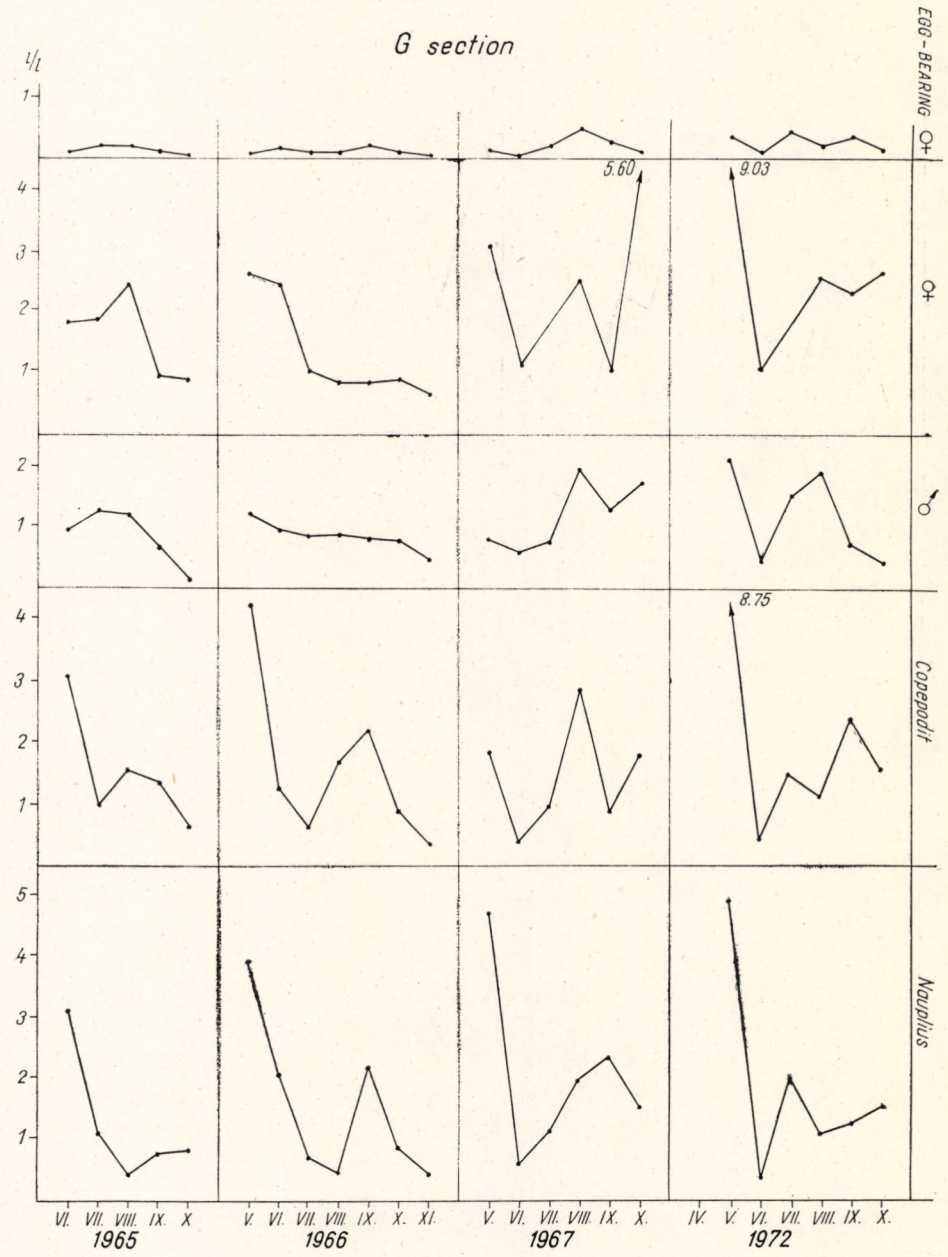

Fig. 4. Variation in the number of the developmental stages of *Eudiaptomus gracilis* at transversal section *G* in Lake Balaton in a period of four years

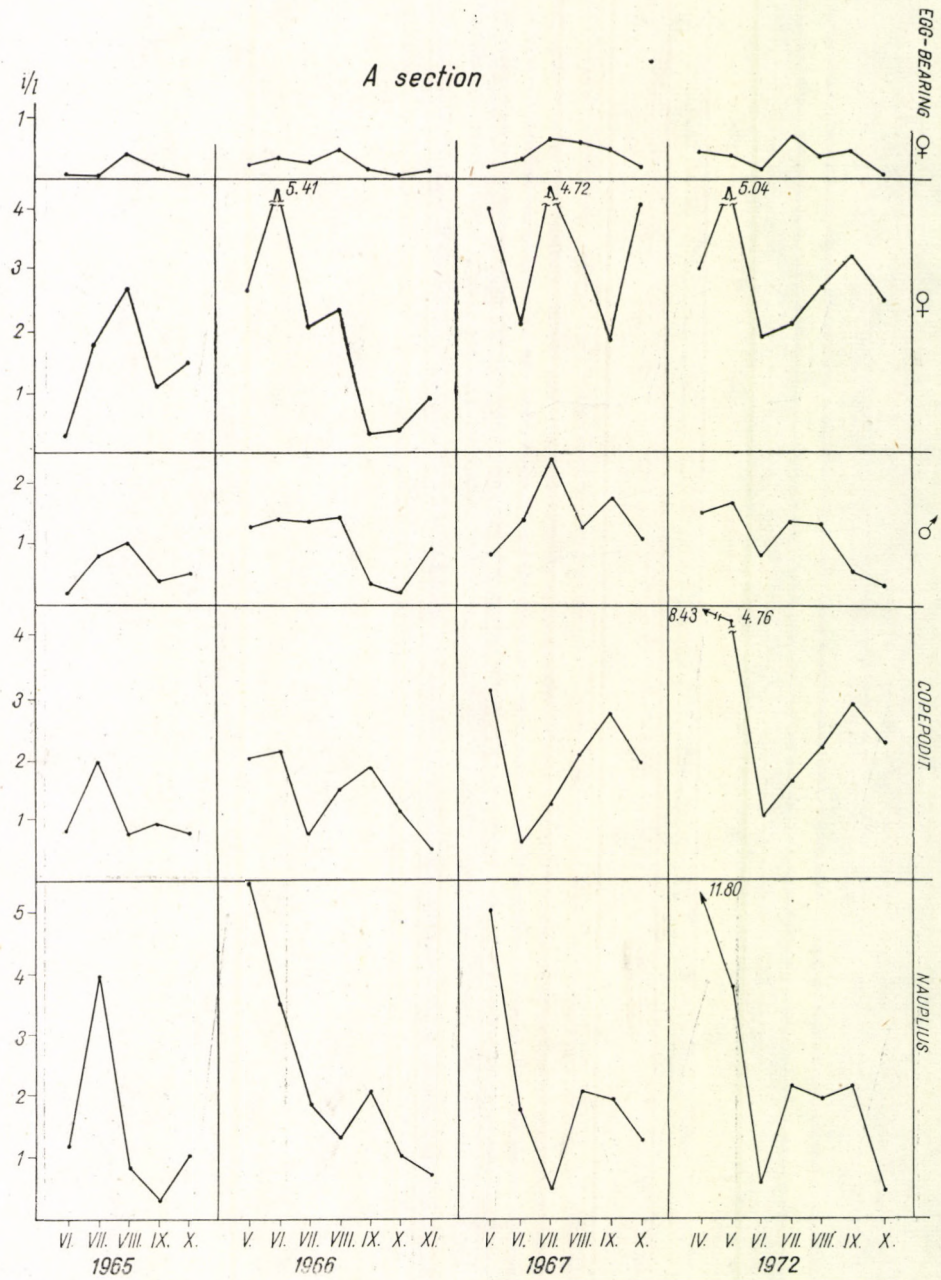

Fig. 5. Variation in the number of the developmental stages of *Eudiaptomus gracilis* at transversal section *A* in a period of four years

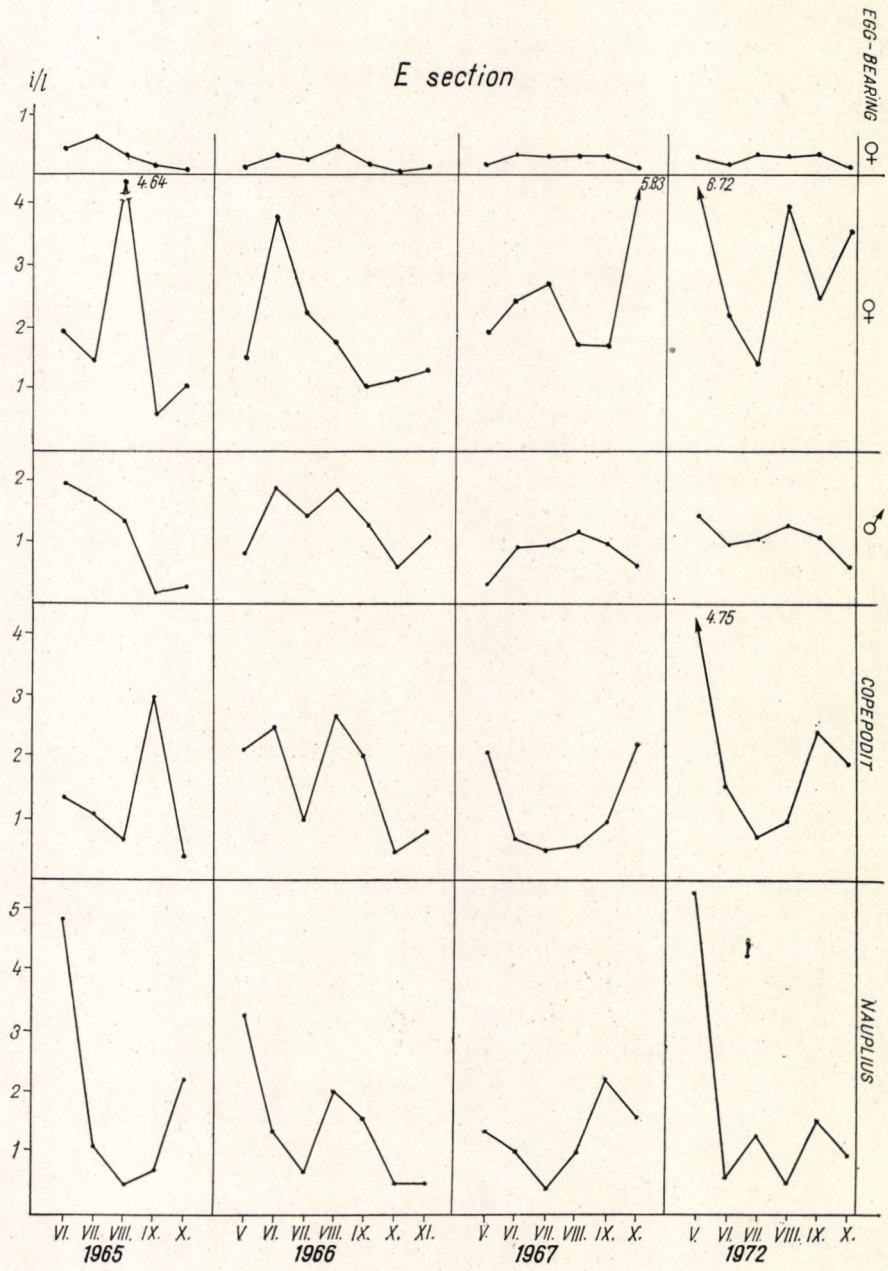

Fig. 6. Variation in the number of the developmental stages of *Eudiaptomus gracilis* at transversal section *E* in a period of four years

TABLE I

Distribution of patterns of the developmental stages of Eudiaptomus gracilis population at the sections investigated

yections		M	K	G	A	E
egg-bearing	1965	Ö	N	B	N	N
	1966	T	T	B	N	N
♀	1967	Ö	B	N	N	B
	1972	T	N	B	N	B
	1973	T	—	—	—	—
	1965	Ö	TN	N	N	N
	1966	TÖ	T	T	TÖ	T
♀	1967	TÖ	TÖ	TNÖ	TNÖ	NÖ
	1972	T	T	TN	TÖ	TN
	1973	TÖ	—	—	—	—
	1965	B	TN	N	N	T
	1966	T	T	T	B	N
♂	1967	N	N	N	N	N
	1972	T	TN	TN	TN	B
	1973	B	—	—	—	—
	1965	TN	T	TN	N	Ö
	1966	TÖ	T	TN	TÖ	TN
copepodite	1967	NÖ	TÖ	TN	TÖ	TÖ
	1972	T	TÖ	TÖ	TÖ	TÖ
	1973	TÖ	—	—	—	—
	1965	T	T	T	NÖ	TÖ
	1966	TN	T	TN	TN	TN
nauplius	1967	TÖ	TÖ	TN	TN	TÖ
	1972	T	TÖ	T	TN	T
	1973	TÖ	—	—	—	—

Explanation: T = peak only in spring N = peak only in summer Ö = peak only in autumn TN = peak both in spring and summer TÖ = peak both in spring and autumn NÖ = peak both in summer and autumn TNÖ = peak both in spring, summer and autumn B = other

populations is characteristic for sections G-A but in other regions of the lake, the spring-autumn peaks (20—28 per cent) are more common.

TABLE II

Percentual frequency of the patterns of the Eudiaptomus gracilis developmental stages at different areas of the lake

Transversal sections	M	K	G	A	E
T	36	40	20	0	15
N	4	15	20	40	25
Ö	12	0	0	0	5
TN	8	15	35	20	15
TÖ	28	25	5	25	20
NÖ	4	0	0	5	5
TNÖ	0	0	5	5	0
B	8	5	15	5	15

The quality of the population composition can be concluded in the best way by comparing the patterns of the copepodite and naupliar stages *(Table III)*. Both stages are mostly characterized by two peaks (62, 72 per cent). Spring peak was also noticed though in lower percentage. On this basis it can be stated that the number of *E. gracilis* increases in Lake Balaton twice in the warm-water period.

TABLE III

Percentual frequency of the patterns of nauplius and copepodite stages of Eudiaptomus gracilis in the whole Lake Balaton (1965—73)

	Copepodite	Nauplius
T	14	33
N	5	0
Ö	5	0
TN	24	33
TÖ	48	29
NÖ	5	5
TNÖ	0	0
B	0	0

When comparing the frequency of the different developmental stages of this population to one another *(Table IV)*, it was observed that the relative quantity of the largest forms (females and egg-bearing females) gradually

TABLE IV

Variation of the developmental stages in the population during the three years investigated (May—October)

	M		K		G		A		E	
	average	%	average	%	average	%	average	%	average	%
1966										
egg-bearing	0.20	1.70	0.18	1.92	0.12	2.01	0.22	2.91	0.21	3.13
female	1.49	12.70	2.17	23.11	1.41	23.66	2.18	28.87	1.89	28.17
male	1.32	11.30	1.29	13.74	0.90	15.10	0.99	13.11	1.30	19.37
copepodite	1.91	16.30	1.86	19.81	1.83	30.70	1.59	21.06	1.77	26.38
nauplius	6.78	57.90	3.89	41.43	1.70	28.52	2.57	34.04	1.54	22.95
1967										
egg-bearing	0.14	1.64	0.17	2.44	0.21	2.84	0.35	3.80	0.24	3.85
female	2.22	26.00	2.22	31.85	2.52	34.05	3.31	35.98	2.70	43.27
male	1.04	12.18	1.02	14.63	1.16	15.68	1.43	15.54	0.85	13.62
copepodite	2.45	28.69	1.54	22.09	1.48	20.00	1.99	21.63	1.18	18.91
nauplius	2.69	31.50	2.02	28.98	2.03	27.43	2.12	23.04	1.27	20.35
1972										
egg-bearing	0.17	1.22	0.27	2.67	0.25	2.83	0.30	3.47	0.24	2.79
female	3.38	24.35	3.07	30.37	3.22	36.47	3.00	34.72	3.37	39.23
male	1.25	9.01	1.24	12.27	1.19	13.48	1.15	13.31	1.24	14.44
copepodite	4.03	29.03	3.21	31.75	2.65	30.01	2.31	26.74	2.04	23.75
nauplius	5.05	36.38	2.32	22.95	1.52	17.21	1.88	21.76	1.70	19.79

decreased from transversal section A to M retaining identical population levels at sections A-E.

The number of the individuals per litre changed inversely. As regards the average of three years, the total number of individuals is highest at section M (11.4) gradually decreasing towards section G (7.4). At section A it shows a low increase (8.4) but declines at section E again (7.2).

The variation in the egg-number/egg-sac of 100—100 animals was also worked up. The samples were netted simultaneously with those for quantitative purposes *(Table V)*. This value was found to decrease in each of the four

TABLE V

Variation in the number of eggs of Eudiaptomus gracilis at the five transversal sections of the lake in the four years of investigations

	M				K			
	1965	1966	1967	1972	1965	1966	1967	1972
IV.	—	—	23.5±4.5	16.9±3.8	—	—	21.8±4.4	—
V.	—	11.4±2.1	17.1±4.1	8.2±2.9	—	7.7±1.5	11.6±2.7	5.8±1.5
VI.	13.1±3.9	11.0±2.2	14.7±2.9	13.3±2.8	5.4±1.2	8.6±1.7	10.5±2.9	11.8±3.7
VII.	14.0±3.3	11.4±2.5	14.8±3.7	15.8±3.8	10.7±2.4	9.5±2.7	15.1±3.4	—
VIII.	13.8±3.3	15.5±4.3	12.8±2.3	18.2±3.8	9.9±2.5	8.3±2.4	9.9±1.7	11.8±4.1
IX.	10.0±2.9	10.7±3.1	11.0±2.9	17.8±5.7	5.6±0.3	4.5±0.0	8.9±2.1	16.6±4.1
X.	17.1±5.1	17.5±3.9	13.2±2.9	18.4±5.0	14.7±3.1	16.2±3.5	10.5±2.5	14.6±3.8
XI.	—	12.1±2.2	—	—	—	11.4±2.8	—	—

TABLE V *(continued)*

	G				A			
	1965	1966	1967	1972	1965	1966	1967	1972
IV.	—	—	13.7±2.9	—	—	—	10.6±2.5	—
V.	—	7.4±1.6	10.4±2.5	5.2±1.1	—	9.2±1.7	7.6±1.5	5.5±1.7
VI.	4.8±1.0	5.0±1.0	10.6±1.6	—	7.6±1.9	3.7±0.2	4.5±1.7	5.9±1.1
VII.	4.6±0.3	6.3±1.2	8.8±2.2	7.9±2.4	5.7±1.3	6.5±1.6	5.4±1.4	6.0±1.4
VIII.	4.1±0.0	5.4±0.0	9.1±2.0	6.0±1.0	5.1±1.1	5.2±1.0	7.0±1.5	6.3±1.3
IX.	6.2±1.1	4.0±1.4	4.6±0.0	8.9±2.4	5.3±1.0	4.4±0.0	5.1±1.1	7.4±1.5
X.	11.4±3.2	12.3±2.4	6.2±1.4	6.8±3.3	8.9±2.2	—	5.7±1.1	6.6±1.3
XI.	—	11.5±2.7	—	—	—	7.9±1.6	—	—

TABLE V *(continued)*

	E			
	1965	1966	1967	1972
IV.	—	—	10.9±2.1	—
V.	—	7.0±1.3	7.4±1.4	4.8±1.0
VI.	7.1±1.6	4.0±1.0	5.9±1.1	—
VII.	5.7±1.4	5.6±1.1	5.5±1.6	6.3±1.5
VIII.	4.4±0.3	5.0±1.0	4.8±0.0	4.9±1.5
IX.	5.3±0.0	5.5±0.0	5.5±0.0	4.9±1.5
X.	8.4±2.0	8.7±2.5	7.5±2.3	5.5±1.3
XI.	—	9.4±1.6	—	—

years in the direction from section M to A. The value of section E was identical with that of section A:

egg-number/egg-sac	M	K	G	A	E
average of 4 years	14.3	10.9	7.9	6.3	6.2

Discussion

Eudiaptomus gracilis is widely distributed and can be found in the British Isles, Scandinavia, the northern region of the USSR as well as in France, Germany, Austria and Eastern-Europe. Its distribution to the South is limited by the Alps (KIEFER, 1968a). In Hungary it is common both in small and large water bodies and fish-ponds (PONYI, 1956).

Its relatively wide distribution in Europe proves that this species has a wide ecological valency. Consequently, the development of its population is very variable. It is mentioned both in the old and the latest studies that this species is represented by its mono-, bi- or polycyclic type in waters of the same type, being geographically near to one another (SPANDL, 1926; ELSTER, 1954; KIBBY, 1971). The overwintering forms of this species as well as the cold- and warm-water forms are well known. In Lake Balaton the nutrition intensity of the cold- and warm-water forms diverges too (P.-ZÁNKAI and PONYI, 1974). The forms of the two seasons show similarly morphological differences. In the cold season a special protruding appendix appears on the third segment of the right antenna of the males, disappearing with the warming up of the water. This seasonal morphological deformation is regarded by some authors as the cyclomorphosis of this species (WOYNÁROVICH, 1938).

Its quantitative importance is underlined by the fact that this species constitutes 50 per cent of total population of plankton crustaceans in the warm-water period (May-October) (PONYI and P.-ZÁNKAI, 1972). Owing to the fact that *E. gracilis* can develop even three cohors in 40 days (WEGLENSKA, 1971), the life stages found in the monthly samples could not be related to each other. Thus the life stages of this population is evaluated separately. At a given place the increase and decrease in the population density of the species is expressed by the pattern formed by the majority of developmental stages.

The quantitative change of the developmental stages of *E. gracilis* in the lake is shown by patterns of 8 different types. Comparing these patterns to each other, it is obvious that even within the same water area different types alternate with one another during the same year. The water areas were compared on the basis of their patterns by adapting the formula of MARCZEWSKI—STEINHAUS:

$$S = \frac{w}{a + b - w} \times 100,$$

where w = number of identical patterns at the sections compared; a and b = number of diverse patterns separately at the two sections.

Accordingly, sections A and G show the greatest similarity, while sections G and K are the most diverse. The rate of similarity was identical between the two sections in the south-wastern basin (M and K) and the other two sections of the north-eastern basin (A and E). Evidently, as regards the develop-

ment of the population, several diverse water areas can be distinguished in Lake Balaton.

TABLE VI

Patterns of the Eudiaptomus gracilis population at the different sampling stations of the lake shown by the formula of MARCZEWSKI—STEINHAUS *(1965—67; 1972—73)*

	M	K	G	A	E
M	—	36.8	9.7	2.7	2.7
K		—	27.3	9.7	5.9
G			—	69.2	14.3
A				—	36.8

Earlier observations (SEBESTYÉN, 1953; PONYI, 1968) showed that the population of *E. gracilis* increased twice a year in the north-eastern basin. With special regard to the nauplii and copepodites, our present studies suggest *(Table III)* that the population may increase 1—3 times a year in Lake Balaton. In the larger lakes (e.g. Lake Constance) summer and autumn peaks were observed (KIEFER, 1968b), while in smaller water bodies only one spring peak was noted (SPANDL, 1926).

Within the population the percentage of egg-bearing females was very low, hardly mounting up to 4 per cent in Lake Balaton. In his study on 42, mostly shallow lakes SMYLY (1968) stated that this value rarely fell under 20 per cent in the warm-water period. In contrary the egg-bearing ones, the percentage of the adult females varied between 1.4 and 43.0 per cent in the population. In the period of four years, the data on individual number/litre and egg-number/egg sacs showed that the "production" of the species is probably the highest at section M, gradually decreasing in the direction to A *(Fig. 7)*. Simultaneously, the percentual distribution of adult and egg-bearing females was inverse. This phenomenon indicates that the degree of grazing by the fish is the highest at section M, gradually decreasing towards the north-east basin. This suggestion is supported by fishery statistics.

Studying the fluctuation in the number of individuals on *E. gracilis* populations from the thirties till recent years (Fig. 8) it is seen that this number (without nauplii) ranged in the period of 1936—38 from 12 to 22, while between 1946 and 1972 it was less than 7 individuals/litre.

When comparing *Figs 7* and *8* it was concluded that the density of fish and fry feeding on *E. gracilis* had increased in the lake. The suggestion that planktonic crustaceans of large body size are grazed intensively by fish is well supported by investigations in fish-ponds (HILLBRICHT-ILKOWSKA et al., 1973), establishing that the decrease in the biomass of crustaceans was followed in some cases by a decrease in the production.

According to the literature the egg-number/egg-sac value of *E. gracilis* is low in oligotrophic lakes and is high in eutrophic waters (CZECZUGA, 1959; 1960; STEEMANN NIELSEN, 1962). THOMAS (1961) compared the egg-number of *E. gracilis* with the total ion-content of the water. He states that the egg-number of this species is more abundant in low ionic concentrations than in high concentrations. Our investigations showed an inverse picture for Lake Balaton *(Table VII)*.

