A MAGYAR TUDOMÁNYOS AKADÉMIA
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S.-Rózsa Katalin

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ELECTRON MICROSCOPIC INVESTIGATIONS OF THE VESICLE POPULATIONS IN THE CENTRAL NERVOUS SYSTEM OF FRESH WATER MUSSEL (*ANODONTA CYGNEA* L.)

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According to GERSCHENFELD (1963), the central nerve terminals of *Vaginula solea* (Pelecypoda) are characterized by three types of vesicle: clear, dense-core and neurosecretory ones. In the neuropile of the ganglia of other bivalves *(Mercenaria mercenaria* and *Spisula solida)* at least four types of vesicle have been distinguished: clear vesicles; dense-core vesicles of 500—800 Å; 900—2000 Å ones with very high electron opacity; and 1000—3000 Å vesicles containing homogeneous material of varying density (LOVELAND 1963; COTTRELL, 1968). These types could be observed (COTTRELL and MASER, 1967) in *Mercenaria* even after differential centrifugation and OsO_4 fixation.

Using OsO_4 fixation, empty, dense-core (full) and transitional (emptying) vesicle forms have been described by ZS.-NAGY (1968b) in the central nervous system of the fresh water mussel. After reserpine treatment the size of the vesicles decreased uniformly and the majority of them turned into empty or emptying state. On this basis the conclusion was drawn corresponding to the former assumptions (ZS.-NAGY, 1964; 1968a) that the three types of vesicle represent the differential functional states of a single element, namely of the full dense-core vesicles. After glutaraldehyde-osmium or single osmium-fixation three types of granule were distinguished in the ganglia of *Anodonta cygnea* (BARANYI, 1971).

On the basis of the above results the reinvestigation of the ultrastructure of the ganglia of *Anodonta cygnea* L. seemed to be necessary in order to reveal the morphology of the vesicles of the axons after fixations differing from the single OsO_4, as well as to decide whether the vesicle populations formerly considered to be homogeneous (ZS.-NAGY, 1968b) can be differentiated into various vesicular profiles.

Material and methods

The investigations were carried out on the ganglia of 15—18 cm long specimens of *Anodonta cygnea* L. The cerebral and pedal ganglia were fixed in two ways: 1. Glutaraldehyde-osmium (GA-Os): fixation with 3% glutaraldehyde diluted with Balaton-water at 20 °C for 2—3 hr, and postfixation with 2% OsO_4 buffered with s-collidine (BENNETT and LUFT, 1959) for 25

min. at 0 °C and for 5 min. at room temperature. 2. Potassium permanganate: the ganglia were fixed in ice-cold 1.5% or 3% $KMnO_4$ dissolved in *Anodonta*-Ringer (MARCZYNSKY, 1959; pH 7.04) for 45 minutes.

Reserpine treatment: 1. 2.5 mg reserpine (Rausedyl) was dissolved in 2 litre Balaton-water and 4 animals were kept in it for 24 hours ("oral treatment"), then the animals were placed in running Balaton-water for one or 20 days; 2. 2.5 mg reserpine (Rausedyl) diluted with 1 ml *Anodonta*-Ringer was injected into the foot and the ganglia were prepared after 12 hours. In all cases of reserpine treatment GA-Os double fixation was used.

After dehydration with ethanol the ganglia were embedded in Araldite (Durcupan, ACM, Fluka). The sections were cut on an LKB Ultrotome III. ultramicrotome, the micrographs were taken with a TESLA BS 413A electron microscope. The sections were contrasted with uranyl acetate and lead citrate (REYNOLDS, 1963).

In all cases, micrographes of a final enlargement of 58.500 were used for measurements of the average diameters and frequency distribution of the vesicles. The size ranges of each column of the histogram increased by one mm beginning at the smallest diameter (3 mm = 513 Å).

Results

Double fixation (GA-Os)

In the axon terminals and axons of the neuropile of both ganglia five types of vesicle can be distinguished on the basis of their morphology and size. *1.* Probably peptridergic neurosecretory vesicles (PNV) with a diameter of 1000—1900 Å. They are characterized by a fine granulated inner content entirely filling the vesicles and by an irregular form *(Fig. 1a). 2.* Large dense-core vesicles (LDCV) with a diameter of 1200—1800 Å. They are spheroid with a core of medium of high density separated only by a narrow halo from the bordering membrane *(Fig. 1b). 3.* Small dense-core vesicles (SDCV) with a diameter of 700—1200 Å and a core of varying electron opacity *(Fig. 1c). 4.* So-called "eccentric" dense-core vesicles (EDCV). Their size strongly varies (1000—2000 Å). They have an ovoid form and a core of varying size and density but always of eccentric localization *(Fig. 1d). 5.* Clear vesicles (CV) with an average diameter of 600—800 Å *(Fig. 1e).*

From the above five types of vesicle, LDCV, SDCV and CV occur predominantly in the axons of neuropile as a rule mixed with each other. All three types can always be observed in the axon terminals recognized by their synaptic-like connections *(Figs 4a* and *4b).* However CV may be absent in other axon-profiles; LDCV or SDCV types may occur alone. The PNV are contained always by a special axon type *(Fig. 2)* occurring rather rarely and representing only a small fragment of all the axon profiles. The EDCV are of varying occurrence. In some axons and terminals they are the only vesicular components *(Fig. 3),* but in other ones they are mixed with LDCV, SDCV and CV.

The majority of the neuronal somas contain no dense-core vesicles. In some of them SDCV and LDCV are observed in relatively small number. Somas showing only EDCV components have also been found *(Fig. 7).*

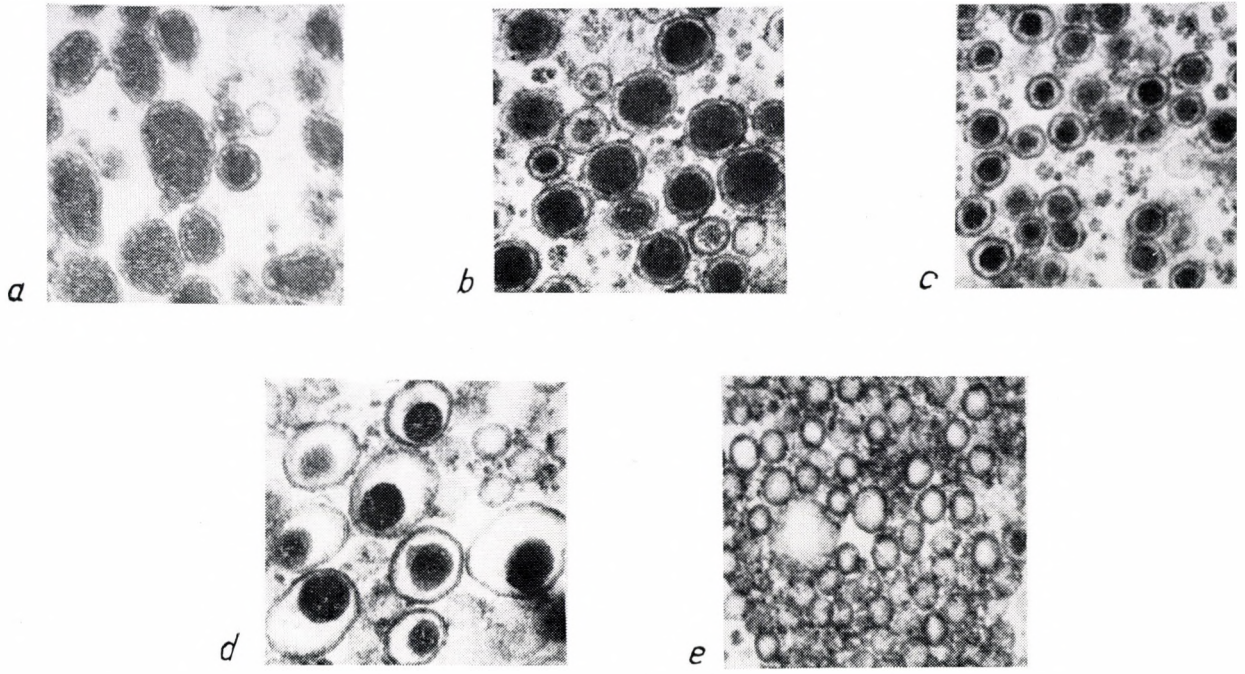

Fig. 1. Different vesicle types in the axons of the neuropile. *a)* Peptidergic neurosecretory — PNV; *b)* large dense-core — LDCV; *c)* small dense-core — SDCV; *d)* eccentric dense-core — EDCV; *e)* clear — CV. GA-Os fixation, ×58.500

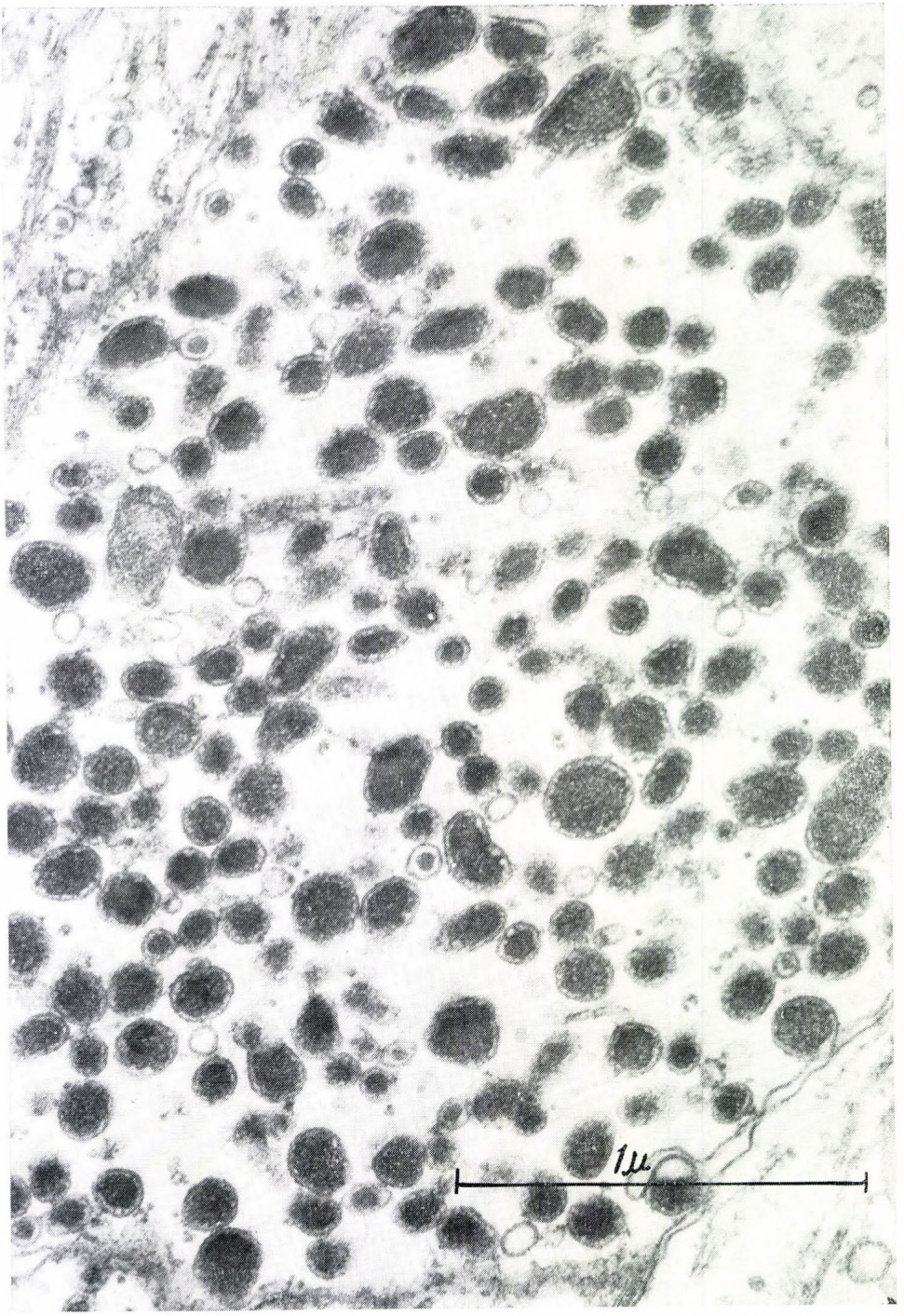

Fig. 2. Axon containing PNV in the neuropile of the pedal ganglion. GA-Os fixation, ×58.500

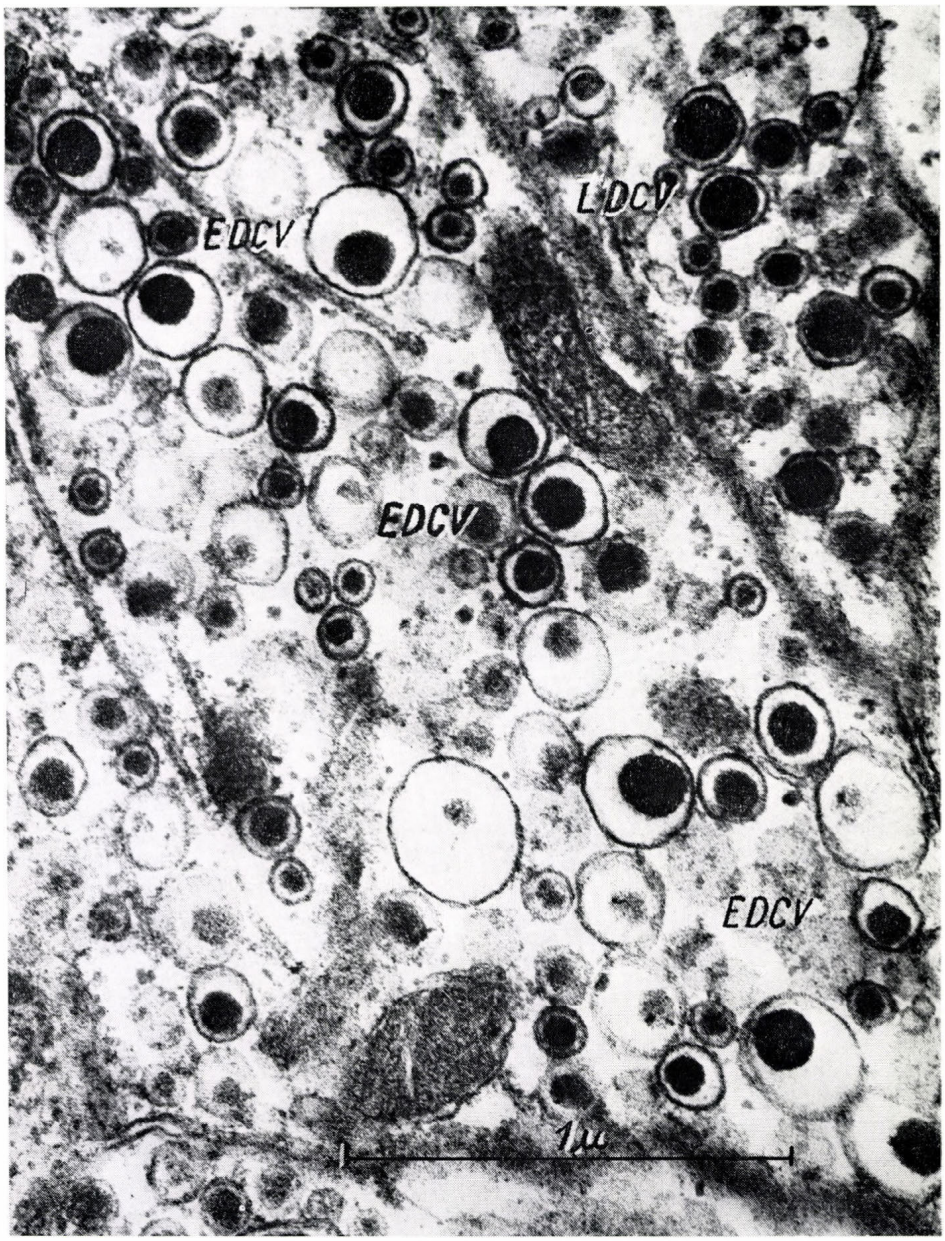

Fig. 3. Axon containing predominantly EDCV, and in the vicinity of it another one showing different types of vesicle. GA-Os fixation, ×58.500

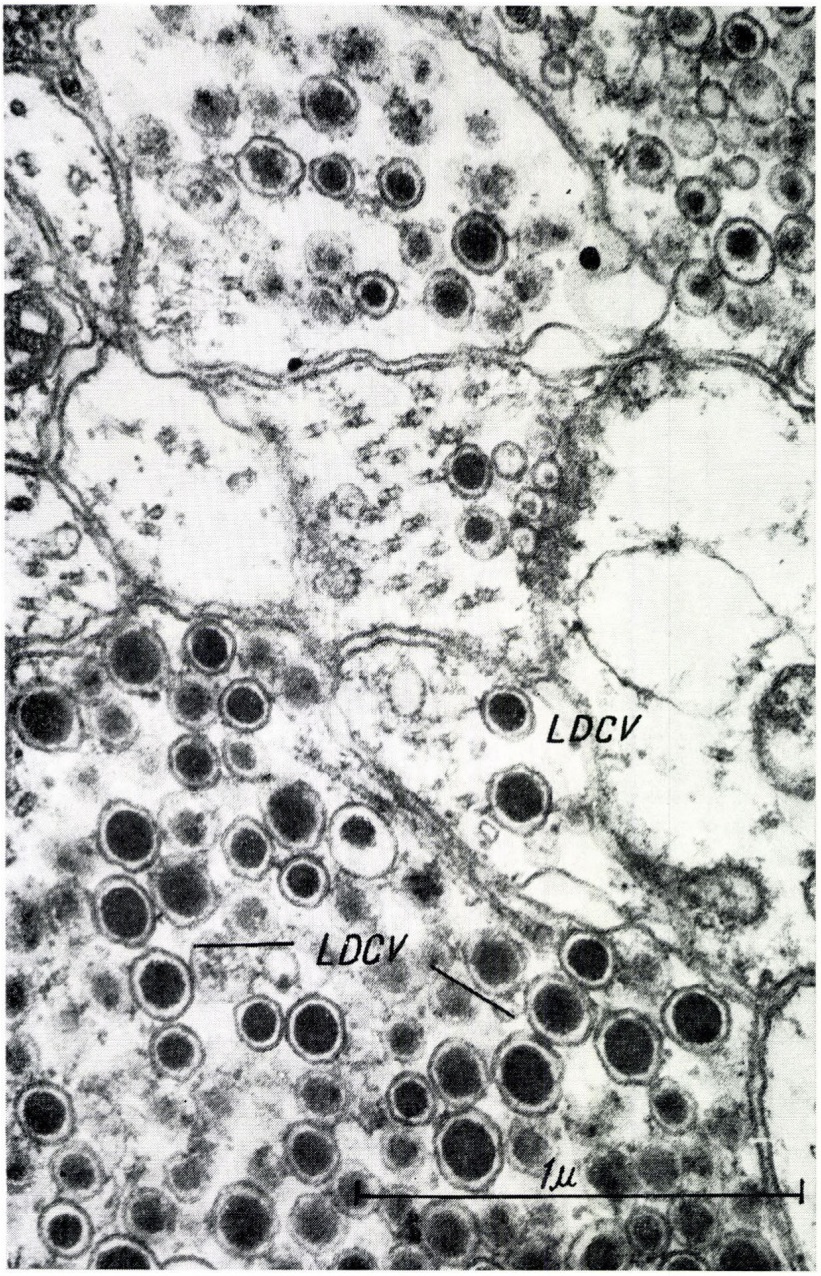

Fig. 4a. Detail from the neuropile of the pedal ganglion. Below an axon containing LDCV. GA-Os fixation. ×58.500

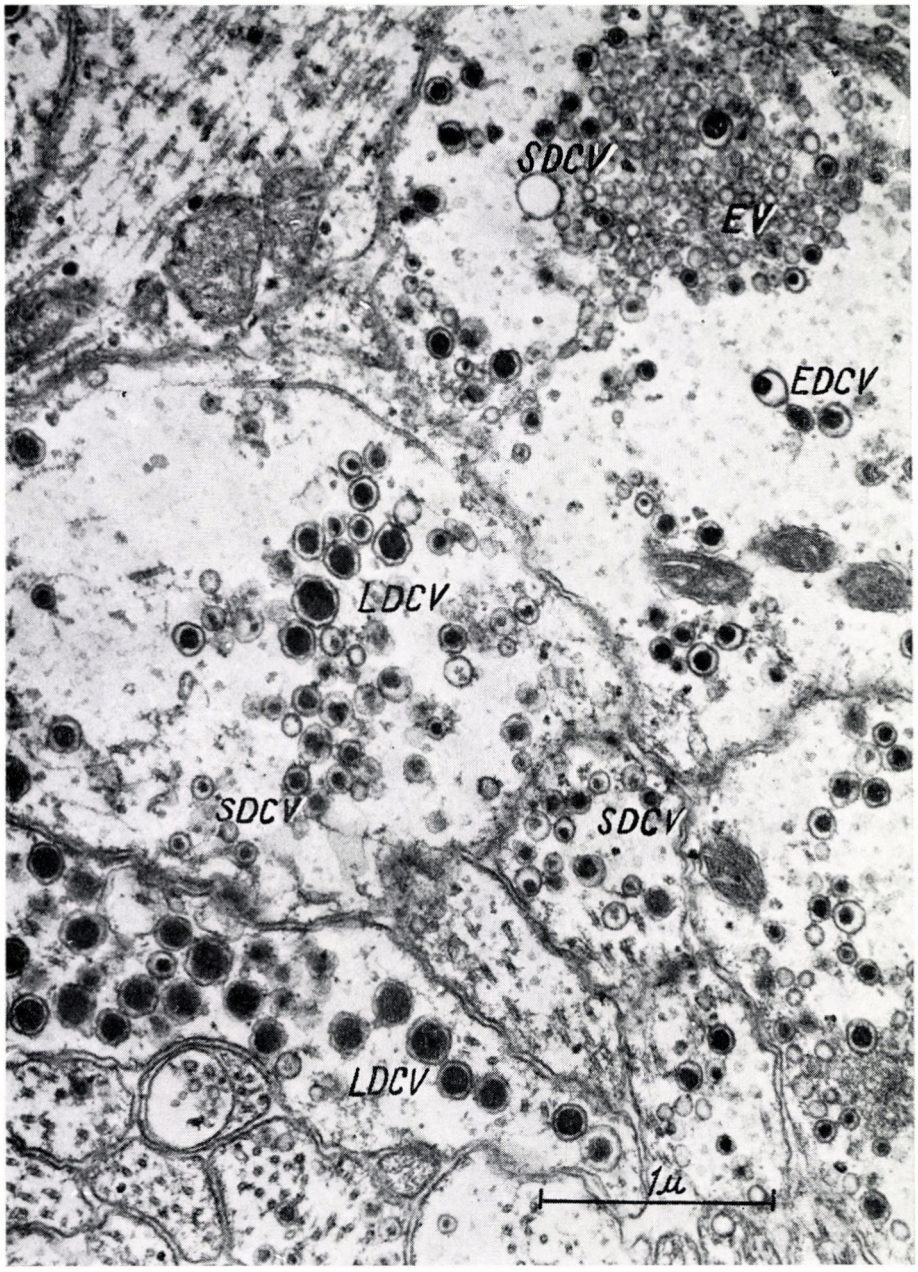

Fig. 4b. Axons transporting different vesicular profiles in the neuropile of the pedal ganglion. GA-Os fixation, ×30.000

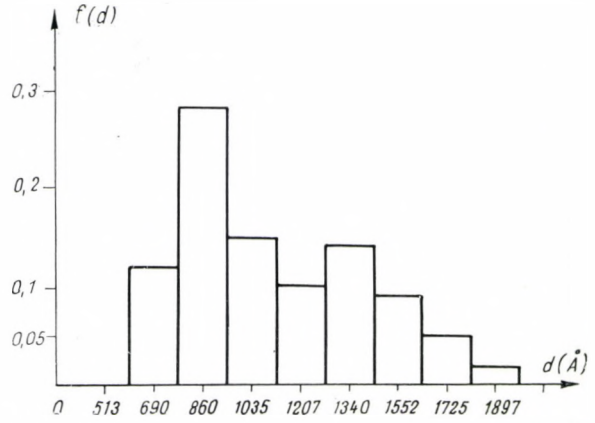

Fig. 5a. Frequency distribution histogram worked out for the CV, SDCV and LDCV

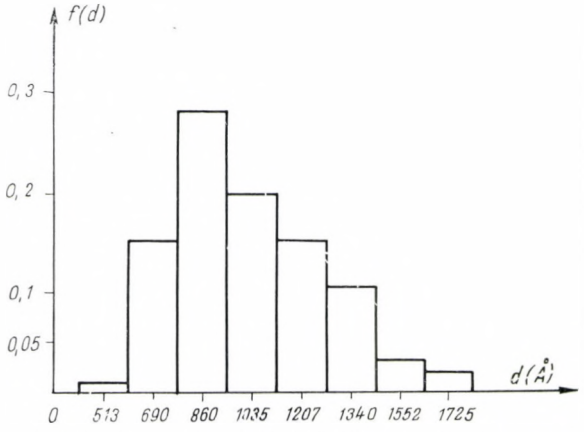

Fig. 5b. Frequency distribution of the CV, SDCV and LDCV after a 12-hour reserpine treatment, injected into the foot

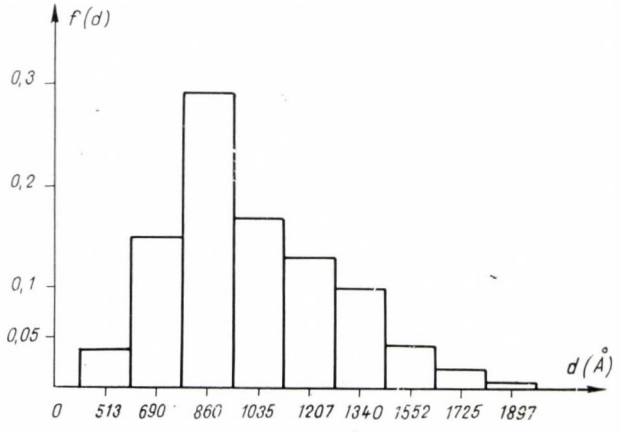

Fig. 5c. Frequency distribution histogram of CV, SDCV and LDCV one day after a 24-hour "oral" reserpine treatment

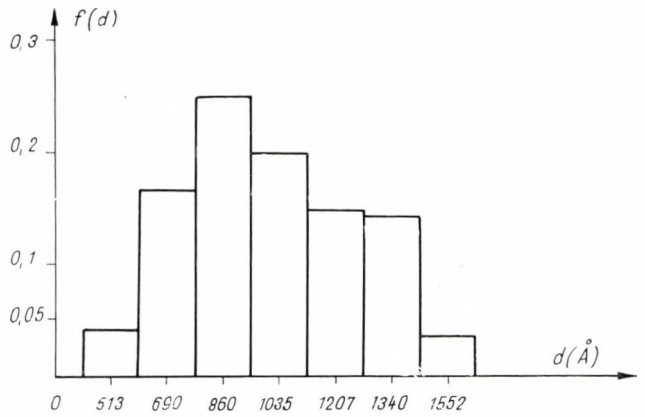

Fig. 5d. Frequency distribution histogram of CV, SDCV and LDCV types 20 days after a 24-hours "oral" reserpine treatment

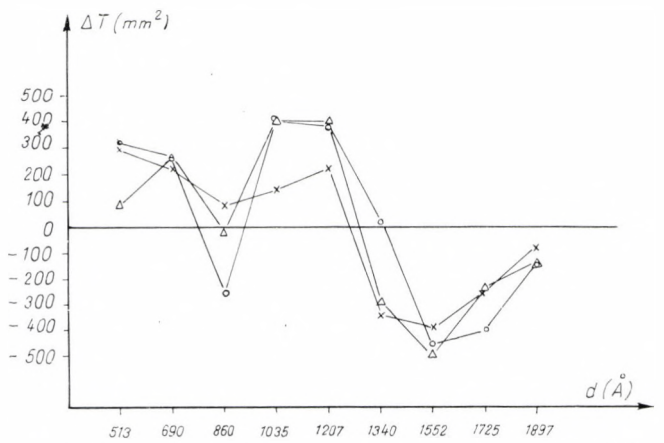

Fig. 6. The proportion of histogram areas compared to that of the control

Taking into consideration the different morphological appearance and relatively rare occurrence of PNV and EDCV profiles, only the LDCV, SDCV and CV types were counted for the frequency distribution measurements.

The frequency distribution histograms were composed for the axon profiles of the neuropile *(Fig. 5a)*. In the case of untreated normal animals, the histogram shows two maxima at the diameters of 860 Å and 1340 Å. This histogram shows certain resemblance to that taken after OsO_4 fixation (Zs.-Nagy, 1968b), but differs from that in the location of the two diameter maxima and in the absence of the smaller diameter groups.

After reserpine treatment the PNV showed no considerable alterations either in frequency or in morphology. The frequency of EDCV decreased. However, the frequency of occurrence of the other three types of vesicle changed 12 hours (injection) and 24 hours ("oral") subsequent to the treat-

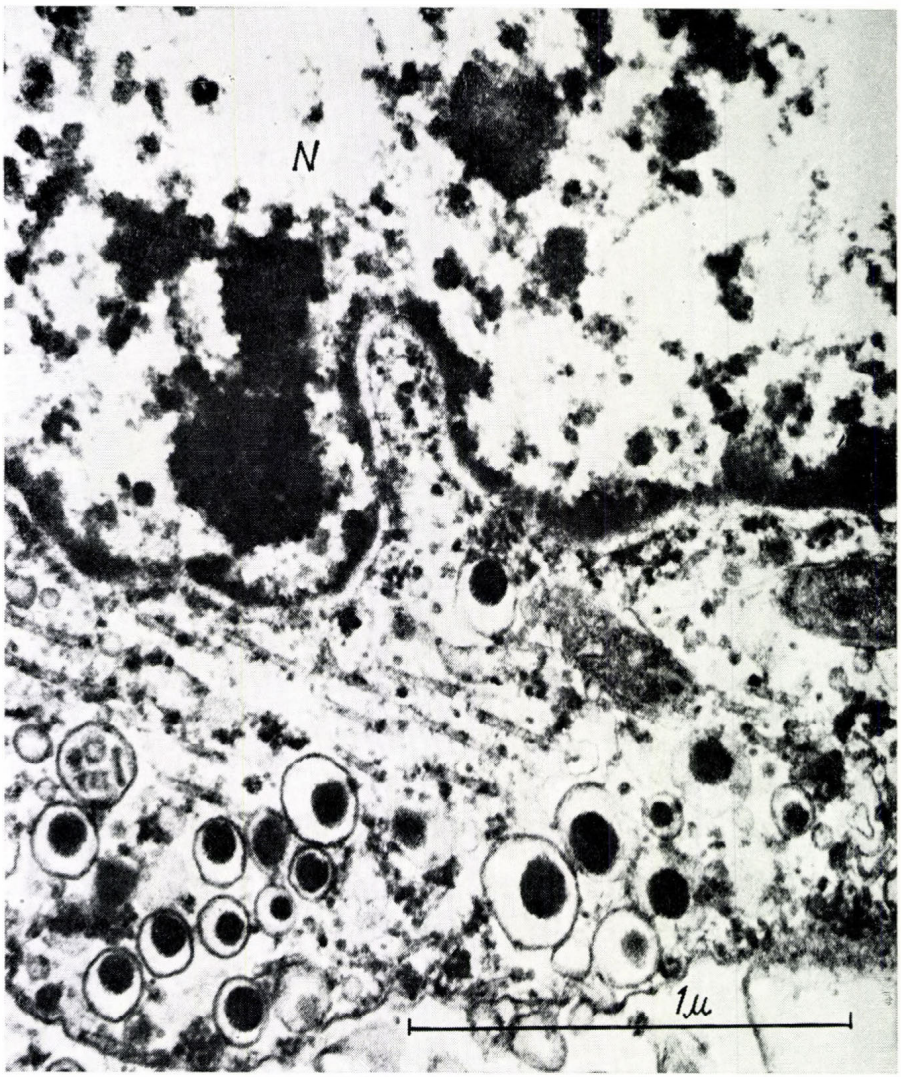

Fig. 7. Perikaryon containing EDCV in the cerebral ganglion. N = nucleus, GA-Os fixation, ×58.500

ment. There was a general diminution in the diameter values, in so far as the normal, 1100 Å average diameter decreased to 956—968 Å, and a shift on the histograms toward the smaller diameters was observed *(Fig. 5b, 5c)*. The maximum of 1340 Å of the normal histogram was absent. At the same time axons completely filled up by LDCV could later be seen *(Fig. 8)*.

Even twenty days subsequent to the reserpine treatment *(Fig. 9)* the average diameter of the vesicles was only 984 Å and the histogram was also altered *(Fig. 5d)*. The summation of the changes caused by the different reserpine treatments are shown in *Fig. 6*, representing the changes of areas belonging to the columns of histograms compared to the normal one.

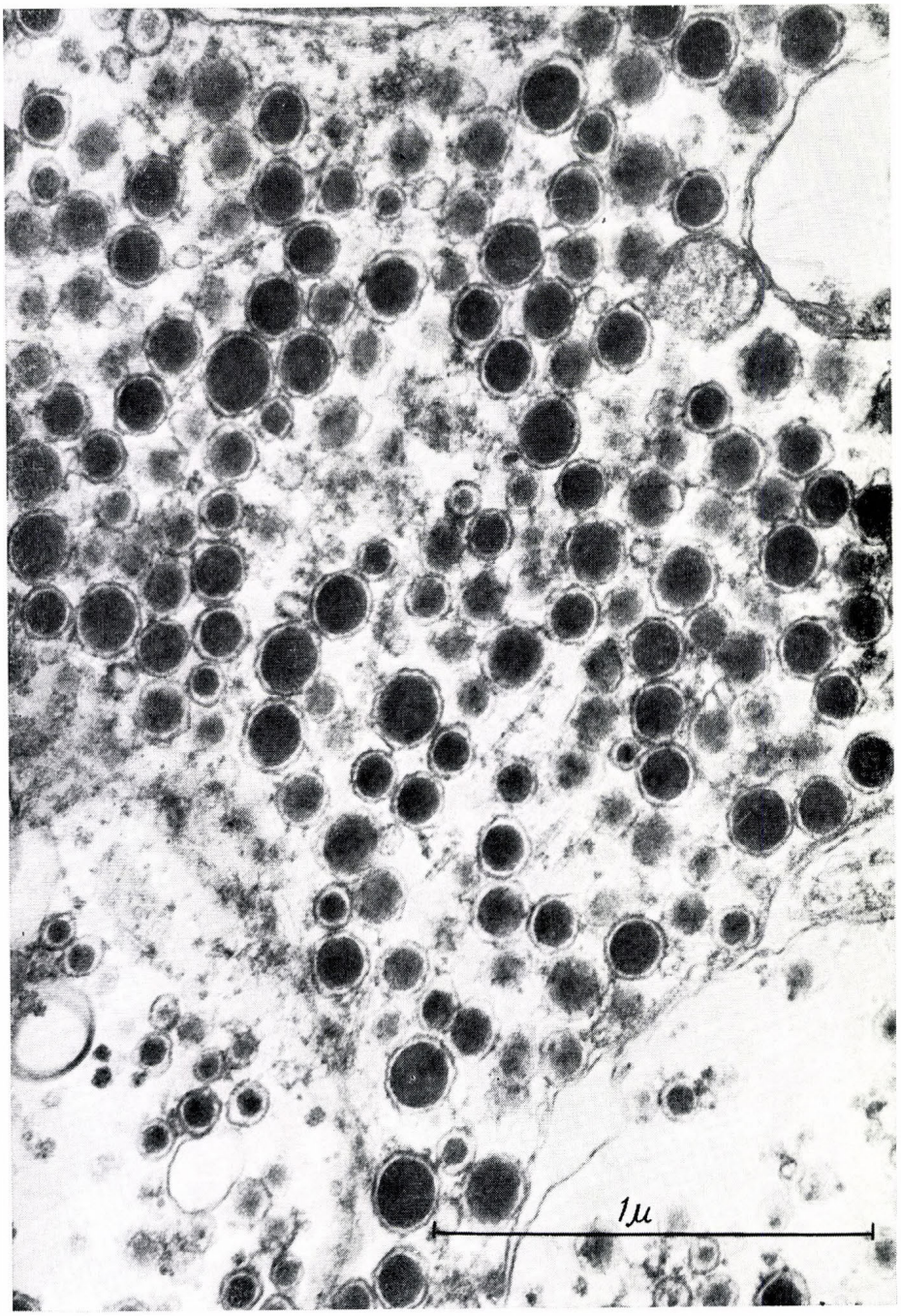

Fig. 8. Axon showing LDCV after a 24-hour reserpine treatment. GA-Os fixation, ×58.500

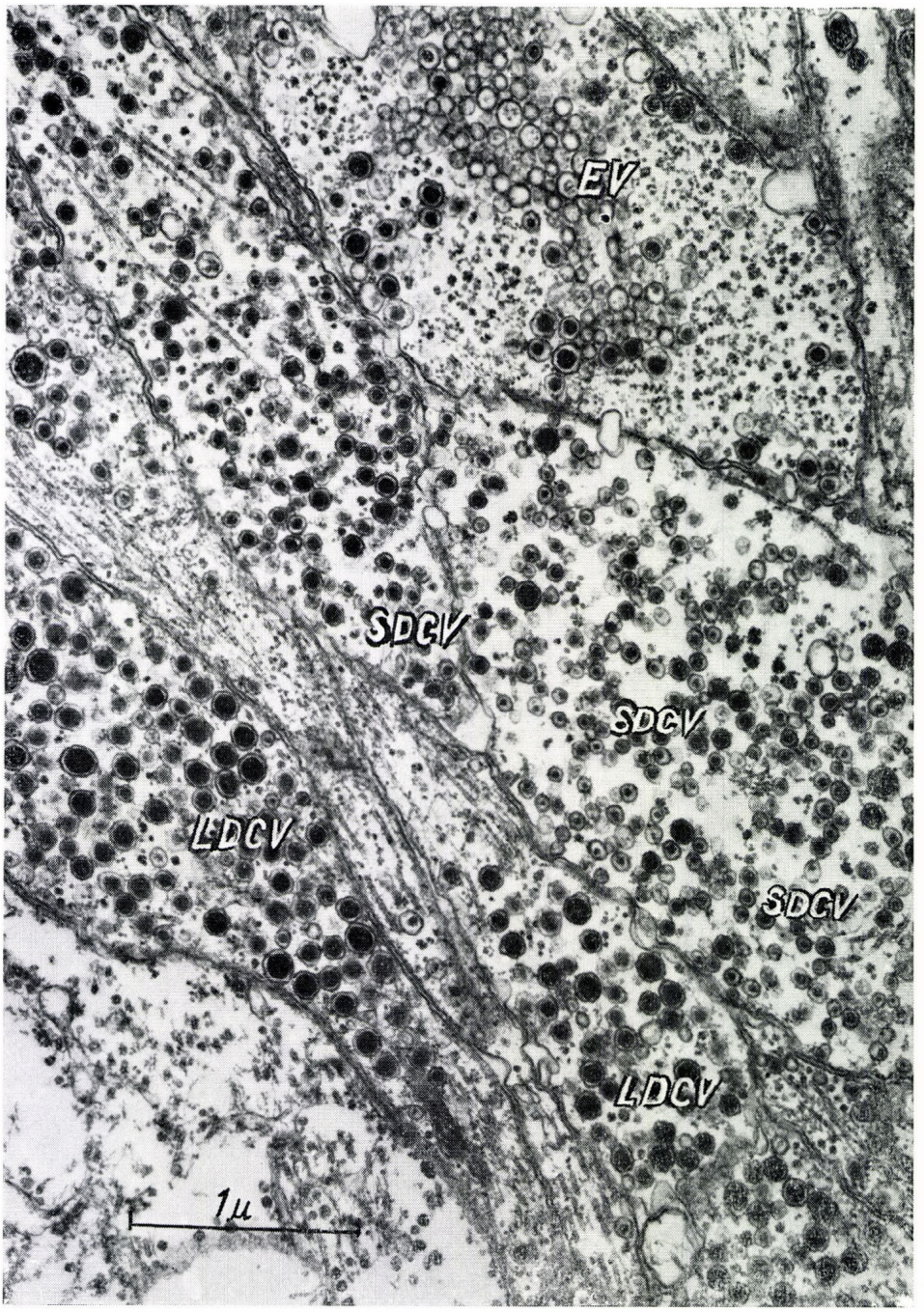

Fig. 9. Detail from the neuropile of the pedal ganglion 20 days after reserpine treatment. GA-Os fixation, ×58.500

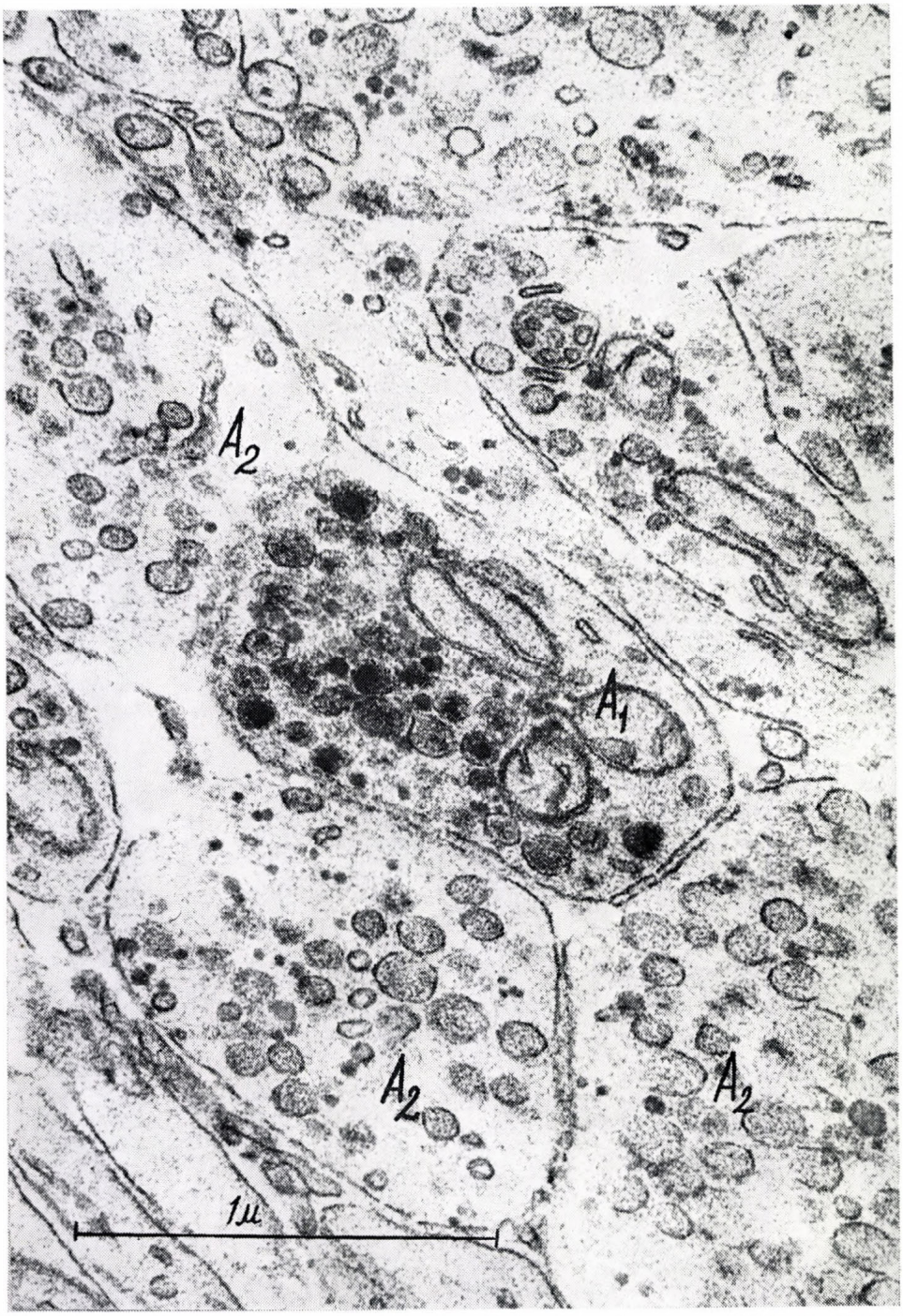

Fig. 10. Detail from the neuropile after $KMnO_4$ fixation. Axon containing granulated vesicles (A_1) is surrounded by other ones showing only "empty" vesicles (A_2). × 58.500

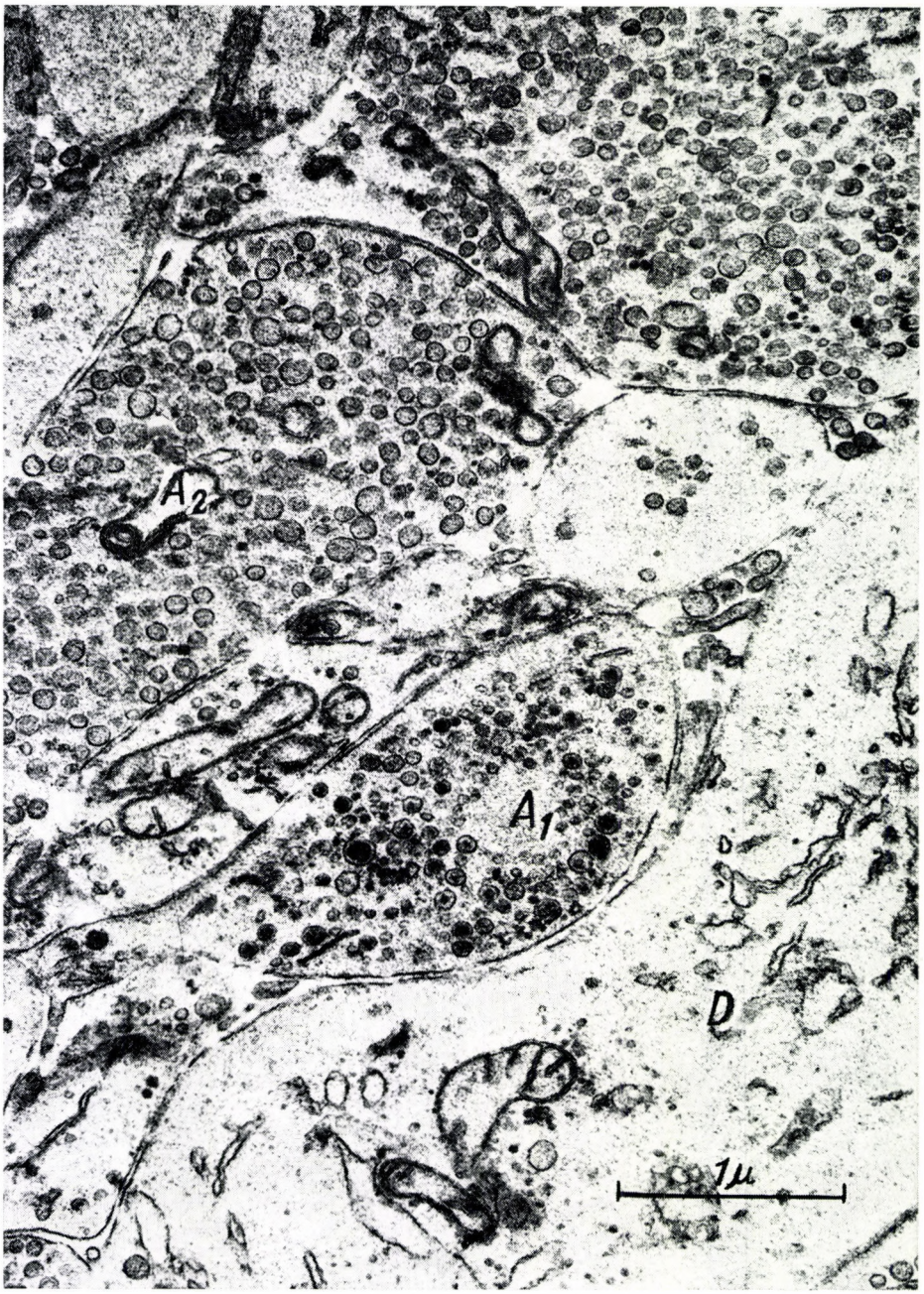

Fig. 11a. Axons differing from each other on the basis of their vesicle content in the neuropile of the pedal ganglion. D = dendrite, ×30.000

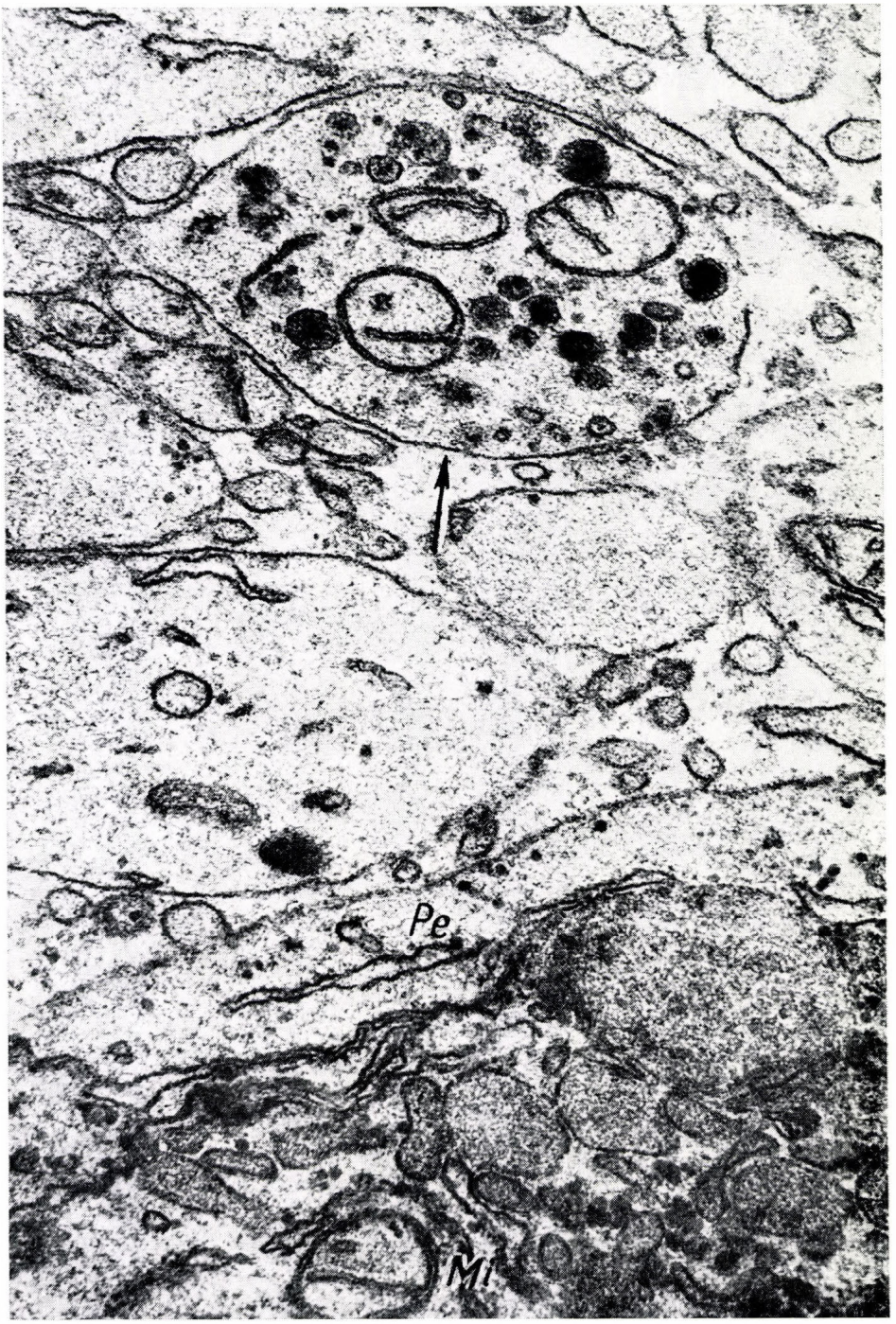

Fig. 11b. Axon with granulated vesicles (arrow) and detail of a perikaryon (Pe) from the cerebral ganglion. Mi = mitochondrium, ×58.500

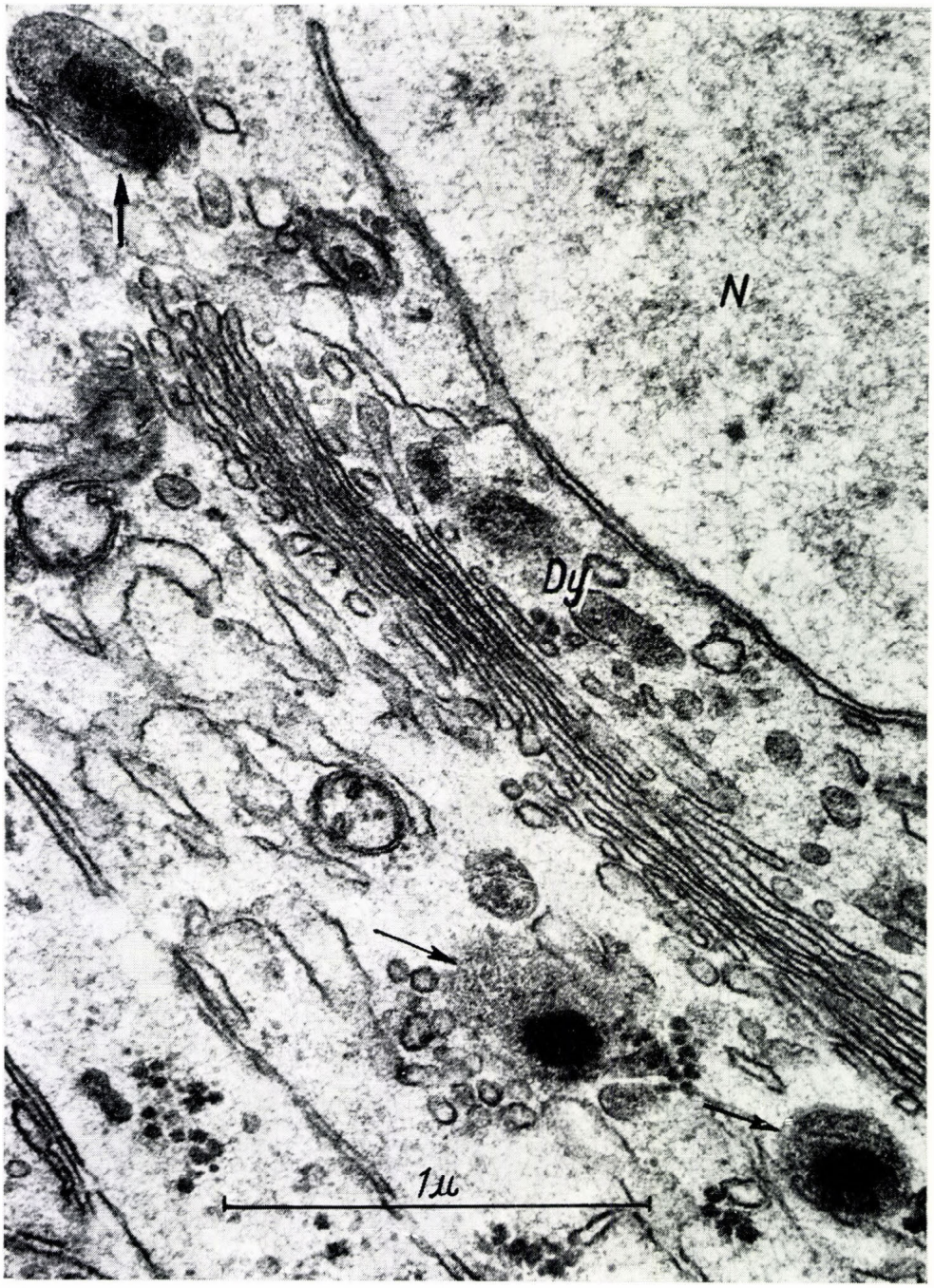

Fig. 12. Special granules of 3000—4000 Å diameters (arrows) in the perikaryon. N = nucleus, Dy = dyctiosome, ×58.500

Potassium permanganate fixation

After the 1.5% and 3% $KMnO_4$ fixation the morphology of both the neuropile and the somas, as well as the appearance and composition of the vesicle population were quite different from those observed after GA-Os fixation. In the axons and terminals two kinds of vesicle can be distinguished: *1.* Granulated vesicles (1050 Å in average) possessing a core of varying density *(Fig. 10); 2.* "Empty" vesicles (averagely 950 Å). Although a homogeneous material of low density can also be found inside this types, granular reaction never occurs *(Fig. 10)*. It can be established that the two types of vesicle are transported by different kinds of axons and the mixture of the two types can rarely be observed *(Figs 11a* and *b)*.