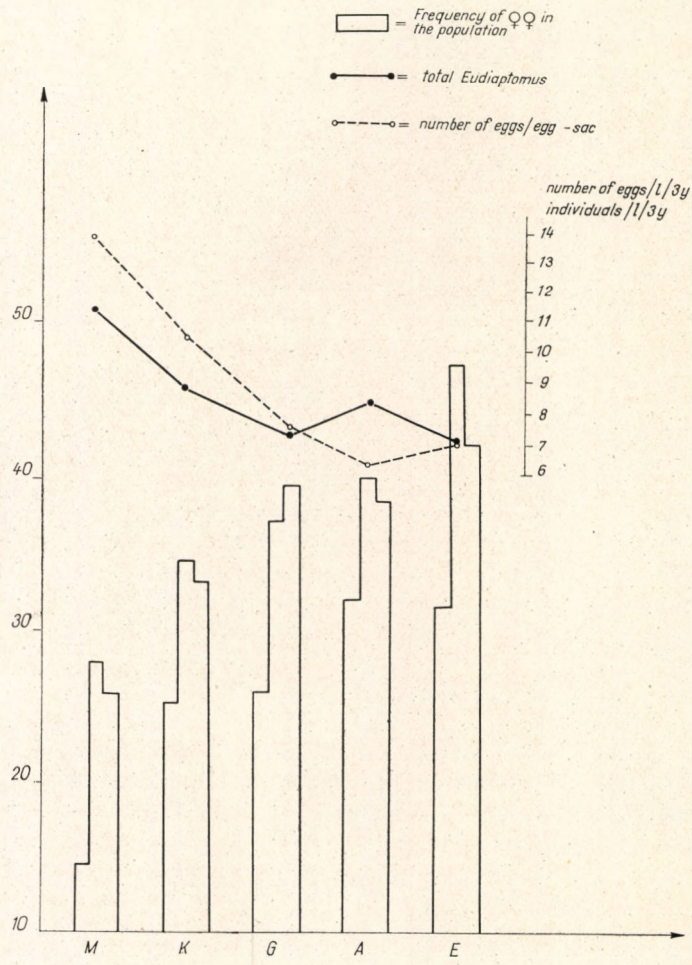

Fig. 7. Comparison of the egg-number/egg-sacs, total individuals and females at different sections of the lake

TABLE VII

Comparison of total ionic content to the egg-numbers of Eudiaptomus gracilis in four English lakes and in different regions of Lake Balaton

	Thomas (1961) Total ion expressed in the % of maximum	egg-number	Orsós (1968) Total ion expressed in % of maximum			Ponyi et al. (1975) egg-number
Windermere	100.0	8.71	BALATON	M	100.00	14.30
Grasmere	88.8	9.30		K	97.74	10.86
Derwent	85.1	6.05		G	95.02	7.41
Ennerdale	64.8	10.53		A	93.52	6.27
				E	91.45	6.19

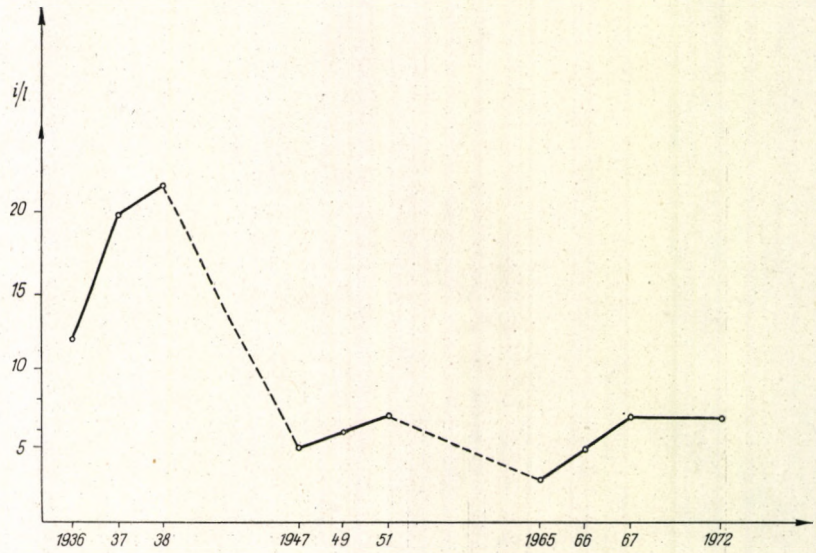

Fig. 8. Variation in the number of *Eudiaptomus gracilis* in May—October at transversal section *A* (without nauplii) on the basis of the data of SEBESTYÉN (1953; 1960), SEBESTYÉN et al. (1951) and ENTZ et al. (1937)

It is suggested that in case of Lake Balaton the increase in the egg-number of *Eudiaptomus gracilis* can be ascribed to the degree of trophyty, and the quantity of salt-content is of secondary part. The highest egg-number was found at the hypertroph region of the lake (HERODEK and TAMÁS, 1975) decreasing parallel with the degree of trophyty.

Summary

In the periods of 1965—67 and 1972—73 the authors investigated the variation of *Eudiaptomus gracilis* populations on the basis of samples taken monthly at water areas of different trophic level of Lake Balaton.

In the course of the investigation the following could be stated:

1. The development of the population of the species differs in space and in seasons. At the south-western end of the lake (section M) where the water quality is hypertrophic a spring peak of 36—40 per cent frequency was found, while in the other basin (sections A-E) summer peaks dominated (25—40 per cent). In the middle of the lake mostly spring-autumn peaks were observed (20—28 per cent).

2. Adapting the formula of MARCZEWSKI—STEINHAUS the patterns of the highest similarity were found at the mesotrophic areas (sections G-A), while those of the less similarity at sections K-G.

3. In the population the relative quantity of forms of large body size (females and egg-bearing females) gradually decreases from section A (mesotrophy) to section M (hypertrophy). Regarding the mean values of the period 1966—67 and 1972, it is seen that the total number of the species is the highest

at the south-west end of the lake (11 individuals/litre) gradually decreasing to the north-east basin (7.2 individuals/litre).

4. The egg-number/egg-sac was found to be the highest in the hypertrophic south-west basin (14.3) decreasing to 6.2 at the other end of the lake.

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CRUSTACEA—PLANKTON VIZSGÁLATOK A BALATONON VI AZ *EUDIAPTOMUS GRACILIS* (G. O. SARS) POPULÁCIÓJÁNAK MENNYISÉGI ALAKULÁSA A TÓ KÜLÖNBÖZŐ TERÜLETEIN

Ponyi Jenő, N.-Horváth Judit és *P.-Zánkai Nóra*

Összefoglalás

A szerzők 1965—67 és 1972—73-ban a Balaton különböző, trofitásban eltérő vízterületein havi gyűjtések alapján vizsgálták az *Eudiaptomus gracilis* G. O. SARS populációjának változását.

A vizsgálatok alapján a következő eredményekre jutottak:

1. A faj populációjának kifejlődése évszakosan és vízterületenként is eltér egymástól. Míg a tó délnyugati végén a hypertróf vízterületen (M szelvény) a vizsgálati esetek 36—40 %-ában csak egy tavaszi maximum alakult ki, addig a tó másik medencéjében (A—E szelvények) egy csúcsú nyári maximumok a jellemzőek (25—40%). A tó középső részein a tavaszi, őszi kettős maximum kialakulása a leggyakoribb (20—28%).

2. A MARCZEWSKI—STEINHAUS-féle hasonlósági index segítségével az *Eudiaptomus gracilis* populációjának alakulását jelölő mintázatok alapján a legnagyobb fokú hasonlóságot a mezotróf vízterületeken (G—A szelvények), a legkisebbet a hypertróf és mezotróf vízterület találkozásánál (K—G szelvény) találtunk.

3. A populáción belül a legnagyobb testű alakok (nőstény és petés nőstény) relatív mennyisége az A szelvénytől (mezotróf) az M szelvényig (hypertróf) fokozatosan csökken. 1966—67 és 1972 év átlagát tekintve a faj összes egyedszáma a tó DNy-i végén a legnagyobb (11 e/lit.), amely fokozatosan csökken a tó másik vége felé (7,2 e/lit.).

4. A petezacskónkénti peteszám a tó DNy-i végén, a hypertróf vízterületen a legmagasabb (14,3), mely a tó másik végén 6,2-re csökken le.

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HORIZONTALLY OCCURRING QUANTITATIVE PHYTOPLANKTON INVESTIGATIONS IN LAKE BALATON, 1974

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As a follow up to the earlier publications on the quantitative and qualitative data of the horizontal phytoplankton of Lake Balaton on the basis of water sample series taken in the sixties, this study summarizes the quantitative and qualitative changes of phytoplankton in space and time in the year 1974. When examining the water samples taken at the transversal sections of the lake in July 1962 it was established that, compared to earlier data, qualitative and quantitative change had occurred in the phytoplankton (TAMÁS, 1965). On the basis of data on the horizontal phytoplankton collected for three years (from spring till autumn) after the great fish kill in 1965 the trend and rate of the change were made known in details (TAMÁS, 1967; 1969; 1972). The local variation in the phytoplankton of the sixties (TAMÁS, 1974) was obtained by multiplying the individual numbers with the mean volume of every single species.

The aim of these studies is to document the qualitative and quantitative changes of phytoplankton that proceed in space and time.

Dates of collecting and methods

Samples were taken fortnightly along section Balatonfüred—Zamárdi from February and in the Keszthely Bay from March. This study presents the mean values of the three deep-water sampling stations. Data on the two nearshore points will be published later *(Fig. 1)*.

The samples were fixed and analysed as described earlier (TAMÁS, 1972; 1974). The identification of algae was carried out on the basis of taxonomic works listed in detail previously (DESIKACHARY, 1959; PRESCOTT, 1962; TAMÁS, 1969; BURRELLY, 1966—1970). The year-long course of the water temperature given in *Fig. 2* is based on the data measured in the Kis-öböl Bay — a small bay on the east shore of the Tihany peninsula. The monthly mean values of the water level given in *Table I* were furnished by the Research Institute for Water Resources Development (Budapest, VITUKI). Data on the temperature, depth and transparency of water and the dates of sampling are summarized in *Table II*.

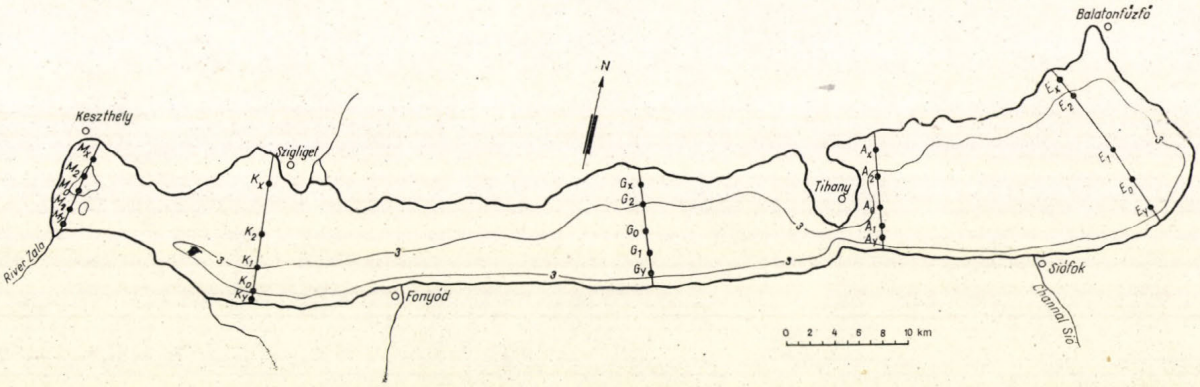

Fig. 1. Map of transversal sections with the sampling stations

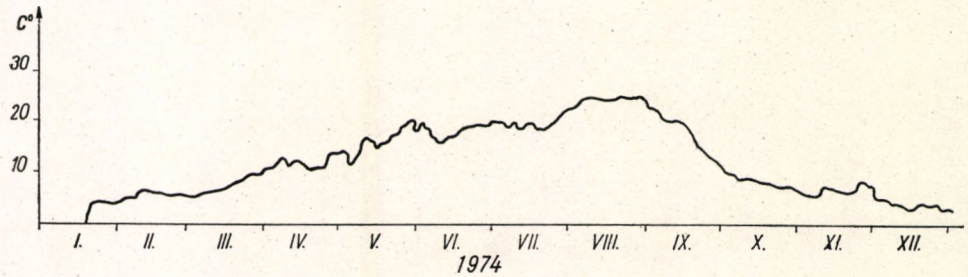

Fig. 2. Fluctuation in the water temperature measured in the Kis-öböl Bay at Tihany throughout the year

TABLE I

Monthly mean values of water level based on the data of Research Institute for Water Resources Development (VITUKI, Budapest)

I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.
77	84	95	94	98	97	93	86	94	103	108	101

Results

177 lifted samples and 100 net-filtrates were analysed, taken at 15 deep water stations of the five transversal sections from February till mid-December 1974. The identified microorganisms were found to belong to the following six taxonomic phyla:

Cyanophyta	22	—
Euglenophyta	11	—
Pyrrophyta	12	—
Chrysophyta	64	2
Chlorophyta	50	4
Caulobacteriales	1	
Together:	160	6

The Cyanophyta phylum was represented by 22 species. *Table III* shows that the filamentous algae of the order Hormogonales were of higher frequency in the Keszthely Bay. The *Anabaena* species made up 61.7 per cent of the individual number of Cyanophyta phylum in early September, 30 per cent on September 18, 44 per cent in early October and 69 per cent at the end of October. The *Aphanizomenon* species contributed 14 per cent of the individual number of the phylum in early September, 29 per cent on September 18, 28 per cent in early October and 12 per cent at the end of the month. Their number gradually decreased in the direction to the north-eastern basin of the lake. In the Keszthely Bay the number of *Lyngbya limnetica* was 850 times higher at mid-September than it had been in 1967. Even in early October an occurrence of 325,000 individuals/litre was found here.

TABLE II

Data on collecting

Collecting place	Collecting date	Water temperature °C	Depth cm	Secchi transparency cm	Notes
M	III. 26	10.7	300	33	Still water, sunshine
	VI. 9	11.4	285	41	*Chironomus* swarm, many *Synedra*
	IV. 23	10	310	49	Sunshine, breeze
	V. 7	12.1	290	47	*Chironomus* swarm, still water
	V. 24	15.8	320	46	
	VI. 4	19	312	45	Few clouds at times, sunshine
	VI. 26	20	295	38	
	VII. 10	20	290	40	
	VII. 25	20	300	50	Many *Melosira-Closterium*
	VIII. 7	22	296	38	Many *Microcystis*
	IX. 3	24	290	31	Algal bloom of *Anabaena spiroides*
	IX. 18	20	295	35	Masses of *Anabaena-Aphanizomenon*
	X. 8	11	300	46	Masses of *Anabaena-Aphanizomenon*
	X. 24	8	308	28	Masses of *Cryptomonas-Cyclotella*
	XI. 13	6.3	310	42	Masses of *Nitzschia acicularis*
	XII. 12	4	305	48	Masses of *Nitzschia-Stephanodiscus*
K	III. 26	10.4	398	40	Many *Cyclotella-Synedra-Ankistrodes.*
	IV. 23	10	410	42	Gloomy weather, breeze
	V. 24	14.7	420	42	Many *Romeria-Cryptomonas*
	VI. 25	18.5	385	57	
	VII. 25	20	412	35	Many *Melosira-Closterium*
	IX. 3	24	410	44	
	X. 9	10	418	67	Masses of *Anabaena-Aphanizomenon*
	X. 24	8	425	54	Many *Cryptomonas-Cyclotella*
	XI. 14	6.3	390	40	Masses of *Nitzschia acicularis*
	XII. 12	4	410	45	Masses of *Nitzschia acicularis*
G	III. 27	10.2	400	48	Many *Synedra-Ankistrodesmus*
	IV. 24	11.1	396	45	Many *Cyclotella-Synedra-Ankistrodes.*
	V. 22	14.7	418	57	Overcast, moderate wind
	VI. 26	19.8	380	56	
	VII. 29	21	415	130	Many *Aphanizomenon-Ceratium-Closter.*
	IX. 6	24	435	50	Many *Cryptomonas*
	X. 10	10	428	108	Many *Lyngbya-Microcystis*
	X. 25	8	430	62	Many *Cyclotella-*
	XI. 15	6.3	440	55	Many *Nitzschia acicularis*
A	II. 20	4	354	58	Many *Nitzschia acicularis*
	III. 27	10	395	43	Many *Cyclotella-Synedra-Ankistrodes.*
	IV. 10	11.9	399	48	Sunshine, breeze
	IV. 24	11.2	390	56	Sunshine, breeze
	V. 8	12	428	50	
	V. 21	15.6	416	59	Overcast
	VI. 24	18.5	420	57	
	VII. 11	19.2	406	45	Many *Aphanizomenon-Lyngbya-Cryptom.*
	VII. 24	18.3	380	33	Many *Ceratium-Closterium*
	VIII. a8	22	370	50	Dead calm, sunshine, many *Closterium*
	IX. 2	24	380	70	
	X. 7	11.2	420	91	Many *Cyclotella-Cryptomonas*
	X. 28	7	430	76	Many *Cryptomonas*
	XI. 19	6	390	65	Many *Cryptomonas-Cyclotella*
	XII. 13	3.5	410	70	Many *Cryptomonas-Cyclotella*
E	III. 28	10.4	470	91	Many *Cyclotella-Synedra*
	IV. 25	10.9	420	52	Overcast, many *Cyclotella*
	V. 22	15.4	434	54	Northeastern, raining, stormy waves
	VI. 27	19.7	426	60	Many *Lyngbya*
	VII. 24	19.5	450	40	Many *Aphanizomenon-Aphanocapsa*
	IX. 2	23	400	83	Many *Cryptomonas-Cyclotella*
	X. 10	11	480	70	Gloomy weather, many *Cyclotella*
	X. 18	7.5	465	82	Many *Stephanodiscus-Cyclotella*
	XI. 20	5	445	64	

Among the filamentous blue-greens, *Romeria elegans* was most abundant from April till June in the Keszthely Bay. The representatives of Cyanophyta phylum made up 13.3 per cent of total algae. Their number varied between 10,000 and 2.5 million (0.2—52.9 per cent) along the sections.

11 species of Euglenophyta phylum (Colaciales 1, Euglenales 10) were noticed in the samples. Species belonging to genera *Euglena* and *Phacus* were several times as numerous as they had been in the sixties in the phytoplankton association. In August and September *Euglena klebsii* was represented in a frequency of 17,000—18,000 individuals/litre in the Keszthely Bay, while in early September 8,000 individuals/litre were noted along the transversal section Szigliget—Balatonmária. It is seen from *Table III* that *Phacus acuminatus*, *Ph. pyrum* and *Trachelomonas volvocina* sometimes produced an abundance of 5,000 individuals/litre. Euglenophyta phylum made up 6.6 per cent of total algae. From June till November, along the two south-western transversal sections (M and K) the samples showed an abundance of 200—52,000 individuals/litre (0.1—1.0 per cent), between Ságpuszta and Balatonszemes (section G) 200—5,400 individuals/litre, while along the two sections of then north-eastern basin (A and E) 200—15,000 individuals/litre. The total mean number of the phylum varied between 0.1 and 2.2 per cent in space and times.

12 species of Pyrrophyta phylum (Cryptophycea 6, Dinophyceae 6) were identified in the samples. Among the Cryptophyceae species of nannoplanktonic size, in addition *Chroomonas*, *Cryptomonas* and *Rhodomonas* were found. The species of these genera were several times as abundant in 1974 as they had been in the sixties. In the samples taken in the Keszthely Bay in late October *Cryptomonas erosa* showed an occurrence of 1 million individuals/litre, *Cryptomonas ovata* 210,000 individuals/litre, while *Cryptomonas caudata*, which is of the smallest size, 2 million indivuduals/litre. Till mid-October the numbers somewhat decreased. At the Szigliget section (K) *Cryptomonas caudata* peaked with 775,000 individuals/litre in mid-October. Simultaneously, also *Cryptomonas erosa* had a maximum of 725,000 individuals/litre. *Ceratium hirundinella* and the other Dinophyceae genera given in *Table III* belong to the algae of large body size. The *Ceratium* population increased along each transversal sections from spring till autumn with maximum numbers of 120,000 individuals/litre at section K in early September and 101,000 individuals/litre at section M in mid-September. At transversal section Ságpuszta—Balatonszemes (G) it had an abundance of 73,000 individuals/litre at the end of July, between Balatonfüred and Zamárdi (7) 19,000 individuals per litre in early September. Simultaneously, a maximum of 22,000 individuals/litre occurred between Balatonalmádi and Balatonvilágos. *Diplopsalis acuta* reached a maximum of 6,000 individuals/litre in the Keszthely Bay at the end of July. *Glenodinium*, *Gonyaulax* and *Peridinium* species also showed July maximum here. The members of this latter genera were as numerous as they had been in the sixties. Pyrrophyta phylum made up 7.2 per cent of total algae. During the sampling period the individual numbers varied between 1,000 and 3.4 million at the transversal sections. The species of this phylum constituted 0.1—43.5 per cent of total algae.

During the investigations 66 species of Chrysophyta phylum (Xantophyceae 2, Crysophyceae 9, Bacillariophyceae 55) were noted in the samples. The species marked with in *Table III* appeared the first time in the series of open water samples taken from Lake Balaton in the seventies (HORTOBÁGYI).

TABLE III

Quantitative data on the phytoplankton of Lake Balaton in 1974 (i/l = 1000 individual per litre; N = net-filtrate (No. 25); D = cell size; o = oligosaprobic; α—m. = α-mesosaprobic; β—m. = β-mesosaprobic)

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
Cyanophyte Chroococcales											
1. *Aphanocapsa delicatissima*	II. 20								+		
W. et G. S. West	III. 26—28		+	10.0	+		+	10.0	+		+
D: 0.5—0.75 μ	IV. 9—10		+						+		
	IV. 23—25	10.0	+	5.0	+	5.0	+	15.0	+		+
	V. 7—8		+					10.0	+		
	V. 21—24	35.0	+	15.0	+	5.0	+	20.0	+	400.0	+
	VI. 4	35.0	+								
	VI. 24—27	20.0	+	5.0	+	10.0	+		+	10.0	+
	VII. 10—11	20.0	+						+		
	VII. 24—25, 29	10.0	+	25.0	+	25.0	+	5.0	+	5.0	+
	VIII. 7—8	15.0	+						+		
	IX. 2—3, 6	10.0	+	5.0	+	10.0	+		+	10.0	+
	IX. 18	10.0	+								
	X. 7—10	25.0	+	5.0	+	100.0	+	40.0	+	20.0	+
	X. 24—25, 28	10.0	+	15.0	+	25.0	+	15.0	+	20.0	+
	XI. 13—15, 19—20	5.0	+	15.0	+		+	5.0	+	5.0	+
	XII. 12—13	5.0	+	20.0	+			10.0	+		
2. *Aphanocapsa grevillei*	VI. 25—26		+		+						
(Hassall) Rabenhorst	VII. 10		+								
D: 3.5—5 μ, β—m.	VII. 25	5.0	+	10.0	+						
	VIII. 7	5.0	+								
	IX. 3		+		+						
3. *Chroococcus limneticus*	II. 20								+		
Lemm.	III. 26—28	40.0	+				+	40.0	+		+
D: 7—10 μ —o, β—m.	IV. 9—10	160.0	+						+		
	IV. 23—25	0.8	+				+		+		+
	V. 7—8		+						+		
	V. 21—24		+			2.0	+		+		+

	VI. 4		+									
	VI. 24—26		+			2.0	+		+			
	VII. 10—11		+						+			
	VII. 24—25, 29	40.0	+				+	4.8	+		+	
	VIII. 7—8		+						+			
	IX. 2—3, 6	1.6	+				+		+	3.2	+	
	IX. 18		+									
	X. 7—10		+			80.0	+		+		+	
	X. 25						+					
4. *Chroococcus minutus* (Kützing) Nägeli	X. 7								+			
	X. 28							40.0	+			
D: 6—8 μ—o, β—m.	XI. 19								+			
5. *Coelosphaerium kuetzingianum* Nägeli	II. 20								+			
	III. 26—28	10.0	+	10.0	+		+		+		+	
D: 3×4 μ	IV. 9—10	5.0	+						+			
	IV. 23—25		+	10.0	+	10.0	+		+		+	
	V. 7—8	5.0	+						+			
	V. 21—24	0.2	+	5.0	+	7.0	+		+		+	
	VI. 4		+									
	VI. 24—27	10.0	+	1.0	+		+	0.8	+	1.4	+	
	VII. 10—11	5.0	+					10.0	+			
	VII. 24—25, 29	5.0	+	10.0	+	5.0	+		+	0.6	+	
	VIII. 7—8	5.0	+					5.0	+			
	IX. 2—3, 6		9		+	5.1	+		+	0.2	+	
	IX. 18		+									
	X. 7—10	5.0	+	0.4	+	10.0	+	10.0	+	10.0	+	
	X. 24—25, 28		+	10.0	+		+	5.0	+	5.0	+	
	XI. 13—15, 19—20		+		+		+		+	5.0	+	
	XII. 12—13		+	10.0	+			10.0	+			
6. *Gomphosphaeria lacustris* Chodat	III. 26—28		+		+		+		+		+	
	IV. 9—10	15.0	+						+			
D: 4×2 μ—o, β—m.	IV. 23—25		+	5.0	+		+		+		+	
	V. 7—8		+						+			
	V. 21—24		+	5.0	+	2.0	+		+		+	
	VI. 4		+									
	VI. 24—27	5.0	+	5.0	+		+	0.4	+	1.4	+	
	VII. 10—11	5.0	+					10.0—	+			

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	VII. 24—25, 29	5.0	+	10.0	+	5.0	+		+	1.0	+
	VIII. 7—8		+						+		
	IX. 2—3, 6		+		+		+		+		+
	IX. 18		+								
	X. 7—10		+		+	5.0	+		+	5.0	+
	X. 24—25, 28		+		+		+		+		+
	XI. 13—15, 19—20	5.0	+		+	15.0	+	5.0	+		+
	XII. 12—13		+		+				+		
7. *Merismopedia glauca* (Ehr.) Näg.	III. 26		+		+						
	IV. 9		+								
D: 3—6μ —0, β—m.	IV. 23		+	20.0	+						
	V. 7	40.0	+								
	V. 24		+		+						
	IX. 3		+								
	IX. 18	10.0	+								
	X. 8		+								
8. *Merismopedia tenuissima* Lemm.	III. 26	80.0	+								
	IV. 8	240.0	+			4.0	+				
D: 1.3—2 μ β—, α—m.	VII. 29					12.8	+				
	IX. 3			320.0	+						
	X. 7							60.0	+		
	X. 28							19.2	+		
9. *Microcystis aeruginosa* Kütz.	II. 20								+		
	III. 26—28		+		+		+		+		+
f. *flos-aquae*	IV. 9—10		+						+		
(Wittr.) Elenkin	IV. 23—25		+		+		+		+		+
[= M. *flosaquae* (Wittr.)	V. 7—8		+						+		
Kirchn.]	V. 21—24		+		+	10.0	+		+		+
D: 3—6 μ β—m.	VI. 4		+								
	VI. 24—27		+	5.0	+	20.0	+	40.0	+	15.0	+

	VII. 10—11	150.0	+						+			
	VII. 24—25, 29		+	20.0	+		+		+			+
	VIII. 7—8	300.0	+						+			
	IX. 2—3, 6	200.0	+	200.0	+	93.0	+	160.0	+	32.0		+
	IX. 18	6.0	+									
	X. 7—10		+	300.0	+	250.0	+		+	150.0		+
	X. 24—25, 28		+	100.0	+	38.0	+	18.0	+			+
	XI. 13—15, 19—20		+	20.0	+		+		+	12.0		+
	XII. 12—13		+		+				+			
Hormogonales												
10. *Anabaena constricta*	VII. 25		+	25.0	+							
(Szafer) Geitl.	VIII. 7	10.0	+		+							
D: 4—6×6—8 μ α—m.	IX. 3	50.0	+	15.0	+							
11. *Anabaena flos-aquae*	VI. 26	0.8	+									
(Lyngb.) Bréb.	VII. 10	7.0	+									
D: 6—7μ β—m.	VII. 25	15.0	+									
	VIII. 7	10.0	+									
	IX. 3, 18	15.0	+									
	X. 8		+									
12. *Anabaena scheremetievi*	V. 22—24		+	0.3	+							
Elenkin	VI. 4	0.5	+									
	VI. 25—26	2.0	+	0.3	+		+					
D: 7—7.5×9—10 μ	VII. 10	28.0	+									
	VII. 25, 29	35.0	+	15.0	+		+					
	VIII. 7	10.0	+									
	IX. 3, 6	50.0	+	30.0	+	5.0	+					
	IX. 18	60.0	+									
	X. 8—9	15.0	+		+		+					
	X. 24—25	80.0	+		+		+					
	XI. 13—15		+		+		+					
13. *Anabaena spiroides*	IV. 9	0.3	+		+		+					
Kleb.	IV. 23—24		+		+		+					
D: 6.5—8μ —o, β—m.	V. 7		+									
	V. 22—24		+		+		+					
	VI. 4	0.1	+									
	VI. 25—26	2.6	+	5.0	+		+					