After $KMnO_4$ fixation no vesicles could practically be seen in the perikarya, nevertheless sometimes big (3000—4000 Å) granules appeared at the Golgi-membranes containing a dark precipitation embedded into a matrix of lower electron density *(Fig. 12)*.

Discussion

In the central and peripheral adrenergic neurons of vertebrates the appearance of the vesicles are influenced by the fixation methods applied (TRANZER and SNIPES, 1968; TRANZER et al., 1969; PELLEGRINO DE IRALDI and GUEUDET, 1969; BLOOM, 1970). The results of HANNEFORTH (1965), RUDE et al. (1969), MYHRBERG (1971, 1972), MANCINI and FRONTALI (1970) show that after OsO_4, GA-Os and $KMnO_4$ fixation the morphology of the vesicles were different even in invertebrates *(Helix pomatia,* RETZIUS-cells of the leech, *Lumbricus terrestris, Periplaneta americana)*. Our results compared to those of OSO_4 fixation (GERSCHENFELD, 1963; ZS.-NAGY, 1968a, b) also indicate that in the neurons of the central nervous system of the fresh water mussel, the composition and the appearance of the vesicle population are affected by the different kinds of fixation (OsO_4, GA-Os, $KMnO_4$). After GA-Os fixation at least five types of vesicles can be distinguished in the neuropile: CV, SDCV, LDCV, EDCV and PNV. The "small empty, small granular and large granular" types of the four vesicular profiles observed in marine species (LOVELAND, 1963; COTTRELL, 1968) can be considered identical with the CV, SDCV and LDCV forms. At the same time, we failed to find the type containing presumably the substance X (COTTRELL and MASER, 1967).

According to some results (GERSCHENFELD, 1963; SAKHAROV et al., 1965), the central and peripheral neurons of Gastropods can be characterized by three types of vesicle after OsO_4 fixation: clear, dense-core and neurosecretory ones. On the other hand, clear synaptic and neurosecretory vesicle were found in the intestinal nerves of *Helix pomatia* (HANNEFORTH, 1965), while both clear and several kinds of dense-core vesicles were present in the abdominal ganglia of *Helix aspersa* (COGGESHALL, 1967; FRAZIER et al., 1967). After GA-Os fixation, four types of vesicle and granules — resembling the morphology of those of *Mercenaria* and *Spisula* — occurred in the central and peripheral nerves of *Glossodoris* (NICAISE et al., 1968) and in the peripheral nerves of *Helix pomatia* (BOGUSCH, 1972). Larger (800—1500 Å) and smaller (500 Å) dense-core vesicles were also be seen among the four types of profile.

In the axons of the neuropile of the central nervous system of *Octopus* five types of vesicular form have been distinguished on the basis of their morphology, frequency and zinc iodide-osmium reactivity (BARLOW and MARTIN, 1971).

In other invertebrate nervous systems we know only a single report (BEST and NOEL, 1969; *Planaria)*, according to which only one type of dense-core vesicle predominates beside the clear ones.

Comparing the results of the single OsO_4 fixation to those of GA-Os in the *Anodonta* ganglia, we can establish that the latter preserves much better the structure of the vesicular components, than the former one. This manifest itself on the one hand, in much rarer occurrence of vesicles showing morphologically "emptying" form, and in the much more expressed dense core; and on the other hand in the lower number of clear vesicles as well as in the larger average diameter of all vesicular profiles. Analyzing the effects of the reserpine treatment those phenomena should be kept in mind. Undoubtly much more noticeable alterations were revealed by the single OsO_4 fixation after reserpine treatment. More than 90 percent of the vesicles appeared as "emptying" and the average diameter decreased markedly (ZS.-NAGY, 1968b), meanwhile the monoamino content became undetectable fluorescence histochemically (ZS.-NAGY, 1967). After GA-Os fixation, the extensive emptying of the vesicles could not be observed, being in accordance with the better preservation of the structure, however, the average diminution of diameter has been detected, though to a considerably less extent than after the single OsO_4 fixation.

The inner content of the dense-core vesicles is evidently not identical with the monoamine content, but is more of the latter. Some carrier proteins (BLOOM and AGHAJANIAN, 1966; 1968), and perhaps enzymes may also be present there. It is also indicated by the fact that after the depletion of the monoamines, the pseudoisocyanine positivity (STERBA, 1961, 1964) showing the presence of certain proteins can still be observed (ZS.-NAGY, 1968b). The protein nature of the dense core might be supported by the result that after glutaraldehyde-dichromate fixation (WOOD, 1966), vesicles showing no precipitation but possessing dense core could be found in the axons (ELEKES, unpublished observation). The internal content of the dense-core vesicles deprived of their monoamines seems to become unstable to such an extent that they appear "emptying" or empty after OsO_4 fixation, while in the same state, they preserve almost completely their intact character using GA-Os fixation. It can therefore be assumed that the alteration of the monoamine content of the dense-core vesicles is indicated more "sensitively" by the single OsO_4 fixation, than by the double one.

Investigating the effect of reserpine on the histograms, first of all the normal state should be analyzed. The normal histogram can be regarded as composed of two symmetrical curves. One with a maximum at 860 Å, the other at 1340 Å, while the group of 1207 Å can be divided between the two main groups. The first group represents 67 percent of the total vesicle population whereas the second 37 percent. On this basis we have to suppose that two different kinds of vesicle are represented by the SDCV and LDCV groups at least morphologically but presumably even functionally. Upon the effect of reserpine treatment the maximum of 1340 Å disappears and the rate of the vesicles of this group decreases from 37 to 24 percent. This change of 13 percent is distributed between the smaller diameters so that e.g. in case of the

reserpine treatment of 24 hours, the rates of the vesicles increase in all groups from 1207 Å toward the smaller ones, moreover a new group with a diameter of 513 Å also appears *(Fig. 5c)*.

Our results show that the vecisles of the LDCV group are able to transform even into smaller ones and then they cannot be distinguished any longer from the original SDCV profiles. Taking into consideration the results of the OsO_4 fixation neither can the former hypothesis (Zs.-Nagy, 1968 a, b) be neglected, according to which all of the dense and clear vesicles, except the PNV and EDCV, represent only one kind of the vesicular element showing only different morphological appearance in the moment of fixation depending on the actual functional state. The present experiments, however, suggest also an other possibility that the dense-core vesicles belonging to the two maxima represent different qualities affected by reserpine differently when using double fixation.

Comparing our results to biochemical and histochemical investigations we have to conclude that after GA-Os fixation the monoamine content of the vesicles is not indicated by the state of the dense centre of the vesicles. In other words, according to both biochemical (Hiripi, 1973) and histochemical (Zs.-Nagy, 1967; 1968 b) investigations the monoamine content of the ganglia was markedly decreased by the reserpine treatment, but the state of the dense-core vesicles showed no correlation with those drastic alterations after double fixation. It has also been concluded in cases of other invertebrates *(Periplaneta, Shistocerca, Blabera)* that after GA-Os fixation the dense core and the catecholamine content of the vesicles are not in direct connection (Mancini and Frontali, 1970; Chanussot et al., 1969). According to Hiripi's (1973) biochemical investigations reserpine applied in the water ("oral" reserpine treatment) considerably diminished the monoamine content of the ganglia by the fifth day (to 10 percent of the original level) and even in 25 days it was at the same low level. This is in accordance with our histochemica-investigations as regards the visceral and pedal ganglia after reserpine treatl ment applied in injection, but in the cerebral ganglia an intense regeneration can be seen already 1 day after the reserpine administration, and in the 8th day the neuropile of the cerebral ganglia showed a monoamine fluorescence quite similar to that of the normal one (Zs.-Nagy, 1967, 1968 b). Further investigations may reveal the reason of regeneration of the fluorescence in the cerebral ganglia, during the period when the biochemical measurements yet fail to indicate the return of dopamine and noradrenaline content.

After $KMnO_4$ fixation, the dense core indicates the monoamine present in the vesicles at the moment of fixation (Hökfelt, 1970, 1971). According to our investigations, a considerably smaller percent of the axons contain dense-core vesicles (granulated vesicles type — 1050 Å) in the neuropile of the fresh water mussel, than after GA-Os fixation. After $KMnO_4$ fixation, the "empty" vesicles (950 Å) occurred in the majority of the axons. High dopamine content of the ganglia has been proved by fluorescence histochemical (Zs.-Nagy, 1967, 1968 b) and biochemical (Hiripi, 1972) investigations. Thus, we are presumably faced with a similar situation encountered in the dopamine-rich regions of the central nervous system of mammals (Hökfelt, 1967, 1968), *Lumbricus terrestris* (Myhrberg, 1972), *Periplaneta americana* (Mancini and Frontali, 1970), according to which dopamine seems to be more loosely bound to the matrix of vesicles and "escapes" before $KMnO_4$ reaches it. At

the same time, the supposition that the dense cores of GA-Os fixation are not identical with the monoamine are supported by the electron microscopic pictures of the $KMnO_4$ fixation.

After GA-Os fixation, the ovoid vesicles possessing always eccentric dense cores and classified into a separate group (EDCV) can be observed in a part of both the somas and the axons, in the latter ones even mixed with CV, SDCV and LDCV types. It cannot be regarded as an independent type following $KMnO_4$ fixation. In vertebrates, that type has been described in the sympathetic fibres of the pineal gland (MACHADO, 1971), being observable at the formation of the small and large granular vesicles from the smooth endoplasmic reticulum. Therefore, according to our opinion three possibilities must be taken into consideration in relation with the EDCV type: *1.* it may be regarded as a variety of LDCV, appearing in a special form after double fixation; *2.* it is a variation of the LDCV being in the state of mobilization; *3.* it is indeed an independent type.

On the basis of the general considerations in relation to the neurosecretory vesicles (MYHRBERG, 1972), the vesicles of 1000—1900 Å diameter (PNV) observed after GA-Os fixation, represent presumably peptidergic neurosecretion. After $KMnO_4$ fixation, the identification of this type is difficult, mainly due to their rare occurrence. They probably loose their dense cores (MYHRBERG, 1972). The possibility can even be raised that the large vesicles occurring in small number in the axons transporting CV, SDCV, and LDCV also represent peptidergic neurosecretion. Their size, morphology and internal content might indicate it. According to BARANYI (1971), two types of neurosecretory vesicle can be observed in the central nervous system of the *Anodonta cygnea* L. The elucidation of the whole problem needs further investigations, so much the more as in the case of the ganglia of Pelecypoda, one cannot speak about well-defined neurosecretory systems or neurohaemal areas, as in Gastropoda (JOOSSE, 1964; RÖHNISCH, 1964; SAKHAROV et al., 1965; NOLTE, 1965; SIMPSON et al., 1966; BOER et al., 1968; WENDELAAR BONGA, 1970).

Summary

1. The ultrastructure of the cerebral and pedal ganglia of freshwater mussel (*Anodonta cygnea* L.) has been investigated in order to analyze the vesicles populations after different fixations (glutaraldehyde-osmium, $KMnO_4$).

2. After glutaraldehyde-osmium fixation five vesicle types can be distinguished: clear vesicles (CV), small dense-core vesicles (SDCV); large dense-core vesicles (LDCV); eccentric dense-core vesicles (EDCV) and presumably peptidergic neurosecretory vesicles (PNV). The latter two types occur rather rarely, the former three represent the majority of the vesicles in the neuropile. The frequency distribution histogram of the main types shows two maxima.

3. On the effect of reserpine treatment the maximum of the type of larger diameter (LDCV) disappeared, and the distribution became more homogeneous. After double fixation the dense cores of the vesicles do not reflect the depletion of the monoamines.

4. After $KMnO_4$ fixation, only two types of vesicle can be distinguished.

5. The functional significance of the vesicle types is discussed.

REFERENCES

B. Baranyi I. (1971): Adatok a tavi kagyló *(Anodonta cygnea)* neuroszekréciós sejtjeinek finom szerkezetéhez. — *Biol. Közl.* **19,** 143—148.

Barlow, J., Martin, R. (1971): Structural identification and distribution of synaptic profiles in the Octopus brain using the zinc-iodide osmium method. — *Brain. Res.* **25,** 241—253.

Bennett, H. S., Luft, J. H. (1959): S-collidine as a basis for buffering fixatives. — *J. Biophys. Biochem. Cytol.* **6,** 113—114.

Best, J. B., Noel, J. (1969): Complex synaptic configurations in Planarian brain. — *Scince* **164,** 1070.

Bloom, F. E. (1970): The fine structural localization of biogenic monoamines in nervous tissue. — *Int. Rev. Neurobiol.* (Ed.: Pfeiffer, C. C. and Smythies, J. R.) *Acad. Press,* **13,** 27—66.

Bloom, F. E., Aghajanian, G. K. (1966): Cytochemistry of synapses: Selective staining for electron microscopy. — *Science* **154,** 1575—1577.

Bloom, F. E., Aghajanian, G. K. (1968): Fine structural and cytochemical analysis of the staining of synaptic junctions with phosphotungstic acid. — *J. Ultrastr. Res.* **22,** 361—375.

Boer, H. H., Douma, E., Koksma, J. M. A. (1968): Electron microscopy study of neurosecretory cell and neurohaemal organs in the pond snail *Lymnaea stagnalis.* — *Studies in the Structure, Physiology and Ecology of Molluscs* (Ed.: Vera Fretter) *Acad. Press* 237—256.

Bogusch, G. (1972): Zur Innervation des glatten Penisretraktormuskels von *Helix pomatia:* Allgemeine Histologie und Histochemie des monoaminergen Nervensystems. — *Z. Zellforsch.* **126,** 383—401.

Chanussot, B., Dando, J., Moulins, M., Laverack, M. S. (1969): Mise en évidence d'une amine biogène dans le système nerveux stomatogastrique des Insectes: étude histochimique et ultrastructurale. — *C. R. Acad. Sci. (Paris)* **568,** 2101—2104.

Coggeshall, R. E. (1967): A light and electron microscopic study of the abdominal ganglion of *Aplysia californica.* — *J. Neurophysiol.* **30,** 1263—1287.

Cottrell, G. A. (1968): Amines in molluscan nervous tissue and their subcellular localization. — In: *Invertebrate Neurobiology* (Ed.: J. Salánki) *Akadémiai Kiadó* 353—364.

Cottrell, G. A., Maser, M. (1967): Subcellular localization of 5-hydroxytriptamine and substance X in molluscan ganglia. — *Comp. Biochem. Physiol.* **20,** 901—906.

Frazier, W. T., Kandel, E. R., Kupfermann, I., Waziri, R., Coggeshall, R. E. (1967): Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica.* — *J. Neurophysiol.* **30,** 1289—1351.

Gerschenfeld, H. M. (1963): Observations on the ultrastructure of synapses in some Pulmonate Molluscs. — *Z. Zellforsch.* **60,** 258—275.

Hanneforth, W. (1965): Struktur und Funktion von Synapsen und synaptischen Grana in Gastropodennerven. — *Z. Vergl. Physiol.* **49,** 485—520.

Hiripi, L. (1972): Catecholamines in the different tissues of fresh water mussel *(Anodonta cygnea* L., Pelecypoda) analysed by thin-layer chromatographic and fluorimetric methods. — *Annal. Biol. Tihany* **39,** 13—20.

Hiripi, L. (1973): Pharmacological analysis on the regulatory mechanisms of the periodic activity of the fresh water mussel *(Anodonta cygnea* L., Pelecypoda). — *Annal. Biol. Tihany* **40** 27—53.

Hökfelt, T. (1967): The possible ultrastructural identification of tubero-infundubular dopamine-containing nerve endings in the median eminence of the rat. — *Brain. Res.* **5,** 121—123.

Hökfelt, T. (1968): In vitro studies on central and peripheral monoamine neurons at the ultrastructural level. — *Z. Zellforsch.* **91,** 1—74.

Hökfelt, T. (1970): Electron microscopic studies on peripheral and central monoamine neurones. — In: *Aspects of Neuroendocrinology* (Eds.: W. Bargmann *and* B. Scharrer) *Berlin—Heidelberg—New York* 79—94.

Hökfelt, T. (1971): Ultrastructural localization of intraneuronal monoamines. Some aspects of methodology. — In: *Progress in Brain Research* **34,** 213—222.

Joosse, J. (1964): Dorsal bodies and dorsal neurosecretory cells of the cerebral ganglia of *Lymnaea stagnalis* L. — *Arch. néerl. Zool.* **16,** 1—103.

LOVELAND, R. E. (1963): Some aspects of cardio regulation of *Mercenaria mercenaria*. — *Ph. D. Thesis, Harvard Univ.*

MACHADO, A. B. M. (1971): Electron microscopy of developing sympathetic fibres in the rat pineal body. The formation of granular vesicles. — *Progr. Brain Res.* **34,** 171—185.

MANCINI, G., FRONTALI, N. (1970): On the ultrastructural localization of catecholamines in the beta lobes (corpora pedunculata) of *Periplaneta americana*. — *Z. Zellforsch.* **103,** 341—350.

MARCZYNSKI, T. (1959): The fresh-water clam *Anodonta cygnea* L. as a test object for serotonin and related compounds. — *Bull. Acad. Polon. Sci. Biol.* **7,** 147—150.

MYHRBERG, H. E. (1971): Ultrastructural localization of monoamines in the epidermis of *Lumbricus terrestris* (L.). — *Z. Zellforsch.* **117,** 139—154.

MYHRBERG, H. E. (1972): Ultrastructural localization of monoamines in the central nervous system of *Lumbricus terrestris* (L.) with remarks on neurosecretory vesicles. — *Z. Zellforsch.* **126,** 348—362.

NICAISE, G., PAVANS, M. DE CECCATTY, BALEYDIER, C. (1968): Ultrastructures de connexions entre cellules nerventes, musculaires et glio-interstitielles chez *Glossodoris*. — *Z. Zellforsch.* **88,** 470—486.

NOLTE, A. (1965): Neurohämal-„Organe" bei Pulmonaten (Gastropoda). — *Zool. Jb. Anat.* **82,** 365—380.

PELLEGRINO DE IRALDI, A., GUEUDET, R. (1969): Catecholamine and serotonin in granulated vesicles of nerve endings in the pineal gland of the rat. — *Int. J. Neuropharmac.* **8,** 9—14.

REYNOLDS, E. S. (1963): The use of lead citrate in electron microscopy. — *J. Cell. Biol.* **17,** 208—212.

RÖHNISCH, S. (1964): Untersuchungen zur Neurosekretion bei *Planorbarius corneus* L. (Basommatophora). — *Z. Zellforsch.* **63,** 767—798.

RUDE, S., COGGESHALL, R. E., VAN ORDEN, L. S. III. (1969): Chemical and ultrastructural identification of 5-hydroxytriptamine in an identified neuron. — *J. Cell. Biol.* **41,** 832—854.

SAKHAROV, D. A., BOROVYAGIN, V. L., ZS.-NAGY, I. (1965): Light, fluorescence and electron microscopic studies on „neurosecretion" of *Tritonia diomedia* BERGH (Mollusca, Nudibranchia). — *Z. Zellforsch.* **68,** 660—673.

SIMPSON, L., BERN, H. A., NISHIOKA, R. S. (1966): Examination of the evidence for neurosecretion in the nervous system of *Heliosoma tenue* (Gastropoda, Pulmonata). — *Gen. Comp. Endocrinol.* **7,** 525—548.

STERBA, G. (1961): Fluoreszenzmikroskopische Untersuchungen über die Neurosekretion beim Bachneunauge *(Lampetra planeri* BLOCH). — *Z. Zellforsch.* **55,** 763—789.

STERBA, G. (1964): Grundlagen des histochemischen und biochemischen Nachweises von Neurosekret (= Trägerprotein der oxytozine) mit Pseudoisozyaninen. — *Acta Histochem.* **17,** 268—292.

TRANZER, J. P., SNIPES, R. L. (1968): Fine structural localization of noradrenaline in sympathetic nerve terminals: A critical study on the influence of fixation. — *Proc. 4th Europ. Reg. Conf. Electr. Micr. Rome* **2,** 519—520.

TRANZER, J. P., THOENEN, H., SNIPES, R. L., RICHARDS, J. G. (1969): Recent developments on the ultrastructural aspect of adrenergic nerve endings in various experimental conditions. — *Progr. Brain. Res.* **31,** 33—46.

WENDELAAR BONGA, S. E. (1970): Ultrastructure and histochemistry of neurosecretory cells and neurohaemal areas in the Pond snail *Lymnaea stagnalis* (L.). — *Z. Zellforsch.* **108,** 190—224.

WOOD, J. G. (1966): Electron microscopic localization of amines in central nervous tissue. — *Nature* **209,** 1131—1133.

ZS.-NAGY, I. (1964): Electron microscopic observations on the cerebral ganglion of the fresh water mussel *(Anodonta cygnea* L.). — *Annal. Biol. Tihany* **31,** 147—152.

ZS.-NAGY, I. (1967): Histochemical demonstration of biogenic monoamines in the central nervous system of the lamellibranch mollusc *Anodonta cygnea* L. — *Acta biol. Acad. Sci. hung.* **18,** 1—8.

ZS.-NAGY, I. (1968 a): Fine structural analysis of the neurons of *Anodonta cygnea* L. (Pelecypoda). — *Annal. Biol. Tihany* **35,** 35—59.

ZS.-NAGY, I. (1968 b): Histochemical and electron microscopic studies on the relation between dopamine and dense-core vesicles in the neurons of *Anodonta cygnea* L. — In: *Neurobiology of Invertebrates* (Ed.: J. SALÁNKI) *Akadémiai Kiadó, Budapest and Plenum Press, New York* 69—84.

VEZIKULAPOPULÁCIÓK ÖSSZETÉTELÉNEK ELEKTRONMIKROSZKÓPOS VIZSGÁLATA TAVI KAGYLÓ (*ANODONTA CYGNEA* L.) KÖZPONTI IDEGRENDSZERÉBEN

Elekes Károly és Zs.-Nagy Imre

Összefoglalás

1. A cerebrális és pedális ganglion ultrastruktúráját vizsgáltuk a vezikulapopulációk elemzése céljából különböző fixálások (Glutáraldehyd-ozmium; $KMnO_4$) után.

2. Glutáraldehyd-ozmium rögzítés után 5 vezikulatípus különíthető el: üres vezikulák (EV), kis dense-core vezikulák (SDCV), nagy dense-core vezikulák (LDCV) excentrikus dense-core vezikulák (EDCV) és feltehetően peptiderg vezikulák (PNV). Utóbbi két típus meglehetősen ritka, az előbbi három képezi a vezikulák nagy többségét a neuropilben, amelyeknek megoszlási hisztogramja két maximumot mutat.

3. Reserpinkezelés hatására a nagyobb átmérőjű (LDCV) típus maximuma eltűnik a hisztogramról, a megoszlás homogénebbé válik. Kettős fixálás után a dense-core vezikulák középső része nem tükrözi a monoaminok depléciójá.

4. $KMnO_4$ fixálás után csak kétféle vezikula, "üres" és granuláris különböztethető meg.

5. Az egyes vezikulatípusok funkcionális jelentőségét diszkutálják szerzők az adatok tükrében.

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PHARMACOLOGICAL INVESTIGATIONS ON THE REGULATION MECHANISMS OF THE PERIODIC ACTIVITY OF THE FRESH WATER MUSSEL (*ANODONTA CYGNEA* L.)

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The synthesis, storage, depletion, inactivation and the pharmacological effects of the monoamines in the central nervous system indicate that these substances perform a complex function in the brain. During the last decade an attempt has been made to reveal the connection between the changes of the monoaminergic systems and the behavioural responses. One of the most useful ways for this work proved to be the alteration of the monoaminergic systems by different pharmacons and the analysis of changes of the behavioural responses. Nevertheless, the quantitative evaluation of these responses encounters significant difficulties and only the general aspects could be followed such as the increase or decrease of responsiveness. The situation has become more complicated due to some recent observations according to which many pharmacons are able to induce significant changes without affecting the monoamine level, only altering their turnover. The results, however, called attention to the role of monoamines, first of all, to that of serotonin (5HT) in the regulation of the activity. Thus e.g. seasonal changes of adrenaline, noradrenaline (NA) and 5HT parallel with the EEG activity have been described in frog by SEGURA et al. (1967). On the basis of experiments carried out also on frogs, the role of 5HT in activity regulation and temperature acclimatization processes has been emphasized in amphibia (HARRI, 1972).

Significant, however not unequivocal changes were found in the brain 5HT level during hibernation (UUSPÄÄ, 1963; SPAFFORD and PENGELLEY, 1971; DRASKOCZY and LYMAN, 1967). At the same time, seasonal changes were detected in mice kept under the same conditions (VALZELLI and GARATTINI, 1968).

The daily change of acitivity is followed by a significant change of 5HT level in rat and turtle (FRIEDMANN and WALKER, 1968; QUAY, 1963; 1967) connected with the conditions of illumination. The most convincing evidence for the regulatory role of 5HT in the activity has been presented by JOUVET (1968; 1969) who demonstrated a higher 5HT level of the brain during sleep than wakefulness.

The nervous systems of invertebrates contain a significant amount of monoamines (WELSH and MOOREHEAD, 1960; DAHL et al., 1966; SWEENEY, 1963), and although the stimulatory transmitter function of 5HT has long

been accepted, unfortunately its role in the activity regulation has hardly been investigated.

CARDOT (1971) described the seasonal change of 5HT level in *Helix pomatia*, whereas our investigations proved that the seasonal change of 5HT level regulates the initiation and maintenance of hibernation (HIRIPI and SALÁNKI, 1972).

The activity of the fresh water mussel *(Anodonta cygnea)* is characterized by a distinct periodicity. This involves a regular alternation of active and rest phases realized in different functioning of the adductor muscles and other organs (SALÁNKI and LUKACSOVICS, 1967; MORTON, 1969). The period of rest is characterized by a prolonged tonic contraction of the adductors, whereas during the active period the adductors are relaxed and perform quick, rhythmic contractions. According to previous investigations, 5HT plays a significant role in the relaxation of the adductors (SALÁNKI, 1963; SALÁNKI and LÁBOS, 1969), and one can assume that the regulation of the mechanisms maintaining the activity takes place through the serotoninergic system.

It has been shown during our previous investigations that the central nervous system of fresh water mussel contains 40—70 μg/g wet weight of 5HT (HIRIPI, 1968), 10—20 μg/g wet weight of dopamine (DA) and 1—2 μg/g wet weight of NA (HIRIPI, 1972). It has also been proved that 5HT may have connection with the regulation of the periodic activity (SALÁNKI, 1963). Further evidences were given by our earlier investigations for that role of 5HT. It is known that the stimulation of the cerebro-visceral connective (CVc) using suitable parameters is of relaxing effect on the adductors in the majority of cases, and the relaxation is especially of expressed degree upon the influence of repeated stimulation (SALÁNKI and LÁBOS, 1963). We determined the 5HT content in both adductors during relaxed state. After two hr of stimulation, the 5HT content of the anterior and posterior adductors was higher than that of the control muscles. The stimulation induced an increase of 33 percent in the anterior and 25 percent in the posterior adductor in the 5HT content, being significant in both cases.

The 5HT content was analyzed in the ganglia and the adductors at the beginning of the active and rest periods, i.e. in the opposite phases of the spontaneous periodic activity.

When measuring the 5HT content of the ganglia, only that of the visceral one changed at the beginning of the active and rest periods. It was 23 percent lower at the beginning of the active period in the visceral ganglion.

Measurements of 5HT content in the anterior and posterior adductors separatedly, revealed that the 5HT level is twice as high in the former than in the latter one. The 5HT contents were higher in both adductors at the beginning of the active state than those in the period of rest. The increase amounted to 30 percent in the anterior adductor and 25 percent in the posterior one, being significant only in the former.

Since the activity of this bivalve can quantitatively be followed on the basis of the characteristic periodicity, it represents a good object for investigating of the connections between the changes of the monoamine level and the activity pattern. The fact that the activity can be recorded not only under laboratory but also under natural conditions, represents a significant contribution to the analyses of the behavioural responses. This way one can

control the effects of the laboratory circumstances on the natural environmental conditions.

The present investigations were intended at answering the question, how far the pharmacons affecting the monoamine metabolism do alter the monoamine level and the activity, and how can we interpret those alterations from the point of view of regulatory mechanisms of the periodic activity?

Methods

Determination of 5HT

The 5HT content of 100—200 mg ganglion tissue was measured after homogenization in 0.1 n HCl using the method of KUNTZMAN et al. (1961).

Determination of catecholamines (CA)

About 200 mg of ganglion tissue was homogenized in acidified buthanol (CHANG, 1964), then centrifuged (5 min, 2000 rpm). The CA content of the supernatant was transferred to 1 ml of 0.1 n HCl in the presence of heptane by shaking (MAICKEL et al., 1968). The pH of this acidic phase was modified to 8.5 by addition of 10 vol Tris-HCl buffer (0.5 M, pH 8.5), whereas the isolation of CA using Al_2O_3 and determination was carried out by the method of ANTON and SAYRE (1962; 1964).

Treatments with pharmacons

Mussels of 150 g body weight were used. Their activity was recorded on actographs (SALÁNKI and BALLA, 1964).

The acitivity had been recorded for 3—5 weeks before the treatment took place, and for 2—5 weeks subsequent to the treatment depending on the effect. The treatments were carried out as follows: the pharmacons were dissolved in filtered Balaton-water, then 4 mussels were placed in 2 l of water containing the pharmacons: the animals were kept in the solutions for 24 hr, except that of tranylcypromine, where the treatment had only a 10 hr duration. After the treatment, the solutions were changed to normal running water. In the cases of control animals the running of the Balaton-water was stopped for the same period of time as that of the treatments. At different points of time, the 5HT and CA contents of the nervous systems of the animals were measured. The pharmacons used were as follows:

5HT-creatinine sulphate	10	μmole/l
NA-bitartarate	10	,,
DA-HCl	10	,,
p-Chlorphenylalanine (pCPA)	250	,,
α-methyl-metathyrosine (α-MMT)	512	,,
Reserpine (Inj. Rausedyl)	2.05	,,
Trans-2-phenyl-cyclopropylamine (tranylcypromine)	188	,,
Para-bromo-metamphetamine (V-111)	19	,,

Evaluation of parameters of the activity

The duration (T_A and T_R) as well as number (n_A and n_R) of active and rest (A and R) periods were measured in each case before and after the treatments *(Fig. 1)*. The sums of durations of active and rest periods were calculated (ΣT_A and ΣT_R) and so was the total duration of the investigation ($\Sigma T_A + \Sigma T_R$). The total duration of active and rest periods was expressed as a percentage of the total duration of investigations:

$$\frac{\Sigma T_A}{\Sigma T_A + \Sigma T_R} \times 100 \text{ and } \frac{\Sigma T_R}{\Sigma T_A + \Sigma T_R} \times 100.$$

The frequency of periodicity was:

$$\frac{n_A}{\Sigma T_A + \Sigma T_R} = \frac{n_R}{\Sigma T_A + \Sigma T_R}.$$

The average length of active and rest periods:

$$\frac{\Sigma T_A}{n_A} \text{ and } \frac{\Sigma T_R}{n_R}.$$

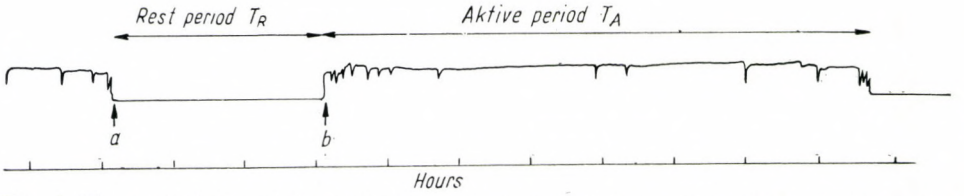

Fig. 1. The periodicity of the activity. a: beginning of the period of rest; b: beginning of the active period

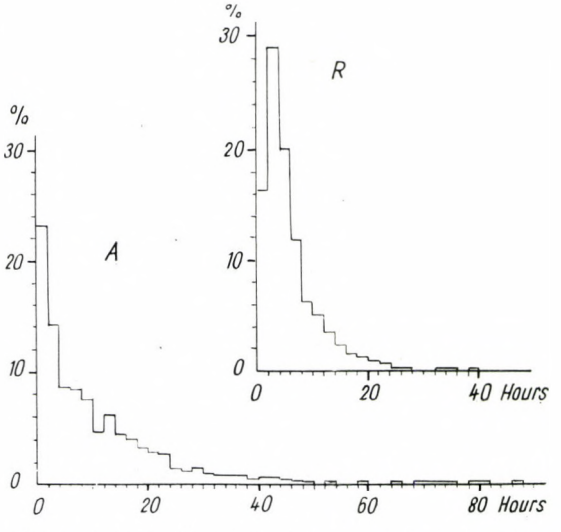

Fig. 2. The frequency distribution of the periods before the treatments

Considering the activity of all mussels before the treatment, we calculated the frequency distribution of the active and rest periods *(Fig. 2)*, and on the basis of activities of mussels after the treatment, the same parameter was determined.

The investigations were carried out in the months of April, May and June. The frequency distributions of the active and rest periods were calculated from 1400 periods before the treatment and from 300—350 periods after the treatment. The thin lines on the figures indicate this parameter before the treatment, whereas the thick ones do the same after the treatment.

Results

The effect of pharmacons affecting the monoamine level

In the first step we investigated whether the activity and monoamine level of the mussels are influenced by stopping of water current for 24 hr. The effect is shown in *Table I* based on the examination of 5 mussels. The parameters of the activity were calculated on the basis of 15 days before and 15 days after the stopping of water current, and the differences are expressed as percentages.

The results show unanimously that the stopping of water current induced no change in the periodic activity. The only change apparently significant (+18 percent) occurred in the average length of the periods of rest. However, this change can be explained by the wide dispersion of the average

TABLE I

The changes of the parameters of the periodic activity.
The changes are expressed in percent of the average values before treatment
(+) = increase (—) = decrease

	Frequency of the periodicyt	Active time	Average length of active period	Average length of rest period
Control	— 0.7	— 4.2	— 1.5	+ 18.0
pCPA	+ 12.5	—12.5	—30.8	+ 26.9
α-MMT	+ 7.3	—12.4	—20.4	+ 51.5
Reserpine	+ 17.9	—30.3	—42.9	+147.9
Tranylcypromine	+ 56.0	— 2.3	—30.7	— 27.3
V-111	+ 36.3	+ 2.7	—20.9	— 11.7
5HT	— 4.7	+ 6.6	+81.2	— 18.2
DA	+114.5	— 0.1	—27.1	— 37.6
NA	+ 44.0	— 4.2	—43.8	+ 50.7

lengths of the periods (the S.D. value amounts to 100—150 percent of the average), and extreme values occurring in certain cases may distort unreally the average length. This was the situation in this case, too, since the change was not reflected in the other parameters of the activity.

The analysis of monoamine concentrations revealed the same result. The concentrations of 5HT, DA and NA showed only less than 10 percent change as compared to the animals kept in running water. In the case of 5HT even 3—5 days of anoxia induces no higher alteration.

Effects of 5HT, NA and DA

The animals become active within a few minutes after the administration of 5HT. If the adductor muscles are in tonic contraction, the 5HT relaxes them, and the frequency as well as amplitude of the quick, rhythmic contractions increase. This activity pattern persists during the treatment, then after the change of the water, the original level of activity is only slowly restored. The treatment and the slow cessation of the effect result in an active period of 20—60 hr. The rest periods following this long active one are of short duration. Already before the first period of rest one can observe short rest states when the tonic contraction of the adductors is of lower level than before the treatment and even later. This effect is more expressed when the monoamino-oxidase is inhibited by tranylcypromine *(Fig. 3)*. The increase of the frequency of rhythmic contractions is also of considerable extent during the active period. The change of distribution of active and rest periods *(Fig. 4)* indicates a decrease in the percentual rate of the short, active periods of 2—4 hr duration in favour of the longer ones. In the case of the rest periods the situation is reversed, namely the percentual rate of the periods of rest shorter than 2 hr significantly increases and the longer ones become considerably less frequent.

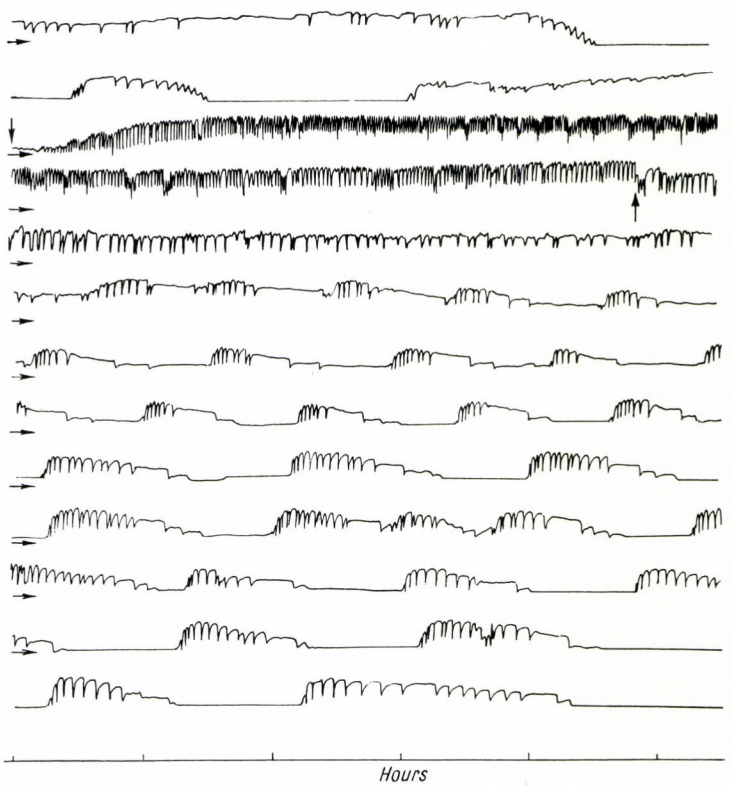

Fig. 3. Effect of tranylcypromine on the activity. Continuous recording. ↓ beginning of the treatment; ↑ end of the treatment; → level of tonic contraction before the treatment

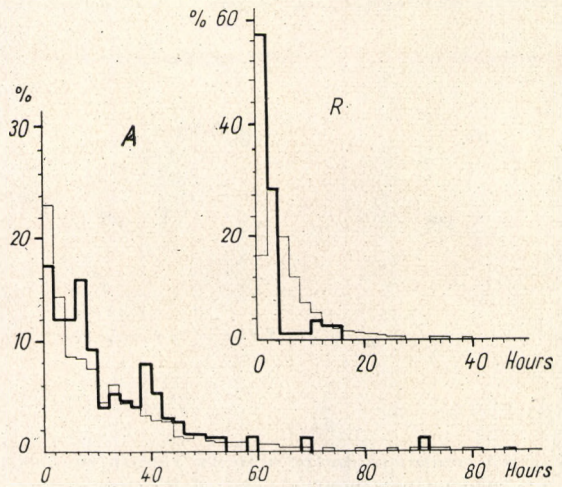

Fig. 4. The frequency distribution of the periods after 5HT treatment

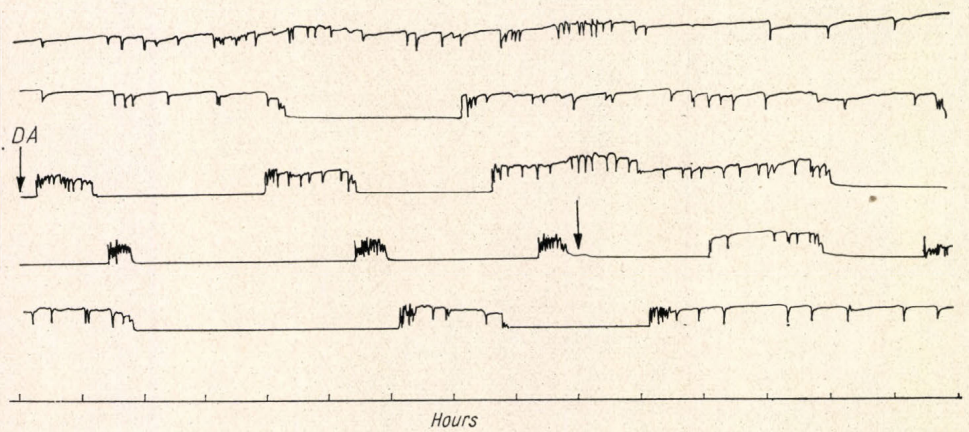

Fig. 5. The effect of DA treatment on the activity. Continuous recorping. DA beginning of the treatment; ↓ end of the treatment ↓

The effects of NA and DA can be observed practically only during the treatments. Both monoamines seem to interrupt the prolonged active periods by inducing short rest ones of 1—2 hr duration *(Figs. 5* and *6)*. This way the frequency of periodicity significantly increases, nevertheless the percentual rate of the rest time remains unchanged *(Table I.)*. This effect manifests itself in the percentual distribution of the active and rest periods *(Figs. 7* and *8)*, in the increase of rate of the short active periods and decrease of that of the longer ones. At the same time, the rate of the rest periods shorter than 2 hr increases to a significant extent and that of the longer ones decreases.

It is of interest that while the DA decreases the average lengths of both active and rest periods, the NA does only that of the active ones, and increases that of the rest periods *(Table I.)*.

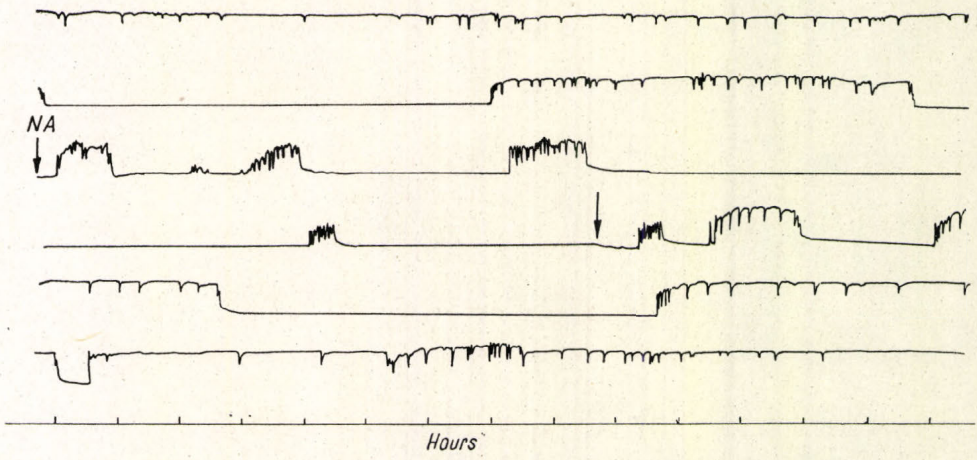

Fig. 6. The effect of NA treatment on the activity. Continuous recording. NA beginning of the treatment; ↓ end of the treatment ↓

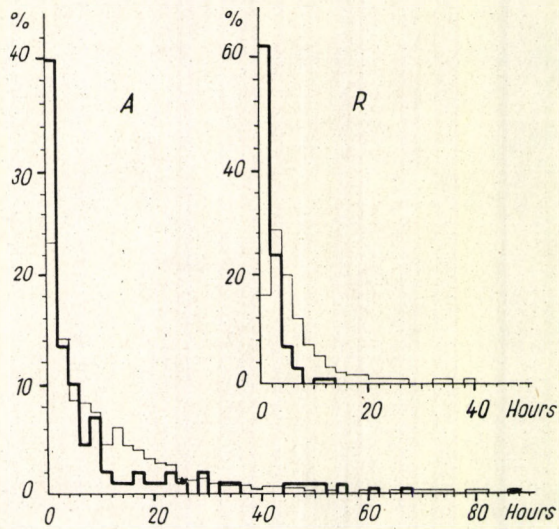

Fig. 7. The frequency distribution of periods after DA treatment

Effect of parachlorphenylalanine (pCPA)

The frequency of the periodicity, the average length of the rest periods as well as their total duration increase, whereas the total time of the active periods as well as the average lengths of them decrease *(Table I.)* upon the effect of the treatment.

The distribution of the active periods after the treatment shows a slight increase of active periods of mean duration (8—10 hr), while both the shorter and longer ones display a lower percentual rate. At the same time, the rate of rest periods shorter than 6 hr decreases, whereas that of the rest periods

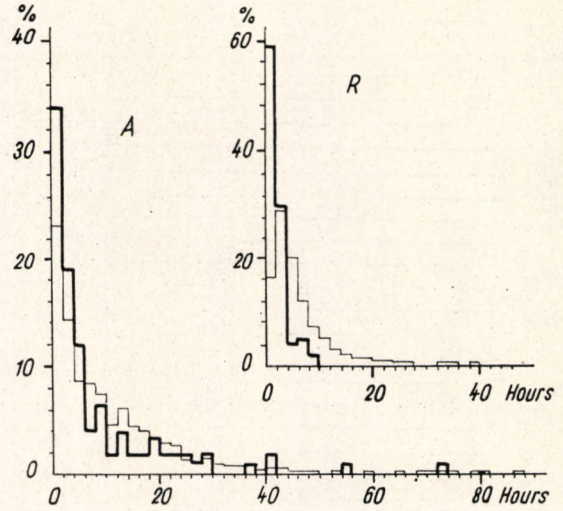

Fig. 8. The frequency distribution of periods after NA treatment

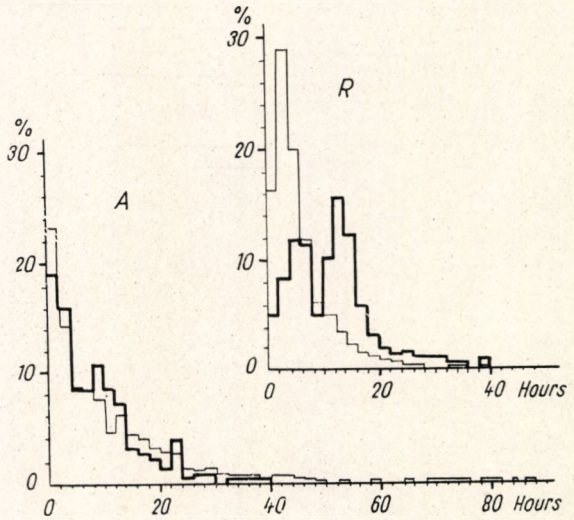

Fig. 9. The frequency distribution of the periods after pCPA treatment

of 10—40 hr duration significantly increases *(Fig. 9)*. The decrease of the average length of the active periods apparently can be attributed to the total disappearance of the active periods longer than 40 hr. At the same time, the increase of the average length of the rest periods originates in the extension of the short periods up to 10—20 hr. The activity of the animals gradually decreases upon the influence of the treatment *(Fig. 10)*. The 5HT content of the ganglia also shows a gradual diminution *(Fig. 11)*.

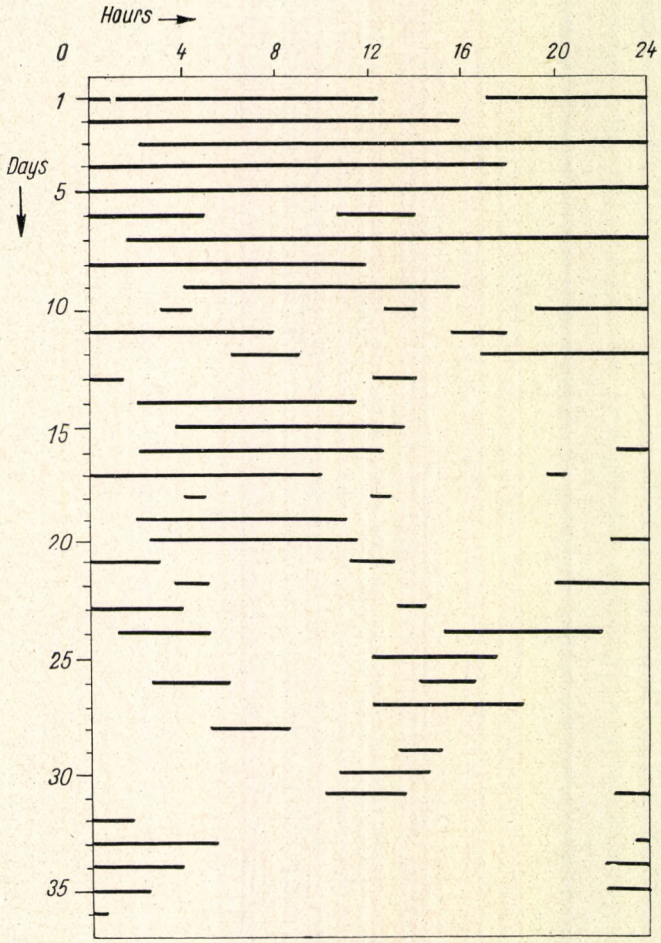

Fig. 10. The gradual decrease of the activity after pCPA treatment. The treatment took place on the 1st day

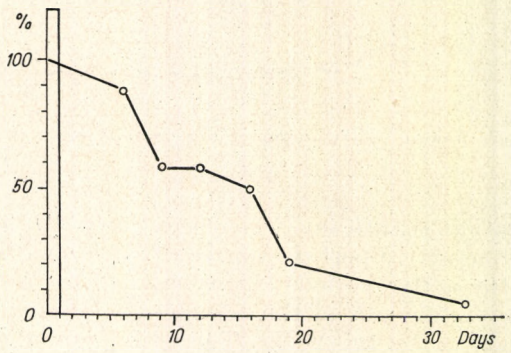

Fig. 11. The decrease of 5HT after pCPA treatment. The 5HT content of the treated animals is shown as a percentage of the control ones

The effect of α-methyl-meta-tyrosine (α-MMT)

The frequency of periodicity and the total duration of the rest periods slightly increased after the treatment, while the average length of the rest periods was 50 percent longer and the total active time and the average length of the active periods decreased *(Table I.)*. The rate of the active periods

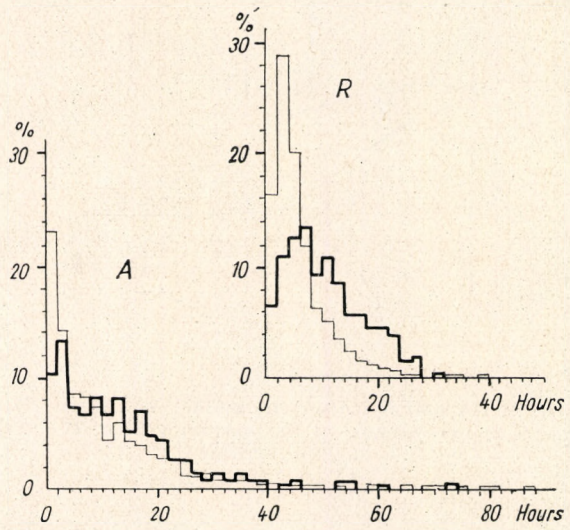

Fig. 12. The frequency distribution of periods after α-MMT treatment

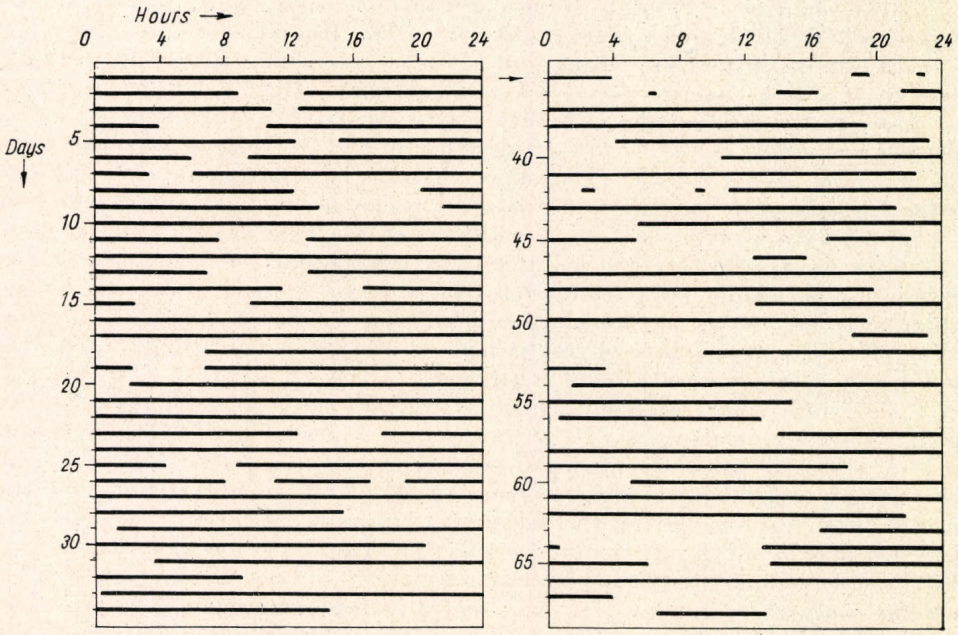

Fig. 13. The effect of α-MMT on the periodic activity. 1—34 days: activity before the treatment; 35th day: treatment; 35—68th days: activity after the treatment

shorter than 10 hr decreases, that of the longer ones (10—30 hr) increases. The distribution of the rest periods shows a more significant change. The rest periods shorter than 8 hr extend and result in a significant increase of the rate of rest periods of 10—30 hr *(Fig. 12)*. The effect of this treatment on the periodic activity is shown by *Fig. 13*. The alteration of CA is presented in *Fig. 14*. At the concentration of α-MMT used, the CA level decreased about 50 percent.