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	VII. 10	28.5	+								
	VII. 25, 29	35.0	+	30.0	+		+				
	VIII. 7	30.0	+								
	IX. 3i 6	500.0	+	25.0	+	2.7	+				
	IX. 18	700.0	+								
	X. 8—9	700.0	+	200.0	+	0.4	+				
	X. 24—25	200.0	+	35.0	+	0.2	+				
	XI. 12—13	25.0	+	20.1	+		+				
	XII. 12		+	4.8	+						
14. *Aphanizomenon flos-aquae* (L.) Ralfs	III. 26—28		+		+		+		+		+
D: 4—6×6—12 μ β—,	IV. 9—10	0.8	+								
α—m.	IV. 23—25	0.1	+	0.2	+		+	0.4	+		+
	V. 7—8		+						+		
	V. 21—24		+		+	2.0	+	1.6	+	0.4	+
	VI. 4		+								
	VI. 24—27	1.4	+	5.0	+	3.0	+	7.3	+	25.0	+
	VII. 10—11	52.5	+					75.0	+		
	VII. 24—25, 29	75.0	+	135.0	+	130.0	+	130.0	+	235.0	+
	VIII. 7—8	40.0	+					50.0	+		
	IX. 2—3, 6	65.0	+	40.0	+	50.0	+	60.0	+	105.0	+
	IX. 18	425.0	+								
	X. 7—10	375.0	+	80.0	+	5.4	+	100.0	+	10.0	+
	X. 24—25, 28	35.0	+	40.7	+	2.8	+	5.4	+	3.0	+
	XI. 13—15, 19—20	0.4	+	0.8	+		+	0.6	+	0.2	+
	XII. 12—13	5.0	+	1.6	+		+	0.4	+		
15. *Aphanizomenon issatschenkoi* (Ussaczew)	VI. 4	0.2	+								
Proschkina—Lavrenko	VI. 24—27	0.6	+	5.0	+		+	1.0	+	0.7	+
D: 7.5—9×3.2—5.4 μ	VII. 10—11	1.2	+					5.0	+		
	VII. 24—25, 29	75.0	+	10.0	+		+		+		+
	VIII. 7—8	10.0	+						+		
	IX. 2—3, 6	75.0	+	50.0	+	1.4	+		+		+

	IX. 18	300.0	+									
	X. 7—10	75.0	+	40.0	+	0.4	+		+			+
	X. 24—25, 28	15.0	+	8.0	+	0.2	+	5.0	+			
	XI. 12—15, 19		+		+		+					
	XII. 12—13		+		+		+					
16. *Lyngbya circumcreta* G. S. West	II. 20								+			
D: 1.8—2×1—2 μ	III. 26—28	20.0	+	7.6	+		+	5.0	+			+
	IV. 9—10	5.1	+						+			
	IV. 23—25	0.1	+		+	5.0	+	2.6	+	0.2		+
	V. 7—8		+					12.6	+			
	V. 21—24	2.8	+	0.3	+	4.0	+	1.6	+	0.4		+
	VI. 4	0.1	+									
	VI. 24—27		+	0.1	+	3.0	+	0.6	+	1.2		+
	VII. 10—11		+					3.2	+			
	VII. 24—25, 29		+		+	2.8	+	6.0	+	7.2		+
	VIII. 7—8		+					2.9	+			
	IX. 2—3, 6		+	1.0	+	2.0	+	1.2	+	1.2		+
	IX. 18	0.2	+									
	X. 7—10	0.4	+		+		+	10.0	+	5.1		+
	X. 24—25, 28		+		+	0.2	+		+	3.4		+
—	XI. 13—15, 19—20		+		+	0.6	+	0.4	+	0.6		+
	XII. 12—13		+	0.4	+			0.2	+			
17. *Lyngbya limnetica* Lemm.	II. 20							20.0	+			
D: 1—1.5×3—5 μ	III. 26—28	10.0	+	30.0	+	25.0	+	75.0	+	165.0		+
	IV. 9—10	60.0	+					90.0	+			
	IV. 23—25	25.0	+	5.0	+	75.0	+	65.0	+	60.0		+
	V. 7—8	25.0	+					275.0	+			
	V. 21—24	20.0	+	5.0	+	50.0	+	105.0	+	160.0		+
	VI. 4	35.0	+									
	VI. 24—27	26.0	+	5.0	+	26.1	+	22.0	+	150.0		+
	VII. 10—11	8.0	+					60.0	+			
	VII. 24—25, 29		+	5.0	+	135.0	+	150.0	+	285.0		+
	VIII. 7—8	10.0	+					250.0	+			
	IX. 2—3, 6	40.0	+	20.0	+	55.0	+	25.0	+	150.0		+
	IX. 18	850.0	+									
	X. 7—10	325.0	+	115.0	+	225.0	+	190.0	+	120.0		+
	X. 24—25, 28	65.0	+	50.0	+	85.0	+	115.0	+	60.0		+
	XI. 13—15, 19—20	35.0	+	50.0	+	85.0	+	130.0	+	35.0		+
	XII. 12—13	40.0	+	20.0	+		+	100.0	+			

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
18. *Oscillatoria tenuis*	IX. 18		+								
AGARDH	X. 8	10.0	+								
D: 5—8×2.6—5 μ α—m.	X. 24		+								
19. *Pseudanabaena catenata*	IV. 23		+								
LAUTERBORN	V. 7		+								
D: 2×3 μ	V. 22—24		+	0.1	+	2.0	+				
	IV. 25—26		+		+		+				
	VII. 25, 29		+		+	5.0	+				
	IX. 18		+								
	X. 8	10.0	+								
	X. 24		+								
20. *Rhaphidiopsis mediterranea*	VI. 25—26		+		+						
SKUJA	VII. 10		+								
D: 8—9×2.5—3 μ	VII. 25	10.0	+		+						
	VIII. 7	10.0	+								
	IX. 3	5.0	+		+						
	IX. 18	125.0	+								
	X. 8—9	60.0	+	45.0	+						
	X. 24		+	20.0	+						
	XI. 13—14		+		+						
21. *Romeria elegans*	II. 20								+		
(WOŁOSZYŃSKA) KOCZWARA	III. 26—28		+		+		+	0.6	+		
[= *Raciborskia elegans*	IV. 9—10	310.0	+						+		
WOŁOSZYŃSKA]	IV. 23—25	130.0	+		+	20.0	+		+		
D: 4—9×1.3—1.5 μ	V. 7—8	300.0	+								
	V. 22, 24		+	145.0	+	20.0	+				
	VI. 4	540.0	+								
	VI. 25—26	80.0	+		+	20.0	+				
	VII. 10		+								
	VII. 25, 29		+		+		+				

	IX. 3, 6		+		+		+					
	IX. 18		+									
	X. 8	15.0	+									
	X. 24		+									
22. *Spirulina laxissima*	IX. 3				+							
G. S. West	X. 9			0.6	+							
D: 0.7—0.8 μ	X. 24				+							
23. Colaciales Euglenophyta												
Colacium vesiculosum	VIII. 7—8		+						+			
Ehr.	IX. 2—3		+					5.0	+			
D: 10—14×8—10 μ β—m.	IX. 18	25.0	+									
	X. 7—8		+						+			
Euglenales												
24. *Euglena acus* Ehr.	III. 27					0.2	+					
D: 100—180×10—14 μ	IV. 23—25		+		+		+		+		+	
β—α—m.	V. 7—8		+						+			
	V. 21—24		+		+	0.1	+		+	0.4	+	
	VI. 4	0.2	+									
	VI. 24—27		+	0.1	+	0.4	+		+		+	
	VII. 10—11		+					0.4	+			
	VII. 24—25, 29	0.6	+	0.2	+		+	0.2	+	3.2	+	
	VIII. 7—8	1.0	+					0.6	+			
	IX. 2—3, 6		+	0.3	+		+	0.2	+	1.0	+	
	IX. 18		+									
	X. 7—10	0.4	+	0.2	+		+	0.4	+		+	
	X. 24—25, 28		+		+		+		+		+	
	XI. 13—14, 19—20		+		+				+		+	
	XII. 12—13		+		+				+			
25. *Euglena ehrenbergii*	II. 20								+			
Klebs	III. 26—28				+	5.0	+	2.5	+		+	
D: 150—200×25—30 μ	IV. 10								+			
	IV. 23—25		+		+		+		+		+	
β—m.	V. 21—24		+		+	2.0	+		+		+	
	VI. 4	0.1	+									
	VI. 24—27	0.2	+	0.1	+	0.2	+		+	0.4	+	
	VII. 10		+									
	VII. 24—25, 29	0.4	+	0.2	+		+	0.4	+	1.2	+	

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	VIII. 7—8	1.2	+						+		
	IX. 2—3, 6	0.2	+	0.4	+		+		+	0.2	+
	IX. 18		+								
	X. 7—10		+	0.4	+	2.6	+	5.0	+	2.6	+
	X. 24—25, 28				+		+		+	0.2	+
	XII. 13								+		
26. *Euglena klebsii* (Lemm.) Mainx	III. 26—28		+	0.2	+	0.2	+		+		+
D: 70—100×6—8 μ	IV. 9—10		+					0.2	+		
	IV. 23—25		+		+	0.1	+		+		+
	V. 7—8		+						+		
	V. 21—24		+	0.2	+	0.4	+	0.4	+		+
	VI. 4	2.5		+							
	VI. 24—27	0.4	+	0.1	+	1.2	+		+	0.3	+
	VII. 10—11		+						+		
	VII. 24—25, 29	3.0	+		+	0.4	+		+	0.4	+
	VIII. 7—8	17.2	+						+		
	IX. 2—3, 6		+	8.4	+		+		+		+
	IX. 18	18.7	+								
	X. 7—10	3.3	+		+	0.4	+		+		+
	X. 24—25, 28	0.2	+	0.2	+		+	0.2	+		+
	XI. 13—14, 19—20		+	0.2	+				+		+
	XII. 12—13		+	0.6	+		+		+		
27. *Euglena limnophila* Lemm. var. *minor* Drez.	III. 26—27		+		+		+	5.0	+		+
D: 28—50×6—12 μ	IV. 9—10		+					0.2	+		
	IV. 23—25		+	0.1	+	0.1	+		+		+
	V. 21—24		+		+	0.1	+	0.8	+		+
	VI. 24—27		+	0.1	+		+	0.6	+	0.2	+
	VII. 10—11	0.4	+						+		
	VII. 24—25, 29	0.4	+	0.2	+		+	0.2	+	0.2	+
	VIII. 7—8	5.0	+					0.4	+		
	IX. 2—3, 6	0.8	+	0.8	+		+	0.2	+	0.2	+

	X. 7—10		+		+		+		+	0 6	+
	X. 24—25		+	0.2	+	0.8	+	0.2	+	0.4	+
	XI. 13—14, 19—20	0.2	+		+		+		+		
	XII. 13							0.2	+		
28. *Euglena oxyuris* SCHMARDA	V. 21—24		+	0.1	+	0.1	+		+		+
D: 130—240×20—40 μ	VI. 4		+								
β—α—m.	VI. 24—27	2.8	+	0.2	+		+	0.2	+	0.2	+
	VII. 10—11	0.2	+					0.4	+		
	VII. 24—25, 29	2.0	+	0.4	+		+		+	2.4	+
	VIII. 7—8	1.4	+					1.2	+		
	IX. 2—3, 6		+	1.6	+		+	1.2	+	2.8	+
	IX. 18	0.6	+								
	X. 9—10					0.2	+			0.2	+
	XI. 14			0.2	+	0.2	+				
29. *Phacus acuminatus* STOKES	IV. 23			0.1	+						
D: 20—25×20—27 μ	V. 21—24		+		+						+
β—α—m.	VI. 4	0.3	+								
	VI. 24—27	0.2	+	0.2	+						+
	VII. 24—25, 29		+	0.2	+				+	0.6	+
	VIII. 7—8	5.1	+						+		
	IX. 2—3, 6	2.6	+	2.8	+			0.2	+	0.2	+
	IX. 18	2.7	+								
	X. 9—10		+	0.2	+				+		+
	X. 24		+		+				+	0.4	+
	XI. 14, 20				+					0.2	+
	XII. 12—13			0.4	+						
30. *Phacus hamelii* ALL. et LEF.	III. 27						+				
D: 25—30×15—20 μ	IV. 24					5.0	+				
	V. 22					2.0	+				
31. *Phacus longicauda*	V. 22, 24		+		+					0.2	+
(EHR.) DUJ.	VI. 4	0.2	+								
D: 150—190×50—70 μ	VI. 24—27	0.2	+	0.2	+						+
β—α—m.	VII. 25		+	0.4	+						
	VIII. 7		+								
	IX. 3		+	0.2	+						
	IX. 18	0.4	+								
	X. 8		+		+						

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
32. *Phacus pyrum* (Ehr.) Stein	VII. 24—25		+						+		
	VIII. 7—8		+					5.0	+		
D: 30—50×10—20 μ	IX. 2—3		+								
	IX. 18	0.2	+								
	X. 8	5.0	+								
	X. 24		+								
	XI. 13	5.0	+								
	XII. 12		+								
33. *Trachelomonas volvocina* Ehr.	VII. 25		+								
	VIII. 7	5.0	+								
D: 15—18 μ β—m.	IX. 3, 18	5.0	+								
	X. 8		+								
Pyrrophyta											
Cryptophyceae											
34. *Chroomonas nordstedtii* Hansg. f. *minor* Nygaard	III. 26				+						
	IV. 23			10.0	+						
D: 8—10×3.5—5 μ	V. 24			75.0	+						
	VI. 25			5.0	+						
	VII. 25				+						
	IX. 3			10.0	+						
	X. 9				+						
35. *Cryptomonas caudata* Schiller	II. 20							25.0	+		
	III. 26—28	175.0	+	25.0	+	75.0	+	35.0	+	125.0	+
D: 14—17×8 μ	IV. 9—10	175.0	+					50.0	+		
	IV. 23—25	65.0	+	75.0	+	10.0	+	50.0	+	80.0	+
	V. 7—8	225.0	+						+		
	V. 21—24	190.0	+	325.0	+	30.0	+	75.0	+	75.0	+
	VI. 4	475.0	+								
	VI. 24—27	75.0	+	300.0	+	50.0	+	50.0	+	50.0	+
	VII. 10—11	425.0	+					45.0	+		

	VII. 24—25, 29	280.0	+	200.0	+	70.0	+	225.0	+	125.0	+
	VIII. 7—8	250.0	+					175.0	+		
	IX. 2—3, 6	400.0	+	350.0	+	275.0	+	100.0	+	275.0	+
	IX. 18	200.0	+								
	X. 7—10	275.0	+	275.0	+	60.0	+	80.0	+	100.0	+
	X. 24—25, 28	1850.0	+	450.0	+	100.0	+	100.0		75.0	+
	XI. 13—15, 19—20	1000.0	+	775.0	+	25.0	+	45.0	+	35.0	+
	XII. 12—13	875.0	+	280.0	+			225.0	+		
36. *Cryptomonas erosa* Ehr.	II. 20							5.0	+		
D: 30—34×18—20 μ	III. 26—27	35.0	+		+	10.0	+		+		
	IV. 9—10	125.0	+						+		
β—α—m	IV. 23—25		+	5.0	+		+		+		+
	V. 7—8	50.0	+						+		
	V. 21—24	15.0	+		+	5.0	+	5.0	+		+
	VI. 4	75.0	+								
	VI. 24—27	60.0	+	10.0	+		+		+		+
	VII. 10—11	60.0	+						+		⑯
	VII. 24—25, 29	30.0	+	65.0	+		+		+		+
	VIII. 7—8	75.0	+						+		
	IX. 2—3, 6	165.0	+	50.0	+	10.0	+	15.0	+	5.0	+
	IX. 18	375.0	+								
	X. 7—10	100.0	+	275.0	+	5.0	+	5.0	+	5.0	+
	X. 24—25, 28	1150.0	+	100.0	+	5.0	+	5.0	+	5.0	+
	XI. 13—15, 19—20	775.0	+	725.0	+	55.0	+	45.0	+	45.0	+
	XII. 12—13	210.0	+	275.0	+			35.0	+		
37. *Cryptomonas ovata* Ehr.	IV. 23—25		+		+		+				
	V. 7		+								
D: 30—70×8—20 μ	V. 21—24		+		+	1.0	+				
	VI. 4	10.0	+								
	VI. 24—27		+	5.0	+		+				
	VII. 10—11		+						+		
	VII. 24—25, 29		+		+		+		+		
	VIII. 7—8		+						+		
	IX. 2—3, 6	10.0	+		+		+	10.0	+		
	IX. 18	25.0	+								
	X. 7—10	35.0	+		+	5.0	+	5.0	+		
	X. 24—25, 28	210.0	+	20.0	+		+	10.0	+		
	XI. 13—15, 19—20	50.0	+	60.0	+		+		+		
	XII. 12—13	25.0	+	65.0	+				+		

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
38. *Cryptomonas pusilla* BACHM.	II. 20							5.0	+		
D: 7×5 μ	III. 26—27		+						+		
	VII. 25		+								
	VIII. 7	5.0	+								
	IX. 3		+								
	X. 8		+								
	X. 24	50.0	+								
	XI. 13		+								
	XII. 12	100.0	+								
39. *Rhodomonas lacustris* PASCHER et RUTTNER	III. 26—28	25.0	+				+			5.0	+
	IV. 9		+								
D: 10—12×5—7 μ	IV. 23—25		+				+				+
	V. 7—8	10.0	+						+		
	V. 21—24	20.0	+			10.0	+		+		
	VI. 4	15.0	+								
	VI. 24—27	10.0	+			15.0	+		+		+
	VII. 10—11		+			10.0	+				
	VII. 24—25, 28		+			25.0	+	25.0	+		+
	VIII. 7—8		+						+		
	IX. 2—3, 6	75.0	+		+	25.0	+	50.0	+		+
	IX. 18	50.0	+								
	X. 7—10	60.0	+		+	60.0	+		+	15.0	+
	X. 24—25, 28	150.0	+	25.0	+		+	10.0	+		+
	XI. 13—15, 19—20	100.0	+		+	20.0	+	10.0	+	10.0	+
	XII. 12—13	100.0	+	100.0	+				+		
Dinophyceae											
40. *Ceratium hirundinella*	II. 20								+		
(O. F. MÜLLER) SCHRANK	III. 26—28		+	0.4	+	0.6	+	0.1	+	0.4	+
D: 150—190×22—24 μ	IV. 9—10	15.0	+					0.2	+		
β—m.	IV. 23—25	0.6	+	1.4	+	0.6	+	1.2	+	0.6	+
	V. 7—8	2.0	+					1.0	+		

	V. 21—24	2.0	+	6.4	+	1.0	+	2.4	+	2.6	+
	VI. 4	2.7	+								
	VI. 24—27	17.0	+	10.0	+	12.0	+	6.0	+	13.0	+
	VII. 10—11	31.4	+					6.2	+		
	VII. 24—25, 28	86.0	+	46.0	+	73.0	+	13.0	+	15.4	+
	VIII. 7—8	88.0	+					6.6	+		
	IX. 2—3, 6	86.0	+	120.0	+	34.0	+	19.0	+	22.0	+
	IX. 18	101.0	+								
	X. 7—10	0.4	+	3.6	+	0.2	+	0.8	+	2.2	+
	X. 24—25, 28	0.2	+	0.5	+	0.2	+	0.8	+	0.2	+
	XI. 13—15, 19—20		+		+		+		+		+
41. *Diplopsalis acuta* ENTZ [= *Entzia acuta* (APST.) LEBOUR., *Glenodinium acutum* APST., *Peridinium latum* PAULSEN] D: 30—50×26—40 μ	V. 21—24		+		+	0.2	+		+		+
	VI. 4		+								
	VI. 24—27	0.4	+	0.4	+	0.4	+	0.2	+	0.1	+
	VII. 10—11	1.4	+					0.2	+		
	VII. 24—25, 28	6.0	+	2.0	+	2.0	+	0.8	+	0.8	+
	VIII. 7—8	0.6	+					0.6	+		
	IX. 2—3, 6	2.4	+	0.4	+	0.2	+	0.4	+	0.1	+
	IX. 18	2.6	+								
	X. 7—10		+	0.4	+		+		+	0.2	+
	X. 24—25, 28		+	0.1	+		+		+		+
	XI. 13—15, 19—20		+	0.5	+						
42. *Glenodinium gymnodinium* PENARD D: 40×35 μ	VI. 24—27		+	0.2	+				+		+
	VII. 10—11		+						+		
	VII. 24—25, 28	2.0	+	0.4	+			0.2	+	0.4	+
	VIII. 7—8		+						+		
	IX. 2—3, 6		+	0.2	+				+	0.1	+
	IX. 18	0.8	+								
	X. 8—10		+		+				+		+
	X. 28										+
43. *Gonyaulax apiculata* (PENARD) ENTZ fil. D: 30—60×30—50 μ	V. 21—24		+		+	0.5	+		+		+
	VI. 4		+								
	VI. 24—27		+	0.2	+		+		+		+
	VII. 10—11		+						+		
	VII. 24—25, 28	4.0	+	0.6	+		+	0.2	+		+
	VIII. 7—8	0.2	+						+		
	IX. 2—3, 6	1.6	+	0.2	+	0.2	+	0.6	+	0.1	+
	IX. 18	1.4	+								
	X. 7—10		+	0.2	+		+		+	0.2	+

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
44. *Peridinium inconspicuum* Lemm. D: 18—28×12—20 μ	VI. 24							3.0	+		
	VII. 10—11		+						+		
	VII. 24—25		+						+		
	VIII. 7	2.8	+								
	IX. 3, 18	0.4	+								
	X. 24	0.1	+								
45. *Peridinium penardii* Lemm. [= *Glenodinium penardii* Lemm., Lindem., *P. andrzejowskii* Wol.] D: 28—30×26—28 μ	X. 24	0.1	+								
	XI. 13	0.2	+								
Chrysophyta											
Xantophyceae	II. 20							5.0	+		
46. *Planctonema lauterborni* Schmidle D: 10—14×2.5—3.5 μ	III. 26—28	1.2	+	10.0	+	1.0	+	5.0	+	6.4	+
	IV. 9—10	2.5	+					1.6	+		
	IV. 23—25	25.0		3.0	+	5.0	+	10.0	+	5.0	+
	V. 7—8	5.0	+					10.0	+		
	V. 21—24	8.6	+	20.0	+	5.0	+	15.0	+	0.2	+
	VI. 4	10.0									
	VI. 24—27	5.0	+	10.0	+	10.0	+	3.4	+	5.0	+
	VII. 10—11	1.7	+					3.6	+		
	VII. 24—25, 29	5.0	+	10.0	+	5.0	+	1.6	+	10.0	+
	VIII. 7—8	0.8	+					5.0	+		
	IX. 2—3, 6		+	5.0	+	30.0	+	0.2	+	15.0	+
	IX. 18	2.6	+								
	X. 7—10	5.0	+	2.4	+	7.5	+	10.0	+	10.0	+
	X. 24—25, 28	5.0	+	3.0	+	5.2	+	8.1	+	1.8	+
	XI. 13—14, 19—20	0.2	+	1.6	+	10.0	+	5.0	+	1.0	+
	XII. 12—13	10.0	+	50.0				2.4	+		
47. *Rhizochrysis limnetica* G. M. Smith	VI. 4		+								
	VI. 25—26	10.0	+	0.5	+						

D: 35—45 μ	VII. 25				+								
	XI. 3		+		+								
	IX. 18	20.0	+										
	X. 7—10	5.0	+	5.0	+			5.0	+				
	X. 24, 28		+	5.0	+				+				
Chrysophyceae													
48. *Amphichrysis compressa* KORSCH.	VIII. 7		+										
	IX. 3	5.0	+	15.0	+								
D: 27—30×15—18 μ	IX. 18		+										
	X. 9				+								
49. *Chrysococcus rufescens* KLEBS	III. 26—28				+	10.0	+					+	
	IV. 23—25				+		+					+	
D: 8—12 μ	V. 21—24		+	25.0	+	5.0	+					+	
	VI. 4	50.0	+										
	VI. 24—27		+	20.0	+		+				10.0	+	
	VII. 10—11		+						+				
	VII. 24—25, 29	25.0	+	50.0	+	15.0	+		+		50.0	+	
	VIII. 7—8	15.0	+					25.0	+				
	IX. 2—3, 6		+	15.0	+	15.0	+		+		90.0	+	
	IX. 18	15.0	+										
	X. 7—10		+		+	15.0	+					+	
	X. 24—25, 28		+				+						
	XI. 13—15	25.0	+			5.0	+						
50. *Chrysoglenz verrucosa* WISL.	VI. 26		+										
D: 30—32×20—25 μ	VII. 10	40.0	+										
	VII. 25		+										
51. *Dinobryon divergens* IMH.	III. 26—28			10.0	+	35.0	+				10.0	+	
D: 40—46×6—9 μ	IV. 23—25			10.0	+	10.0	+					+	
—o, β—m.	V. 22, 25				+	7.0	+						
	VI. 25, 26				+	6.0	+						
	VII. 25, 29		+	1.0	+								
	VIII. 7	640.0	+										
	IX. 3, 6		+		+		+						
	X. 25						+						
	XI. 15					25.0	+						

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
52. *Dinobryon sertularia* EHR.	VII. 25		+								
D: 30—40×10—14 μ	VIII. 7	670.0	+								
β—m.	IX. 3		+								
53. *Dinobryon sociale* EHR.	III. 27						+				
D: 33—36×8—9 μ	IV. 24					5.0	+				
β—m.	V. 22					3.0	+				
	VI. 26						+				
	VII. 25		+								
	VIII. 7	570.0	+								
	IX. 3		+								
54. *Mallomonas acaroides* PERTY	V. 24		+		+						
	VI. 4	5.0	+								
D: 25—40×10—16 μ	VI. 25—26		+	1.0	+						
	VII. 25		+								
	IX. 3			5.0	+						
	X. 9			5.0	+						
	X. 24				+						
55. *Salpingoeca frequentissima* (ZACH.) LEMM.	III. 28		+		+				+	15.0	+
	IV. 23—25		+		+			20.0	+		
D: 10—12×3.8—4.5 μ	V. 7—8		+						+		
	V. 24	10.0	+	50.0	+						
	VI. 4		+								
	VI. 25			20.0	+						
	VII. 25				+						
	IX. 3			25.0	+						
	X. 9				+						
56. *Synura uvella* EHR.	IV. 23		+								
D: 25—35×10—15 μ	V. 7	10.0	+								
—o, β—m.	V. 24		+								