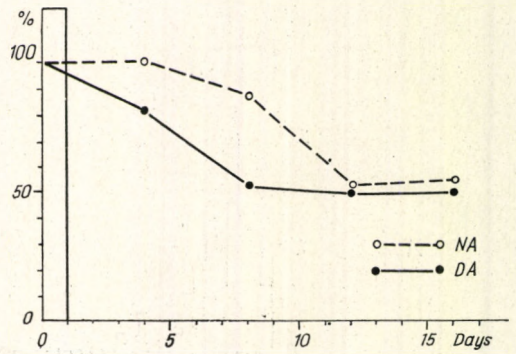

Fig. 14. The decrease of DA and NA after the α-MMT treatment. The concentrations of monoamines are given as percentages of the normal values. The treatment took place on the 1st day

Effect of reserpine

The changes induced by reserpine are more significant than those observed after pCPA and α-MMT treatments. The frequency of periodicity increases almost 20 percent during and after the reserpine treatment of 24 hr. The total active time decreases about 30 percent, while the total rest time increases to the same extent. The decrease of the average lengths of the active periods originates in the significant decrease of the active periods longer than 25 hr *(Fig. 15)*. Active periods longer than 25 hr occur mainly during and after the treatment, and in some cases the prolonged rest state is interrupted by an activity of 1—2 days 3—4 weeks after the treatment (*Fig. 16*, 63—64—65th days). The average length of the rest periods increases nearly 150 percent. This should be attributed to the increase of rate of periods longer than 15 hr as well as to the decrease of rate of the periods of short duration *(Fig. 15)*. The appearance of rest periods of 40—60 hr is of significance, this has never been observed in the controls.

The reserpine treatment causes significant and quick changes in the ganglionic concentrations of all the three monoamines *(Fig. 17)*. The depletion of DA and 5HT is of the highest speed. By the end of the treatment, the concentrations of both amines decrease to about 50 percent. Ninety percent of DA becomes depleted by the 4th day, whereas the same rate of depletion is reached by the 6th day in the case of 5HT. The depletion of NA is slower. Fifty percent depletion is to be measured after 3.5 days, while 90 percent is reached only after 15 days. The concentrations of monoamines remain permanently low after the treatment and neither the monoamine level nor the activity seem to be restored during the first month subsequent to the treat-

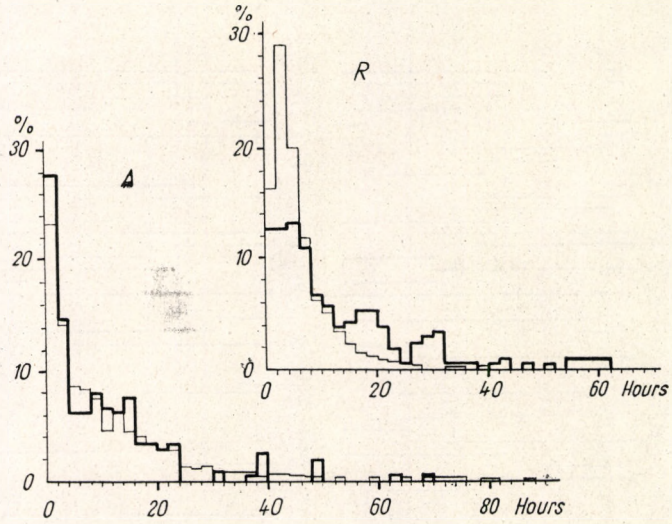

Fig. 15. The frequency distribution of periods after reserpine treatment

ment. What is more, the activity of the animal demonstrated in Fig. 16 remained at a low level even 2.5 months after the treatment.

Although systematic investigations concerning the quick, rhythmic adductor functions within the active periods have not been carried out, it deserves interest that the frequency of the quick, rhythmic activity increases to a high extent during the treatment as it was observed in the case of 5HT treatment, too. This frequency, however, strongly decreases during the 2nd and 3rd weeks following the treatment and is of lower value than before the treatment.

The effect of tranylcypromine

This treatment induces a very significant alteration of the activity of the animals resembling the effect of 5HT during the initial phase. This pharmacon increases the frequency of the periodicity to a very high extent, by 56 percent, whereas the rates of total active and rest times remain unchanged. The average lengths of both the active and rest periods decrease uniformly about 30 percent. The percentual distributions of both periods significantly change. The rates of short active and rest periods (less than 2 hr) markedly increase, while the rates of those longer than 6 hr become minimal *(Fig. 18)*. The response of the animals resembles to the effect of 5HT even from the point of view that during the treatment and cessation of the effect, prolonged active periods appear (between 30—70 hr), representing, however, only 0.1—0.2 percent of the number of periods. Similarly as after the administration of 5HT, the frequency of quick, rhythmic contractions greatly increases during the active periods appearing in the course of the treatment *(Fig. 3)*. The 5HT concentration of the nervous system is altered only to a low extent by the treatment *(Fig. 19)*.

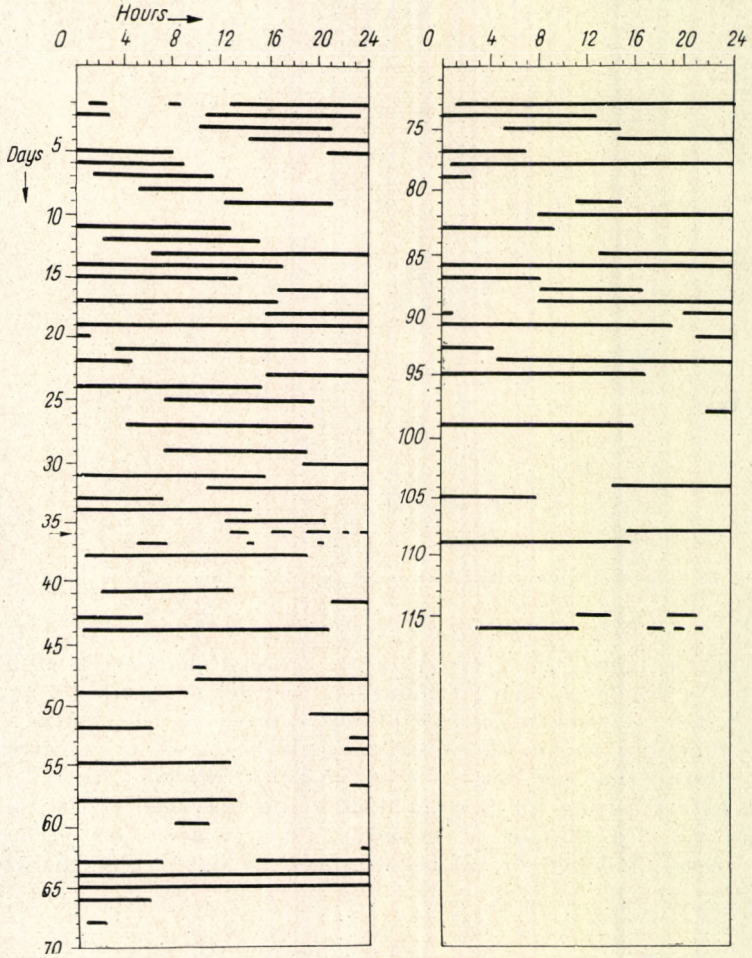

Fig. 16. The effect of reserpine on the periodic activity. 1—35th days: activity before the treatment; 36th day: treatment; 36—115th days: activity after the treatment. The lines following each other indicate the duration of the active periods, the interruptions do that of the passive ones

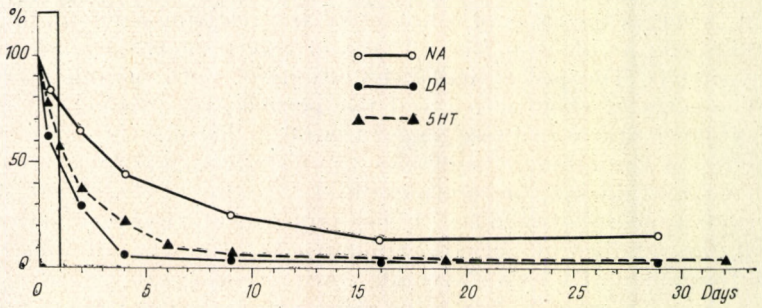

Fig. 17. The decrease of 5HT, DA and NA after reserpine treatment. The treatments took place on the 1st day. The concentrations of monoamines are expressed as percentages of those of normal ones

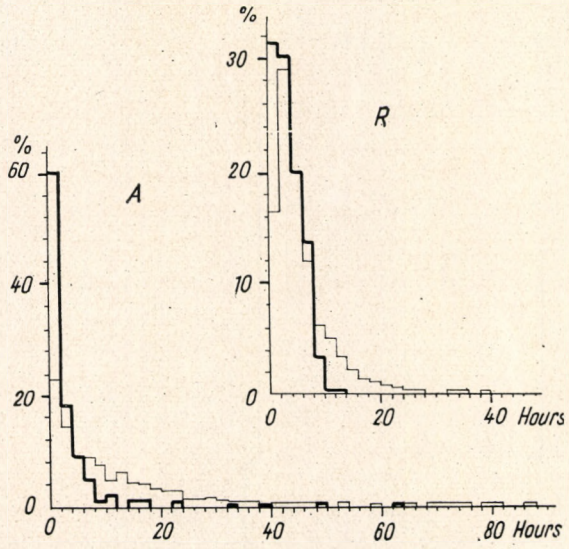

Fig. 18. The frequency distribution of periods after tranylcypromine treatment

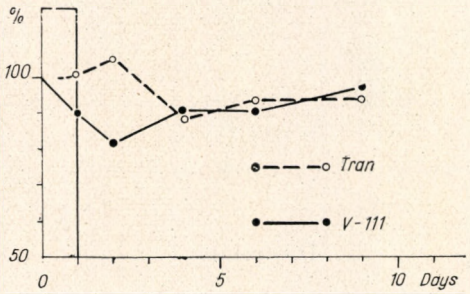

Fig. 19. The decrease of 5HT after tranylcypromine and V-111 treatments. The treatment took place on the first day. The concentrations of monoamines are given as percentages of those of the control animals

Para-bromo-metamphetamine treatment

The frequency of periodicity increases 36 percent after the treatment, the average lengths of both periods decrease, whereas the percentual rates of total active and rest times show only a minimal alteration *(Table I)*. The percentual distribution of duration of both the active and rest periods displays a considerable change. The rate of active periods shorter than 8 hr increases, while that of the longer ones decreases. The rate of the rest periods shorter than 4 hr also increases, whereas that of the longer ones significantly decreases *(Fig. 20)*. The 5HT level shows an unequivocal decrease (maximally 18 percent), restored by the 8th day *(Fig. 19)*

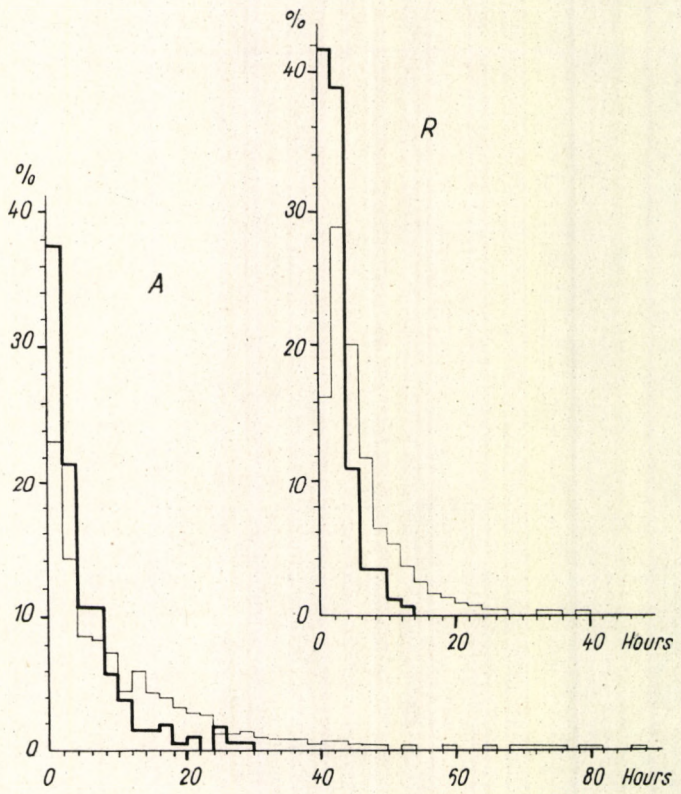

Fig. 20. The frequency distribution of periods after V-111 treatment

Discussion

The analysis of the possible mechanism of rhythm regulation revealed that centres of "activity" and "rest" localized in the central nervous system are responsible for its determination and maintenance (SALÁNKI, 1970). These centres function through the transmitter system or systems.

Earlier investigations have rendered probable that in the fresh water mussel the transmitter system of the centre of "activity" is of serotoninergic nature, since 5HT relaxes the adductor muscles on both ganglionic (SALÁNKI, 1963) and muscular (SALÁNKI and LÁBOS, 1969) levels, furthermore, significant concentrations of it are present in both the nervous system and the adductors (HIRIPI, 1968).

The functional presence of this system in the relaxation of the adductors from the tonic contraction is supported by the earlier findings when stimulating the CVc (SALÁNKI and HIRIPI, 1970). The hypothesis was obvious that if the 5HT caused relaxation not only applied to the ganglia but also at the level of the adductors (SALÁNKI and LÁBOS, 1969), the stimulation of the CVc resulting in the relaxation, too, (SALÁNKI and LÁBOS, 1963) should influence the 5HT level of the adductors. The increase of 33 and 25 percent in the 5HT

concentration of the adductors upon the influence of the stimulation proved the correctness of that hypothesis. Since the adductors do not synthesize 5HT, this process takes place only in the nervous system (HIRIPI and SALÁNKI, 1969) and mainly so does the storage of it (HIRIPI, 1968; HIRIPI et al., 1972), the 5HT demand of the relaxation of the adductors as well as the increase of the 5HT level should originate in the ganglia. The 5HT from the ganglia reaches the adductors probably by means of an active transport independent from the concentration, where it takes part in the relaxation of the adductors depleting at the neuromuscular level.

Since the periodic activity of the mussel consists of an alternation of tonic contraction and relaxation of the adductors, it seemed to be reasonable to investigate, whether any change of the 5HT level can be observed in the adductors during the spontaneous, physiological active and passive periods, being in accordance with those observed after the stimulation of the CVc. According to our results, the 5HT level was higher in the adductors at the beginning of the active period, i.e. at the spontaneous relaxation of the adductors, than at the beginning of the rest state (SALÁNKI and HIRIPI, 1970). The increase of 5HT level (30 percent in the anterior and 25 percent in the posterior adductor) is practically identical with the values observed after the stimulation of the CVc, although this increase was not significant statistically in the posterior adductor at the beginning of the spontaneous relaxation. The increase of 5HT level of the adductors was followed by a change of 5HT level only in the visceral ganglion among the three ganglia. Nevertheless, the change amounting to 23 percent in the visceral ganglion was of opposite direction as compared to that of the adductors. If according to the chain of ideas mentioned above, the 5HT content of the adductors originates in the ganglia, a significant difference should exist between the cerebral and visceral ganglia as regards their regulatory mechanism toward the anterior and posterior adductors, respectively, connected with the alteration of 5HT level. Namely, the decrease of 5HT level of the visceral ganglion at the beginning of the activity can be interpreted in the way that the higher 5HT demand of the adductor relaxation is satisfied from the visceral ganglion at an unchanged rate of synthesis even during the active transport, resulting in a decrease of the ganglionic 5HT level. At the same time, in the cerebral ganglion a periodic change of the synthetic speed may assure a constant 5HT level independently from the periodic activity. This way the cerebral ganglion possesses a primary role in the regulation of the periodicity of the activity, which is in accordance with the conclusions drawn from other experiments that the cerebral ganglion regulates primarily the relaxation of both adductors (PAVLOV, 1885; VERESCHAGIN, 1960; SALÁNKI et al., 1968).

Our results prove the hypothesis with a high probability that the serotoninerg system functions as a chemical regulatory system of the centre of "activity".

The question arises what system regulates the centre of "rest"? The results of the pharmacological analysis of the serotoninergic system as well as the independence of the 5HT level from the state of activity in the cerebral ganglion exclude the possibility that the decrease of 5HT in itself can induce the appearance of rest period. Pharmacological data show that among the CA NA and adrenaline are able to induce tonic contraction of the adductors at the muscle level of *Anodonta* (SALÁNKI and LÁBOS, 1969), however, at the

ganglionic level they induce the relaxation of the adductors similarly to the effect of 5HT (SALÁNKI, 1963). Nevertheless, this effect is produced by concentrations of them an order of magnitude higher than that of 5HT. At the same time electrophysiological results revealed that both DA and NA cause prolonged inhibition of certain nerve cells of Gastropoda (GERSCHENFELD and TAUC, 1964; GLAIZNER, 1968; KISS and SALÁNKI, 1971; WALKER et al., 1971), and what is more, the NA blocks the activity of the Br-cell showing a characteristic rhythm in the nervous system of *Aplysia* (BOISSON and CHALAZONITIS, 1972). The different chemical sensitivity as well as the different effects of the same transmitter on various neural pathways have also been demonstrated in the ganglia of *Anodonta* (SALÁNKI and VARANKA, 1971). In the light of these pharmacological results one cannot exclude the catecholaminergic system as being the transmitter system of the centre of "rest", so much the more as significant concentrations of DA and NA are present in the nervous system of the fresh water mussel (HIRIPI, 1972).

The pharmacological investigations influencing the function of the above transmitter systems proved to be suitable to draw further conclusions concerning the existence and function of the centres of "activity" and "rest".

The prolonged active state, induced by exogenous 5HT can be interpreted that the increase of 5HT level significantly altered the dynamic balance of centres of "activity" and "rest" and the effect of the former became realized. The 5HT acts probably both periphperically at the level of the adductors and centrally on the ganglia. The effect manifests itself even after the treatment when the 5HT acting peripherically is not present any more. We assume that the effect is prolonged by the part of 5HT picked up and stored by the nervous elements. The subcellular localization of 5HT showed that a significant part of the endogeneous 5HT is bound to synaptosomes (HIRIPI et al., 1972). It has also been shown by our earlier investigations that 5HT injected into the foot musculature is taken up by the nervous system, and apart from the increase of activity, this 5HT maintains a higher 5HT level of the ganglia for several days (HIRIPI and SALÁNKI, 1971). The exogeneous 5HT is distributed among the subcellular fractions to the rates identical with those of the endogeneous one (unpublished observations). This way the exogeneous 5HT taken up mainly by the synaptosomes shifts the balance toward the centre of "activity" for several days. The effect of the centre of "rest" becomes limited in time so that it can maintain only short periods of rest besides the long active periods. This is also indicated by the change of distribution of durations of the active and rest periods, namely by the increase of the rate of the short, rest periods of 2 hr as well as that of the more prolonged active ones.

The serotoninergic system is probably one of the regulatory factors of the quick, rhythmic contraction activity, too. Namely, all pharmacological treatments flooding the neuromuscular and ganglionic serotoninergic receptors with 5HT, increased the frequency of the quick rhythm to a high extent. This effect appeared during the treatment with 5HT, the MAO-inhibitor tranylcypromine and the reserpine.

The results of DA and NA treatments indicate that the dopaminergic and noradrenergic systems functioning in the centre of "rest" are not able to overcompensate the serotoninergic one permanently to such a degree that the rest period would be able to get a significant predominance. The DA can

activate the function of the centre of "rest" by inducing short rest periods interrupting this way the function of the centre of "activity". This results in the shortening of both the active and rest periods as well as the 100 percent increase of the frequency demonstrated well by the change of distribution of the periods.

The NA stimulates the "rest centre" by interrupting the prolonged active periods with more prolonged rest ones than existed before, increasing this way the frequency and, as against to the DA, also the average length of the rest periods. This difference in the effects indicates that DA acts not only as a precursor increasing the concentration of NA, but also as an independent dopaminergic component. The justification of this assumption is supported by the pharmacological investigations cited formerly, evidencing the specific, prolonged inhibitory effect of DA on certain neurons. Notwithstanding, the two systems cannot be completely separated, since DA as the precursor of NA may also act through the alteration of concentration of NA. Since there are no data at our disposal concerning the change of the CA level at the spontaneous change of periods, any further analysis of the mechanism would only be mere speculation. However, on the basis of our results one can assume that the catecholaminergic system is able to function as a transmitter system of the centre of rest.

In the further experiments we attempted to reveal the mechnaisms of the two systems mentioned above by means of pharmacological influences. The function of the centre of "activity" was attempted to be inhibited through the blocking of synthesis of its transmitter.

It has been evidenced by KOE and WEISSMANN (1968) that pCPA inhibits specifically the synthesis of 5HT in vertebrates without any considerable influence on the catecholamines. The slow but significant decrease of 5HT appears even in the fresh water mussel. The interpretation of the alterations of activity fits well the model constructed for the explanation of regulations.

The analysis of the pCPA effects leads also to the former conclusion that 5HT plays a decisive role in the function of the centre of "activity". However, it is conspicuous that even in spite of the considerable decrease of 5HT level, the centre of "rest" does not reach that degree of predominance which could be expected on the basis of the decrease of 5HT level. This may be explained by assuming that 5HT is present in two pools in the nervous system. One of them is an active pool stored mainly in the synapses and easily mobilized, the other one is of inactive state and forms a reserve. The balance of them assures the level of the active pool even in cases of wide variations of the reserved 5HT. Probably, the ganglionic 5HT is stored in the reserve pool in a greater concentration where the decrease of 5HT level does not alter the function of the active pool. Of course, this is true only for a critical level.

The decrease of synthesis distorts the balance of the active and reserve pools to such a degree that the alteration of the active pool will damage the serotoninergic mechanism. This way the centre of activity seems to be exhausted and the effect of centre of rest becomes predominant. This is why the average length of the active periods decreases and that of the passive ones increases.

The p-brom-metamphetamine, as KNOLL and MAGYAR (1971) described, specifically affects the serotoninergic system so that it does not inhibit the

synthesis but does the uptake of 5HT and causes 5HT liberation. The concentration used altered the 5HT level only to a slight extent, namely decreased it. Notwithstanding, a characteristic change appeared in the activity. The increase of the frequency and decrease of the active and rest periods are reflected even in the significant change of the percentual distribution of the periods. This effect of V-111 differs from that of pCPA and the difference can be explained just by the various mechanisms of effect. While the effect of pCPA inhibiting the synthesis is prolonged and appears to be considerable at the critical level of the reserve pool, that of V-111 appears quickly. The inhibition of incorporation into the synaptosomes may result in the damage of both pools, thus the active transport may be influenced through the stabile pool. The effect of V-111, however, induces no prolonged passive period, since both pools quickly regenerate during the rest, resulting in the disconnection of the effect of the centre of rest.

It is of interest that the alteration of the catecholaminergic system assumed to be the transmitter system of the centre of "rest" by α-MMT induces an effect like the pCPA. However, it increases the frequency only to a lower extent, the decrease of the average length of the active periods is smaller, but the increase of average length of the rest periods is far more significant. While in the case of pCPA treatment the rest periods being shorter than 8 hr are extended to 15—20 hr at the expense of the active ones longer than 15 hr, in the case of α-MMT treatment the rest periods shorter than 8 hr increase to 15—25 hr so that the duration of the active periods hardly changes. This effect of α-MMT supports further the hypothesis that the catecholaminergic system might be the transmitter of the centre of rest. CARLSSON and his group demonstrated (CARLSSON, 1964) in vertebrates that the depletion of NA caused by α-MMT is mediated by a corresponding decarboxylated compound of this pharmacon. Namely, after α-MMT treatment one can measure both the α-methyl-metatyramine formed by decarboxylation and metaraminol formed by dopamine β-hydroxylase from the latter compound. The binding of these compounds is stronger than that of NA in the brain.

The competition plays important role in the depletion mechanism of NA caused by α-MMT, since the appearance of the α-methylated amine is of the same order of magnitude as that of the disappearing NA. However, the inhibition of the synthesis may also contribute to this process.

The effect of α-MMT in *Anodonta* may well be interpreted in the light of the data obtained in vertebrates and fits well the basic hypothesis.

The catecholaminergic system assures the predominance of the centre of rest so that the depleted NA stimulates the inhibitory neurons. The decrease of concentration caused by the depletion, however, results in no damage of the centre of rest, since the methylated products substitute the NA and are able to maintain the noradrenergic transmission (CARLSSON, 1964; SHORE et al., 1966).