Bacillariophyceae													
57. *Amphora ovalis* Kütz.	II. 20									5.0	+		
D: 40—80×20—40 μ	III. 26—28	40.0	+		+		+				+		+
—0, β—m.	IV. 9—10	10.2	+								+		
	IV. 23—25	10.0	+	20.0	+	15.0	+	2.6	+	5.0	+		
	V. 7—8		+					10.0	+				
	V. 21—24		+		+	0.4	+	1.2	+	0.2	+		
	VI. 4		+										
	VI. 24—27	0.2	+	1.0	+		+	0.2	+		+		
	VII. 10—11	10.0	+						+				
	VII. 24—25, 29	5.0	+	5.0	+								
	VIII. 7—8		+						+				
	IX. 2—3		+	0.2	+			0.5	+		+		
	IX. 18		+										
	X. 7—10	10.0	+		+			0.4	+	5.0	+		
	X. 24—25, 28	2.2	+		+		+		+	0.4	+		
	XI. 13—15, 19—20		+		+	0.2	+	5.0	+	0.6	+		
	XII. 12—13			1.6	+				+				
58. *Amphora ovalis* var. *pediculus* Kütz.	II. 20							30.0	+				
	III. 26—28	20.0	+	15.0	+		+		+				
D: 10—30×10—20 μ	IV. 9—10		+					5.0	+				
	IV. 23—25		+		+	15.0	+		+				
	V. 7—8	10.0	+					10.0	+				
	V. 21—24		+		+	5.0	+		+				
	VI. 24—26			1.0	+		+		+				
	VII. 10—11		+					5.0	+				
	VII. 24—25	10.0	+	5.0	+				+				
	VIII. 7—8		+					5.0	+				
	IX. 2—3		+	10.0	+				+				
	IX. 18	10.0	+										
	X. 7—10		+	5.0	+				+				
	X. 24—25, 28			2.9	+			0.4	+	0.2	+		
59. *Asterionella formosa* Hassal	III. 26—28	6.4	+	0.8	+	2.4	+	1.6	+				
D: 60—100×1—2 μ	IV. 9—10	3.1	+						+				
β—m.	IV. 23—25	1.3	+	0.8	+	0.4	+						
	V. 7	2.4	+										
	V. 21—24	1.8	+	0.6	+	1.0	+				+		
	VI. 4	8.8	+										
	VI. 24—26		+		+	6.4	+			0.4	+		
	VII. 10		+										
	VII. 24—25, 29	1.6	+		+					1.6	+		

TABLE III *(continued)*

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
60. *Attheya zachariasii* J. BRUN.	VI. 24—26			0.5	+			5.0	+		
D: 52—56×14—16 μ	VII. 25				+						
—o, β—m.	IX. 3			25.0	+						
61. *Caloneis schumanniana* (GRUN.) CLEVE var. *biconstricta* GRUN.	VII. 10	0.2	+								
	X. 10									0.2	+
D: 50—70×10—14 μ											
62. *Cocconeis placentula* EHR.	VII. 10	5.0	+								
D: 50×30μ —0, β—m.											
63. *Cyclotella bodanica* EULENST.	II. 20							50.0	+		
	III. 26—28	375.0	+	375.0	+	375.0	+	150.0	+	110.0	+
D: 30—40 μ	IV. 9—10	400.0	+					100.0	+		
	IV. 23—25	190.0	+	200.0	+	375.0	+	135.0	+	135.0	+
	V. 7—8	400.0	+					150.0	+		
	V. 21—24	60.0	+	110.0	+	80.0	+	25.0	+	50.0	+
	VI. 4	175.0	+								
	VI. 24—27	120.0	+	70.0	+	35.0	+	50.0	+	50.0	+
	VII. 10—11	90.0	+					40.0	+		
	VII. 24—25, 29	50.0	+	35.0	+	10.0	+	35.0	+	20.0	+
	VIII. 7—8	10.0	+					5.0	+		
	IX. 2—3, 6	25.0	+	35.0	+	5.0	+	15.0	+	10.0	+
	IX. 18	10.0	+								
	X. 7—10	250.0	+	25.0	+	25.0	+	20.0	+	20.0	+
	X. 24—25, 28	30.0	+	35.0	+	40.0	+	50.0	+	20.0	+
	XI. 13—15		+	5.0	+	25.0	+	35.0	+	15.0	+
	XII. 12—13	175.0	+	60.0	+			140.0	+		
64. *Cyclotella glomerata* BACHM.	II. 20							25.0	+		
D: 4—10 μ	III. 26—28	550.0	+	300.0	+	300.0	+	75.0	+	225.0	+
	IV. 9—10	600.0	+					90.0	+		

	IV. 23—25	35.0	+	150 0	+	225.0	+	25.0	+	50.0	+
	V. 7—8	475.0	+						+		
	V. 21—24	20.0	+		+	30.0	+		+	30.0	+
	VI. 4	50.0	+								
	VI. 24—27	40.0	+	80.0	+	10.0	+	20.0	+	20.0	+
	VII. 10—11		+					15.0	+		
	VII. 24—25, 29	25.0	+	100.0	+	10.0	+	25.0	+	55.0	+
	VIII. 7—8	25.0						25.0	+		
	IX. 2—3, 6	230.0	+	75.0	+	35.0	+	60.0	+		+
	IX. 18	175.0	+								
	X. 7—10	2600.0	+	350.0	+	150.0	+	410.0	+	250.0	+
	X. 24—25, 28	650.0	+	225.0	+	125.0	+	25.0	+	70.0	+
	XI. 13—15, 19—20	350.0	+	475.0	+	110.0	+	275.0	+	60.0	+
	XII. 12—13	1250.0	+	675.0	+			175.0	+		
65. *Cyclotella ocellata* PANT.	II. 20							60.0	+		
[= *C. kützingiana* (THWAIT.)	III. 26—28	375.0	+	225.0	+	350.0	+	375.0	+	165.0	+
Chauvin var. *planetophora*	IV. 9—10	575.0	+					200.0	+		
FRICKE]	IV. 23—25	320.0	+	200.0	+	350.0	+	350.0	+	300.0	+
D: 6—20 μ	V. 7—8	475.0	+					125.0	+		
	V. 21—24	190.0	+	150.0	+	100.0	+	60.0	+	40.0	+
	VI. 4	320.0	+								
	VI. 24—27	250.0	+	50.0	+	75.0	+	50.0	+	50.0	+
	VII. 10—11	215.0	+					35.0	+		
	VII. 24—25, 29	275.0	+	25.0	+	25.0	+	65.0	+	75.0	+
	VIII. 7—8	225.0	+					20.0	+		
	IX. 2—3, 6	65.0	+	50.0	+	25.0	+	10.0	+	50.0	+
	IX. 18	100.0	+								
	X. 7—10	325.0	+	125.0	+	20.0	+	225.0	+	110.0	+
	X. 24—25, 28	245.0	+	175.0	+	25.0	+	150.0	+	75.0	+
	XI. 13—15, 19—20	25.0	+	100.0	+	40.0	+	135.0	+	150.0	+
	XII. 12—13	250.0	+		+			200.0	+		
66. *Cyclotella quadriiuncta*	IV. 23—25							10.0	+	10.0	+
SCHRÖTER	V. 8							0.4	+		
D: 20—40 μ	VI. 27									15.0	+
	X. 7—10		+			75.0	+	235.0	+	95.0	+
	X. 24—25, 28	30.0	+			25.0	+	20.0	+	21.5	+
	XI. 15, 19					90.0	+	10.0	+	40.0	+
	XII. 13							35.0	+		

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
67. *Cymatopleura elliptica* (Bréb.) W. Smith D: 75—160×40—70 μ —o, β—m.	III. 26—28		+	0.6	+	0.6	+	0.1	+		+
	IV. 9—10	0.2	+					0.2	+		
	IV. 23—25	0.1	+	0.2	+		+	0.2	+	0.2	+
	V. 7—8		+					0.4	+		
	V. 21—24	0.2	+	0.2	+	0.2	+	1.6	+	0.2	+
	VI. 4		+								
	VI. 24—27	2.8	+	0.4	+	0.8	+	0.8	+		+
	VII. 10—11	3.0	+					0.2	+		
	VII. 24—25, 29	0.4	+	0.8	+	0.2	+	0.6	+		+
	VIII. 7—8	1.4	+						+		
	IX. 2—3, 6	1.4	+	0.4	+	0.2	+			0.2	+
	IX. 18	0.4	+								
	X. 7—10	3.2	+	0.4	+		+		+	0.5	+
	X. 24—25, 28	0.4	+	0.4	+		+	0.1	+	0.4	+
	XI. 14—15, 19—20			0.2	+	0.4	+	0.1	+		+
	XII. 12—13	0.2	+					0.2	+		
68. *Cymatopleura solea* (Bréb.) W. Smith D: 120—140×20—24 μ β—α—m.	III. 26—28			0.1	+						+
	IV. 23—25				+				+	0.2	+
	V. 21—24				+				+	1.0	+
	VI. 24—27				+			0.2	+		+
	VII. 11							0.6	+		
	VII. 24—25			0.2	+				+	0.2	+
	VIII. 7—8		+					0.2	+		
	IX. 2—3, 6	0.6	+						+	0.2	+
	IX. 18	0.2	+								
	X. 8	0.4	+								
	X. 24, 28	0.2	+					0.1	+		
	XI. 13		+			0.2	+				
	XII. 13							0.4	+		
69. *Cymbella cymbiformis* (Kütz.) V. Heurck D: 60—100×10—14 μ	IX. 3			5.0	+						

70. *Cymbella lanceolata* (Ehr.) V. Heurck D: 100—200×24—34 μ —o, β—m.	VII. 25			0.2	+								
71. *Cymbella prostrata* (Berk.) Cleve D: 20—26×9—13 μ β—m.	V. 22, 24	5.0	+									5.0	+
72. *Diatoma elongatum* Ag. var. *tenuis* (Ag.) Kütz. D: 58—78×3—4 μ —o, β—m.	IV. 9—10 IV. 24 V. 8 V. 24	15.0 5.0	+ +						15.0 20.0	+ +			
73. *Diatoma vulgare* Bory var. *brevis* Grun. D: 30—40×10—13 μ —o, β—m.	IX. 2											1.2	+
74. *Diploneis domblittensis* (Grun.) Cleve D: 30—45×15—20 μ	IX. 2								5.0	+			
75. *Diploneis elliptica* D: 20—60×10—30 μ	V. 8 VII. 24 XI. 19								10.0 5.0 5.0	+ + +			
76. *Diploneis puella* (Schum.) Cleve D: 20—24×11—12 μ	XI. 19								5.0	+			
77. *Epithemia sorex* Kütz. D: 20—40×8—12 μ	IV. 24								5.0	+			
78. *Fragilaria construens* (Ehr.) Grun. D: 10—25×5—12 μ	II. 20 IV. 23 V. 22 VI. 4	1.6 20.0	+ +			2.0	+		10.0	+			

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	VI. 25			0.1	+						
—o, β—m.	VII. 10	75.0	+								
	VIII. 7—8	50.0	+					25.0	+		
	IX. 3	5.0	+	29.0	+						
	IX. 18	2.0	+								
	X. 8—10	26.0	+	10.0	+	5.0	+			5.0	+
	XI. 14			75.0	+						
	XII. 12			4.0	+						
79. *Fragilaria crotonensis* KITTON	V. 7	50.0	+								
D: 40—50×3—4 μ	X. 24	50.0	+								
—o, β—m.											
80. *Gomphonema olivaceum* (LYNGB.) KÜTZ.	VII. 10	10.0	+								
β—, α—m											
D: 10—30×5—10 μ											
81. *Gyrosigma acuminatum*	III. 28									0.4	+
(KÜTZ.) RABH.	IV. 24	0.1	+								
o: 100—120×15—18 μ											
—o, β—m.										3	
82. *Gyrosigma distortum*	II. 20							5.0	+		
(W. SMITH) CLEVE var.	IX. 3	0.3	+								
parkeri HARRIS.	IX. 18	0.4	+								
D: 70—100×15—17 μ	X. 7—8	0.8	+					0.2	+		
	XII. 12—13	0.2	+					0.2	+		
83. *Gyrosigma kuetzingii* (GRUN.)	IV. 24							0.3	+		
CLEVE	V. 21—22					1.0	+	0.8	+	0.2	+
D: 80—100×12—15 μ	VII. 24									0.2	+

D: 80—100×12—15 μ	IX. 3			0.2	+						
	X. 9					5.0	+				
	X. 28							.01	+	0.4	+
	XI. 19							0.2	+		
	XII. 13							0.2	+		
84. *Gyrosigma prolongatum* (W. Smith) Cleve	IX. 18	1.0	+								
D: 120—200×5—10 μ											
85. *Melosira granulata* (Ehr.) Ralfs	IV. 9	0.5	+								
	IV. 23—25	3.0	+	6.0	+		+		+		+
D: 5—20 μ	V. 7—8		+					7.0	+		
β—m.	V. 21—24		+	6.0	+	5.0	+		+	5.0	+
	VI. 4	2.0	+								
	VI. 24—27	17.5	+	10.0	+	21.5	+	1.0	+	11.5	+
	VII. 10—11	10.0	+					58.0	+		
	VII. 24—25, 29	16.0	+	60.0	+	4.0	+	20.0	+	29.0	+
	VIII. 7—8	14.5						79.0	+		
	IX. 2—3, 6	17.0	+	23.0	+	1.0	+	30.5	+	28.0	+
	IX. 18	55.0	+								
	X. 7—10	150.0	+	16.0	+	0.4	+	5.5	+	0.6	+
	X. 24—25, 18		+	2.0	+		+		+		+
	XI. 14—15, 19—20		+		+			3.0	+	10.0	+
	XII. 12—13	0.6	+	2.0	+				+		
86. *Melosira granulata* var. *angustissima* O. Müll.	IV. 9	3.0	+								
	IV. 23—24		+	4.0	+		+				
D: 3—5 μ	V. 22—24		+	5.0	+	2.0	+				
—o, β—m.	VI. 4	9.8	+								
	VI. 25, 26	9.0	+	10.0	+	6.0	+				
	VII. 10—11	10.0	+						+		
	VII. 24—25, 29	131.0	+	34.0	+		+	16.0	+	34.0	+
	VIII. 7—8	35.5	+					4.0	+		
	IX. 2—3, 6	58.0	+	46.0	+	3.0	+	12.0	+	8.0	+
	IX. 18	64.0	+								
	X. 7—10	25.0	+		+		+		+		+
	X. 24—25	4.0	+					4.0	+		
	XI. 13, 15		+						+		
	XII. 12	10.0	+								

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
87. *Navicula costulata* GRUN.	II. 20							5.0	+		
D: 20×6 μ	III. 26	10.0	+								
	IV. 23	10.0	+								
	IX. 3	5.0	+								
	IX. 18	10.0	+								
	X. 8	10.0	+								
88. *Navicula cryptocephala* KÜTZ.	II. 20							20.0	+		
	III. 27—28					25.0	+	35.0	+	5.0	+
D: 25×6 μ	IV. 9—10	15.0	+						+		
β—, α—m.	IV. 23—25	15.0	+	10.0	+		+	20.0	+	15.0	+
	V. 7—8	50.0	+					10.0	+		
	V. 21—24	20.0	+		+	10.0	+	15.0	+		+
	VI. 4	10.0	+								
	VI. 24—27		+		+	5.0	+	5.0	+		+
	VII. 10—11	5.0	+					5.0	+		
	VII. 24—25, 29		+	15.0	+	5.0	+	60.0	+	20.0	+
	VIII. 7—8	5.0	+					5.0	+		
	IX. 2—3, 6	10 0	+		+		+	25.0	+	10.0	+
	X. 7—10	25.0	+	10.0	+		+		+	5.0	+
	X. 24—25, 28		+	20.0	+	15.0	+			5.0	+
	XI. 14—15, 19				+	15.0	+		+		+
	XII. 12—13	5.0	+	10.0	+			10.0	+		
89. *Navicula dicephala* (EHR.) W, SMITH	III. 28									5.0	+
	IV. 25									5.0	+
D: 30×10 μ	V. 24	5.0	+								
90. *Navicula gracilis* EHR.	II. 20							10.0	+		
D: 50×10 μ	III. 26	5.0	+								
—o, β—m.	IV. 9	5.0	+								
	IV. 23	5.0	+								
	VI. 26	5.0	+								

	IX. 3	5.0	+								
	X. 28	5.0	+								
91. *Navicula hungarica* var.	IV. 10							5.0	+		
capitata (Ehr.) Clev	V. 23			5.0	+						
D:20×6 μ	VII. 10	5.0	+								
	X. 24	5.0	+								
92. *Navicula placentula* (Ehr.)	IV. 9	5.0	+								
Grun.	VI. 23—25	10.0	+	5.0	+			5.0	+		+
D: 60×20 μ	V. 7—8	15.0	+						+		
	V. 21—24		+		+		+	5.0	+	0.5	+
	VI. 24—27	10.0	+	5.0	+	5.0	+		+		+
	VII. 24—25, 29	5.0	+	10.0	+		+		+		
	VIII. 8							5.0	+		
	IX. 3	10.0	+		+				+		
	X. 8	5.0	+								
	X. 24, 28	5.0	+					5.0	+		
93. *Navicula pupula* Kütz.	III. 27							5.0	+		
D: 30×8 μ	IV. 23			5.0	+						
	VI. 24					5.0	+				
	VII. 25			5.0	+						
	X. 28									0.5	+
94. *Navicula scutelloides*	VII. 24							0.5	+		
. Smith											
D: 20×10 μ											
95. *Navicula tuscula* (Ehr.)	IV. 9	0.1	+								
Grun.											
D: 50×20 μ											
96. *Nitzschia acicularis*	II. 20							150.0	+		
W. Smith	III. 26—28	15.0	+	50.0	+	35.0	+	25.0	+		+
D: 50—80×3 μ	IV. 9—10	75.0	+						+		
β—, α—m.	IV. 23—25	25.0	+	175.0	+	65.0	+	10.0	+	5.0	+
	V. 7—8	85.0	+					25.0	+		
	V. 21—24	30.0	+		+	35.0	+	20.0	+	15.0	+
	VI. 4	10.0	+								
	VI. 24—27		+	30.0	+	25.0	+		+		+

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	VII. 10—11	25.0	+					25.0	+		
	VII. 24—25, 29	105.0	+	25.0	+		+	10.0	+	10.0	+
	VIII. 7—8	35.0	+						+		
	IX. 2—3, 6	20.0	+	20.0	+	5.0	+		+	10.[1]	+
	IX. 18	325.0	+								
	X. 7—10	7050.0	+	525.0	+	10.0	+	45.0	+	10.0	+
	X. 24—25, 28	9800.0	+	1 550.0	+	4.0	+	4.0	+	20.0	+
	XI. 14—15, 19—20	7150.0	+	16,000.0	+	200.0	+	45.0	+	10.0	+
	XII. 12—13	15,800.0		17,850.0				60.0	+		
97. *Nitzschia amphibia* GRUN. D: 20—40×3—5 μ	III. 26—28	60.0	+	10.0	+		+	25.0	+		+
	IV. 9—10	125.0	+						+		
	IV. 23—25	45.0	+	85.0	+	85.0	+	10.0	+	20.0	+
	V. 7—8	150.0	+					60.0	+		
	V. 21—24	20.0	+	15.0	+	25.0	+	15.0	+	20.0	+
	VI. 4	40.0	+								
	VI. 24—27	30.0	+	20.0	+	50.0	+	5.0	+	25.0	+
	VII. 10—11	60.0	+					25.0	+		
	ÅII. 24—25, 29	50.0	+	55.0	+	5.0	+	35.0	+	15.0	+
	VIII. 7—8	100.0	+					15.0	+		
	IX. 3—3, 6	150.0	+	25.0	+		+		+	15.0	+
	IX. 18	150.0	+								
	X. 7—10	100.0	+	70.0	+	5.0	+	10.0	+		+
	X. 24—25, 28	75.0	+	15.0	+	10.0	+		+		+
	XI. 14—15, 19—20	5.0	+	20.0	+	10.0	+	30.0	+	15.0	+
	XII. 12—13	15.0	+	25.0	+			10.0	+		
98. *Nitzschia hungarica* GRUN. D: 20—80×6—9 μ	IV. 25									5.0	+
	VII. 25	5.0	+	10.0	+						
	IX. 18	75.0	+								
	X. 8	5.0	+								
99. *Nitzschia sigmoidea* (EHR.) W. SMITH	III. 26—27			1.8	+			0.1	+		
	IV. 9—10	1.0	+						+		

D: 160—500×8—10 μ	IV. 23—25	0.2	+	0.2	+	0.3	+	0.6	+	0.5	+
	V. 7—8		+					1.0	+		
	V. 21—24	0.2	+	0.3	+	0.2	+	6.0	+	1.6	+
	VI. 24—27	1.0	+	0.1	+	2.4	+	1.4	+	0.2	+
	VII. 10—11	2.9	+					0.4	+		
	VII. 24—25, 29	1.0	+	5.0	+		+	0.2	+	0.2	+
	VIII. 7—8	0.2	+					5.0	+		
	IX. 2—3, 6	45.0	+	1.0	+	0.2	+		+	0.2	+
	IX. 18	0.8	+								
	X. 7—10	22.7	+	1.4	+		+			1.2	+
	X. 24, 28	3.0	+	0.8	+					1.3	+
	XI. 14, 20			0.6	+					1.4	+
	XII. 12—13	2.6	+	4.8	+			0.2	+		
100. *Nitzschia subrostrata* HUST.	II. 20										
D: 38—45×3 μ	III. 26—28	70.0	+	60.0	+	65.0	+	25.0	+		+
	IV. 9—10	125.0	+					25.0	+		
	IV. 23—25	115.0	+	105.0	+	50.0	+	20.0	+	25.0	+
	V. 7—8	850.0	+					150.0	+		
	V. 21—24		+	25.0	+	10.0	+	35.0	+	40.0	+
	VI. 4	125.0	+								
	VI. 24—27	100.0	+		+	10.0	+	10.0	+		+
	VII. 10—11	75.0	+					25.0	+		+
	VII. 24—25, 29	110.0	+	100.0	+	10.0	+	20.0	+	15.0	
	VIII. 7—8	50.0	+						+		
	IX. 2—3, 6	80.0	+	110.0	+	5.0	+	10.0	+	10.0	
	IX. 18	25.0	+								
	X. 7—10	135.0	+	190.0	+	5.0	+		+		+
	X. 24—25, 28	10.0	+	60.0	+		+		+		
	XI. 14—15, 19				+	5.0	+	15.0	+		+
	XII. 12—13			50.0	+			50.0	+		
101. *Nitzschia tryblionella* HANTZSCH. var. *debilis* (ARNOTT) A. MAYER	VII. 24									5.0	
D: 15—20×7—8 μ	IX. 18	15.0	+								
102. *Opephora martyi* HÉRIBAUD	III. 26	10.0									
D: 20×6 μ											

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		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
103. *Rhoicosphenia curvata* (KÜTZ.) GRUN. D: 20—30×6 μ	XII. 12	5.0	+								
104. *Stenopterobia pelagica* HUST. D: 100—200×5—6 μ	II. 20								+		
	III. 26—28		+	0.2	+			0.1	+	490.0	+
	IV. 9—10	0.1	+					0.2	+		
	IV. 23—25		+		+			0.2	+	3.5	+
	V. 8							0.4	+		
	V. 21—23						+	1.6	+	0.4	+
	VI. 24—27				+	0.2	+	0.4	+	0.1	+
	VII. 10—11	1.0	+					0.2	+		
	VII. 24—25, 29	2.0	+	0.8	+		+	0.2	+	0.4	+
	VIII. 7—8		+						+		
	IX. 2—3	1.0	+	0.8	+						+
	IX. 18	1.4	+								
	X. 8—9	2.4	+		+						
	X. 24—25	1.4	+	1.0	+		+				
	XI. 13—15		+		+	0.2	+				
	XII. 12—13	0.6	+	0.6	+						
105. *Stephanodiscus binderanus* (KÜTZ.) KRIEGER [= *Melosira binderana* KG.] D: 4—20×3 μ	VIII. 7		+								
	IX. 3, 6	20.0	+			10.0	+				
	IX. 18	85.0	+								
	X. 8		+								
106. *Stephanodiscus dubius* (FRICKE) HUST. D: 15—20 μ	VI. 4	25.0	+								
	VI. 24—26		+		+			5.0	+		
	VII. 10—11		+						+		
	VII. 24—25	65.0	+	10.0	+				+		
	VIII. 7	20.0	+								
	IX. 3	10.0	+	15.0	+						
	IX. 18	5.0	+								

	X. 7—9	75.0	+	50.0	+				+				
	X. 24, 28	550.0		50.0	+			30.0	+				
	XI. 13—14, 19	40.0	+	250.0	+				+				
	XII. 12—13	35.0	+	725.0	+				+				
107. *Stephanodiscus hantzschii* Grun	IX. 2—3	20.0	+										+
	X. 7—10		+		+		+		+	30.0			+
D: 8—12 μ	X. 24—28		+		+		+		+	125.0			+
β—, α—m.	XI. 14—15, 19	475.0	+	2050.0	+	35.0	+		+				+
	XII. 12—13	950.0	+	2300.0	+			150.0	+				
108. *Surirella biseriata* Bréb.	X. 10									0.2			+
D: 150—300×40—70 μ													
109. *Surirella robusta* Ehr. var.	IV. 24—25						+	5.0	+	0.1			+
splendida (Ehr.)	V. 8							0.2	+				
V. Heurck	V. 21—24		+		+	0.1	+	0.4	+				
D: 80—200×40—60 μ	VI. 4		+										
	VI. 24—26	0.2	+		+	0.2	+		+				
	VII. 10—11		+						+				
	VII. 24—25, 29	1.2	+	1.0	+	0.4	+	0.2	+				
	VIII. 7—8		+						+				
	IX. 3—6		+	1.8	+		+						
	X. 8—9	0.4	+		+								
110. *Surirella turgida* W. Smith	VI. 27									0.1			+
D: 50—60×40 μ	VII. 10—11	0.5	+					0.2	+				
	IX. 18	0.4	+										
111. *Synedra acus* Kütz. var.	II. 20							10.0	+				
angustissima Grun.	III. 26—28	587.5	+	712.5	+	927.5	+	925.0	+	980.0			+
D: 200—400×3 μ	IV. 9—10	725.0	+					160.0	+				
	IV. 23—25	425.0	+	30.2	+	175.0	+	5.0	+	10.0			+
	V. 7—8	60.0	+					15.0	+				
	V. 21—24	20.0	+		+	30.0	+	15.0	+	15.0			+
	VI. 4	2.5	+										
	VI. 24—27	110.6	+		+	13.5	+	2.7	+	13.0			+
	VII. 10—11	5.0	+						+				
	VII. 24—25, 29	5.0	+		+		+	20.0	+				+
	VIII. 7—8		+						+				

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	IX. 2—3, 6	10.0	+	3.4	+	0.8	+		+	1.0	+
	IX. 18	150.0	+								
	X. 7—10	25.0	+		+		+	0.6	+	5.0	+
	X. 24—25, 28	300.0	+	3.0	+	6.2	+	3.0	+	1.4	+
	XI. 13—15, 19—20		+		+	2.6	+	25.0	+	5.5	+
Chlorophyta	XII. 12—13	15.0	+		+			15.4	+		
Volvocales											
112. *Chlamydomonas dangeardii*	IV. 24							5.0	+		
CHMIEL.	V. 22					1.0	+				
D: 14—20×8—14 μ	VI. 4	10.0	+								
	VI. 24, 26							15.0	+		
	VII. 24, 29					10.0	+			5.0	+
	VII. 11							5.0	+		
	IX. 3	50.0	+	5.0	+						
	IX. 18	15.0	+								
	X. 24	15.0	+								
	XI. 13	5.0	+								
113. *Chlamydomonas media*	III. 28							40.0	+		
KLEBS. var. *minor* PASCH.	V. 22					1.0	+				
D: 12—14 μ	VI. 4	10.0	+								
	VII. 24—25, 29	10.0	+							10.0	+
	IX. 3	50.0	+				+				
	X. 7—9	5.0	+			5.0	+		+		
	X. 24,28		+				+	5.0	+		
114. *Chlamydomonas sp.*	X. 24							5.0	+		
D: 18—22×14—16 μ	XI. 14							5.0	+		
115. *Phacotus lenticularis*	VIII. 7		+								
(EHR.) STEIN	IX. 3	10.0	+								
D: 13—20 μ	IX. 18		+								

Tetrasporales												
116. *Elakatothrix lacustris* Korsch.	III. 27					20.0	+					
D: 20—22×4.4—4.8 μ	IV. 9—10	0.6	+					0.4	+			
β—m.	IV. 23—25	10.0	+	15.0	+	50.0	+		+	5.0	+	
	V. 7—8	10.0	+					15.0	+			
	V. 22—24	1.6	+	0.5	+	30.0	+	5.0	+		+	
	VI. 4	15.0	+									
	VI. 24—27	15.0	+	1.0	+	15.0	+	3.1	+	10.0	+	
	VII. 24—25, 29	10.0	+		+		+		+		+	
	VIII. 7—8		+						+			
	IX. 2—3, 6	10.0	+	2.9	+	5.0	+		+		+	
	X. 7—8		+					3.2	+		+	
	X. 24—25, 28	15.0	+	5.0	+	0.8	+	5.4	+	6.1	+	
	XI. 15, 19—20					10.0	+		+	10.0	+	
	XII. 12—13							1.0	+			
117. *Gloeococcus schroeteri* Lemm.	III. 26—28		+		+		+		+			
D: 6—10 μ	IV. 9—10	20.0	+					5.0	+			
	IV. 23—25		+		+	7.5	+		+		+	
	V. 7—8	40.0	+						+			
	V. 22—24	15.0	+	20.0	+	1.0	+			5.0	+	
	VI. 4	50.0	+									
	VI. 24—27	20.0	+	30.0	+		+		+		+	
	VII. 10—11	25.0	+					5.0	+			
	VII. 24—25, 29	10.0	+	50.0	+	1.6	+		+		+	
	VIII. 7—8		+					5.0	+			
	IX. 2—3, 6, 18		+		+		+		+			
	X. 7—10	15.0	+	5.0	+		+					
	X. 24—25, 28		+		+	20.0	+					
	XI. 15						+					
118. *Stylosphaeridium stipitatum* Geitler et Gimesi (Bachm.)	III. 27								+			
	IV. 10							30.0	+			
	VI. 24								+			
D: 8—10×5—8 μ	X. 8		+									
	X. 24	0.5	+									
	XI. 13		+									
Chlorococcales												
119. *Actinastrum hantzschii* Lagerh.	X. 24	0.8	+									
D: 12—20×3—5 μ												

Species	Date of collection	Localit'es									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
120. *Actinastrum hantzschii* var. *fluviatile* SCHROED. D: 40—43×3 μ —o, β—m.	X. 8	40.0	+								
121. *Ankistrodesmus braunii* BRUNNTHALER D: 20—40×8—10 μ	V. 22									5.0	+
	VI. 4	5.0	+								
	VI. 26	10.0	+								
	IX. 3	5.0	+								
122. *Ankistrodesmus convolutus* CORDA D: 8—10×2—3 μ	III. 26—27	25.0	+					30.0	+		
	IV. 10, 24							5.0	+		
	V. 22					10.0	+				
	VI. 26					15.0	+				
	VII. 24							5.0	+		
	X. 25					5.0	+				
123. *Ankistrodesmus falcatus* (CORDA) RALFS D: 25—80×2—4 μ	II. 20							15.0	+		
	III. 26—28	15.0	+	10.0	+		+		+	15.0	+
	IV. 9—10	30.0	+						+		
	IV. 23—25	5.0	+	5.0	+	20.0	+				+
	V. 7	60.0	+								
	V. 22, 24	5.0	+	10.0	+	10.0	+				
	VI. 4	5.0	+								
	VI. 24—27	7.9	+				+				+
	VII. 24—25, 29	5.0	+		+	15.0	+	5.0	+	5.0	+
	VIII. 7—8	15.0	+					3.3	+		
	IX, 2—3, 6	20.0	+	10.0	+		+	10.0	+	5.0	+
	IX. 18	.50	+								
	X. 7—10	20.0	+	10.0	+		+	2.0	+		+
	X. 24—25, 28	10.0	+	5.0	+	1.0	+	5.0	+	0.6	+
	XI. 13—15, 19—20	5.0	+	50.0	+	25.0	+		+	5.0	+
	XII. 12—13			25.0	+			0.2	+		

124. *Ankistrodesmus falcatus* var. *mirabilis* (West et West) G. S. West D: 100—150×2—3 μ	V. 7	5.0	+									
	V. 22—24	5.0	+			1.0	+					+
	VI. 24—27	5.0	+					3.0	+	10.0		+
	VII. 24									5.0		+
	XI. 15					0.5	+					
125. *Ankistrodesmus falcatus* var. *spirilliformis* G. S. West D: 25—35×2—3 μ β—, α—m.	II. 20							150.0	+			
	III. 26—28	675.0	+	675.0	+	800.0	+	350.0	+	400.0		+
	IV. 9—10	140.0	+					90.0	+			
	IV. 23—25	20.0	+	10.0	+	150.0	+	60.0	+	60.0		+
	V. 7—8	200.0	+					225.0	+			
	V. 22—24		+		+	100.0	+	5.0	+	50.0		+
	VI. 4		+									
	VI. 24—27	15.0	+	5.0	+	10.0	+	3.0	+			+
	VII. 24—25, 29	15.0	+	5.0	+		+	5.0	+			
	VIII. 7—8	5.0	+					5.0	+			
	IX. 2—3, 6		+		+		+		+			+
	X. 7—10	150.0	+	90.0	+				+	15.0		+
	X. 24—25, 28	110.0	+	35.0	+	35.0	+	35.0	+	17.6		+
	XI. 13—15, 19—20	50.0	+	110.0	+	30.0	+	35.0	+	10.0		+
	XII. 12—13	90.0	+	400.0	+			65.0	+			
126. *Botryococcus breunii* Kütz. D: 6—12×3—6 μ	IX. 2—3, 6			1.0	+	4.2	+	3.2	+	6.0		+
	X. 7, 9					30.0	+	1.6	+			
127. *Chodatella balatonica* Scherffel D: 6×3 μ	V. 22					1.0	+			10.0		+
	VII. 24									5.0		+
	IX. 26					5.0	+	5.0	+			
	VIII. 8							10.0	+			
	IX. 2, 6					5.0	+	5.0	+			
	X. 7—10					10.0	+		+			+
	X. 24—25, 28	5.0	+	5.0	+		+		+	5.0		+
	XI. 13—15, 19—20	5.0	+		+	5.0	+	5.0	+			
128. *Coelastrum microporum* Naeg. D: 8—16 μ	IX. 2—3	5.0	+		+					.02		+
	IX. 18	5.0	+									
	X. 8—10	5.0	+	2.5	+							+
	X. 24—25, 28		+		+							+
	XI. 19—20								+	2.5		+
	XII. 13							5.0	+			

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
129. *Crucigenia quadrata* MORREN D: 3—4 μ β—m.	V. 7—8	10.0	+						+		
	V. 22—24		+		+		+		+		+
	VI. 4	5.0	+								
	VI. 24—27		+		+		+		+		+
	VII. 10	5.0	+								
130. *Crucigenia tetrapedia* (KIRCH.) W. et G. S. WEST D: 6—7×3—5 μ β—m.	II. 20								+		
	III. 26—28		+	4.1	+	14.4	+	1.6	+	8.0	+
	IV. 9—10	20.0	+					2.4	+		
	IV. 23—25	3.6	+	2.8	+	20.0	+		+	20.0	+
	V. 7—8	40.0	+					0.4	+		
	V. 22—24		+	35.0	+	10.0	+		+	20.0	+
	VI. 4	15.0	+								
	VI. 24—27	5.4	+	10.0	+	5.8	+	15.0	+	0.4	+
	VII. 10—11	20.4	+					40.0	+		
	VII. 24—25, 29	1.6	+	5.0	+	20.0	+	20.0	+	20.0	+
	VIII. 7—8	30.0	+						+		
	IX. 2—3, 6		+		+		+	0.8	+	1.6	+
	IX. 18	20.0	+	16.4	+	1.6	+	5.8	+	40.0	+
	X. 7—10		+								
	X. 24—25, 28	20.4	+		+		+	8.7	+	2.4	+
	XI. 13—15, 19—20		+	22.9	+	1.6	+	20.0	+	2.0	+
	XII. 12—13		+		+			1.6	+		
131. *Dictyosphaerium pulchellum* WOOD D: 3—10 μ	II. 20								+		
	III. 26—28	390.0	+	100.0	+	160.0	+	140.0	+	60.0	+
	IV. 9—10	100.0	+					20.0	+		
	IV. 23—25	400.0	+	40.0	+	40.0	+	20.0	+	60.0	+
	V. 7—8	160.0	+					80.0	+		
	V. 22—24	40.0	+	40.0	+	30.0	+	20.0	+	30.0	+
	VI. 4	20.0	+								
	VI. 24—27	210.0	+	80.0	+	25.0	+			90.0	+
	VII. 10—11	30.0	+					100.0	+		

	VII. 24—25, 29	75.0	+	140.0	+	20.0	+	80.0	+	60.0	+
	VIII. 7—8	20.0	+					60.0	+		
	IX. 2—3, 6	20.0	+	20.0	+		+	20.0	+	20.0	+
	IX. 18	100.0	+								
	X. 7—10	860.0	+	120.0	+	40.0	+	145.0	+	120.0	+
	X. 24—25, 28	420.0	+	320.0	+	60.0	+	70.8	+		+
	XI. 13—15, 19—20	80.0	+	170.0	+	130.0	+	100.0	+	10.0	+
	XII. 12—13	265.0	+	245.0	+			60.0	+		
132. *Gloeoactinium limneticum* G. M. Smith	II. 20							80.0	+		
D: 4—4.3×2—2.6 μ	III. 26—28		+	120.0	+	20.0	+	140.0	+		
	IV. 9—10	380.0	+						+		
	IV. 23—24	20.0	+				+		+		
	IX. 2—3, 6	30.0	+				+		+		
	IX. 18	38.0	+								
	X. 7—10	160.0	+			40.0	+	30.0	+		
	X. 24—25, 28		+			20.0	+	70.0	+		
	XI. 13—15, 19—20		+				+		+		
	XII. 13							100.0	+		
133. *Kirchneriella lunaris* (Kirchn.) Moebius	VI. 24							5.0	+		
D: 7—13×3—8 μ	VII. 11								+		
	VIII. 8								+		
	IX. 2							5.0	+		
	X. 7—8		+						+		
	X. 24, 28	3.2	+						+		
134. *Kirchneriella obesa* (W. West) Schmidle	V. 8								+		
D: 10—14×4—6 μ	V. 21—24		+					5.0	+		+
	VI 4		+								
	VI. 24—27	5.0	+						+	5.0	+
	VII. 10—11		+						+		
	VII. 24—25, 28	5.0	+								+
	VIII. 7		+								
	IX. 3		+								
	IX. 18	0.8	+								
	X. 7—10	10.0	+								+
	X. 24, 28		+						+	10.0	+
	XI. 19—20								+		+
	XII. 13							10.0	+		

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
135. *Lagerheimia genevensis* CHOD. D: 5—6×3—3.5 μ	IV. 9	25.0	+								
	IV. 23		+		+						
	V. 7		+								
	V. 24		+		+						
	VI. 4	10.0	+								
	VI. 24—27		+	3.0	+						
	VII. 10		+								
	V. 25		+	5.0	+						
	VIII. 7	10.0	+								
	IX. 3		+		+						
	IX. 18		+								
	X. 8—9	10.0	+		+						
	X. 24	30.0	+	10.0	+						
	XI. 13—14	5.0	+		+						
	XII. 12		+	75.0	+						
136. *Lagerheimia wratislaviensis* * SCHROED. D: 7.8—9×4—7 μ	VI. 4		+								
	VI. 26	5.0	+								
	VII. 10	5.0	+								
	VII. 25	2.5	+								
	VIII. 7	2.0	+								
	IX. 3, 18	5.0	+								
	X. 8	5.0	+								
	X. 24	10.0	+								
	XI. 13		+								
137. *Oocystis solitaria* WITTR. D: 7—18×3—8 μ	III. 26—28			20.0	+	20.0	+				
	IV. 23—25		+		+	30.0	+				
	V. 7	20.0	+								
	V. 21—24		+	20.0	+	20.0	+		+		+
	VI. 4		+								
	VI. 24—27	10,4	+	20.0	+	20.0	+	0.8	+	20.0	+
	VII. 10—11		+						+		

	VII. 24—25, 29	20.0	+	30.0	+		+	20.0	+	10.0	+
	VIII. 7—8	40.0	+					5.0	+		
	IX. 2—3		+		+			10.0	+		+
	X. 7—10		+		+		+	10.0	+	10.0	+
	X. 24—25, 28	20.0	+	20.0	+	20.0	+		+		+
	XI. 13—15, 19—20		+		+		+	20.0	+		+
	XII. 12—13		+	40.0	+				+		
138. *Oocystis submarina* LAGERH.	III. 26—28		+	20.0	+	80.0	+				+
D: 7—18×3—8 μ	IV. 9	40.0	+								
	IV. 23—25		+		+	20.0	+			40.0	+
	V. 7		+								
	V. 21—24	10.0	+		+	20.0	+			40.0	+
	VI. 24—27	20.0	+	10.0	+		+				+
	VII. 10		+								
	VII. 24—25, 29		+		+	20.0	+				
	VIII. 7—8		+						+		
	IX. 2—3, 6		+	20.0	+		+	10.0	+		
	IX. 18	40.0	+								
	X. 7—10		+		+	10.0	+		+		
	X. 24—25	10.0	+				+				
	XI. 13		+								
139. *Pediastrum boryanum*	III. 26			0.4	+						
(TURP.) MENEGH.	IV. 23—24			0.2	+		+				
D: 5—20 μ	V. 22—24		+		+	0.1	+				
β—, α—m.	VI. 4	0.2	+								
	VI. 25—26		+	0.1	+		+				
	VII. 25	0.2	+		+						
	VIII. 7		+								
	IX. 3, 18	5.0	+	0.2	+						
	X. 8—9	6.4	+	0.2	+						
β—, α—m.	X. 24—25	0.8	+		+		+				
	XI. 13, 20	0.1	+			0.2	+				
	XII. 12	0.1	+								
140. *Pediastrum duplex* MEYEN	IV. 23—24		+		+		+				
var. *reticulatum* LAGERH.	V. 7—8	0.8	+				+				
D: 14—16 μ	V. 21—24		+	0.7	+	0.2	+		+		+
	VI. 4	1.3	+								

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
	VI. 24—27	0.4	+	0.6	+		+	0.4	+	0.2	+
	VII. 10—11		+						+		
	VII. 24—25, 29	1.4	+		+	0.6	+				+
	VIII. 7—8		+						+		
	IX. 2—3, 6		+	0.2	+	0.6	+	0.8	+		
	X. 7—9	6.4	+		+		+		+		
	X. 24—25, 28	0.4	+		+	0.4	+				
	XI. 13, 20			0.2	+		+			0.4	+
41. *Pediastrum simplex* (MEYEN) LEMM. (f. *clathratum*)	V. 22					0.1	+				
	VI. 26—27					0.2	+				+
D: 12—18 μ	VII. 10—11		+						+		
	VII. 24—25, 29	0.6	+			0.2	+		+	0.4	+
	VIII. 7—8							0.8	+		
	IX. 2—3, 6	0.2	+				+		+	0.8	+
	IX. 18	0.7	+								
	X. 8—10	0.1	+							0.4	+
	X. 24, 28	0.2	+							0.4	+
	XI. 13, 20		+								+
142. *Scenedesmus acuminatus* (LAGERH.) CHOD.	V. 22					0.2	+				
	X. 24—25	10.0	+				+				
D: 10—30×3—6 μ	XI. 13, 20		+			0.8	+				
143. *Scenedesmus arcuatus* LEMM. forma UHERKOV.	IV. 23	0.4	+								
	V. 7		+								
D: 8—15×3—8 μ	V. 24	1.6	+		+						
	VI. 4	40.0	+								
	VI. 24—27			15.0	+						+
	VII. 10		+								
	VII. 24—25, 29	20.0	+	20.0	+					1.6	+
	VIII. 7		+								
	IX. 2—3, 6		+		+						

	IX. 18		+									
	X. 8	20.0	+									
	X. 24		+									
144. *Scenedesmus balatonicus* Hortob.	III. 26—28		+		+		+					
D: 12—30×3—9 μ	IV. 9	1.6	+									
	IV. 23—25		+	1.6	+		+					+
	V. 21—24		+		+	1.0	+					+
	VI. 4	0.8	+									
	VI. 24—27		+	2.0	+		+		+	1.6		+
	VII. 10—11		+					3.2	+			
	VII. 24—25, 29				+		+		+			+
	VIII. 7—8		+						+			
	IX. 2—3, 6		+	3.2	+	3.2	+	3.2	+	6.4		+
	IX. 18	1.6	+									
	X. 7—10		+	3.2	+		+	5.6	+			+
	X. 24—28	3.2	+	1.2	+				+			
	XI. 13—14		+		+							
145. *Scenedesmus ecornis* (Ralfs) Chod.	III. 26—28		+	10.0	+		+			30.0		+
	IV. 9	30.0	+									
D: 8—16×3—6 μ	IV. 23—25	30.0	+		+	10.0	+		+			+
	V. 7—8		+						+			
	V. 21—24		+		+	15.0	+	10.0	+			+
	VI. 4	20.0	+									
	VI. 24—27	60.0	+	30.0	+	20.0	+		+	10.0		+
	VII. 10—11	90.0	+					30.0	+			
	VII. 24—25, 29	20.0	+	50.0	+	90.0	+	30.0	+	10.0		+
	VII. 7—8	40.0	+					10.0	+			
	IX. 2—3, 6	90.0	+	10.0	+	60.0	+	40.0	+	10.0		+
	IX. 18	40.0	+									
	X. 7—10	80.0	+	60.0	+	30.0	+	10.0	+			+
	X. 24—25, 28	40.0	+	30.0	+	35.0	+	10.0	+			+
	XI. 13—14, 19—20	10.0	+	20.0	+	30.0	+	10.0	+	100		+
	XII. 12—13		+	10.0	+			40.0	+			
146. *Scenedesmus granulatus* W. * et G. S. West f. *disciformis* Hortob.	VIII. 7		+									
	IX. 3	20.0	+									
D: 6.5—10×4—7 μ	IX. 18		+									

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
147. *Scenedesmus intermedius* Chod.	III. 26	10.0									
	IV. 9		+								
D: 6—12×3—7 μ	IV. 23—25	10.0	+		+		+		+		
	V. 7—8	20.0	+					10.0	+		
	V. 21—24		+		+	10.0	+		+		+
	VI. 4		+								
	VI. 24—27	20.0	+	30.0	+	20.0	+		+		+
	VII. 10—11	20.0	+						+		
	VII. 24—25, 29	50.0	+	60.0	+		+	20.0	+	10.0	+
	VIII. 7—8	40.0	+					30.0	+		
	IX. 2—3, 6		+		+		+		+		+
	IX. 18	30.0	+								
	X. 7—10	10.0	+	30.0	+	20.0	+		+		
	X. 24—25, 28	40.0	+	20.0	+		+		+		
	XI. 13—14, 19—20		+		+	30.0	+	20.0	+		
	XII. 13								+		
	IX. 10								+		
148. *Scenedesmus quadricauda* (Turp.) Bréb.	III. 26—28	100.0	+	20.0	+	5.4	+	10.0	+		+
	IV. 9—10	220.0	+						+		
D: 8—25×3—10 μ	IV. 23—25	60.0	+	5.2	+		+	10.0	+		+
	V. 7—8	170.0	+					20.0	+		
	V. 21—24	20.0	+	40.0	+	20.0	+	10.0	+	30.0	+
	VI. 4	160.0	+								
	VI. 24—27	10.0	+	20.0	+	25.0	+	20.0	+		+
	VII. 10—11	60.0	+					20.0	+		
	VII. 24—25, 29	30.0	+	10.0	+	20.0	+	20.0	+	20.0	+
	VIII. 7—8	130.0	+					20.0	+		
	IX. 2—3, 6	90.0	+		+		+	10.0	+		+
	IX. 18	10.0	+								
	X. 7—10	230.0	+	50.0	+	10.4	+	5.0	+	20.0	+
	X. 24—25, 28	180.0	+	40.0	+	20.0	+	5.4	+		+
	XI. 13—14, 19	10.0	+	90.0	+		+	10.0	+		
	XII. 12	10.0	+	70.0	+			40.0	+		

149. *Scenedesmus longispina* Chod. D: 8—22×3—6 μ	IX. 18		+									
	X. 8	40.0	+									
	X. 24	60.0	+									
	XI. 13		+									
150. *Scenedesmus spinosus* Chod. D: 6—12×3—4 μ	V. 24		+		+							
	VI. 4	30.0	+									
	VI. 25—26		+	5.0	+							
	VII. 10		+									
	VII. 25	20.0	+	10.0	+							
	VIII. 7		+									
	IX. 3	20.0	+		+							
	IX. 18		+									
	X. 8—9		+		+							
	X. 24	90.0	+	20.0	+							
	XI. 13—14	10.0	+		+							
	XII. 12	10.0	+	30.0	+							
151. *Schroederia setigera* (Schroed.) Lemm. D: 17—52×2.8—6 μ β—m.	III. 27—28		+					20.0	+	10.0	+	
	IV. 9—10		+						+			
	IV. 23—24		+		+				+			
	V. 7—8	20.0	+						+			
	V. 21—24	5.0	+		+	0.5	+	5.0	+		+	
	VI. 4	5.0	+									
	VI. 24—27		+	5.0	+		+		+		+	
	VII. 10—11	20.0	+					15.0	+			
	VII. 24—25, 29	30.0	+	10.0	+		+	25.0	+	15.0	+	
	VIII. 7—8	5.0	+					5.0	+			
	IX. 2—3, 6		+	10.0	+	5.0	+	5.0	+	5.0	+	
	IX. 18		+									
	X. 7—10	10.0	+	10.0	+		+		+		+	
	X. 24	10.0	+	10.0	+							
	XI. 13		+									
152. *Selenastrum gracile* Reinsch D: 20—26×4—5 μ	II. 20							5.0	+			
	III. 27								+			
	VII. 25		+									
	VIII. 7	5.0	+									
	IX. 3		+									
	IX. 18	10.0	+									
	X. 8	10.0	+									
	X. 24		+									

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N	i/l	N	i/l	N	i/l	N	i/l	N
153. *Tetraëdron caudatum* Corda (Hansg.) var. *incisum* Lagerh. D: 12—15 μ	VI. 25			1.0	+						
	IX. 3		+								
	IX. 18	15.0	+								
	X. 9				+						
	X. 24		+	5.0	+						
	XI. 13	5.0	+								
	XII. 12		+		+						
154. *Tetraëdron regulare* Kütz. D: 16—30 μ	VI. 4		+								
	VI. 26	5.0	+								
	VII. 10	0.5	+								
	VII. 25		+								
155. *Tetrastrum hastiferum* * (Arn.) Korschik D: 6—10 μ	III. 26	5.0	+								
	IV. 9, 23		+								
	V. 7	20.0	+								
	V. 24		+								
	V. 24		+								
	VI. 16		+								
	VII. 10	0.5	+								
	X. 24		+								
	XI. 13	120.0	+								
	XII. 12		+								
156. *Tetrastrum heteracanthum* (Nordst.) Chod. D: 4—8 μ	XI. 13		+								
	XII. 12	5.0	+								
157. *Tetrastrum staurogeniaeforme* (Schroed.) Lemm. D: 5—6 μ	III. 26—28		+		+		+		+		
	IV. 9	20.0	+								
	IV. 23—25	5.0	+	5.0	+	5.0	+				
	V. 7	20.0	+								
	V. 21—24		+	5.0	+	1.0	+				

	VI. 4	140.0	+										
	VI. 24—27		+	10.0	+		+						
	VII. 10—11	15.0	+						+				
	VII. 24—25, 29	20.0	+	20.0	+				+				
	VIII. 7—8	10.0	+					5.0	+				
	IX. 2—3, 6	40.0	+		+				+				
	IX. 18		+										
	X. 8—9	10.0	+	40.0	+								
	X. 24	40.0	+	20.0	+								
	XI. 13—14		+		+								
	XII. 12			5.0	+								
Zygnematales													
158. *Closterium acerosum*	VI. 24—27					0.2	+	0.4	+	0.3	+		
(Schrank.) Ehr.	VII. 10—11		+						+				
D: 300—500×40—60 μ	VII. 24—25, 29	0.2	+				+				+		
	VIII. 7		+										
	IX. 2, 6					0.4	+				+		
	X. 9					5.0	+						
	X. 25						+						
159. *Closterium aciculare*	II. 20								+				
T. West	III. 26—28			0.8	+	0.4	+	0.4	+		+		
D: 400—700×4—8 μ	IV. 10							0.4	+				
	IV. 23—25			0.3	+	0.3	+	0.7	+	0.8	+		
	V. 7—8		+					0.1	+				
	V. 22—24	0.2	+		+	6.0	+	0.4	+	1.2	+		
	VI. 4		+										
	VI. 24—27	1.0	+	60.0	+	12.0	+	14.0	+	22.5	+		
	VII. 10—11	7.0	+					79.0	+				
	VII. 24—25, 29	184.0	+	132.0	+	158.0	+	65.0	+	113.0	+		
	VIII. 7—8	21.0	+					125.0	+				
	IX. 2—3, 6	3.4	+	2.0	+	2.0	+	12.4	+	20.6	+		
	IX. 18	2.8	+										
	X. 7—10	0.8	+	2.2	+	2.6	+	4.2	+	4.0	+		
	X. 24—25, 28	6.0	+	1.6	+	1.2	+	6.2	+	7.0	+		
	XI. 13—15, 19—20	0.8	+	1.6	+	1.2	+	1.0	+	1.2	+		
	XII. 12—13	1.2	+	3.4	+			0.2	+				
160. *Closterium acutum* Bréb.	III. 26—28			0.2	+			0.2	+				
var. *variabile* (Lemm.)	IV. 9		+					0.4	+				
Krieger	IV. 23—25	5.0	+	0.2	+		+	0.1	+	0.6	+		