Comparing the effects of α-MMT and the two catecholamines investigated, the extension of the rest periods caused by α-MMT seems to be realized through the effect of endogeneous NA, which may be modulated by the decrease of dopamine level by means of a yet unknown mechanism. It is of interest to compare the effect of chlorpromazine observed in vertebrates and mussels from the view-point of the role of catecholamines. Besides other effects, it induces a supersensitivity toward CA in vertebrates by blocking

the inactivation performed by binding, therefore, a higher concentration of the free CA is present at the receptor sites (AXELROD, 1964).

If the mechanism of the effect is the same even in the nervous system of *Anodonta*, the prolonged tonic contraction induced by chlorpromazine (SALÁNKI, 1963) and the effects of CA, mainly the extension of the passive periods caused by NA may be the consequences of increase of CA-saturation of the inhibitory neurons.

When analyzing the effects of reserpine, the conclusion can be drawn that the effects are brought about by common influences of both serotoninergic and catecholaminergic systems, however, the change of the former is of decisive role. Upon the influence of reserpine the depletion of 5HT and DA is quick and nearly exponential, whereas that of NA is much more prolonged. Comparison of the effects and the depletion shows that during the treatment the prolonged active periods and the quick rhythmic activity are caused first of all by the quick depletion of 5HT. This effect predominates so that the centre of rest is only rarely able to sustain the activity during the treatment. The reserpine causes depletion of the labile 5HT pool with the inhibition of incorporation in that pool (COSTA and BRODIE, 1964; CARLSSON, 1964). However, the depletion is so quick during the treatment that the liberated 5HT cannot be inactivated by MAO completely, therefore the ganglionic level of free 5HT increases. This may stimulate the neuronal activity inducing the active transport and may increase even the speed of the passive diffusion toward the effector organs. Because of the short distance between the ganglia and the adductor muscles, the diffusion of 5HT can be so intense that it may substitute even the active transport. The receptors of the adductors become greatly saturated the effect of which resembles that caused by 5HT and tranylcypromine. After the quick depletion of 5HT, the activity of the centre of rest is induced not only by the significant concentration of NA but also by the exhaustion of the centre of activity. After the extensive decrease of 5HT level, the labile pool drops below the critical level and becomes unable to maintain the function of the stabile pool. The prolonged rest periods appear and at minimal levels of the amine concentration and not infrequently periods of rest occur of even several days, duration. This is indicated by a 150 percent increase of length of rest periods and by the change of the percentual distribution of the periods. In this state the limiting factor of appearance of an active period is the speed of recovery of the stabile pool. This way from the point of view of reserpine effects not the level of depletion but the speed of it is of decisive role, which has been evidenced in vertebrates by BRODIE and RAID (1968).

This is indicated also by our observations that two different doses may induce the same level of depletion at different points of time, and the larger one causing a much quicker depletion increases the frequency of the quick rhythm to a high extent, whereas the smaller one shows only minimal effect of this type. It has earlier been mentioned that the MAO-inhibiting tranylcypromine induces an effect resembling those of 5HT and reserpine. The administration of it causes a prolonged active period during which even the rhythmic activity increases, meanwhile the 5HT level of the ganglion remains practically unchanged. It has been shown in vertebrates that the inhibition of MAO increases the 5HT level (GARATTINI and VALZELLI, 1965). The presence of MAO has been demonstrated in the nervous system of *Anodonta* and it

takes part in the inactivation of 5HT (HIRIPI and SALÁNKI, 1971). However the investigations of different tissues of *Anodonta* led to the conclusion that most part of 5HT is not inactivated in the ganglia but in the kidney. The first step of this way of inactivation is the depletion of 5HT from the ganglia toward the kidney taking place through diffusion and the participation of the circulatory system. This agrees with the results obtained on other objects (GERSCHENFELD and STEFANI, 1968; CARDOT, 1964; MIROLLI, 1968), indicating at the same time that in some species the MAO does not take part in the inactivation of 5HT in the ganglia at all. This has also been evidenced by the investigations demonstrating that MAO-inhibition induces no increase in the ganglionic 5HT level (KERKUT and COTTRELL, 1963).

The inhibition of the enzymatic inactivation, however, results in the increase of the 5HT level only in the case if that is the single way of elimination. The inhibition of MAO in *Anodonta* resulted in the increase of 5HT content in the peripheral organs. The 5HT content of the ganglia does not increase, since the 5HT uninactivated by MAO is eliminated by diffusion and circulation. The rate of increase may be much more higher in the adductors and the saturation of 5HT receptors of adductors may come into being. Reaching the critical level, the adductors relax. However, the 5HT not inactivated in the ganglia may increase also the active axonal transport by stimulating the neurons and this further increases the 5HT concentration of the adductors. The restitution, i.e. the appearance of periods of rest depends on the rate of regeneration of MAO. The 5HT inactivation increases depending on the speed of regeneration of the enzyme molecules and below a critical 5HT level, the adductors may again show a tonic contraction.

The administration and effect of MAO-inhibitors can be interpreted in a different way in case of CA as in that of 5HT. Since the catechol-o-methyl-transferase is able to inactivate the CA not inactivated by MAO, the unchanged CA level indicates that the centre of rest is not affected by the MAO-inhibition.

The pharmacological investigations revealed at the same time that apart from the change of ganglionic 5HT level, first of all, the 5HT transport toward the adductors plays a decisive role in the activity.

According to our assumption, the fast axonal transport existing in the nerves of *Anodonta* (HESLOP and HOWES, 1972) may represent the active transport. According to the data of the above authors the axonal transport slightly differs from that of vertebrates. This transport is controlled and independent from the pressure and concentration gradients. It is inhibited by much lower concentrations of dinitrophenol and cianid, indicating its metabolic dependence. The most striking difference manifests itself in the effect of temperature, since it is independent from the temperature between 4 and 12° C, and linearly increases with the further increase of the temperature. The temperature dependence of the axonal transport agrees with that of the activity of the fresh water mussel. The investigation of seasonality and temperature dependence of the activity as well as of 5HT level (unpublished results) indicates that under natural circumstances the activity observed at low temperatures, begins to be normalized at 8—10° C, after which the axonal transport linearly increases with the temperature.

Summary

On the basis of an earlier working hypothesis we investigated whether the pharmacological influences on the transmitter systems of the assumed centres of "activity" and "rest" responsible for the regulation of the rhythm of fresh water mussel (*Anodonta cygnea* L.) act on the monoamine levels and the periodicity of the activity. The serotoninergic system of the centre of activity was influenced by inhibition of synthesis (pCPA) and the inactivating enzyme (tranylcypromine) by depletion of 5HT (Reserpine, V-111) as well as by exogeneous 5HT.

The catecholaminergic system of the centre of rest was altered by exogeneous dopamine and noradrenaline, α-methyl-metatyrosine, reserpine and tranylcypromine.

Investigating the periodic activity and the monoamine levels, it was found:

1. The 5HT level plays a decisive role in the function of the centre of activity. The prolonged activity caused by exogeneous 5HT is a consequence of prolonged increase of 5HT level, manifesting itself primarily in the increase of the average length of active periods.

2. The ganglionic 5HT level was not increased by the MAO-inhibiting tranylcypromine, however, prolonged active periods and great increase of rhythmic contractions were induced by this drug through the absence of the peripheric inactivation. After the treatment the frequency of rhythmicity was increased by decreasing the average lengths of active and rest periods.

3. The decrease of 5HT level caused by pCPA and reserpine, resulted in the decrease of average length of active periods and the increase of that of the rest ones. The p-brom-metamphetamine increases mainly the frequency of rhythmicity through a slight decrease of 5HT level.

4. The dopamine decreases the average length of both the active and rest periods, whereas the noradrenaline increases that of the inactive ones. The effect of α-MMT is probably realized through the effect of NA and the significant increase of the rest periods is caused by the liberation of NA.

5. After reserpine treatment the predominance of the centre of rest is induced partly by the significant decrease of 5HT level and partly by the considerable NA level persisting because of the slower depletion of NA.

6. The 5HT level in itself cannot determine the function of the centre of activity. The active transport toward the adductors takes also part in the relaxation of the adductors, i.e. in the regulation of the active period. The decrease of 5HT level influences the active transport only below a certain critical level.

REFERENCES

ANTON, A. H., SAYRE, D. F. (1962): A study of factors affecting the aluminium oxide-trihydroxyindole procedure for analysis of catecholamines. — *J. Pharmacol. Exp. Ther.* **138,** 360—372.

ANTON, A. H., SAYRE, D. R. (1964): The distribution of dopamine and DOPA in various animals and a method for their determination in diverse biological material. — *J. Pharmacol. Exp. Ther.* **145,** 326—336.

AXELROD, J. (1964): The uptake and release of catecholamines and the effect of drugs. —

In: *Progress in brain research* (Eds.: HIMWICH, H. E., HIMWICH, W. A.) *Elsevier* **8,** 81—89.

BOISSON, M., CHALAZONITIS, N. (1972): Abolition by noradrenaline of the waving bursting neuronal activity (Br neuron of *Aplysia fasciata).* — *Comp. Biochem. Physiol.* **41A,** 883—886.

BRODIE, B. B., REID, W. D. (1968): Serotonin in brain: Functional Consideration. — *Adv. Pharmac.* **6B,** 97—113.

CARDOT, J. (1964): Considérations sur le métabolisme de la 5-hydroxytryptamine et de la tryptamine chez le Mollusque *Helix pomatia.* — *C. R. Acad. Sci. Paris* **258,** 1103—1105.

CARDOT, J. (1971): Variations saisonnières de la 5-hydroxytryptamine dans les tissus nerveux et cardiaque chez le Mollusque *Helix pomatia.* — *C. R. Soc. Biol. Paris* **165,** 338—341.

CARLSSON, A. (1964): Functional significance of drug-induced changes in brain monoamine levels. — In: *Progress in brain research* (Eds.: HIMWICH, H. E., HIMWICH, W. A.) Elsevier **8,** 9—27.

CHANG, C. C. (1964): A sensitive method for spectrophotofluorometric assays of catecholamines. — *Int. J. Neuropharmac.* **3,** 643—649.

COSTA, E., BRODIE, B. B. (1964): Concept of the neurochemical transducer as an organized molecular unit at sympathetic nerve endings. — In: *Progress in brain research* (Eds.: HIMWICH, H. E., HIMWICH, W. A.) *Elsevier* **8,** 168—185.

DAHL, E., FALCK, B., von MEKLENBURG, C., MYHRBERG, H., ROSENGREEN, E. (1966): Neuronal localization of dopamine and 5-hydroxytryptamine in some Mollusca. — *Z. Zellforsch.* **71,** 489—498.

DRASKOCZY, P. R., LYMAN, C. P. (1967): Turnover of catecholamines in active and hibernating ground squirrels. — *J. Pharmac. exp. Ther.* **155,** 101—111.

FRIEDMAN, A. H., WALKER, C. A. (1968): Circadian rhythms in rat mid-brain and caudate nucleus biogenic amine levels. — *J. Physiol.* **197,** 77—85.

GARATTINI, S. L., VALZELLI, L. (1965): Serotonin. — *Elsevier, Amsterdam.*

GERSCHENFELD, H. M., TAUC, L. (1964): Differentes aspects de la pharmacologie des synapses dans le système nerveux central des Mollusques. — *J. Physiol. Paris* **56,** 360—361.

GERSCHENFELD, H. M., STEFANI, E. (1968): Evidence for an excitatory transmitter role of serotonin in molluscan central synapses. — *Adv. Pharmac.* **6A,** 369—392.

GLAIZNER, B. (1968): Pharmacological mapping of cells in the suboesophageal ganglia of *Helix aspersa.* — In: *Neurobiology of Invertebrates.* (Ed.: J. SALÁNKI) *Akadémiai Kiadó, Budapest* 1968, pp. 267—284.

HARRI, M. (1972): Effect of season and temperature acclimation on the 5-hydroxytryptamine level and utilization in the brain and intestine of the frog, *Rana temporaria.* — *Comp. gen. Pharmac.* **3,** 11—18.

HESLOP, J. P., HOWES, E. A. (1972): Temperature and inhibitor effects on fast axonal transport in a molluscan nerve. — *J. Neurochem.* **19,** 1709—1716.

HIRIPI, L. (1968): Paper chromatographic and fluorimetric examination of the serotonin content in the nervous system and other tissues of fresh-water molluscs. — *Annal. Biol. Tihany* **35,** 3—11.

HIRIPI, L. (1972): Catecholamines in the different tissues of fresh-water mussel *(Anodonta cygnea* L., Pelecypoda) analysed by thin-layer chromatographic and fluorimetric methods. — *Annal. Biol. Tihany* **39,** 13—20.

HIRIPI, L., SALÁNKI, J. (1969): 5HTP-DOPA decarboxylase in the nervous system and other tissues of *Anodonta cygnea* L. (Pelecypoda). — *Annal. Biol. Tihany* **36,** 19—24.

HIRIPI, L., SALÁNKI, J. (1971): The role of monoamino oxidase in the inactivation of serotonin in the nervous system and other tissues of *Anodonta cygnea* L. — *Annal. Biol. Tihany* **38,** 31—38.

HIRIPI, L., SALÁNKI, J. (1971): Role of monoamines in the central regulation of periodic activity in *Anodonta cygnea* L. (Pelecypoda). — *Symposium on Invertebrate Neurobiology, Mechanisms of Rhythm Regulation* (Ed. J. SALÁNKI) *Akad. Kiadó, Budapest* pp. 391—401.

HIRIPI, L., SALÁNKI, J. (1972): Szezonális és aktivitásfüggő szerotonin szint változások molluskák központi idegrendszerében. — *X. Biológus Vándorgyűlés, Szeged,* 1972.

HIRIPI, L., SALÁNKI, J., ZS.-NAGY, I., B.-MUSKÓ, I. (1972): Biogen monoaminok szubcelluláris lokalizációja *Anodonta cygnea* L. központi idegrendszerében. — *MÉT XXXVIII. Vándorgyűlése, Budapest,* 1972.

JOUVET, M. (1968): Insomnia and decrease of cerebral 5-hydroxytryptamine after destruction of the raphe system in the cat. — *Adv. Pharmac.* **6B,** 265—279.
JOUVET, M. (1969): Pharmacological and neurophysiological studies suggest a relationship between brain serotonin and sleep. — *Science* **163,** 32—41.
KERKUT, G. A., COTTRELL, G. A. (1963): Acetylcholine and 5-hydroxytryptamine in the snail brain. — *Comp. Biochem. Physiol.* **8,** 53—63.
KISS, I., SALÁNKI, J. (1971): The heterogenic chemical sensitivity of the central neurones of *Lymnaea stagnalis* L. — *Annal. Biol. Tihany* **38,** 39—52.
KNOLL, J., MAGYAR, K. (1971): p-bromo-methamphetamine (V-111) a strong inhibitor of H^3-5HT uptake in the synaptosomes. — *Proc. 3rd Internat. Meeting, Internat. Soc. Neurochem.* pp. 231, *Akadémiai Kiadó, Budapest.*
KOE, B. K., WEISSMAN, A. (1968): The pharmacology of para-chlorophenylalanine a selective depletor of serotonine stores. — *Adv. Pharmac.* **6B,** 29—47.
KUNTZMAN, R., SHORE, P. A., BOGDANSKI, D. F., BRODIE, B. B. (1961): Microanalitical procedures for fluorimetric assay of brain DOPA-5HTP decarboxilase, norepinephrine and serotonin and a detailed mapping of decarboxylase activity in brain. — *J. Neurochem.* **6,** 226—232.
MAICKEL, R., COX, R. H., SAILLANT, J., MILLER, F. P. (1968): A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. — *Int. J. Neuropharmac.* **7,** 275—281.
MIROLLI, M. (1968): Discussion of evidence for an excitatory transmitter role of serotonin in molluscan central synapses. — *Adv. Pharmac.* **6A,** 393—394.
MORTON, B. (1969): Studies on the biology of *Dreissena polymorpha.* II. Correlations of the rhythms of adductor activity, feeding, digestion and excretion. — *Proc. Malac. Soc. London,* **38,** 401.
PAVLOV, J. (1885): Wie die Muschel ihre Schaale öffnet. — *Pflügers Arch. ges. Physiol.* **37,** 6—31.
QUAY, W. B. (1963): Circadian rhythm in rat pineal serotonin and its modifications by estrous cycle and photoperiod. — *Gen. Comp. Endocrinol.* **3,** 473—479.
QUAY, W. B. (1967): Twenty-four-hour rhythms in cerebral and brainstem contents of 5-hydroxytryptamine in a turtle, *Pseudemys scripta elegans.* — *Comp. Biochem. Physiol.* **20,** 217—221.
SALÁNKI, J. (1963): The effect of serotonin and catecholamines on the nervous control of periodic activity in fresh-water mussel *(Anodonta cygnea).* — *Comp. Biochem. Physiol.* **8,** 163—171.
SALÁNKI J. (1970): Endogén ritmusok szabályozása. *Doktori értekezés.*
SALÁNKI, J., LÁBOS, E. (1963): Studies of the double innervation of adductor muscle tone in the clam *Anodonta cygnea* L. — *Acta Physiol. Acad. Sci. Hung.* **24,** 55—66.
SALÁNKI, J., BALLA, L. (1964): Ink-lever equipment for continuous recording of activity in mussels (mussel-actograph). — *Annal. Biol. Tihany* **31,** 117—121.
SALÁNKI, J., LUKACSOVICS, F. (1967): Filtration and O_2 consumption related to the periodic activity of fresh-water mussel *(Anodonta cygnea* L.). — *Annal. Biol. Tihany* **34,** 85—98.
SALÁNKI, J., PÉCSI, T., LÁBOS, E. (1968): On the role of ganglia in regulation of the activity of tonic molluscan *(Anodonta cygnea* L.) muscle. — *Acta Biol. Acad. Sci. Hung.* **19,** 391—406.
SALÁNKI, J., LÁBOS, E. (1969): On the role of cholinergic, adrenargic and tryptaminergic mechanism in the regulation of a "catch" muscle *(Anodonta cygnea* L.). — *Annal. Biol. Tihany* **36,** 77—93.
SALÁNKI, J., HIRIPI, L. (1970): Increase of serotonin in the adductor of *Anodonta cygnea* L. (Pelecypoda) relaxed by nerve stimulation and relation to the periodic activity. — *Comp. Biochem. Physiol.* **32,** 629—636.
SALÁNKI, J., VARANKA, I. (1971): Differences in the chemical sensitivity of nerve pathways in the central nervous system of fresh-water mussel *(Anodonta cygnea* L.). — *Annal. Biol. Tihany* **38,** 87—96.
SEGURA, E. T., BISCARDI, A. M., APELBAUM, J. (1967): Seasonal variations of brain epinephrine, norepinephrine and 5-hydroxytryptamine associated with changes in the EEG of the toad, *Bufo arenarum* HENSEL. — *Comp. Biochem. Physiol.* **22,** 843—850.
SHORE, D. A., ALPERS, H. S., BUSFIELD, D. (1966): On the mechanism of norepinephrine depletion by reserpine, metaraminol and related compounds and antagonism by monoamine oxidase inhibition. — In: *Mechanisms of release of biogenic amines.* (Eds.: von EULER, U. S., ROSELL, S., URNÄS, B.) *Pergamon Press.*

SPAFFORD, D. C., PENGELLEY, E. T. (1971): The influence of the neurohumor serotonin on hibernation in the golden-manteled ground squirrel, *Citellus lateralis*. — *Comp. Biochem. Physiol.* **38A,** 239—250.

SWEENEY, D. (1963): Dopamine: its occurrence in molluscan ganglia. — *Science* **139,** 1051.

UUSPÄÄ, V. J. (1963): The 5-hydroxytryptamine content of the brain and some other organs of the hedgehog *(Erinaceus europaeus)* during activity and hibernation. — *Experientia,* **19,** 156—158.

VALZELLI, L., GARATTINI, S. (1968): Behavioral changes and 5-hydroxytryptamine turnover in animals. — *Adv. Pharmac.* **6B,** 249—260.

VERESCHAGIN, S. M. (1960) Верещагин С. М. К вопросу о роли церебральных ганглиев в сократительной деятельности заднего мускула у анодонты. — *Нервная Система* **I.** 68—7—2.

VÉRÓ, M., SALÁNKI, J. (1969): Inductive attenuator for continuous registration of rhythmic and periodic activity of mussels in their natural environment. — *Med. Biol. Engng.* **7,** 235—237.

WALKER, R. J., RALPH, K. L., WOODRUFF, G. N., KERKUT, G. A. (1971): Evidence for a dopamine inhibitory post-synaptic potential in the brain of *Helix aspersa*. — *Comp. gen. Pharmac.* **2,** 15—26.

WELSH, J. H., MOORHEAD, M. (1960): The quantitative distribution of 5-hydroxytryptamine in the invertebrates, especially in the nervous system. — *J. Neurochem.* **6,** 146—169.

A PERIODIKUS AKTIVITÁS SZABÁLYOZÁSI MECHANIZMUSÁNAK— FARMAKOLÓGIAI VIZSGÁLATA TAVI KAGYLÓN (*ANODONTA CYGNEA* L.)

Hiripi László

Összefoglalás

Egy korábbi munkahipotézisünkből kiindulva vizsgáltuk, hogy a tavikagyló (*Anodonta cygnea* L.) ritmusának szabályozásáért felelős „aktivitási" és „nyugalmi" központok feltételezett transzmitter rendszerének farmakológiai befolyásolása hogyan hat a monoamin szintre és az aktivitás periodicitására. Az aktivitási központ szerotoninerg rendszerét a szerotonin szintézis gátlásával (pCPA), a lebontó enzim gátlásával (tranilcipromin), a szerotonin kiürítésével (reserpin, V-111) és exogén szerotoninnal befolyásoltuk.

A nyugalmi központ katecholaminerg rendszerét exogén dopaminnal és noradrenalinnal, α-metil-meta-tirozinnal, reserpinnel és tranilciprominnal befolyásoltuk.

A periodikus aktivitást és monoamin szintet vizsgálva azt találtuk, hogy:

1. A szerotonin szint meghatározó szerepet játszik az aktivitási központ működésében. Az exogén szerotonin által okozott tartós aktivitás a szerotonin szint növekedésének következménye, mely elsősorban az aktív periódusok átlaghosszának növekedésében jelentkezik.

2. A monoamino-oxidáz gátlószer tranilcipromin nem növeli a ganglionáris szerotonin szintet, de a perifériás inaktiváció hiányán keresztül tartós aktív periódust és a ritmikus kontrakciók nagymértékű növekedését eredményezi. A kezelés után pedig a ritmicitás frekvenciáját növeli az aktív és nyugalmi periódusok átlaghosszának csökkentésén keresztül.

3. A szerotonin szint csökkenése pCPA és reserpin hatására az aktív periódusok átlaghosszának csökkenését és a nyugalmi periódusok hosszának növekedését eredményezi A p-bróm-methamphetamin a szerotonin szint kismértékű csökkentésén keresztül elsősorban a ritmicitás frekvenciáját növeli.

4. Míg a dopamin mind az aktív, mind a nyugalmi periódusok átlaghosszát csökkenti, addig a noradrenalin a nyugalmi periódust növeli. Az α-MMT hatása valószínű-

leg a NA hatásán keresztül realizálódik és a felszabaduló NA okozza a nyugalmi periódus jelentős növekedését.

5. A reserpin kezelés alkalmával a nyugalmi központ túlsúlyát a szerotonin szint jelentős csökkenésén túl a NA lassúbb kiürülése folytán még meglevő jelentős NA szint is okozza.

6. A szerotonin szint önmagában nem meghatározója az aktivitási központ működésének. Elsősorban a záróizom irányába történő aktív transzport az a folyamat, amely a záróizom relaxációjában, azaz az aktív periódus szabályozásában részt vesz. Az 5HT szint csökkenése csak bizonyos kritikus szint alatt befolyásolja az aktív transzportot.

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THE ROLE OF IONIC ENVIRONMENT IN THE POTENTIAL GENERATION OF THE GIANT NEURONES OF *LYMNAEA STAGNALIS* L.

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In our earlier papers (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971) a number of identifiable neurones were described, which exhibited a marked variation in activity, but the type of activity was always characteristic of a given neurone. Then the question arose, what delicate mechanisms are responsible for the differences in the generation of the pacemaker activity of individual neurones? In examining this question it has to be considered that the pacemaker activity of giant neurones is generated by a different mechanism (WAZIRI et al., 1965) and in a different part of the membrane (TAUC, 1962) than the activity evoked either by natural or artificial stimulation. On the other hand, there are some differences in the special ionic conductances underlying the pacemaker generation of the spike in the nervous system of various species (KOSTYUK, 1968), furthermore the individual neurones of the same animal can also have different properties in this respect. As the maintenance of the membrane potential as well as the generation of the action potential can be explained on the basis of the concentration gradients and conductances for Na^+, Ca^{++}, K^+ and Cl^- ions (HODGKIN and HUXLEY, 1952), it might be supposed that different ionic processes underlie the variety of the spontaneous generation of activity. In close relation to this the possibility of electrogenic ion-currents has to be considered, too (THOMAS, 1972).

Concerning the CNS of *Lymnaea stagnalis* only a few contradictory data have been reported on this problem (MAGURA et al., 1971; JERELOVA et al., 1972; SATELLE and LANE, 1972). In the present paper experiments were performed in order to study the effect of Na^+-, Ca^{++}-, K^+- and Cl^--free solutions on some identified neurones of *Lymnaea stagnalis*. In the first place we wished to elucidate whether the ionic requirements for the potential generation of different neurones are different, and to obtain convincing data referring to some questions like the problem of ions involved in the generation of membrane and action potentials and the problem of the ionic dependence of spontaneous and synaptically driven activity.

Material and methods

Examinations were conducted on the abdominal and right parietal ganglia of isolated CNS of *Lymnaea stagnalis* using the identifiable giant neurones demonstrated in *Fig. 1.* All the experiments were performed at room

temperature. The examined neurones are located on the dorsal surface of ganglia, whose most important electrophysiological parameters have already been described (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971).

Membrane and action potentials were recorded by glass microelectrodes filled with 2.5 M KCl. The current for polarizing the membrane was transmitted through the same electrode. The membrane potential and its changes were measured after the compensation of tip potential of the electrode using a digital voltmeter. For recording we used an amplifier with FET input (KISS et al., 1972).