Species	Date of collection	Localities									
		M		K		G		A		E	
		i/l	N		N		N		N		N
D: 50—140×2.5—5 μ	V. 7—8		+					0.2	+		
	V. 22—24	0.6	+	0.6	+	6.0	+		+	0.6	+
	VI. 4	3.0	+								
	VI. 24—27	13.5	+	3.0	+	8.4	+	4.7	+	8.5	+
	VII. 10—11	2.0	+					14.0	+		
	VII. 24—25, 29	2.8	+	5.5	+	4.0	+	4.0	+	8.0	+
	VIII. 7—8	0.2	+					6.1	+		
	IX. 2—3, 6	0.4	+	5.0	+	2.8	+	5.0	+	10.2	+
	IX. 18	0.4	+								
	X. 7—10	0.2	+	3.4	+	5.5	+	10.0	+	8.5	+
	X. 24—25, 28	1.4	+	1.2	+	11.1	+	7.2	+	7.3	+
	XI. 13—15, 19—20	3.2	+	1.6	+	7.3	+	7.0	+	1.4	+
	XII. 12—13	0.6	+	3.0	+			2.6	+		
161. *Closterium parvulum* NAEG.	III. 26			0.2	+						
	IV. 10								+		
D: 100—130×10—15 μ	IV. 23—25				+		+	0.1	+		+
	V. 7—8		+						+		
	V. 22—24		+	0.6	+	1.0	+		+	0.6	+
	VI. 4	0.4	+								
	VI. 24—27		+	0.8	+	2.0	+	0.4	+	0.6	+
	VII. 10—11	1.0	+					1.0	+		
	VII. 24—25, 29	1.0	+	1.0	+		+	0.2	+	0.6	+
	VIII. 7—8		+						+		
	IX. 2—3, 6		+	0.6	+		+		+		+
	IX. 18	0.2	+								
	X. 7—10	0.4	+	0.2	+	1.0	+		+		+
	X. 24—25, 28	0.8	+	0.2	+	0.4	+	0.2	+		+
	XI. 13—15, 19—20	0.2	+	0.6	+	0.4	+		+	0.4	+
	XII. 12	1.0	+	1.2	+						
162. *Closterium polystictum* NYGAARD	VI. 24								+		
	VII. 10—11		+					0.4	+		
D: 450—580×9—11 μ	VII. 24—25, 29	0.2	+		+			0.2	+		+

	VIII. 7		+								
	IX. 2—3		+	0.6	+					0.2	+
	IX. 18	0.4	+								
	X. 24	0.4	+								
163. *Closterium pronum* BRÉB.	V. 22									0.2	+
D: 260—400×5—12 μ											
164. *Staurastrum gracile*	III. 27					0.2	+				
REINSCH	IV. 23—24		+		+	0.1	+				
D: 60×30 μ	V. 7—8	0.2	+						+		
	V. 21—24	0.2	+	0.2	+	0.2	+	0.4	+		+
	VI. 4		+								
	VI. 24—27	0.5	+	0.1	+	0.4	+	0.6	+	1.0	+
	VII. 10—11		+					1.0	+		
	VIII. 7—8		+						+		
	IX. 2—3, 6		+		+		+		+	0.1	+
	IX. 18	0.2	+								
	X. 7—10	0.3	+	0.2	+	0.8	+	0.4	+	1.0	+
	X. 24—25, 28		+		+	0.2	+	0.8	+	0.2	+
	XI. 19—20					0.2	+		+		+
165. *Staurastrum paradoxum*	V. 21—24				+	0.4	+		+		+
MEYEN	VI. 4		+								
D: 45×20 μ	VI. 24—27	0.4	+	0.1	+	0.6	+	1.0	+	0.3	+
	VII. 10—11	0.2	+					1.0	+		
	VII. 24—25, 29	0.2	+	0.2	+	0.2	+	0.2	+	0.2	+
	VIII. 7—8		+					2.6	+		
	IX. 2—3, 6		+		+		+	0.4	+		+
	IX. 18	2.7	+								
	X. 7—10	2.5	+				+	0.8	+		+
	X. 24—25, 28		+			0.6	+		+		
	XII. 12			0.2	+						
Caulobacteriales											
166. *Planctomyces bekefii*	VII. 10	40.0	+								
GIMESI	X. 8—9	40.0	+	600.0	+						
D: 1—1.2 μ	XII. 12—13		+	220.0	+			0.2	+		

* = The occurrence of this species in Lake Balaton is new to the flora
** = The occurrence of this species is new to the flora of Hungary

TABLE IV

Number of species and individuals of phytoplankton along the transversal sections of the phyla

Phyla	Date of collection	Localities					
		M			K		
		sp	i/l	%		i/l	%
Cyanophyta (22)	II. 20						
	III. 26—28	5	160.0	4.3	4	57.6	2.0
	IV. 9—10	9	796.2	16.5			
	IV. 23—25	6	166.0	8.2	6	45.2	3.7
	V. 7— 8	4	370.0	9.0			
	V. 21—24	4	58.0	7.4	8	175.7	15.1
	VI. 4	7	610.9	23.4			
	VI. 24—27	10	148.4	10.1	10	36.4	3.5
	VII. 10—11	10	305.2	16.8			
	VII. 24—25, 29	10	305.0	14.2	11	295.0	17.1
	VIII. 7— 8	12	455.0	12.1			
	IX. 2—3, 6	10	996.6	33.2	10	706.0	37.6
	IX. 18	11	2 501.2	49.1			
	X. 7—10	12	1 615.4	11.0	9	786.0	21.0
	X. 24—25, 28	6	405.0	2.4	8	278.7	8.0
	XI. 13—15, 19—20	5	70.4	0.7	6	106.3	0.5
	XII. 12—13	3	65.0	0.3	7	61.9	0.2
Euglenophyta (11)	II. 20						
	III. 26—28				1	0.2	0.0
	IV. 9—10						
	IV. 23—25				2	0.2	0.0
	V. 21—24				2	0.3	0.0
	VI. 4	5	3.3	0.1			
	VI. 24—27	4	3.6	0.2	7	1.0	0.1
	VII. 10—11	2	0.6	0.1			
	VII. 24—25, 29	5	6.4	0.3	6	1.6	0.1
	VIII. 7—8	7	35.9	1.0			
	IX. 2—3, 6	3	3.6	0.1	7	15.0	0.7
	IX. 18	7	52.6	1.0			
	X. 7—10	3	8.7	0.0	3	0.8	0.0
	X. 24—25, 28	1	0.2	0.0	2	0.2	0.0
	XI. 13—15, 19—20	2	5.2	0.1	2	0.4	0.0
	XII. 12—13	1	0.6	0.0	2	1.0	0.0
Pyrrophyta (12)	II. 20						
	III. 26—28	3	235.0	6.3	2	25.4	0.9
	IV. 9—10	3	315.0	6.5			
	IV. 23—25	2	65.0	3.2	4	91.4	7.4
	V. 7—8	4	287.0	7.0			
	V. 21—24	4	227.0	28.9	3	406.4	35.0
	VI. 4	5	577.7	22.1			
	VI. 24—27	5	162.4	11.0	8	330.8	31.6
	VII. 10—11	4	517.8	28.5			
	VII. 24—25, 29	6	408.0	18.9	6	314.0	18.2
	VIII. 7—8	7	421.6	11.2			
	IX. 2—3, 6	8	740.4	24.7	7	530.8	28.2
	IX. 18	9	711.2	14.0			
	9. 7—10	5	470.4	3.2	5	554.2	14.0
	X. 24—25, 28	8	3 410.4	20.4	6	595.6	16.6
	XI. 13—15, 19—20	5	1 927.0	18.5	4	1 560.5	7.4
	XII. 12—13	5	1 310.0	6.5	4	720.0	3.0

in 1974 = i/l-1000 individuls per litre % = individuals expressed in per cent of total algae

G			A			E		
sp	i/l	%	sp		%	sp	i/l	%
			1	10.0	1.4			
1	25.0	0.7	5	130.6	5.2	1	165.0	5.8
5	115.0	6.2	4	83.0	9.4	2	60.2	6.6
			3	297.6	23.7			
11	108.0	13.3	4	128.2	25.7	4	560.8	52.9
7	84.1	13.3	7	72.1	19.0	8	204.7	31.5
			6	163.2	20.9			
8	320.6	33.2	5	295.8	32.3	6	533.8	40.3
			4	307.9	30.3			
9	224.2	28.3	4	246.2	32.4	7	301.6	32.0
9	676.2	50.2	6	410.0	24.0	7	320.1	26.4
7	151.4	21.3	8	222.0	25.3	5	91.4	16.0
3	100.6	9.6	5	141.0	13.3	6	57.8	11.2
			5	120.6	7.8			
			1	15.0	2.2			
3	5.4	0.2	2	7.5	0.3			
			2	0.4	0.0			
3	5.2	0.3						
6	4.7	0.6	2	1.2	0.2	2	0.6	0.1
3	1.8	0.3	2	0.8	0.2	4	1.1	0.2
			2	0.8	0.1			
1	0.4	0.0	3	0.8	0.1	6	8.0	0.6
			4	7.2	0.7			
			5	7.0	0.9	5	4.4	0.5
3	3.2	0.2	2	5.4	0.3	3	3.4	0.3
1	0.8	0.1	2	0.4	0.1	3	1.0	0.2
1	0.2	0.0				1	0.2	0.1
			1	0.2	0.0			
			1	90.0	10.4			
			3	35.0	5.0			
3	87.6	2.6	2	35.1	1.4	3	130.4	4.5
			2	50.2	6.0			
2	10.6	0.5	2	51.2	5.8	2	80.6	8.8
			1	1.0	0.1			
7	47.7	5.9	3	82.4	16.5	2	77.6	7.3
4	77.4	12.3	4	59.2	15.5	3	63.1	9.7
			4	61.4	7.9			
4	170.0	17.6	6	4.2	0.5	4	141.6	10.7
			3	182.2	18.0			
6	344.4	43.5	7	195.0	25.7	6	302.3	32.0
5	130.2	9.6	4	95.8	5.6	7	122.6	10.1
3	105.2	14 7	5	125.8	14.3	3	80.2	14.0
3	100.0	9.6	3	100.0	9.4	3	90.0	17.5
			2	260.0	16.6			

TABLE IV *(continued)*

Phyla	Date of collection	Localities					
		M			K		
		sp	i/l	%	sp	i/l	%
Chrysophyta (66)	II. 20						
	III. 26—28	14	2 125.1	56.8	15	1 771.0	62.5
	IV. 9—10	20	2 685.7	55.7			
	IV. 23—25	18	1 236.2	60.7	19	1 014.4	82.0
	V. 7—8	15	2 657.4	64.6			
	V. 21—24	15	395.8	50.4	12	407.1	35.0
	VI. 4	16	863.1	33.1			
	VII. 24—27	16	710.7	48.2	21	330.6	31.6
	VII. 10—11	21	649.3	35.7			
	VII. 24—25, 29	23	898.4	41.7	23	562.0	32.5
	VIII. 7—8	18	2 467.4	65.7			
	IX. 2—3, 6	22	788.2	26.2	25	540.8	28.8
	IX. 18	27	1 470.4	28.9			
	X. 7—10	24	10 855.9	73.8	16	1 390.2	37.0
	X. 24—25, 28	19	11 766.2	70.3	16	2 148.1	60.0
	XI. 13—15, 19—20	8	8 070.2	77.8	11	18 977.4	89.9
	XII. 12—13	17	18 524.2	91.3	14	21 758.0	91.8
Chlorophyta (54)	II. 20						
	III. 26—28	7	1 220.0	32.6	13	980.7	34.6
	IV. 9—10	13	1 027.2	21.3			
	IV. 23—25	12	569.0	27.9	11	85.3	6.9
	V. 7—8	16	796.0	19.4			
	V. 21—24	12	104.2	13.3	12	172.6	14.9
	VI. 4	21	555.7	21.3			
	VI. 24—27	20	449.5	30.5	25	346.7	33.2
	VII. 10—11	16	306.1	16.8			
	VII. 24—25, 29	27	535.1	24.9	17	553.7	32.1
	VIII. 7—8	15	373.2	10.0			
	IX. 2—3, 6	19	474.0	15.8	14	87.8	4.7
	IX. 18	25	354.8	7.0			
	X. 7—10	28	1 722.1	11.7	18	446.2	12.0
	X. 24—25, 28	30	1 157.6	6.9	19	554.2	15.4
	XI. 13—15, 19—20	14	304.3	2.9	11	471.9	2.2
	XII. 12—13	9	382.9	1.9	14	930.7	4.0
Caulobacteriales (1)	VII. 10	1	40.0	2.1			
	X. 8	1	40.0	0.3	1	600.0	16.0
	XII. 12—13				1	220.0	1.0
Total algae (166)	II. 20						
	III. 26—28	29	3 740.1	100	35	2 834.7	100
	IV. 9—10	45	4 824.1	100			
	IV. 23—25	38	2 036.8	100	42	1 236.5	100
	V. 7—8	39	4 110.4	100.			
	V. 21—24	35	785.0	100	37	1 162.1	100
	VI. 4	54	2 610.7	100			
	VI. 24—27	55	1 474.6	100	71	1 045.5	100
	VII. 10—11	54	1 819.0	100			
	VII. 24—25, 29	71	2 152.9	100	63	1 701.3	100
	VIII. 7—8	59	3 753.1	100			
	IX. 2—3, 6	62	3 002.8	100	63	1 880.4	100
	IX. 18	79	5 090.2	100			
	X. 7—10	73	14 712.5	100	52	3 747.4	100
	X. 24—25, 28	64	16 739.4	100	51	3 577.0	100
	XI. 13—15, 19—20	34	10 377.1	100	34	21 226.5	100
	XII. 12—13	35	20 282.7	100	42	23 691.6	100.
	Average	52	6 094.4		49	6 199.3	

G			A			E		
sp	i/l	%	sp	i/l	%	sp	i/l	%
			13	385.0	55.4			
12	2 126.5	63.2	14	1 646.9	65.6	11	2 011.8	70.1
			10	572.0	66.0			
14	1 375.7	74.0	21	649.0	73.2	17	584.4	64.1
			19	604.7	48.2			
22	356.9	43.8	15	226.6	45.4	17	228.8	21.6
18	282.0	44.8	17	163.1	42.7	13	200.3	30.8
			15	238.2	30.6			
11	89.6	9.3	17	314.3	34.4	17	340.6	25.8
			14	223.2	22.0			
13	135.2	17.0	10	168.2	22.2	15	248.8	26.4
12	322.9	24.0	12	966.7	56.6	17	548.0	45.1
10	260.4	36.5	14	300.8	34.2	15	342.9	59.9
17	573.6	54.8	15	593.3	55.8	12	313.5	60.9
			16	849.0	54.6			
			4	250.0	36.0			
10	1 120.4	33.3	9	692.2	27.5	7	563.0	19.6
			9	153.6	17.6			
12	352.9	19.0	10	103.4	11.6	7	186.4	20.5
			8	350.7	28.0			
28	296.7	36.4	9	60.8	12.2	12	192.6	18.1
17	184.6	29.3	15	86.4	22.6	15	180.8	27.8
			14	315.0	40.5			
15	384.7	39.9	16	299.8	32.7	19	298.9	22.6
			15	292.8	29.0			
11	88.6	11.2	17	142.8	18.8	14	86.3	9.1
16.0	215.3	16.0	14	233.6	13.6	10	220.1	18.1
15	195.7	27.4	13	229.7	26.1	10	56.6	9.9
16	272.3	26.0	10	228.0	21.5	11	52.9	10.3
			12	325.6	21.0			
			1	0.2	0.0			
			22	695.0	100			
29	3 364.9	100	32	2 512.3	100	22	2 870.2	100
			24	866.2	100			
36	1 859.4	100	37	866.6	100	28	911.6	100
			31	1 254.0	100			
74	814.0	100	33	499.2	100	37	1 060.4	100
49	629.9	100	45	381.6	100	43	650.0	100
			41	778.6	100			
39	965.3	100	47	914.9	100	52	1 322.9	100
			40	1 013.3	100			
39	792.4	100	43	759.2	100	47	943.4	100
45	1 347.8	100	38	1 711.5	100	44	1 214.2	100
36	713.5	100	42	879.3	100	36	572.1	100
40	1 046.7	100	33	1 062.3	100	33	514.4	100
			37	1 555.6	100			
43	1 281.5		36	1 050.0		38	1 117.7	

In August the species of genus *Dinobryon* were found in an abundance of 600,000 individuals/litre in the Keszthely Bay. Of the diatoms the *Cyclotella* species were most common in the cold-water periods, i.e. in March-April and October December. Consequently, in the Keszthely Bay the total number of *Cyclotella* made up 61 per cent of the phylum in March, 58.6 per cent in early April, 50.8 per cent in early May, 21 per cent in early October and 9 per cent in December. Like in the sixties, *Melosira granulata* and its variety had a population of 100,000 individuals/litre here. At this section the cell number of *Nitzschia acicularis* ranged from 7 million to 15.8 million from early October till mid-December. In that month this diatom had the same abundance between Szigliget and Balatonmária reaching its maximum of 17.8 million cells/litre in mid-December. At the south-western transversal sections (M and K) the frequency of *Nitzschia amphibia* species was found to be 100,000—150,000 individuals/litre. In early May *Nitzschia subrostrata* reached a maximum population of 850,000 individuals/litre. In the Keszthely Bay the *Stephanodiscus* species altogether were found to be more than 1 million individuals/litre in the period of October-December. The population of almost 1 million individuals/litre of *Synedra acus* var. *angustissima* was conspicuous in the March samples taken from each of the sections. Even at the end of October it was represented by 300,000 individuals/litre, with its individual number varying between 89,000 and 21.7 million individuals/litre. Chrysophyta phylum contributed 39.8 per cent of total algae. In terms of percentage it ranged from 9.3 to 91.8 per cent.

Chlorophyta phylum was represented by 54 species in the samples (Volvocales 4, Tetrasporales 3, Chlorococcales 39, Zygnematales 8). In the April samples it was conspicuous that *Ankistrodesmus falcatus* var. *spirilliformis*, *Dictyosphaerium pulchellum*, *Gloeoactinium limneticum* and *Scenedesmus quadricauda* varied between 100,000 and 800,000 individuals/litre. In the Keszthely Bay *Closterium aciculare* peaked with 184,000 individuals/litre at the end of July, i.e. a 76 times higher value than in the sixties. This time its abundance exceeded 100,000 individuals/litre at the other sections, too. Chlorophyta phylum made up 32.5 per cent of total algae. Its individual number varied between 52,000 and 1.7 million in the period investigated and contributed 1.9—40.5 per cent of total species number. *Planctomyces* belonging to order *Caulobacteriales* occurred sporadically in the samples. Along transversal section K (Szigliget—Balatonmária) it had a higher population density of 600,000 individuals/litre early in October, while in mid-October this value was 220,000 individuals/litre. *Planctomyces* formed 0.6 per cent of total algae species.

It is seen from *Table IV* that the highest number of species (79) occurred in the sample taken in the Keszthely Bay on September 18. Along the other sections of the south-western basin a May (section G, 74) and a June peak of species (section K, 71) occurred. In the north-eastern basin much lower peaks were noted (section A, 47; section E, 52). A minimum of species (22) was observed also here in February and March.

The highest individual numbers (20 million and 23 million) as well as the number of species, were found along the south-western sections in December. The lowest number of individuals (381,600 individuals/litre) was noted at the end of June along the transversal section Balatonfüred—Zamárdi. In that month this value was found to be twice as high between Ságpuszta

and Balatonszemes and three times as high between Szigliget and Balatonmária. A minimum of individuals/litre was noted in the sample taken at the end of May (785,000 individuals/litre) along the south-westernmost section, while another minimum of 514,400 individuals/litre occurred in the north-eastern basin between Balatonalmádi and Balatonvilágos in November.

Table V gives the biomass of phytoplankton expressed in 10^6 μ^3 at the dates of sampling (WILLÉN, 1970; KRISTIANSEN, 1971). The highest biomass value of 13.5 mg/litre was noted in the sample taken in the Keszthely Bay on September 18. Both in early and late October total biomasses of 10 mg were found at this section. This high number includes the biomass values of *Anabaena*, *Aphanizomenon* and *Ceratium*, too. The dates of biomass maxima sectionally varied. The December value at section Szigliget—Balatonmária was found to be 9.4 mg/litre including both the mass of *Nitzschia acicularis* and the biomass values of *Stephanodiscus* and *Cyclotella* species. The diatoms made up 75 per cent of the weight of biomass. Between Ságpuszta and Balatonszemes the total biomass was 6 mg/litre in the sample taken on July 29, of which *Ceratium hirundinella* itself constituted 3.8 mg/litre and *Closterium aciculare* 1.4 mg/litre. At transversal section Balatonfüred—Zamárdi (A) the highest biomass value was 2.5 mg/litre in early August comprising *Closterium aciculare* of 1.1 mg/litre. Between Balatonalmádi and Balatonvilágos the total biomass was noted to be 6.4 mg/litre, of which phylum Chrysophyta constituted 6.1 mg/litre, *Stenopterobia* 4.8 mg/litre and *Synedra acus* var. *angustissima* 0.5 mg/litre.

The lowest values of total biomass ranged from 0.5 to 1.4 mg/litre at the sections in the different months *(Table V)*.

TABLE V

Biomass values of phytoplankton in Lake Balaton in 1974
($10^6\mu^3$ = wet weight per litre)

Date of collection	M	K	G	A	E
	$10^6\mu^3$/l	$10^6\mu^3$/l	$10^6\mu^3$/l	$10^6\mu^3$/l	$10^6\mu^3$/l
II. 20				615.0	
III. 26—28	3 213.3	3 641.3	3 523.4	1 957.9	6 471.5
IV. 9—10	4 801.4			949.2	
IV. 23—25	1 904.2	1 760.3	2 843.6	1 871.5	1 274.4
V. 7—8	4 407.6			1 689.0	
V. 21—24	876.5	1 451.9	1 094.4	1 623.6	1 110.4
VI. 4	2 226.0				
VI. 24—27	3 208.8	2 103.7	1 531.7	1 162.4	1 620.1
VII. 10—11	4 381.9			2 027.4	
VII. 24—25, 29	9 108.3	5 795.3	6 003.2	2 431.0	3 368.8
VIII. 7—8	7 224.4			2 578.7	
IX. 2—3, 6	9 531.4	8 364.2	2 355.5	1 932.0	2 481.8
IX. 18	13 514.8				
X. 7к10	10 299.7	3 059.7	1 084.4	1 316.3	1 040.8
X. 24—25, 28	11 252.8	2 097.0	529.1	934.5	516.5
XI. 13—15, 19—20	5 251.4	8 593.9	739.4	807.7	1 133.6
XII. 12—13	7 022.1	9 495.5		870.5	
Average	6 151.5	4 636.2	2 189.4	1 517.8	2 113.1

Discussion

The qualitative and quantitative changes of phytoplankton continued in the seventies. Species, new to the flora of Lake Balaton, are marked in *Table III*. Data on their size and occurrence in Hungary will be published later. In the Keszthely Bay the number of alga species coming from River Zala and other inlets was higher than it had been in the sixties. Of the diatoms the *Nitzschia acicularis*, *Cyclotella*, *Stephanodiscus* and *Cryptomonas* species were of very frequent occurrence in the whole lake turning the colour of the water into brownish-green in the Keszthely Bay and along transversal section Szigliget—Balatonmária from October till December. In the north-eastern basin along the transversal section Balatonalmádi—Balatonvilágos the appearance of *Synedra acus* var. *angustissima* and *Stenopterobia pelagica* in great quantities caused an opalescent water coloration. At this time the latter phenomenon was observed to be present in an increased degree in the ports and nearshore shallow waters of the southern shore. Making a comparison with the data of the sixties it may be established that some species that had occurred earlier in great quantities (e.g. *Coelosphaerium kuetzingianum*, *Gomphosphaeria lacustris*, *Lyngbya circumcreta*, *Attheya zachariasi*, *Asterionella formosa*, *Melosira granulata* and its variety, the *Dinobryon*, *Closterium* and *Staurastrum* species) decreased in numbers in the seventies.

The quantitative change is similarly confirmed by the data on the seventies (Herodek and Tamás, 1973; 1974; 1975) which are several times as high, especially in the south-western basin of the lake, as those of the biomass of the sixties. In the Keszthely Bay the population density was found to be 4.2 million individuals/litre in August 1965 and 1.3 million individuals per litre in 1966. From early October till mid-December 1974 these values varied between 10 and 20 million individuals/litre. Between Szigliget and Balatonmária the population maximum was found to be 1.1 million individuals/litre in August 1965, 1.7 million individuals/litre in 1967 and 1 million individuals/litre in September 1967. In 1974 this latter value (1 million individuals/litre) was noted as minimum here with a maximum of 23.6 million individuals/litre in December (population density in November 21.1 million individuals/litre). Along the other sections the highest values of the sixties correspond to the lowest ones of the seventies. At sections M and K the frequency of 6 million individuals/litre is a several times higher value than those of the sixties.

The same can be stated about the biomass. The qualitative and quantitative changes of phytoplankton are significantly influenced by physiological as well as environmental factors (Sebestyén, 1963; Dussart, 1966). As a result of River Zala several brooks, channels domestic and other sewage-waters flowing into the lake, the qualitative distribution showed changes in space and in time. The intensive sunshine in early spring (March) and the calm period favourably affected the population density of *Synedra acus* var. *angustissima* which likes water temperature around 10°C. The cold, windy and rainy summer and the permanently rippling water prevented the populations of *Dinobryon* — *Asterionella* and *Attheya* — *Melosira* from becoming abundant. At the end of the summer and in autumn the *Anabaena-Aphanizomenon* population reached high density in the barely 20°C water only for a short time. Significant water blooms were noted in the nearshore bays (especially in the

south-western basin). The nutriments getting into the lake in the rainy period from mid-September (washed into the lake by rain, etc.) were favourable to the *Cryptomonas*, *Stephanodiscus* and *Nitzschia acicularis* species. In the south-western basin of the lake the water was coloured brownish-green by *Nitzschia acicularis* of a population density of several million cells/litre. This phenomenon developed gradually from the end of September and spread over to the north-eastern region of the lake by December.

To determine the degree of trophyty, the primary production was examined between Balatonfüred and Zamárdi at the beginning of the seventies (Herodek and Tamás, 1973; 1974; 1975). The annual phytoplankton production was determined with ^{14}C-method and was found to be as high as 114 g C/m^2. The biomass value ranged from 1.0 to 19.5 g/m^2 throughout the year. These values are characteristics of the production of moderately eutrophic waters. In the south-western region of the lake investigations on primary production were carried out from June 1973 till June 1974. At the deepest point of the Keszthely Bay the annual production was noted to be 831 g C/m^2. Compared to that of the data of the sixties the phytoplankton density increased significantly (13 g/m^2). The south-western basin of the lake is highly hypertrophic.

During the investigations carried out in the fifties on the weed-detritus in the littoral zone (Gellért and Tamás, 1958; 1959; 1960) and on the alga periphyton of the coastal stones (Tamás and Gellért, 1958; 1959; 1960) the β-mesosaprobic organisms were found in large numbers. In sixties α- and β-mesosaprobic organisms occurred more and more frequently in the open water too (water bloom, discoloration).

Summary

The author examined 177 lifted samples and 100 netfiltrates taken from 15 stations along the 5 transversal sections of Lake Balaton in the period of February December 1974. It was established that the 160 species and 6 varieties belong to the following six taxonomic phyla: Cyanophyta 22, Euglenophyta 11, Pyrrophyta 12, Chrysophyta 66, Chlorophyta 54, Caulobacteriales 1.

The highest number of species (79) was found in the Keszthely Bay on September 18 and the lowest ones (22) at section Balatonfüred—Zamárdi in late June and at section Balatoalmádi—Balatonvilágos in March.

Maxima of individuals were noted in December at transversal sections M (20 million) and K (23 million). Minimum of individuals (381,600) was found between Balatonfüred and Zamárdi at the end of June.

In the Keszthely Bay the number of filamentous blue-greens (*Anabaena* and *Aphanizomenon* species) increased from mid-September till the end of October. On September 18, in addition to the filamentous blue-greens the biomass value of 13.5 mg/litre was significantly contributed by the *Ceratium hirundinella* population too. The lowest biomass values of the sections varied between 0.5 and 1.4 mg/litre.

The great quantity of *Synedra acus* var. *angustissima* produced an opalescent water coloration in March, while in the period of September October a water bloom of blue-greens *(Anabaena, Aphanizomenon)* occurred at the

south-western region of the lake. Just here a frequency of 7.0—15.8 million individuals/litre of *Nitzschia acicularis* with the *Cryptomonas* species made the water brownish-green from October till December.

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HORIZONTÁLIS MENNYISÉGI FITOPLANKTON VIZSGÁLATOK A BALATONBAN 1974. ÉVBEN

Tamás Gizella

Összefoglalás

1974. évben februártól decemberig a tó 5 harántszelvényének 15 pontjáról 177 merített és 100 hálószüredék mintát vizsgált. Ennek eredményeként a 160 faj és 6 változat rendszertani törzsbe sorolható: Cyanophyta 22, Euglenophyta 11, Pyrrophyta 12, Chrysophyta 66, Chlorophyta 54, Caulobacteriales 1.

A harántszelvények gyűjtőhelyei közül fajszámban a leggazdagabb (79) a Keszthelyi-öböl szeptember 18-i mintája, a legalacsonyabb (22) pedig a Balatonfüred—Zamárdi februári és Balatonalmádi—Balatonvilágos márciusi mintája volt.

Egyedszám maximumot a tó legdélnyugatibb szelvényein (*M* és *K*) decemberben 20 és 23 milliós értékkel jegyzett fel. Az egyedszám minimum Balatonfüred—Zamárdi szelvényen június végén 381 600 egyed volt literenként.

A Keszthelyi-öbölben szeptember közepétől a fonalas kékalgák (*Anabaena, Aphanizomenon* fajok) száma október végéig emelkedett. A szeptember 18-i 13,5 mg/l biomassza maximum kialakulásában a fonalas kékalgák mellett a *Ceratium hirundinella* tömegnek is jelentős szerep jutott. A legalacsonyabb biomassza értékeket a tó különböző szelvényein 0,5—1,4 mg/l között talált.

A víz színét márciusban opálossá tette a *Synedra acus* var. *angustissima* tömeg, szeptember—októberben pedig a fonalas kékalgák *(Anabaena, Aphanizomenon)* vízvirágzása következett be a tó délnyugati részén. Októbertől decemberig ugyanitt a *Nitzschia acicularis* 7—15,8 milliós egyedszáma a *Cryptomonas* fajokkal együttesen barnászöldre színezte a vizet.

DR. TAMÁS GIZELLA

1975. augusztus 19-én, életének 49-ik évében, rövid betegség után hirtelen elhunyt dr. Tamás Gizella, a Magyar Tudományos Akadémia Tihanyi Biológiai Kutatóintézetének tudományos munkatársa.

Tamás Gizella Budapesten született 1926. december 21-én. Középiskoláit Budapesten végezte és ugyanott folytatta egyetemi tanulmányait 1944—1949 években a Budapesti Tudományegyetemen. Hallgató korában „Parti algavegetáció vizsgálata a budapesti Dunaszakaszon" c. tanulmányával pályadíjat nyert.

1949-ben elnyerte az egyetemi doktori címet. Bölcsészdoktori értekezésének tárgya is a Duna algavegetációjának vizsgálata volt. E két említett tanulmányával már tudományos pályafutása legelején eljegyezte magát a scientia amabilis egy különösen vonzó ágával, az algológiával. Egyetemi tanulmányainak befejezése után egy ideig az Egyetemi Növényrendszertani Intézetben dolgozott Budapesten.

1950 októberétől új munkahelyén, a Tihanyi Biológiai Kutatóintézetben dolgozott csaknem 25 éven keresztül. Két beömlő patakból, a tihanyi Belsőtóból és más vizekből származó minták mellett kiemelten foglalkozott balatoni anyaggal.

Jó szakmai felkészültségével, kitűnő szakismeretével, rendkívüli szorgalmával, kitartással párosult alapos lelkiismeretes munkájával, maradandó és a Balatonkutatás számára alig felbecsülhető értékű tanulmányokkal nagymértékben növelte a Balaton algaflórájára vonatkozó újabb ismereteinket. Ezek során balatoni vonatkozásban tisztázta azt a szerepet, amit az egysejtű csillósok táplálkozásában a kovamoszatok játszanak.

Eredményei időtállóak, melyek a jövő kutatói számára is biztos összehasonlító bázist jelentenek a Balatonban eddig bekövetkezett és a jövőben várható változások megítélése szempontjából. E tekintetben külön kiemelendő az 1945—51-es fitoplankton mintasorozatok feldolgozása (planktonsűrűség, bio-

massza). Ezek folytatásaként az 1965—67 és 1972—74-es mintasorozatok eredményei alapján nyomon követhetők a fitoplankton és mikrofitobentosz összetételének, sűrűségének és biomasszájának regionális évszakos és évtizedes változásai. Ezeknek a változásoknak az ismerete döntő jelentőségű a gyakorlat, a Balaton eutrofizálódása elleni védekezés megszervezése érdekében.

Egyik legutóbbi tanulmánya 1974-ben Akadémiai kutatási jutalomban részesült.

50 tudományos dolgozata jelent meg, jórészük az Intézeti Évkönyvben, továbbá számos más hazai és nemzetközi tudományos folyóiratban. Munkásságáról gyakran számolt be tudományos előadások keretében. Tagja volt a Magyar Hidrológiai Társaságnak, a Magyar Biológiai Társaságnak és a Nemzetközi Limnológiai Egyesületnek (S. I. L.). Eredményeit nemcsak a magyar algológus és limnológus szakkörökben értékeli, de gyakorlatilag is hasznosítják. Nevét külföldön is jól ismerik és becsülik, amit külföldi szakemberekkel folytatott széleskörű tudományos cserekapcsolatai is bizonyítanak.

25 éven keresztül folytatott értékes balatoni tanulmányai egyre újabb és még értékesebb eredményekkel kecsegtettek, amiről számos kézirata vagy részben feldolgozott tanulmánya is tanúskodik.

Egyetemi hallgatók és fiatal algológusok gyakran felkeresték Tamás Gizellát, akiket türelemmel párosult szeretettel vezetett be az algahatározás kérdéseibe és a mennyiségi algavizsgálatok módszereibe.

Két súlyos szívműtéte ellenére töretlenül folytatta munkáját gyakran még betegen is. Munkássága delelőjén, szinte az íróasztal mellől ragadta e körünkből a halál. Elvesztése az Intézet számára, de különösen a hidrobiológiai kutatás terén nagy űrt hagyott hátra.

Halála mély fájdalommal tölti el mindazokat, akik Vele munkatársként vagy éppen évtizedekig együtt dolgoztak, vagy hosszabb-rövidebb ideig tudományos kapcsolatban álltak és mindazokat, akik őt mint embert tisztelték, becsülték és szerették.

DR. GIZELLA TAMÁS

On the 19th of August 1975 suddenly died Dr. Gizella Tamás, scientific research worker of the Hungarian Academy of Sciences after a short sickness.

The deceased was born at Budapest on the 21st December 1926. She went to secondary school in Budapest continuing her studies between 1944—1949 at the Budapest University of Sciences. As student she gained a prize with her paper "Studies on the littoral vegetation on the Budapest section of the Danube".

In 1949 she obtained a title of University Doctor. Her thesis dealt with „Investigation of the alga vegetation of the Danube River". By these two studies she got involved in problems of algology, a very attractive branch of the Scientia Amabilis, already at the beginning of her career. After graduation she worked for a while at the Institute for Systematic Botany at the University of Budapest.

In October 1950 Gizella Tamás became staff-member of the Biological Research Institute at Tihany where she was working for nearly 25 years.

Besides working up samples from two tributaries of Lake Balaton, the Belső-tó Lake in Tihany, in the course of her work the role of Diatoms plaid as food for Ciliates in Lake Balaton could be cleared.

Her main interest was towards problems of Lake Balaton itself. Deep professional knowledge combined with persistence, extraordinary diligence and thorough, conscientious work gave her studies remarkable results enlarging our newest knowledge on the algaflora of Lake Balaton in a very valuable way. The results obtained are long-lasting, giving a reliable base for comparisons to valuate the present and coming changes of the lake environment.

In this respect studies working up phytoplankton samples collected during the period 1945—1951 (biomass, density) should be emphasized in particular. As a continuation of these studies based on results of the samples collected during 1965—1967 and 1972—1974, the regional, seasonal and decennial changes of the composition, density and biomass of phytoplankton as well as microphytobenthos can be followed. The knowledge of these changes is of crucial importance for the praxis in preparing protective arrangements against the eutrophication of Lake Balaton.

One of her last studies in 1974 was awarded by a research prize of the Hungarian Academy of Sciences.

Gizella Tamás published 50 scientific papers mostly in the Annales of the Tihany Institute but several in other Hungarian or international journals. She was member of the Hungarian Hydrological Society, the Hungarian Biological Society and the Societas Internationalis Limnologiae.

The results obtained by her were not only valuated by Hungarian algologists and limnologists but had a good international reputation, as demonstrated by an expanded international exchange of her scientific papers with scientists at home and abroad.

25 years of her scientific activities gave more and more promising results testified as well by several manuscripts under preparation.

Gizella Tamás was visited several times by students and young algologists and she taught with patience and love, introducing them into alga-determinations and methodics in the field of quantitative alga studies.

Despite two serious heart operations, she continued her work with undiminished energy, often even being ill. She has been taken away on the summit of her activities, almost from beside her desk. Her loss left a vacuum for the Institute and the more for the hydrobiological studies.

All of us who were in human, scientific or collegial contact with her for years or even for decades, and who honored, respected and loved her, share the grief inflicted by her early and sudden death.

CHRONICLE

The research activity of the Institute was conducted during 1974 according to the middle-distance research plan approved for 1972—1975 being part of the main topics of research of the Hungarian Academy of Sciences in the themes "*Bioregulation*" and "*Biosphere*". Accordingly, the researches carried out at the *Department of Experimental Zoology* were focused on the neurohumoral regulation in invertebrate animals within the frame-work of the theme "*Regulatory mechanisms of the physiological processes*", while at the *Department of Hydrobiology* the hydrobiological investigations on Lake Balaton were conducted in the theme "*Investigations on Lake Balaton and on its catchment area*" being connected also with the main topic "*The protection of human beings and the natural environment*".

Results of the research work performed by the members of the two departments were published partly in *Annal. Biol. Tihany* Vol. **42**, and partly in various Hungarian and foreign journals (See *Annal. Biol. Tihany*, 1975, **42**, p. 299). The scientific lectures held in 1974 by the staff of the Institute were published in *Annal. Biol. Tihany* **42**, pp. 300—301.

In 1974 on the competition for the "Prize of Research Work" proposed by the Secretary General of the Hungarian Academy of Sciences the following scientific workers won the prize on the basis of their work entitled: HIRIPI L.: Pharmacological investigations on the regulation mechanisms of the periodic activity of the fresh-water mussel (*Anodonta cygnea* L.). — *Annal. Biol. Tihany* 1973. **40**, 27—53; HERODEK S. and G. TAMÁS: The primary production of phytoplankton in Lake Balaton, April-September 1972. — *Annal. Biol. Tihany* 1973, **40**, 207—218.

Scientific degree: Dr. KATALIN S.-RÓZSA senior scientific research worker on the basis of her dissertation entitled "Elementary and complex mechanisms at the regulation of heart beats on the invertebrate animals" won the degree of doctor of biological sciences on the 2nd of December, 1974.

This year M. VÉRÓ, technical councillor, was awarded with the Bronze Medal of the Order of Labour and J. ADAMIK, mechanic, was honoured with the Order of Outstanding worker in appreciation of his prominent work.

The Institute's permanent staff comprises 56 persons as follow: 20 scientific research workers, 1 technical councillor, 14 technical assistants, 6 administrative and 15 other workers.

The following changes took place in the scientific staff of the Institute: Dr. JÁNOS OLÁH scientific research worker of the Hydrobiological Department left on the 1st of March for the Research Station of Fish production at Szarvas. ISTVÁN VADÁSZ electrical engineer, assistant scientific worker, on the 15th of March was appointed as a scientific research worker to the Department of Experimental Zoology. ISTVÁN TÁTRAI, ichthyologist, on the 15th of August was appointed as an assistant scientific worker to the Hydrobiological Department. JÁNOS NEMCSÓK, biologist, scholar assistant scientific worker on the 1st of September was appointed as an assistant scientist to the Department of Experimental Zoology.

Inland scientific connections

Similarly to previous years, the Institute had inland connections with several scientific Institutes of the Hungarian Academy of Sciences and departments of universities, realized by cooperations, consultations and teaching.

The cooperations have been realized with the following Institutes:

1. Analytical Department of the University of Chemical Industry of Veszprém for photometric measurement of seasonal changes in the Na, K, Ca, Cl-ion contents of the haemolymph of *Anodonta*. The results are published in this volume of the Annales.

2. With the Institute of Biochemistry of the Biological Research Center (Szeged) of the Hungarian Academy of Sciences the investigations were carried out in order to study the accumulation of ^{14}C, ^{3}H dopamine, noradrenaline and 5-hydroxytryptamine in the central nervous system of Molluscs. These investigations initiated the use of isotopes in our institute and the results are published also in this volume.

3. Using computer analysis common research was done for clearing up the connections between planktonic organisms of the Lake Balaton with the Department of Zoology and Department of the Productivity of the University of Agricultural Sciences (Keszthely).

4. Investigations of the pesticides were done with the Department for DDD studies at OKI.

5. With the Bacteriological and Chemical Laboratory of KÖJÁL (Veszprém) investigations on water-chemistry were carried out.

6. Our Institute supplied from our locust breeding insects for research work performed by NEVIKI (Veszprém).

Participations at the university education:

Dr. JÁNOS SALÁNKI, Director of the Institute, held lectures at the Department of Comparative Physiology of Eötvös Loránd University, Budapest, for students-biologists, under the title "*Excitation at membrane level*". Dr. JENŐ PONYI Deputy Director of the Institute held lectures at a summer course on the "*Basic problems of hydrobiology*" for the students of Agricultural High School (Kaposvár).

PÉTER GARÁDI, student of the University of Agricultural Sciences (Gödöllő), prepared his thesis to be submitted for certification at the Hydrobiological Department of the Institute entitled: "Investigations of the growth

and population of *Abramis brama* L. in Lake Balaton considering the utilization of the populations by fishing."

In the summer months 11 university students joined in the experimental work of the two Departments, and 5 students from the Agricultural High School (Kaposvár) took part in field work organized by the Hydrobiological Department. The students of the third course of the University of Agricultural Sciences (Keszthely) and the student-engineers of the Department for Environment Protection of the University of Agricultural Sciences (Gödöllő), and also the participants of the training course, organized by KISS LAJOS Elementary School (Veszprém) for teacher-biologists visited our Institute. In the frame-work of a movement "One School-one factory" some help was extended to the Elementary School in Tihany how to organize a practical course in biology for pupils at our Institute.

About 45 Hungarian scientific workers visited our Institute in 1974 especially to carry out methodical studies. Several workers spent a longer period of time at our Institute:

Dr. I. ÁGOSTON, from the Department of Ophthalmology of the Medical University of Pécs; J. HEGEDŰS from the Department of Biophysics of the Medical University of Pécs; G. KOTTRA, from the IInd Department of Internal Diseases of the Semmelweis Medical University, Budapest; M. RÁGYANSZKY from the Research Station of Fish-production, Szarvas; Dr. J. SERFŐZŐ, from the Kossuth Lajos University, Debrecen; Dr. I. SZILASSI from the Chemical Department of the Medical University of Debrecen; Dr. M. VARSÁNYI, from the Chemical Department of the Medical University of Debrecen; E. VIZKELETY, from VIZIG, Szombathely; Dr. G. UHERKOVICS, from the PAK Laboratory of Hydrobiology, Pécs.

The Institute attended the International Fair in Budapest joining in the exhibition of the Hungarian Academy of Sciences with the topic "Investigations of the changes in Lake Balaton".

In 1974 two external themes were investigated at our Institute. For Chemicals Works of Richter Gedeon Ltd. (Kőbánya) the researches: "Studying the ability for depleting of the noradrenaline by M16 inhibiting the activity of dopamine beta-hydroxylase at the central nervous system of *Anodonta cygnea*" were completed, while for the Balaton Fishery Company (Siófok) the investigations on the theme "Relations of the growth and nourishment of plant-feeding fishes in Lake Balaton" were studied, to be examined until 1976.

Scientific connections with foreign institutes and research workers:

A. In 1974 collaborative work was done in the following themes:

1. According to official agreement, mutual work was done with the Institute of Evolutionary Physiology and Biochemistry of the Academy of Sciences of the USSR (Leningrad) on the theme "Evolution, physiology and morphology of sense organs" and in the frame-work of the above theme Prof. V. L. SVIDERSKY spent a week in our Institute.

2. The mutual work was done also according to bilateral agreement with the Institute of Ecology of the Polish Academy of Sciences (Warsawa) on the topic "Studying the productivity of the Lakes Balaton and Mazur"and

Dr. HILLBRICHT-ILKOWSKA spent 1 week, while Dr. A. STANCZYKOWSKA spent 2 weeks in our Institute, then from our side A. FRANKO, assistant scientific research worker, visited the companion Institute.

3. Within the frame-work of bilateral agreement the collaboration has been continued with the Institute of Development Biology of the Academy of Sciences of the USSR (Moscow) on "Comparative studies on the role of transmitters in the intracellular and intercellular regulations" in the frame-work of which Dr. A. AREFJEVA spent 2 weeks in our Institute.

4. Joint work was done according to the bilateral agreement with the Physiological Institute of the Czechoslovakian Academy of Sciences (Prague) on "Quantitative analysis of the nerve processes of invertebrates" and Dr. T. J. SKVARIL, engineer, spent 2 weeks in our Institute.

5. Within the frame-work of the Council of Mutual Economic Aid the joint work has been continued with the Physiological and Pathological Institute of the Slovakian Academy of Sciences (Bratislava) and Dr. JÁNOS SALÁNKI, Director of our Institute, was invited for 1 week to the companion Institute.

6. According to bilateral agreement mutual work has started on the theme "Neurobiology of Invertebrates" between our Institute and the Brain Research Laboratory of the Institute of Biology and Medicine of Montenegro belonging to the Serbian Academy of Sciences (Kotor). The possibilities of the mutual work were discussed by of Dr. R. SIMINOFF and Dr. J. IVANUS visiting our Institute and repaying the visit, Dr. J. SALÁNKI went to the Kotor Laboratory. Dr. G. KONJEVIC from Kotor spent a month in our Institute.

7. Common researches were done also with the Physiological Institute of the Ukrainian Academy of Sciences (Kiev) on the "Investigations of the transport mechanisms of ions at the generation of activity patterns in excitable cells" in the frame-work of INTERMOZG. I. VADÁSZ scientific research worker spent two months in the companion Institute and Dr. I. S. MAGURA from Kiev paid a short visit to our Institute. In the first theme of INTERMOZG on the "Physiology of synapses and neurone" work-meeting was organized in Kiev between the 16th and 19th of April which was attended by Dr. J. SALÁNKI.

8. Within the frame-work of the Council of Mutual Economic Aid we collaborated also with the Institute of Biophysics (Puschino) of the Academy of Sciences of the USSR. The coordination meeting of this organization was attended by Dr. J. SALÁNKI between the 4th and 7th of February. T. FORRÓ Deputy Director of the Institute also visited the above Institute in Puschino.

B. Travels abroad from our Institute besides the above mentioned trips in 1974:

1. Dr. B. ENTZ, senior scientific research worker, finished his work as a FAO expert in UAR on the 15th of November, 1974.

2. A. FRANKÓ, assistant scientific research worker, took part in a one-month study trip in the Department of Bioenergetic and Production of the Institute of Experimental Ecology of the Polish Academy of Sciences (Warsawa).

3. Dr. L. HIRIPI, scientific research worker, was invited for a three-month study trip, between the 15th September and 15th December, to the Neurochemical Department of the Max-Planck Institute for Experimental Medicine in Göttingen (German Federal Republic).

4. Dr. T. KISS, scientific research worker, attended the international symposium on the "Physiology of smooth muscle" held in Kiev between the 14th and 18th of October.

5. J. NEMCSÓK, assistant scientific research worker, took part in a study trip in the Zoological Department of Friedrich-Schiller University (Jena) between the 27th of February and 25th of March.

6. Dr. KATALIN S.-RÓZSA, senior scientific research worker, attended the coordination meeting of the research organizing in the frame-work of the Council of Mutual Economic Aid at the Institute of Biophysics (Puschino) of the Academy of Sciences of the USSR between the 17th and 23rd of February.

7. Dr. J. SALÁNKI, Director of the Institute, attended the Vth Interdisciplinary Conference on Rhythms at Noordwijk, then visited the Biological Department of Free University in Amsterdam (Netherland) between the 23rd and 29th of June. He attended also the meeting of the Executive Committee of IUBS at Paris (France) as a delegate of the Hungarian Organization between the 23rd and 27th of October.

8. Dr. J. PONYI, Deputy Director of the Institute, took part in the study trip to the Hydrobiological Department of the Zoological Institute of the Bulgarian Academy of Sciences (Sofia, Bulgaria).

9. Dr. NÓRA P.-ZÁNKAI, scientific research worker, visited the Institute of Limnology at Lunz and Wien (Austria) between the 7th and 21st of October.

10. M. VÉRÓ, technical councillor spent one month in France between the 18th of November and 18th of December visiting the following Institutes: Cellular Neurophysiological Laboratory of C.N.R.S. in Marseille, Neurophysiological Laboratory of C.N.R.S. in Gif-sur-Yvette and Neurophysiological Laboratory of Ecole Normale Supèrieure in Paris.

11. Dr. I. ZSOLNAI-NAGY, senior scientific research worker, has continued his working-study trip at the Center of Experimental Gerontology of I.N.R.C.A. at Ancona (Italy).

C. The following scientific workers paid a short visit to our Institute during 1974:

Prof. K. ACHTE (Medical University of Helsinki, Helsinki, Finland); A. S. BATUEV, Department of Higher Nervous Activity of the Leningrad State University, Leningrad, USSR; S. D. BECK (University of Wisconsin, USA); S. BENEDEK M.B.B.S. F.A.N.Z.C.O. M.R.C. Psych (London) D.P.M. Sydney, Australia); E. BERNAYS (Centre for Overseas Pest Research, London, Great Britain); W. H. BESADA (Faculty of Sciences, Alexandria University, UAR); W. BLANEY (London University, London, Great Britain); T. W. BLACKSTAD (Anatomical Institute, Aarhus University, Aarhus, Denmark); G. BONDE (Swedish ambassador to Budapest); A. COOK (Centre for Overseas Pest Research, London, Great Britain); J. DE WILDE Prof. (Department of Entomology, Waageningen, Netherland); Z. DOBROWSKI (Department of Entomology of the Agricultural University, Warsawa, Poland); W. EBERT (Research Institute for Plant Protection, Eberswalde, DDR); I. ECKERSTEIN (Swedish under-secretary); E. FALCK (Ludwig Georgs-Gymnasium, Darmstadt, FRG); G. P. GEORGIEV (Academy of Sciences of the USSR, Moscow, USSR); J. E. HALVER (FAO-UNDP manager director); G. E. HANIOTAKY

(Nuclear Research Center "Democritos" Athen, Greece); P. HARREWY (University of Cambridge, Cambridge, Great Britain); J. IVANUS (Central Laboratory of the University of Belgrad, Yugoslavia); I. KIRJAKOV (University of Plovdiv, Plovdiv, Bulgaria); M. A. KUYPER (Laboratory of Chemical Cytology of the University of Nijmegen, Netherland); V. S. LESSE (Los Angeles University, Los Angeles, California, USA); Dr. P. P. LOBANOV (president of VASHNILL, Moscow, USSR); S. LUNDKVIST (Swedish Minister of Agriculture); T. MILLER (Department of Entomology of the University of California, Berkeley, USA); A. MOHAMED (Faculty of Agriculture, AlAzhar University, Nasr City Cairo, UAR); R. F. MULLER (Section of Biology of the University of Rostock, DDR); J. T. NIEBES (University of Leiden, Netherland); R. OLSON (Stockholm, Sweden); PHAM KHAK LAM (State Medical University, Hanoi, Viet-Nam); A. A. PIROGOV (Institute of Biophysics, Academy of Sciences of the USSR, Leningrad, USSR); Prof. M. A. RONKIN (Moscow, USSR); Prof. H. A. ROSENTHAL (St. Louis, Washington University, Missouri, USA); J. A. RUDINSZKY (Oregon State University, Corvallis, Oregon, USA); K. RUSS (Oregon State University, Corvallis, Oregon, USA); P. SCHETTES (Institute Centre of Insect Physiology and Ecology, Nairobi, Kenya); R. SIMINOFF (Brain Research Laboratory of the Institute of Biology and Medicine of Montenegro, Kotor, Yugoslavia); B. SWEDMARK (Swedish Royal Academy of Sciences, Stockholm, Sweden); H. SELEGIEWICZ (Institute of Zoology of the Polish Academy of Sciences, Warsawa, Poland); K. TOTH (Research Station for Biology, Geography and Geology, Pingarati, Roumania); J. H. VISSER (Department of Entomology, Waageningen, Netherland); J. WLODEK and J. WROBEL (Hydrobiological Institute of the Polish Academy of Sciences, Warsawa, Poland); D. I. WOOD (University of California, Berkeley, USA).