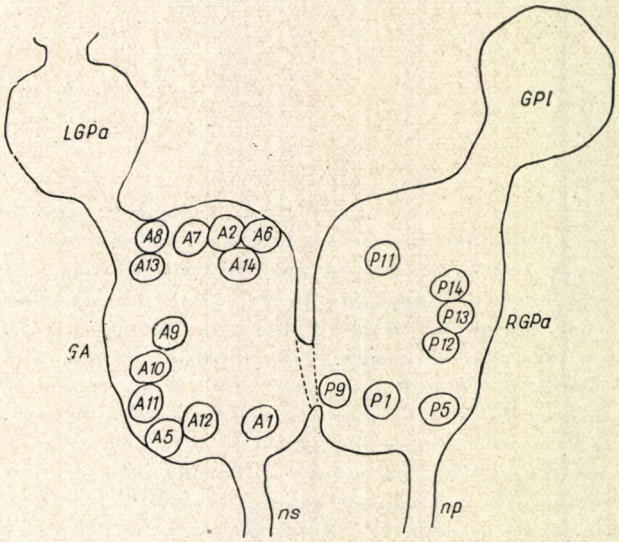

Fig. 1. Localization of the identified neurones. GPl — ganglion pleurale; LGPa — left ganglion parietale; RGPa — right ganglion parietale; GA — ganglion abdominale; ns — nervus splanchnicus; np — nervus pallialis

The isolated ganglion ring, from which the thick connective tissue was removed was placed in a chamber containing 3 ml physiological saline. The composition of this saline was: NaCl 46.2 mM; KCl 4 mM; $CaCl_2$ 5.6 mM (JULLIEN and RIPPLINGER, 1948).

Test solutions with no content of a given ion were made as follows:

NaCl was replaced in 3 different ways: by osmotically equivalent quantity of cholin-Cl, Tris-Cl or saccharose alternatively. No significant differences could be found between these solutions so they are to be described collectively.

$CaCl_2$ was replaced by osmotically equivalent quantity of saccharose.

In the K^+-free solution equivalent concentration of NaCl was used instead of KCl.

For making a Cl^--free solution various combinations of the following compounds were used: Na-, K-, and Ca-propionate, Na- and K-acetate, Na- and K-sulphate. As to replace $CaCl_2$, Ca-propionate was used in every case the effect of Ca-propionate was separately tested under normal conditions at normal Cl^--concentration.

After recording the control activity in physiological solution the whole volume of this solution was exchanged for a given ion-free one. Following

this the chamber was continuously perfused with the latter solution at least for 5 min.

In some of the K^+-free solution experiments the microelectrodes were filled with 3 M NaCl instead of KCl.

For examining the excitability of the soma depolarizing current or chemical stimulation was applied to the membrane. For the chemical stimulation ACh was added to the bath at a similar concentration and in similar way to that described in one of our earlier papers (KISS and SALÁNKI, 1971).

During recording the bioelectric signals were fixed on a magnetic tape and the desired portions were photographed later by means of an EMG oscilloscope and MF 1—1 photorecorder.

Results

On the basis of the reaction to the removal of Na^+ the neurones can be classified into the following categories: The spontaneous activity of the cells belonging to the first category is abolished within 2—3 min. in Na-free solution It is most frequently preceded by a continuous reduction of the spike amplitude and simultaneously the firing rate decreases, too *(Figs 2* and *3)*. This type of reaction was shown by A1, A5 and P5 neurones, whose activity seemed to be under compound synaptic control.

The neurones included in another category cease their activity within 6—8 min. *(Fig. 4)*, e.g. the P12 pacemaker and the A11 driven neurone belong into this group. In addition two pacemaker neurones were found, which continued to generate action potential more than 10 min. (A10 and P1 cell). From the latter cell sometimes pacemaker activity can be registered even 30—40 min. after the removal of Na^+. The neurones included in the latter two categories are less sensitive to the absence of Na^+, their action potential is characterized by only a slight reduction of amplitude, furthermore after a transitional decline it frequently tends to be normalized *(Fig. 5)*.

Immediately after the application of the Na^+-free solution a transitional hyperpolarization of the membrane of 8—10 mV can be registered and during this period the spontaneous activity is often completely blocked, but it returns again in 4—5 min. *(Fig. 6)*. However the final disappearance of activity does not yet occur in this period, on the contrary, sometimes it is preceded by a depolarization of the membrane.

After the spontaneous activity ceased in the Na^+-free solution all the examined neurones can be activated by depolarization indicating that the excitability of the soma membrane is maintained *(Fig. 7)*. However, after prolonged treatment with the Na^+-free solution also the electric excitability of membrane disappears. The time required for this is only slightly variable ranging between 18 and 20 min. except P1 cell, which can be activated by depolarization for undefined time within the period of the investigation.

The question raised, whether a neurone of such condition is able to react to electric stimulation only. As the majority of the identified neurones can be excited by ACh, this substance seemed to be suitable for trying to activate the neurones became silent in the absence of Na^+. Some of the cells — for example A10 — are depolarized and generate a short train of spikes following

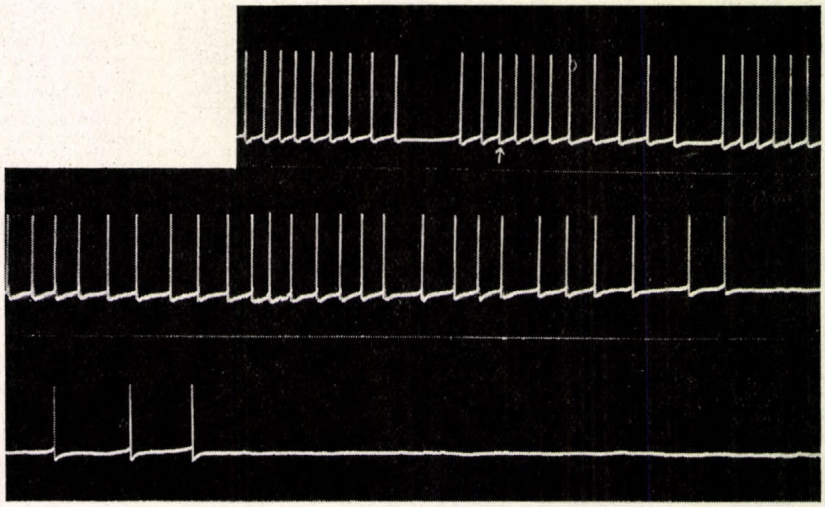

Fig. 2. Course of the abolition of spontaneous activity in the Na-free solution on P5 neurone. Arrow marks the exchange of physiological solution

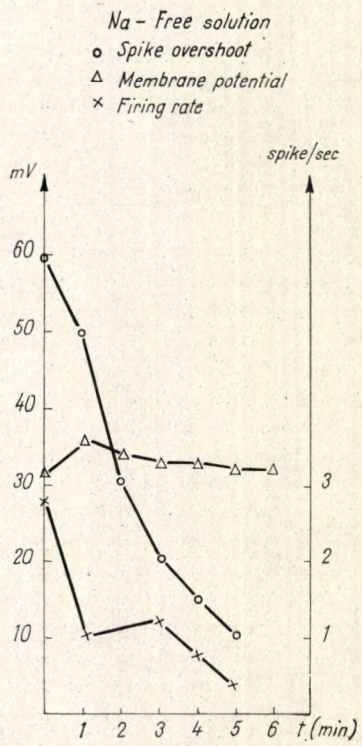

Fig. 3. Changes in the parameters of spontaneous activity of A1 cell as a function of time

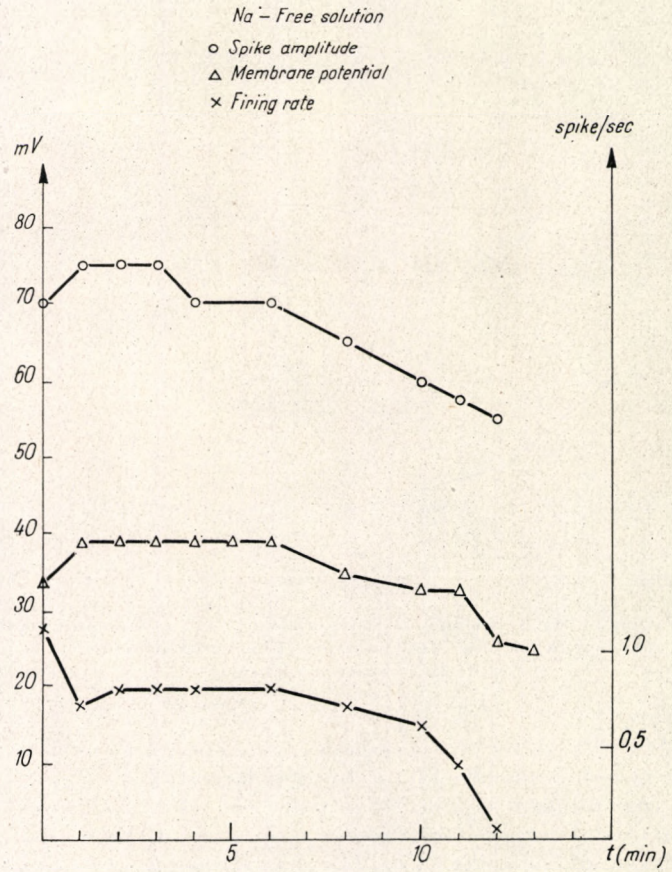

Fig. 4. Changes in the parameters of spontaneous activity of A10 cell as a function of time

the application of ACh even in Na^+-free solution *(Fig. 8)*. But the other neurones cannot be activated by ACh.

On the cell A10 mentioned above a special effect of Na^+-free solution was occasionally found. Its firing rate — diverging from normal — increased in the early period. It can be attributed to an early abolition of ILD-s determining the general character of the activity when the firing rate of rhythmic pacemaker activity does not yet significantly declined *(Fig. 9)*.

Effect of the removal of Ca^{++}

In Ca^{++}-free solution the spontaneous activity generally disappears in 5—10 min. The changes in the membrane potential are not unequivocal, although in most of the cases a transitional depolarization can be observed in the early phase. It is accompanied by a marked increase in the firing rate giving place to a decreased frequency a few minutes before the activity is abolished. There is a continuous marked reduction in the amplitude of the action

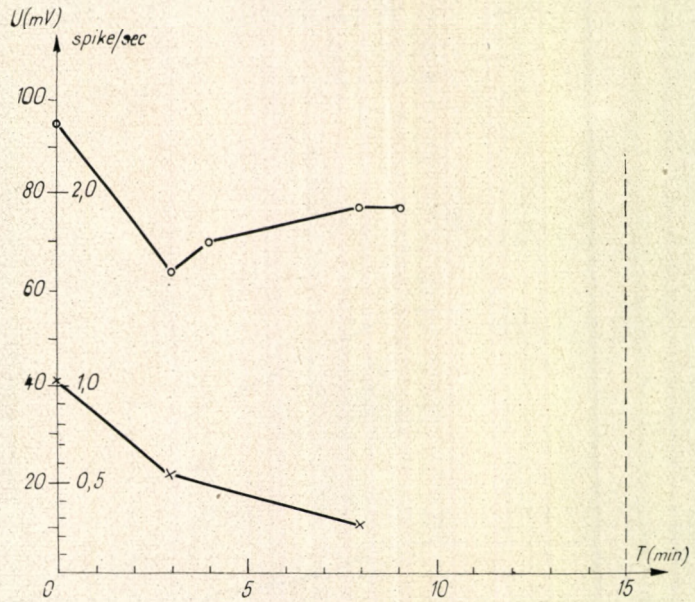

Fig. 5. Alteration of the spontaneous firing rate (— x —) and amplitude of the spike produced by A11 cell in the Na^+-free solution. The vertical broken line marks the time of the cessation of activity

potential *(Fig. 10)*. After the cessation of the spontaneous activity it cannot be restored either spontaneously or by depolarization.

Ca^{++}-free solution is able to activate the silent neurones, although for only a short time, because later there is a damage of the mechanism involved in the generation of the spike *(Fig. 11)*. There was only two neurones (A5 and P12) whose spontaneous activity was maintained in Ca^{++}-free solution for as long as 15—20 min. or even longer *(Fig. 12)*.

On an individual neurone we failed to demonstrate any definite correlation between time courses of the capability for producing spikes in Na^+- and Ca^{++}-free solution.

Effect of the removal of K^+

In K-free solution the membrane potential of the neurones increases by 5—10 mV. It is to be expected because of the increased K^+-concentration gradient between the two sides of the membrane. This hyperpolarization is accompanied by an increase in the amplitude of action potential and a decrease in the frequency of spontaneous activity *(Fig. 13)*. The rate of decrease in the frequency is rather variable, in most of the cases it is below 50%, in some other cases, it can be much greater until the activity is eliminated. Sometimes this elimination occurs in such a way that the spontaneous, continuous activity is broken off by a hyperpolarization of 10—20 mV *(Fig. 14)*.

In a general given identified neurone cannot be characterized by any definite type or degree of the above changes, as there is a great variability

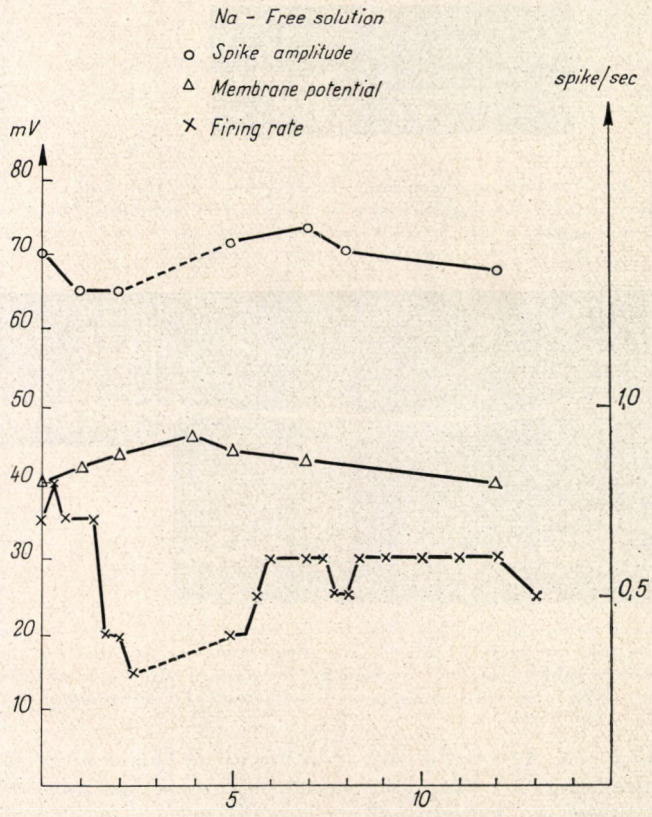

Fig. 6. A10 cell; time relations of the temporary cessation and recovery of the spontaneous activity in the Na^+-free solution. During the interval marked by broken line the neurone is silent. Notice, that this interval coincides with a transitional hyperpolarization of the membrane

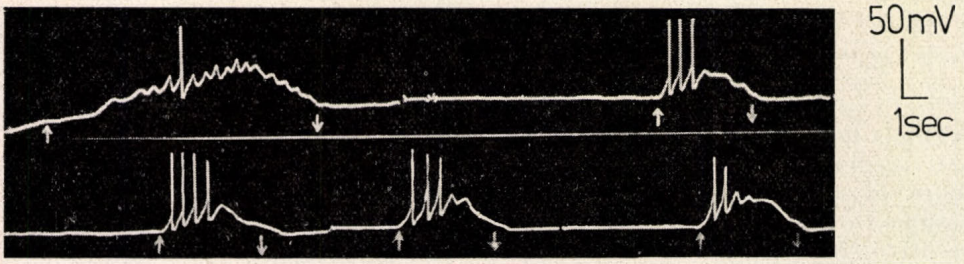

Fig. 7. Activation of P5 neurone by an artificial depolarization in the Na^+-free solution after the complete cessation of the spontaneous activity. Arrows mark the onset and interruption of depolarizing DC in order at 6; 10; 14; 18 and 22 min. from the application of the Na^+-free solution. Notice, that in the 6th min. only local potentials are produced in addition to a single spike, after this there is a continuous recovery of the generation of spike, but later it is blocked again

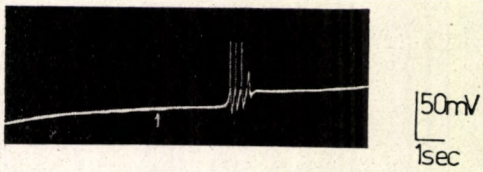

Fig. 8. A10 cell lost the spontaneous activity in the Na^+-free solution is activated by ACh in the 10th min. after the application of the ion-free solution. Note: the shift in DC level preceding the arrow is an artifact resulted from an error in the function of the magnetic tape-recorder

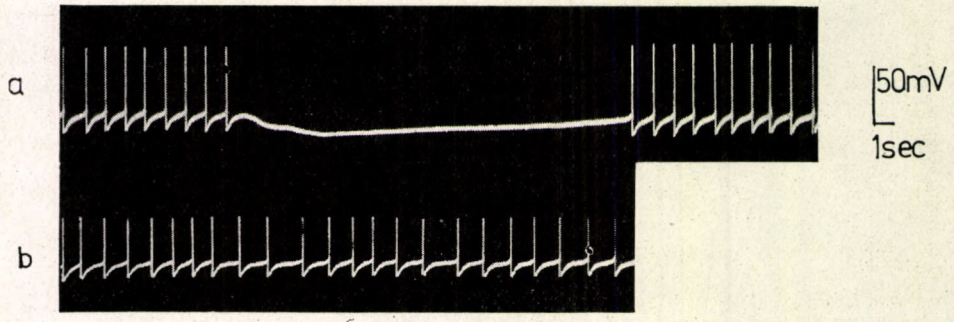

Fig. 9. Transitional effect of the Na^+-free solution on A10 cell realized in an increased firing rate. *a)* control activity; *b)* in the 3rd min. after the application of the Na^+-free solution it is fairly visible that the increase in frequency observed simultaneously with a reduction in the amplitude is resulted from the elimination of ILD

even on the same cell. A10 cell is an exception as on this cell the sudden hyperpolarization breaking off the continuous spontaneous activity can most frequently be observed. Occasionally it results in a permanent cessation of firing, on another occasion the silence appears to be transitional with a time course quite similar to that of ILD *(Fig. 15)*.

A series of the measurements was done by means of microelectrodes filled with NaCl. Under these conditions the K^+-free solution caused much greater hyperpolarization *(Fig. 16)*. At the same time no significant difference was found in the changes observed in the amplitude and frequency of the spikes compared with that measured by means of the KCl-filled microelectrodes.

Effect of the removal of Cl^-

Cl^--free solution results in characteristic changes in the type of activity within a few minutes. The discharge pattern becomes irregular, the continuous activity tends to be replaced by groups of the spikes *(Fig. 17 a, b)*. Furthermore, in some measurements performed in the absence of Cl^- the activity assumed a bursting character *(Fig. 17 c)*, where the duration of the consecutive spikes also showed peculiar changes *(Figs 17 d, e)*. Changes of this nature were produced for example on A10 cell. On this cell a burst-like grouping of spikes had been observed also in the control state *(Fig. 18 a)*, and this character became more pronounced upon the removal of Cl^- *(Fig. 18 b)*. It can frequently be observed that the potential generation during a burst is inactivated for a short time at a depolarized level *(Fig. 19)*.

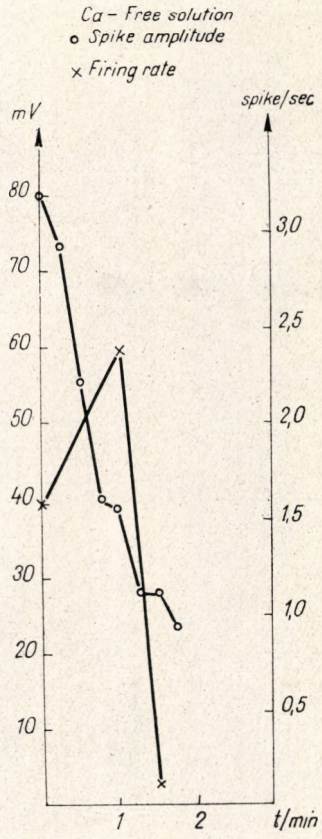

Fig. 10. The most frequently observable influence of the Ca++-free solution on the spontaneous activity: a fast reduction the firing rate shows a biphasic change

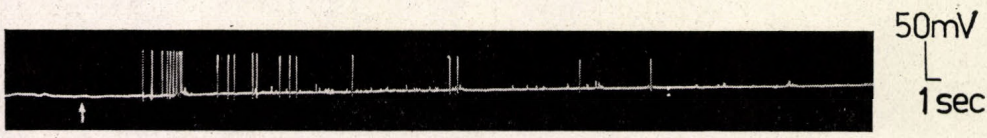

Fig. 11. Activation of a silent neurone by Ca++-free solution. Arrow marks the exchange of physiological solution. Notice, that after a temporary activation the firing rate and the amplitude rapidly decrease, following this the activity disappears at a depolarized level

In the absence of Cl^- all the neurones are characterized by a decrease in the spike amplitude and an increase in the impulse duration.

If ACh was applied to the preparation in a solution containing no Cl^- the burst-like sequence of the potentials was considerably prolonged *(Fig. 18 c)*.

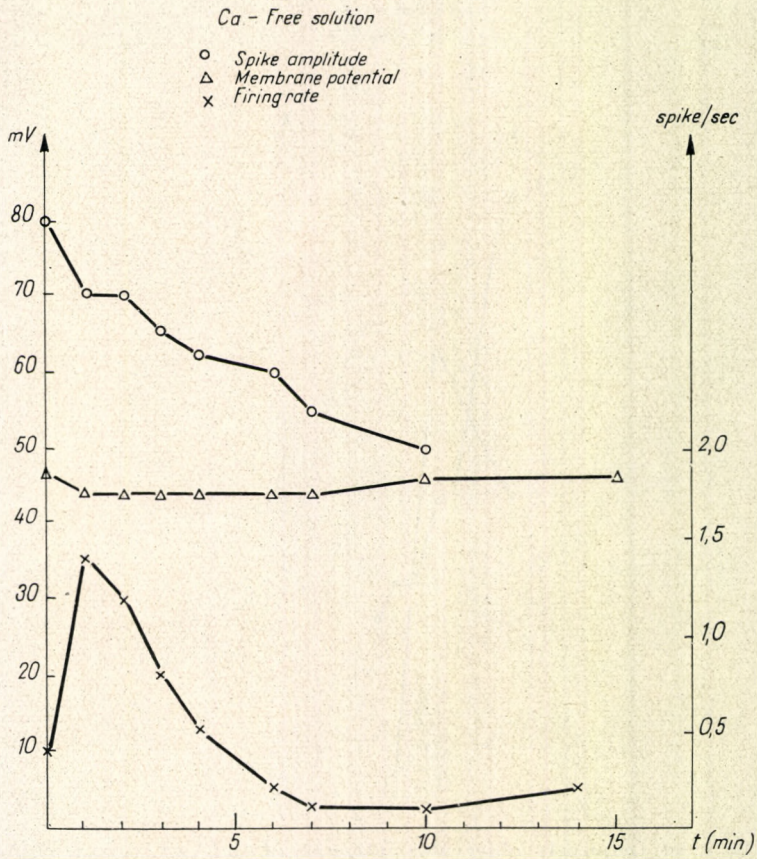

Fig. 12. Changes in the parameters of spontaneous activity in the Ca^{++}-free solution on P12 neurone, which continues to produce spikes more than 15 min. after exchanging the physiological solution

Discussion

It has been described by a number of investigators that the activity of some giant neurones is maintained in Na^+-free solution for quite a long time. In these cases it was supposed that the rising phase of the spike is produced by a Ca^{++} current (Gerasimov et al., 1965; Oomura et al., 1961; Jerelova et al., 1972). In some of the cases the "calcium-spike" hypothesis was reconsidered and either a contribution of both Na^+ and Ca^{++} to the generation of action potential was suggested, (Meves, 1968; Geduldig and Junge, 1968; Chamberlain and Kerkut, 1969), or a passive retention or active depot of Na^+ was conceived, which might be able to assure the Na^+-requirement for the spike generation even if the extracellular space is apparently free from Na^+. (Krasts and Veprintzev, 1972; Moreton, 1972). In our examinations the spontaneous activity of most neurones ceased in the Na^+-free solution within several minutes suggesting that Na^+ appears to be the main carrier of the current involved in the generation of action

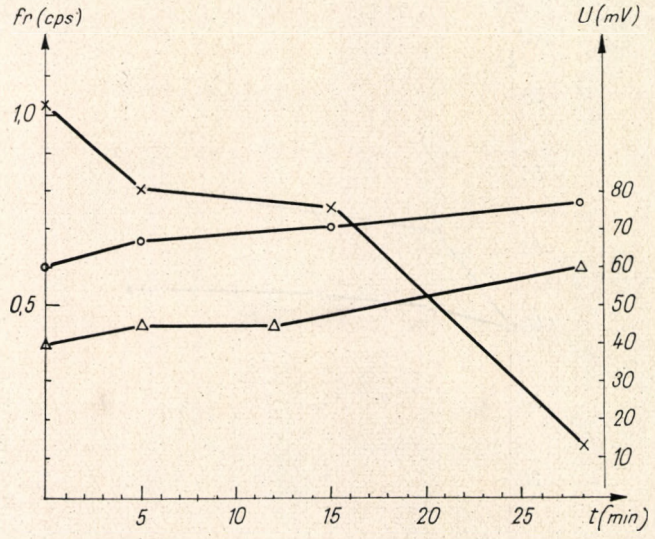

Fig. 13. Influence of the K^+-free solution on the parameters of spontaneous activity of Pl neurone

Fig. 14. Sudden hyperpolarization under the influence of the K^+-free solution in the 6th min. after exchanging the physiological solution on Pl neurone

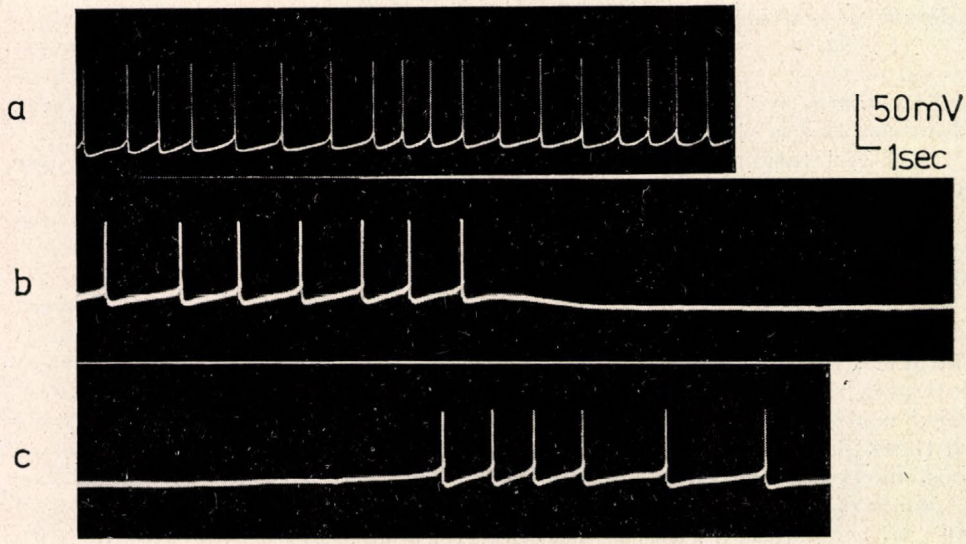

Fig. 15. Effect of the K^+-free solution on A10 cell. *a)* control activity which is not interrupted by ILD-s; *b)* and *c)* in the 4th min. after the application of the K^+-free solution, the continuous spontaneous activity is broken off by a hyperpolarizing phase similar to ILD

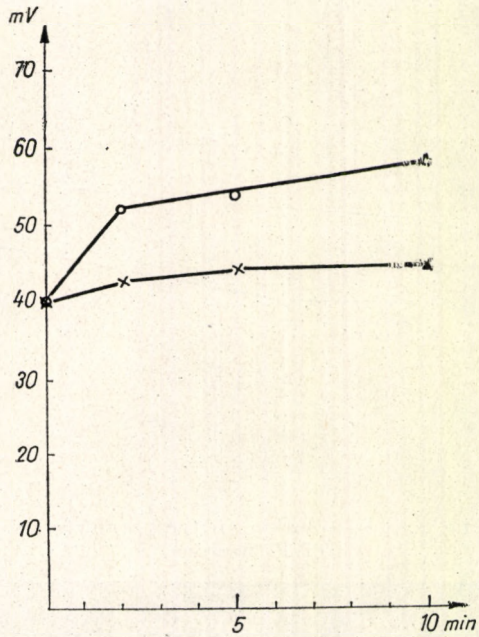

Fig. 16. Development of the hyperpolarizing effect of the K^+-free solution in the case of recording with a KCl-filled (—x—) and a NaCl-filled (—o—) electrode. Both curves show an average of values obtained on the examined neurones

potential. Generally, considering especially the case, when the spike generation in the Na^+-free solution is abolished after a considerable time only, some considerations contradict the presumption of a "calcium-spike":

1. If Ca^{++} were the main carrier of current, one would expect the removal of Ca^{++} to block the generation of the spike most rapidly on those cells whose activity is less sensitive to the removal of Na^+, e.g. P1 neurone. However, this is not supported by the experimental results.