Besides scientists having arrived within the frame-work of collaborative work for a longer period of time, the following scientists stayed at our Institute: Dr. R. KILLIAS, The Natural History Museum of the Humbold University, Berlin, DDR; Dr. T. KUSCH, Zoological Department of the Friedrich-Schiller University, Jena, DDR; Dr. F. SIMALCSIK, Research Station for Biology, Geography and Geology, Pingarati, Roumania.

Meetings

In 1974 the following meetings were held at the Institute:

1. Research School for Statics organized by the Central Institute of Physics of the Hungarian Academy of Sciences between the 13th and 17th of May with 11 participants.

2. Two courses for studying theoretical and practical questions of objective examinations organized by the Center of Higher Education and Pedagogy between 30th of May and 1st of June, then between the 28th and 31st of October with 20 and 24 participants, respectively.

3. International symposium on "The host plant in relation to insect behaviour and reproduction" between the 11th and 14th of June, organized by the Research Institute for Plant Protection of the Ministry of Agriculture and Food (Budapest) and sponsored by the Hungarian Academy of Sciences and the Ministry of Agriculture and Food (40 participants).

4. International Course for Hydrobiological Training organized by the Institute for Water Researches with the aid of FAO between the 28th of June and 5th of July.

5. Symposium on Neurobiology organized by the Committee on Neurobiology and our Institute between the 21st and 24th of August (50 participants).

6. Round-Table Conference on Transport Across Membranes organized by the Hungarian Biochemical Society and our Institute between the 3rd and 7th of September (45 participants).

7. Conference on Interferon researches organized by the Department of Microbiology of the Hungarian Academy of Sciences in the frame-work of cooperations between the Institutes of the countries of the Council of Mutual Economic Aid between the 11th and 14th of September (26 participants).

8. IIIrd Interdisciplinary Symposium on the "Investigations on structure of movements in different sport technics" organized by the sport section of the Hungarian Biological Society and the Biomechanical Committee of the Society for Physical Training and Sport-researches, between the 27th and 29th of September (40 participants).

9. The "Hydrobiological Days" organized by the Hydrobiological Society and the Hydrobiological Department of the Institute between the 3rd and 5th of October (55 participants).

Improvements of research facilities

The equipment park was completed among others in 1974 with ISOCAP (300 typ. Liquid Scintillation System Nuclear Chicago); TTT 2b typ. Automatic titrator (Radiometer, Denmark); Hunor 81B typ. calculator (EMG); 175 typ. four-channel write projection (KUTESZ); 3 MF-1 typ. photorecorder (MEDICOR); 3 Digital displays (MKKL); OP-205 typ. pH-meter; 2 (Radelkisz) -DC-minimatic digital voltmeter (EMG).

In 1974 in our workshop 7 FET input operation amplifiers were constructed.

Library

At the end of the year the Institute's Library comprised 13,568 volumes. Books 4938 units, journals 8630 units.

The Institute's Year Book — *Annal. Biol. Tihany* (1974), Vol. **41** was sent to 659 Institutes and private persons allover the world. In exchange the Library received 204 different journals (volumes) and publications.

KRÓNIKA

1974-ben az Intézetben a kutatómunka az 1972—1975 évekre elfogadott középtávú kutatási tervnek megfelelően folyt, mely az országos, ill. MTA kutatási főirányokhoz „*Bioreguláció*", ill. „*Bioszféra*" témakörökben kapcsolódik. A *Kísérletes Állattani Osztály* munkája az „*Életfolymatok szabályozási mechanizmusai*" c. főfeladaton belül a neurohumorális szabályozás törvényszerűségeinek feltárására irányult gerinctelen állatokon, a *Hidrobiológiai Osztály* pedig folytatta a Balaton hidrobiológiai kutatást „*A Balaton és vízgyűjtőterületeinek kutatása*" c. témában, mely kapcsolódik az „*Ember és természeti környezetének védelme*" c. tárcaszintű főirányhoz is.

A két osztály tudományos tevékenységét tükröző tanulmányok részben az *Annal. Biol. Tihany 41* kötetében, részben más hazai és külföldi folyóiratokban kerültek közlésre (lásd: *Annal. Biol. Tihany* 1975, *42*, 299. oldal). Az 1974-ben tartott tudományos előadások jegyzéke az *Annal. Biol. Tihany 42.* kötetének 300—301 oldalán kerül felsorolásra.

1974-ben „A távlati tudományos kutatási terv kutatásaiban elért jelentős eredmények" elnevezésű, a Magyar Tudományos Akadémia Főtitkára által meghirdetett pályázaton három kutató részesült jutalomban az alábbi pályamunkák benyújtása és díjazása alapján:

Hiripi László: Pharmacological investigations on the regulation mechanism of the periodic activity of the fresh-water mussel (*Anodonta cygnea* L.). — *Annal. Biol. Tihany*, 1973, **40**, 27–53, valamint Herodek Sándor és Tamás Gizella: The primary production of phytoplankton in Lake Balaton April-September 1972. — *Annal. Biol. Tihany* 1973, *40*, 207–218.

Tudományok fokozat: Dr. S.-Rózsa Katalin 1972. december 2-án „Elemi és komplex mechanizmusok a szívműködés szabályozásában gerinctelen állatokon" c. doktori értekezésének megvédésével elnyerte a biológiai tudományok doktora címet.

Az év folyamán kiváló munkájáért Véró Mihály műszaki tanácsadó elnyerte a Munkaérdemrend bronz fokozatát, Adamik József mechanikus pedig „Kiváló Dolgozó" kitüntetésben részesült.

Az Intézet személyi állománya 56 fő, mely a következőkcppen oszlott meg: kutató 20, műszaki tanácsadó 1, kutatási segéderő 14, adminisztratív dolgozó 6, egyéb foglalkozású 15.

Az Intézet kutatóállományában az alábbi változások történtek: Dr. Oláh János a Hidrobiológiai Osztály tudományos munkatársa a Szarvasi Haltenyésztési Kutató Állomásra távozott az Intézetből 1974. március 1-én. Vadász István tudományos segédmunkatárs 1974. március 15-től tudományos munkatársi kinevezést nyert az Intézet Kísérletes Állattani Osztályán. Tátrai István halbiológus augusztus 15- vel felvételt nyert az Intézet Hidrobiológiai Osztályára, tudományos segédmunkatársi állásra. Nemcsók János, biológus, tudományos gyakornok 1974. szeptember 1-vel segédmunkatársi kinevezést nyert a Kísérletes Állattani Osztályra.

Belföldi tudományos kapcsolatok

A korábbi évekhez hasonlóan számos Intézettel és Egyetemmel állt kapcsolatban Intézetünk. Együttműködés folyt az alábbi Intézetekkel:

1. A Veszprémi Vegyipari Egyetem Analitikai Tanszékén fotometriás meghatározások folytak *Anodonta* kemolimfa Na, K, Ca, Cl-iontartalmának szezonális változásaira vonatkozóan. Az eredmények ugyanitt közlésre kerülnek.
2. Az MTA Biológiai Központjának Biokémiai Intézetével (Szeged) ^{14}C és ^{3}H jelzett dopamin, noradrenalin és 5-hydroxytryptamin beépülésének vizsgálata folyt puhatestűek központi idegrendszerében. E vizsgálatok előkészítették izotópok alkalmazását Intézetünkben, az eredmények ugyanezen kötetben kerülnek publikálásra.
3. A Keszthelyi Agrártudományi Egyetem Állattani és Termelésfejlesztési Intézetével közös kutatás folyt a Balaton planktonját alkotó szervezetek egymásközti kapcsolatának számítógépes vizsgálatára.
4. Az OKI DDD osztályával peszticid-kutatás folyt balatoni szervezeteken.
5. A Veszprémi KÖJÁL Bakteriológiai és Vízkémiai Laboratóriumával közös vízkémiai kutatások folytak.
6. A Veszprémi NEVIKI rovarkutatásaihoz Intézetünk sáska-tenyészetéből állatok biztosításával segítséget nyújtott.

Oktató munkában való részvétel

Dr. Salánki János igazgató speciálkollégiumot tartott az ELTE biológia szakos hallgatóinak „*Elemi ingerület*" címmel. Dr. Ponyi Jenő tudományos igazgatóhelyettes pedig „*A hidrobiológia alapjai*" címmel a Kaposvári Mezőgazdasági Főiskola hallgatóinak tartott speciálkollégiumot az Intézetben nyári kurzus keretein belül.

Az Intézet Hidrobiológiai Osztályán készítette Garádi Péter a Gödöllői Agrártudományi Egyetem V. éves hallgatója szakdolgozatát „A dévérkeszeg (*Abramis brama* L.) növekedésének és állományösszetételének vizsgálata a Balatonban, tekintettel az állomány halászati kihasználására" címmel.

A nyári hónapokban 11 biológia szakos egyetemi hallgató dolgozott Intézetünk laboratóriumaiban, e mellett a Kaposvári Mezőgazdasági Főiskola 5 hallgatója a Hidrobiológiai Osztály által szervezett gyakorlaton vett részt. Az Intézet munkájával megismertettük a Keszthelyi Agrártudományi Egyetem III. éves hallgatóit, és a Gödöllői első Környezetvédelmi Szakmérnök

évfolyam hallgatóit, valamint a Veszprémi Kiss Lajos Általános Iskola által szervezett biológus tanártovábbképző részvevőit. Az „Egy iskola egy üzem" mozgalom keretében patronáltuk a Tihanyi Állami Általános Iskolát és biológiai óra keretében gyakorlati bemutatást szerveztünk a tanulóknak.

Az Intézetben mintegy 45 hazai kutató töltött hosszabb-rövidebb időt, elsősorban metodikai tanulmányok céljából. Az Intézetet felkereső kutatók közül az alábbiak töltöttek itt hosszabb időt:

Dr. Ágoston Irén, Pécs, POTE Szemészeti Klinika; Hegedüs János, Pécs, POTE, Biofizikai Intézet; Kottra Gábor, Budapest, SOTE, II. Belgyógyászati Klinika; Rágyanszki Mária, Szarvas, Haltenyésztési Kutatóállomás; Dr. Serfőző József, Debrecen, KLTE, Állattani Intézete; Dr. Szilassi Ildikó, Debrecen, DOTE, Orvosvegytani Intézete; Dr. Varsányi Magdolna, Debrecen, DOTE, Orvosvegytani Intézete; Vizkelety Éva, Szombathely, VIZIG; Dr. Uherkovics Gábor, Pécs, PAK Hidrobiológiai Laboratóriuma.

Az Intézet részt vett az MTA megrendezésében a Budapesti Nemzetközi Vásáron, ahol a „Balaton változásainak kutatása" címmel állított ki.

Az Intézetnek 1974-ben két külső megbízásos munkája volt. A Kőbányai Gyógyszerárugyár részére „M-16 jelzésű dopamin-béta-hidroxiláz gátló anyag noradrenalin-depletáló hatásának vizsgálata tavikagyló idegrendszerében" c. téma kidolgozása befejezést nyert. A Balatoni Halászati Vállalat (Siófok) részére „A fehér busa növekedési és táplálkozási viszonyai a Balatonban" címmel folytatódtak az 1976-ig tartó vizsgálatok.

Külföldi tudományos kapcsolatok

A. 1974-ben az alábbi témákban folyt együttműködés:

1. Kétoldalú együttműködés a SZUTA Evolúciós és Fiziológiai Intézetével (Leningrád) „Gerinctelenek érzékszervi evolúciójának, fiziológiájának és morfológiájának tanulmányozása" c. téma keretében Prof. V. L. Svidersky 1 hetet töltött Intézetünkben.

2. A Lengyel Tudományos Akadémia Ecológiai Intézetével (Varsó): „A Balaton és a Mazúri tavak produktivitásának kutatása" címmel folyt közös vizsgálat, kétoldalú együttműködés keretében. Dr. A. Hillbricht-Ilkowska 1 hétig, Dr. A. Stanczykowska 2 hétig volt tanulmányúton az Intézetben, az Intézet részéről Frankó András tudományos segédmunkatárs tett látogatást a partnerintézetben.

3. A SZUTA Fejlődésbiológiai Intézetével (Moszkva) „Mediátorok sejten belüli és sejtközti szabályozásában játszott szerepének összehasonlító vizsgálata" c. témában folyt kétoldalú együttműködés, melynek keretében Dr. A. M. Arefjeva 2 hetet töltött az Intézetben.

4. A Csehszlovák Tudományos Akadémiai Fiziológiai Intézetével (Prága) „Gerinctelenek idegi folyamatainak kvantitatív analízise" címmel folyt közös kutatás, kétoldalú együttműködés keretében. Dr. J. T. Skvaril 2 hetet töltött Intézetünkben.

5. KGST együttműködés keretében a Szlovák Tudományos Akadémia Patológiai-Fiziológiai Intézetével (Bratislava) „Ingerületgenerálás és ingerület-kontrakció kutatása" c. témában van együttműködés, melynek keretében

meghívásra Dr. SALÁNKI JÁNOS intézeti igazgató 1 hetet töltött a partnerintézetben.

6. Kétoldalú együttműködés keretében a Szerb Tudományos Akadémia Montenegrói Biológiai és Orvosi Kutatóintézet Agykutató Laboratóriumával „Gerinctelen neurobiológiai kutatások" címmel indult együttműködés. Dr. SALÁNKI JÁNOSnak a kotori Agykutató Laboratóriumban tett látogatása, valamint Dr. J. IVANUS és Dr. R. SIMINOFF azt megelőző, a Biológiai Kutatóintézetben, Tihanyban tett látogatása során megvitatták a neurobiológia területén végzendő tudományos kutatásokban való folyamatos együttműködés lehetőségeit. E téma keretében Dr. G. KONJEVIČ 1 hónapot töltött az Intézetben.

7. A kijevi, Bogomoletzről elnevezett Élettani Intézettel „Ionok átviteli mechanizmusai kölcsönhatásának tanulmányozása az ingerlékeny sejtek alapvető aktivitási formái generálásának folyamatában" c. témában folyt közös kutatás INTERMOZG együttműködés keretében, melynek kapcsán VADÁSZ ISTVÁN tudományos munkatárs 2 hónapot töltött a partnerintézetben, és Dr. I. S. MAGURA rövid látogatást tett Intézetünkben. „A neuron és szinapszis fiziológiája" INTERMOZG I. sz. témájának munkaértekezletén Dr. SALÁNKI JÁNOS igazgató vett részt április 16—19 között Kijevben.

8. A SZUTA Biofizikai Intézetével (Puschino) KGST Együttműködés koordinációs értekezletén Dr. SALÁNKI JÁNOS igazgató vett részt Moszkvában február 4–7 között. Továbbá FORRÓ TIBOR gazdasági igazgatóhelyettes látogatást tett a SZUTA Puschinói Biológiai Intézeteiben.

B. Az egyezményes kutatások keretein belül megvalósuló kiutazásokon túl az alábbi tanulmányutak lebonyolítására került sor 1974-ben:

1. Dr. ENTZ BÉLA tudományos főmunkatárs 1974. november 15-én befejezte 5 éves FAO szakértői munkáját az Egyesült Arab Köztársaságban.

2. FRANKÓ ANDRÁS tudományos segédmunkatárs 1 hónapos tanulmányúton vett részt Varsóban a Lengyel Tudományos Akadémia Kísérletes Ecológiai Intézetének Bioenergetikai és Termelésbiológiai Osztályán.

3. Dr. HIRIPI LÁSZLÓ tudományos munkatárs 1974. szeptember 15 és december 15 között meghívásra 3 hónapot töltött a Max Planckról elnevezett Kísérletes Orvostudományi Intézet Neurokémiai Osztályán, Göttingenben (NSZK).

4. Dr. KISS TIBOR tudományos munkatárs részt vett Kijevben a „Simaizom élettana" c. nemzetközi szimpoziumon október 14—18 között.

5. NEMCSÓK JÁNOS tudományos segédmunkatárs február 27 és március 25 között egy hónapos tanulmányúton volt Jénában a Friedrich Schiller Egyetem Állattani Tanszékén (NDK).

6. Dr. S.-RÓZSA KATALIN tudományos főmunkatárs 1974. február 17–23 között a KGST keretein belül folyó kutatások Koordináló Értekezletén vett részt a SZUTA Puschinói Biofizikai Intézetben.

7. Dr. SALÁNKI JÁNOS igazgató június 23—29 között Hollandiában, Noordwijkban részt vett az V. Nemzetközi Interdiszciplináris Ritmus Konferencián. Amszterdamban meglátogatta a Free University Biológiai Tanszékét. Október 23–27 között részt vett az IUBS Végrehajtó Bizottság ülésén Párizsban mint a Magyar Nemzeti Bizottság képviselője.

8. Dr. PONYI JENŐ tudományos igazgatóhelyettes április 16—május 7-ig tanulmányúton volt Szófiában (Bulgária), a Bolgár Tudományos Akadémia Zoológiai Intézetének Hidrobiológiai Osztályán.

9. Dr. P.-ZÁNKAI NÓRA tudományos munkatárs október 7–21-ig tanulmányúton volt Ausztriában, ahol a Lunzi és Bécsi Limnológiai Intézeteket látogatta meg.

10. VÉRÓ MIHÁLY műszaki tanácsadó 1974. november 18-tól december 18-ig 1 hónapot töltött Franciaországban, ahol a C.N.R.S. Neurobiológiai Intézeteit látogatta meg Párizsban, Gif-sur-Yvette-ben és Marseílle-ban.

11. Dr. ZSOLNAI-NAGY IMRE tudományos főmunkatárs folytatta munkatanulmányútját az I.N.R.C.A. Anconai Kísérletes Gerontológiai Központjában, Olaszországban.

C. Az Intézet külföldi látogatói 1974-ben:

Prof. K. ACHTE (Orvostudományi Egyetem, Helsinki, Finnország); Prof. A. S. BATUEV (Leningrádi Állami Egyetem Felső Idegtevékenység Tanszék, Leningrád, SZSZSZR); S. D. BECK (Wisconsini Egyetem, USA); S. BENEDEK (MBBS, FANZCOMPC, Pzych [London], DPM, Sidney, Ausztrália); E. BERNAYS (Tengertúli Pesticid Kutatás Központja, London, Anglia); W. H. BESADA (Alexandriai Egyetem Természettudományi Kara, Alexandria, Egyiptom); W. BLANEY (Londoni Egyetem, London, Anglia); T. W. BLACKSTAD (Aarhusi Egyetem Anatómiai Intézete, Aarhus, Norvégia); G. BONDE (Budapesti svéd nagykövet); A. COOK (Tengerentúli Pesticid Kutatás Központja, London, Anglia); J. DE WILDE Prof. (Entomológiai Intézet, Wageningen, Hollandia); Z. DOBROWSKI (Agrártudományi Egyetem Entomológiai Részlege, Varsó, Lengyelország); W. EBERT (Növényvédelmi Kutatóintézet, Eberswalde, NDK); I. ECKERSTEIN (Svéd államtitkár); E. FALCK (Ludwig-Georgs-Gimnázium, Darmstadt, NSZK); G. P. GEORGIEV (akadémikus, SZUTA Moszkva, SZSZSZR); J. E. HALVER (FAO-UNDP program igazgatója); G. E. HANIOTAKY (Nukleáris Kutató Központ „Democritos" Athén, Görögország); P. HARREW (Cambridge-i Egyetem, Cambridge, Anglia); J. IVANUS (Belgrádi Egyetem Központi Laboratóriuma, Belgrád, Jugoszlávia); I. KIRJAKOV (Plovdivi Tudományegyetem, Plovdiv, Bulgária); M. A. KUYPER (Kémiai-Citológiai Laboratórium, Nijmegen, Hollandia); V. S. LESSE (Los Angelesi Egyetem, California, Los Angeles, USA); P. P. LOBANOV (VASZHNILL elnöke, Moszkva, SZSZSZR); S. LUNDKVIST (Svéd mezőgazdasági miniszter); T. MILLER (Californiai Egyetem, Entomológiai Részlege, Berkeley, USA); A. MOHAMED (AlAzhari Egyetem Mezőgazdasági Kara, Nasv City Kairó, Egyiptom); R. F. MULLER (Rostocki Egyetem Biológiai Kara, Rostock, NDK); J. T. NIEBES (Leideni Egyetem, Leiden, Hollandia); R. OLSON (Stockholm, Svédország); PHAM KHAK LAM (Állami Egyetem Hanoi, Vietnami Demokratikus Köztársaság); A. A. PIROGOV (SZUTA Biofizikai Intézete, Leningrád, SZSZSZR); Prof. M. A. RONKIN (Moszkva, SZSZSZR); Prof. H. A. ROSENTHAL (St. Louis, Washington Egyetem, Missouri, USA); J. A. RUDINSZKY (Oregon Állami Egyetem, Corvallis, Oregon, USA); K. RUSS (Oregon Állami Egyetem, Corvallis, Oregon, USA); P. SCHETTES (Rovar Élettani és Ekológiai Intézet, Nairobi, Kenya); R. SIMINOFF (Tengerbiológiai Intézet Agykutató Laboratóriuma, Kotor, Jugoszlávia); B. SWEDMARK (Svéd Tudományos Akadémia, Stockholm, Svédország); H. SELEGIEWICZ (Lengyel Tudományos

Akadémia Zoológiai Kutatóintézete, Varsó, Lengyelország); K. Tóth („Stejarul" Biológiai, Földrajzi és Geológiai Kutató Állomás, Pingarati, Románia); J. H. Visser (Entomológiai Intézet, Wageningen, Hollandia); J. Wlodek és J. Wrobel (A Lengyel Tudományos Akadémiai Hidrobiológiai Kutatóintézete, Varsó, Lengyelország); D. I. Wood (Californiai Egyetem, Berkeley, USA).

Hosszabb ideig tartózkodtak az Intézetben (egyezményes témán kívül): Dr. R. Kilias: Humbold Egyetem Természettudományi Múzeuma, Berlin (NDK) (2 hét); Dr. T. Kusch: Friedrich Schiller Egyetem Állattani Intézete, Jena (NDK) (1 hónap); Dr. F. Simalcsik: „Stejarul" Biológiai, Földrajzi és Geológiai Kutató Állomás, Pingarati, Románia (2 hónap).

Rendezvények

Az 1974 év folyamán az Intézetben 9 konferencia rendezésére került sor:

1. Szilárdtest kutatási iskola, május 13—17 között, 11 fő részvételével, MTA Központi Fizikai Kutató Intézet rendezésében.

2. Az objektív vizsgáztatás elméleti és gyakorlati kérdései c. tanfolyam, május 30—június 1 között, és október 28—31 között 24—24 fő részvételével a Felsőoktatási Pedagógiai Kutatóközpont szervezésében.

3. Tápnövények szerepe rovarok magatartásában és szaporodásában c. nemzetközi szimpozium, június 11—14 között 40 fő részvételével, a Növényvédelmi Kutató Intézet szervezésében, az MTA és MÉM támogatásával.

4. Nemzetközi Hidrobiológiai Továbbképző Tanfolyam, június 28—július 5 között, a Vízgazdálkodási Kutató Intézet szervezése alatt, a FAO támogatásával.

5. Neurobiológiai Kollokvium, augusztus 21—24 között, 50 fő részvételével a Neurobiológiai Bizottság és az Intézet rendezésében.

6. Membrán Transzport Konferencia szeptember 3—7 között, 46 fő részvételével a Magyar Biokémiai Társaság és az Intézet rendezésében.

7. Interferon konferencia szeptember 11—14 között, 26 fő részvételével, az MTA Mikrobiológiai Kutató Csoportjának szervezése alatt, KGST Együttműködés keretében.

8. III. Interdiszciplináris szimpozium: a sporttechnikák mozgásszerkezetének vizsgálata címmel, szeptember 27—29 között, 40 fő részvételével, a Magyar Biológiai Társaság Mozgásbiológiai Szekciója és a Testnevelési és Sporttudományos Tanács Biomechanikai Bizottsága rendezésében.

XVI. Hidrobiológus Napok október 3—5 között 55 fő részvételével a Hidobiológiai Társaság és az Intézet Hidrobiológiai Osztályának rendezésében.

Kutatási feltételek fejlődése 1974-ben

1974-ben vásárolt jelentősebb műszerek, kutatási eszközök:

ISOCAP (300 tip Liquid Scintillation System) (Nuclear Chicago); TTT 2 b tip Automata titrator (Radiometer, Denmark); HUNOR 81B tip. számológép (EMG); 175 tip. négycsatornás vonalíró (KUTESZ); MF-1 tip. Fotorecorder, 3 db (MEDICOR); Digimet egyenfeszültségmérő, 3 db (MKKL);

OP-205 tip. precíziós pH-mérő, 2 db (Radelkisz); DC minmatic digital voltméter (EMG).

1974-ben az Intézetben készült kutatási eszközök: 7 db FET bemenetű biológiai erősítő.

Könyvtár

Az évvégi összesítés alapján az Intézet leltározott állománya 13 568 egység. Ebből könyv 4938 db, folyóirat 8630 db.

Az Intézeti Évkönyv — *Annal. Biol. Tihany 41.* kötetét 659 címre küldtük meg, melyért cserébe — kötetben számolva — 204 kiadvány érkezett.

LIST OF PAPERS PUBLISHED ELSEWHERE AS IN VOL. 41 OF OUR ANNALES

Biró, P. (1974): A balatoni fogassüllő energiatranszformálása. — *Halászat* **67**, 138—140.

Ponyi, J. (1974): A Balaton élővilága. — *Balaton monográfia, Panoráma, Budapest*, 95—107.

S.-Rózsa, K. (1974): Relation of the second messenger system to the electrogenesis and regulation of the contraction in the heart cell membrane of Insecta. — *Comp. Biochem. Physiol.* **49**A, 81—88.

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