2. Activation of the silent neurones by exposure to the Ca^{++}-free solution is hardly compatible with a "Ca-spike" hypothesis.

3. It was previously shown, that the inward current which had disappeared in Na^+-free solution did not reappear by raising the Ca^{++} concentration.

The question arose, what causes the different sensitivity of different identified neurones to the removal of Na^+? In our earlier papers (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971) it was described that the membrane potential, rhythmic activity and chemical sensitivity are specific to an identified neurone, thus it is conceivable that the ionic mechanisms underlying all these phenomena may also be characteristic of a given cell. Nevertheless, most likely a retention of Na^+ near the membrane has to be considered, too — similarly to other objects. MORETON (1972) attributed the retention of Na^+ to the function of an active pump, which requires some time to turn into an active state under Na^+-free conditions. This suggestion is based on the observation that after an early transitional decrease in the amplitude the spike can be restored. In that cases, when the spontaneous activity of the

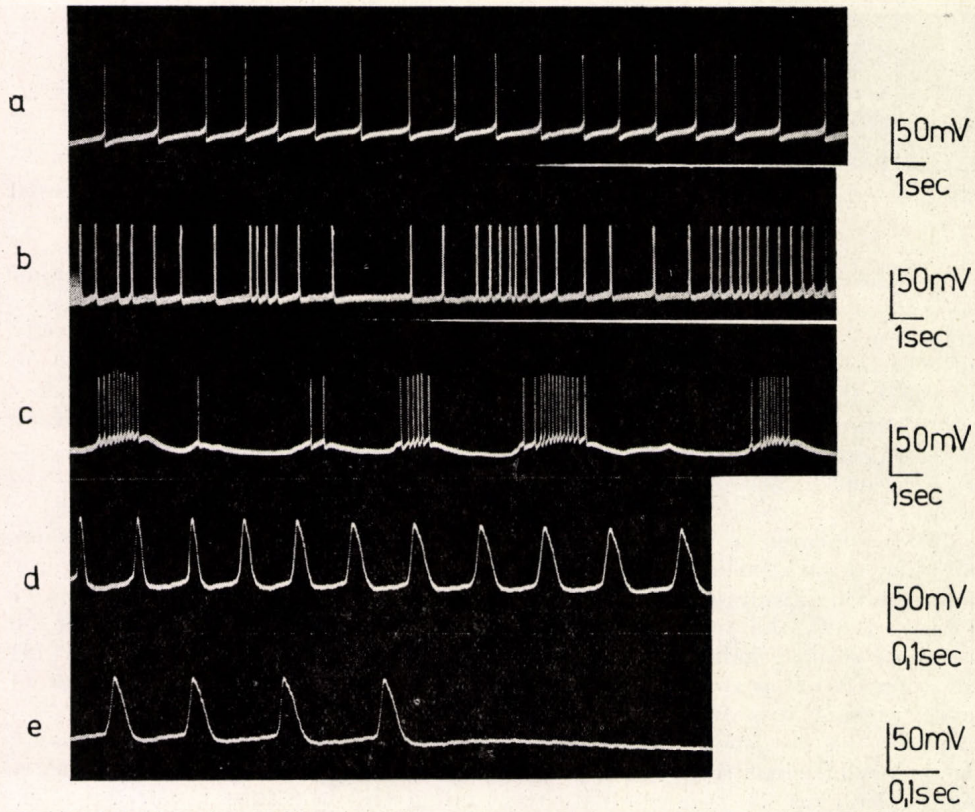

Fig. 17. Effect of the Cl^--free solution. *a)* control activity of A11 neurone; *b)* activity of the same neurone in the 3rd min. following the application of Cl^--free solution. *c)* in the 16th min. the activity assumes a bursting character *d)* and *e)* (continuously) changes in the shape of the action potentials during a burst

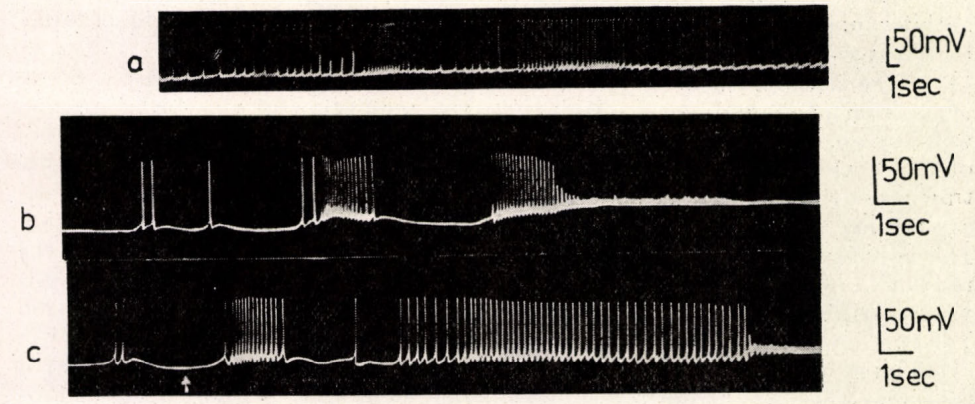

Fig. 18. Effect of the Cl^--free solution on A10 neurone. *a)* control activity demonstrating well the burst-like grouping of the spikes; *b)* in the Cl^--free solution the bursting activity becomes more pronounced; *c)* in the Cl^--free solution ACh applied at the arrow prolongs the time course of the burst

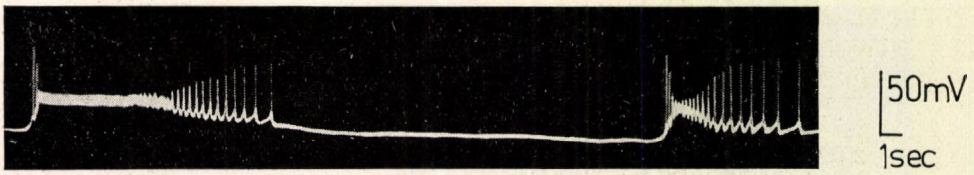

Fig. 19. Effect of the Cl^--free solution on A10 cell. It is fairly visible that the potential generation is inactivated within a burst at a depolarized level

cells examined in the present experiments ceased within 10 min., we found no sign of this phenomenon thus, in this case one can consider a passive retention of Na^+ at the most, variations of which depending on the anatomy of cells may account for the variability of the time required for the abolition of the activity of different identified neurones. However, on the cells firing more than 6—8 min. in absence of Na^+ one can frequently observe a recovery after an early decrease in the spike amplitude and in the firing rate consequently if an active depot exists, it requires at least 6—8 min. to turn into an active state.

It appeared to be characteristic of all the neurones that the spontaneous activity ceased earlier than the excitability of the soma. As on the giant neurones the spontaneous activity is generated in a distant part of the axon (TAUC, 1962), the present observation has from a new side confirmed the results obtained indirectly by voltage clamp technic (MAGURA et al., 1971) showing that it is the soma which reacts later to the removal of Na^+. However, present investigations do not allow to formulate a definite decision, whether this fact shows an actual difference in the ionic mechanisms, or there is a more efficient retention of Na^+ near to the soma membrane. The latter assumption can be supported by the finding that the time required for the abolition of the excitability of the soma does not considerably vary from neurone to neurone, it is 18—20 min. after removing the external Na^+. On the basis of the latter assumption it means the time required for a total running out of Na^+-depot.

The difference in the Na^+ requirement for spontaneous and evoked activity observed in our experiments is in contrast with the data obtained by CARPENTER and GUNN (1970).

The ionic permeability of the membrane is an important factor of the generation of spontaneous activity. In the regulation of this Ca^{++} plays a well-known role. After the elimination of the membrane-stabilizing action of Ca^{++} the alteration of Na^+- and K^+-permeability can account for the transitional depolarization, increased firing rate and finally a complete disappearance of the excitability of the membrane in the Ca^+-free solution. The individual identified neurones show differentiated behaviour also in this respect, however, the time required for the abolition of spontaneous activity varies within a more limited interval compared with the Na^+-free solution. For the explanation of the observed deviation of data concerning Ca^{++} it is even more obvious to suppose a certain degree of retention, because after removing the Ca^{++} from the solution some membrane-bound Ca^{++} remains.

Recently a number of authors have proved the role of an electrogenic Na^+-pump in addition to the unequal distribution of the ions in the maintenance of the membrane potential of giant neurones (KERKUT and THOMAS,

1965; CARPENTER and ALVING, 1968; KOSTYUK et al., 1972; CHRISTOFFERSEN, 1972). On the basis of our results there is a reason to suppose a contribution of electrogenic Na^+-pump to the membrane potential also of the examined neurones of *Lymnaea*. This is indicated by the fact that in the K^+-free solution there is only a slight increase in the resting membrane potential although the $[K]_o - [K]_i$ concentration gradient has increased considerably. It can be explained if the resting potential measured under physiological conditions is not a pure K^+-potential but it is resulted also from the simultaneous function of an electrogenic Na^+-pump. The latter can be blocked by the removal of external K^+, thus in the K^+-free solution the increase in the resting potential cannot reach the theoretically expected value. On *Aplysia* neurones CARPENTER (1970) obtained a pronounced depolarization under K^+-free conditions at 25° C and explained this phenomenon in a similar way.

The above explanation is confirmed by our examinations with NaCl-filled electrodes. The microelectrodes with tip-diameter of about 1 μ had been kept in the cell for 5—10 min. before the K^+-free solution was applied. During this time some spontaneous outflow of Na^+ probably occurred, which might have been of extremely small amount though, nevertheless, sufficient to stimulate the Na^+-pump. The fact that on increasing the intracellular concentration of Na^+ this stimulating effect can be obtained has been described by several authors (KERKUT and THOMAS, 1965; THOMAS, 1969; CHRISTOFFERSEN, 1972).

One possible explanation of the transitional hyperpolarization observed in the Na^+-free solution is a high resting Na^+-permeability of the membrane. In consequence of the removal of Na^+ the voltage opposed to K^+-potential resulted from the Na^+-gradient is eliminated, thus the membrane potential can much better approximate the value to be expected on the basis of the concentration gradient of K^+.

The reason of the burst-like activity developed in the Cl^--free solution may be approximated by the following assumption: In the Cl^--free solution HODGKIN and HOROWITZ (1959) demonstrated an about 20 mV transitional depolarization of the membrane, which lasted for 15—20 min. This coexisted with an increased outflow of K^+. The decrease in resting potential may temporary activate the potential generation. It might be supposed, that the K^+-conductance of the membrane is also damaged resulting in a delayed repolarization phase and an inactivation of generation of the spike at a depolarized level as the increased Na^+-influx during a burst cannot be balanced by the outward K^+-current. Besides, after a certain time a hyperpolarizing phase is resulted by stimulating the activity of the electrogenic Na^+-pump.

Concerning the aberrant behaviour of some neurones obviously it has to be considered that the majority of the examined neurones has synaptic inputs. On the cell A10 and on several other cells sometimes it can fairly be observed that the bursts produced in the Cl^--free solution are preceded by EPSP-s. The possibility cannot be disregarded that on the cells having excitatory synaptic input the effect of the absence of Cl^- may be realized through the postsynaptic membrane, too.

The reactions given to depolarization and ACh in different ion-free solutions generally did not differ from the control. However, in the Na^+-free solution there were some cells which could be activated only by depolarization but were uneffected by ACh. In these cases the mediator effect probably is

realized by changing the Na^+-permeability. CHIARANDINI et al. (1967) suggested the existence of such a mechanism on a cell of CILDA type. In our earlier experiments (KISS and SALÁNKI, 1971) performed on CNS of Lymnaea one of the neurones — marked A10 — was identified as a cell of CILDA type. However, the absence of Na^+ has no influence on the reaction to ACh on this neurone, consequently, the above suggestion cannot be generalized.

Summary

On changing the ionic environment of CNS of *Lymnaea stagnalis* it has been established that

1. On removing the Na^+ the activity of several neurones ceased within a short time, while that of the other neurones ceased later. Even such a neuron was found, which continued to generate action potentials for as long as 30 min. in the absence of Na^+.

After the activity stopped the cells could be activated by depolarization, while only a part of the neurones was affected by ACh under such conditions.

2. In Ca^{++}-free solution the spontaneous activity of the cells was abolished within a relatively short time, but the activity of several cells was maintained for 15—20 min.

3. In the absence of K^+ a relatively small hyperpolarization was accompanied by a decrease in the firing rate.

4. In the absence of Cl^- the rhythm of activity of the cells became irregular, sometimes it assumed a bursting character.

The obtained data can be accounted for partly by different changes in the ionic concentration gradients and in the permeability of the membrane, partly by an influence on the electrogenic Na^+-pump.

REFERENCES

CARPENTER, D. O. (1970): Membrane potential produced directly by the Na^+ pump in *Aplysia* neurons. — *Comp. Biochem. Physiol.* **35,** 371—385.

CARPENTER, D. O., ALVING, B. O. (1968): A contribution of an electrogenic Na^+ pump to membrane potential in *Aplysia* neurons. — *J. Gen. Physiol.* **52,** 1—19.

CARPENTER, D. O., GUNN, R. (1970): The dependence of pacemaker discharge of *Aplysia* neurons upon Na^+ and Ca^{++}. — *J. Cell. Physiol.* **75,** 121—128.

CHAMBERLAIN, S. G., KERKUT, G. A. (1969): Voltage clamp analysis of the sodium and calcium inward currents in snail neurones. — *Comp. Biochem. Physiol.* **28,** 787—801.

CHIARANDINI, D. J., STEFANI, E., GERSCHENFELD, H. M. (1967): Ionic mechanism of cholinergic excitation in molluscan neurons. — *Science* **156,** 1597—1599.

CHRISTOFFERSEN, G. R. J. (1972): Steady state contribution of the Na^+- K^+- pump to the membrane potential in identified neurons of *Helix aspersa*. — *Acta Physiol. Scand.* **86,** 498—514.

GEDULDIG, D., JUNGE, J. (1968): Sodium and calcium components of action potential in the *Aplysia* giant neurone. — *J. Physiol. (London)* **199,** 347—365.

GERASIMOV, V. D., KOSTYUK, P. G., MAISKI, V. A. (1965): Excitability of giant nerve cells of various pulmonate molluscs in sodium-free solutions. — *Fed. Proc.* **24,** T676.

HODGKIN, A. L., HOROWITZ, P. (1959): The influence of K^+ and Cl^- ions on the membrane potential of single muscle fibres. — *J. Physiol.* **148,** 127—160.

HODGKIN, A. L., HUXLEY, A. F. (1952): Currents carried by Na^+ and K^+ ions through the membrane of the giant axon of *Loligo*. — *J. Physiol.* **116,** 449—472.

JERELOVA, O. M., KRASTS, I. V., VEPRINTZEV, B. N. (1972): The effect of Na^+, Ca^{++} and Mg^{++} on the amplitude of the action potential from giant neurons of *Lymnaea stagnalis*. — *Comp. Biochem. Physiol.* **40,** 281—293.

JULLIEN, A., RIPPLINGER, J. (1948): Sur l'automatisme du isolé du coeur de Lymnée. — *C. R. Acad. Sci. (Paris)* **226,** 1396.
KERKUT, G. A., THOMAS, R. C. (1965): An electrogenic sodium pump in snail nerve cells. — *Comp. Biochem. Physiol.* **14,** 167—183.
KISS, I., SALÁNKI, J. (1971): The heterogenic chemical sensitivity of the central neurones of *Lymnaea stagnalis.* — *Annal. Biol. Tihany* **38,** 39—52.
KISS, I., SALÁNKI, J., VÉRÓ, M. (1972): Dependence of reaction to ACh on the membrane potential of neurones of *Lymnaea stagnalis.* — *Annal. Biol. Tihany* **39,** 21—27.
KOSTYUK, P. G. (1968): Ionic background of activity in giant neurons of Molluscs. — *Neurobiology of Invertebrates* (Ed.: J. SALÁNKI). *Akadémiai Kiadó, Budapest and Plenum Press, New York* pp. 145—167.
KOSTYUK, P. G., KRYSHTAL, O. A., PIDOPLICHKO, V. I. (1972): Potential dependent membrane current during the active transport of ions in snail neurones. — *J. Physiol.* **226,** 373—392.
KRASTS, I. V., VEPRINTZEV, B. N. (1972): The giant neurons of *Tritonia:* Its electric properties and the ionic dependence of the action potential. — *Comp. Biochem. Physiol.* **41,** 289—296.
MAGURA, I. S., KISS, I., KRYSHTAL, O. A. (1971): Current-voltage relations of the giant neurone soma membrane of *Lymnaea stagnalis.* — *Acta physiol. Acad. Sci. hung.* **40,** 221—228.
MEVES, H. (1968): The ionic requirements for the production of action potentials in *Helix pomatia* neurons. — *Pflügers Arch.* **304,** 214—241.
MORETON, R. B. (1972): Electrophysiology and ionic movements in the central nervous system of the snail, *Helix aspersa.* — *J. Exp. Biol.* **57,** 513—541.
OOMURA, Y., OZAKI, S., MAENO, T. (1961): Electrical activity of a giant nerve cell under abnormal conditions. — *Nature (Lond.)* **191,** 1265—1267.
SALÁNKI, J., KISS, I. (1969): Identified cells in the central nervous system of *Lymnaea stagnalis* L. — *Annal. Biol. Tihany* **36,** 63—75.
SATTELLE, D. B., LANE, N. J. (1972): cit. in: Moreton, R. B. (1972): Electrophysiology and ionic movements in the central nervous system of the snail, *Helix aspersa.* — *J. Exp. Biol.* **57,** 513—541.
TAUC, L. (1962): Site of origin and propagation of spike in the giant neuron of *Aplysia.* — *J. Gen. Physiol.* **45,** 1077—1097.
THOMAS, R. C. (1969): Membrane current and intracellular sodium changes in a snail neurone during extrusion of injected sodium. — *J. Physiol.* **201,** 496—514.
THOMAS, R. C. (1972): Electrogenic sodium pump in nerve and muscle cells. — *Physiol. Rev.* **52,** 563—595.
WAZIRI, R., FRAZIER, W., KANDEL, E. R. (1965): Analysis of pacemaker activity in an identifiable burst generating neuron in *Aplysia.* — *Physiologist* **8,** 300—310.

AZ IONMILIŐ SZEREPE *LYMNAEA STAGNALIS* L. ÓRIÁS NEURONJAINAK POTENCIÁL GENERÁLÁSÁBAN

Kiss István és Salánki János

Összefoglalás

Az ionmiliő változtatása során megállapítást nyert:

1. Na^+ megvonás esetén egyes neuronok aktivitása hamarabb, másoké később szűnik meg. Találtak olyan idegsejtet is, amely Na^+-hiányban 30 perc múlva is generál akciós potenciált.

A sejtek leállás után is aktiválhatók voltak depolarizációval, míg ACh-ra ilyenkor csak a neuronok egy része válaszolt.

2. Ca^{++}-mentes oldatban a sejtek spontán aktivitása viszonylag rövid időn belül megszűnt, de néhány sejt ilyenkor is 15—20 percen át aktív maradt.

3. K^+-hiány esetén viszonylag kismértékű hiperpolarizáció mellett az aktivitás frekvenciája csökkent.

4. Cl^--hiányban a sejtek aktivitási ritmusa szabálytalanná vált, egyes esetekben burst-ölő jelleget vett fel.

A kapott eredmények részben az ion koncentrációgradiensek és a membrán permeabilitás megváltozásával, részben az elektrogén Na^+-pumpa befolyásolásával magyarázhatók.

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THE EFFECTS OF LOCAL ANAESTHETICS ON THE ACTIVITY GENERATION AND CHEMICAL SENSITIVITY OF GIANT NEURONES

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According to the literature the effect of local anaesthetics can be attributed at least to two different mechanisms. On the one hand they can stabilize the membrane similarly to Ca^{++} preventing the changes in Na^{+} and K^{+} permeability involved in the spike generation. On the other hand, their chemical structure is similar to that of ACh, so they can cause a competitive inhibition of the cholinergic transmission similarly to d-TC (ARIENS, 1964). According to NACHMANSSON's (1961) theory the membrane receptor protein taking part in the axonal spike propagation appears to be the same, that is involved in the cholinergic transmission, thus the site of the two actions essentially is the same. Recently MAUTNER et al. (1972) have a similar conception. However the examinations of some other authors have shown that sites, where the inhibition of neural activity and the blockade of synaptic transmission are produced are independent (BRYANT, 1958; USUBIAGA and STANDAERT, 1967), but they are dependent on the concentration of drugs (RIKER and KOSAY, 1970).

For the pharmacological investigations of localanaesthetics procain and various derivatives of lidocain were most frequently used. Under the influence of these drugs the abolition of spike generation on the giant axon of squid has been demonstrated (SHANES et al., 1959). This result has been interpreted as a consequence of a decrease in potassium and sodium conductances, which has been supported by some other works (BLAUSTEIN and GOLDMAN, 1966; NARAHASHI et al., 1967). The block of transmission has been investigated in particular on the end-plate potential and it has been shown, that procain influences mainly the K^{+}-chanel independently of the Na^{+}-chanel, while both are similarly affected by lidocain (MAENO et al., 1971).

It is known, that the mechanism as well as the conditions for generation of the spontaneous pacemaker activity are somewhat different from those of the evoked activity (ALVING, 1968; WAZIRI et al., 1965). Only a few investigations have been reported so far with respect to the effect of local anaesthetics on the pacemaker activity. On the Purkinje fibers of heart muscle lidocain inhibits the spontaneous potential generation (BIGGER and MANDEL, 1970). On spinal neurones procain blocks the generation of all kinds of action potential, while in the same concentration it does not affect the appearance of EPSP-s (CURTIS and PHILLIS, 1961.) WOOD (1972) proved in an indirect

manner, that some bursting neurons sending inhibitory impulses to the intestinal smooth muscle of the cat stopped generating activity in the presence of lidocain.

As far as we know the effect of local anaesthetics have not yet been examined on the giant neurones of Gastropods. As the neurones of *Lymnaea stagnalis* are suitable objects for studying the pacemaker activity generation and the chemical sensitivity of the soma simultaneously (SALÁNKI and KISS, 1969; KISS and SALÁNKI, 1971), it appeared to be reasonable to investigate the effect of local anaesthetics on these cells. Beside the lidocain a compound marked as RG-1812 (1-piperidinopropanol,3-methoxybenzoate,hydrochloride) (KÁRPÁTI and SZPORNY, 1971; SZPORNY and KÁRPÁTI, 1972) was also tested. The main questions we wished to elucidate were as follows:

a) Are the effects similar to those described on other objects?

b) Whether the drugs exert their actions both on the spontaneous activity and on the chemical sensitivity by affecting the same membrane site or are there independent sites?

c) What are the qualitative and quantitative differences between the two local anaesthetics on the basis of the tests performed?

Material and methods

Examinations were conducted on the giant neurones of abdominal and right parietal ganglia of *Lymnaea stagnalis*. The thick connective tissue was removed from the dorsal surface of the ganglia, thus the cells became fairly visible through the thin tissue located under the thick one. The preparation was placed in a chamber *(Fig. 1)* containing 3 ml physiological saline (JULLIEN and RIPPLINGER, 1948). For changing the solutions a perfusion vessel and a pump were used. The chamber was fixed to the basis of a micromanipulator. A glass microelectrode filled with 2.5 M KCl was fixed to one arm of the micromanipulator, while an other arm was used for holding a capillar filled with ACh or 5HT solution, which could be placed instantaneously into the solution to the vicinity of the ganglionic surface.

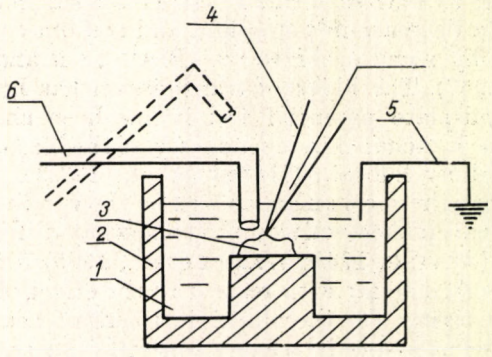

Fig. 1. Scheme of experimental arrangement
1 — physiological solution; 2 — experimental chamber ; 3 — preparation; 4 — microelectrode; 5 — reference electrode; 6 — capillar for the application of substances

During recording the glass microelectrode was connected with a FET negative capacitance high input impedance amplifier (VÉRÓ, 1971). In the course of the experiments the biological signals were fixed on a magnetic tape and the desired portions were registered later by means of a DISA Universal Indicator and photorecorder. The effects of lidocain and RG-1812 on the spontaneous activity were tested in such a way, that after recording the control activity the whole volume of the Ringer solution filling the experimental chamber was exchanged for a drug solution of appropriate concentration. For examining the chemical sensitivity first the control effects of ACh and 5HT were registered at concentration proved to be effective in our earlier work (KISS and SALÁNKI, 1971). Following this the preparation was incubated in drug solution for an appropriate time, then the effects of ACh and 5HT were tested again.

Results

Effects of lidocain and RG-1812 on the spontaneous activity of the neurones

$2\times10^{-3}-5\times10^{-4}$ g/ml concentrations of drugs were used. In several minutes marked changes occur becoming more pronounced in time and finally the spike generation ceases. This changes does not refer to the membrane potential, which was reduced by several mV-s at the most. The most pronounced effects have been shown on the following parameters:

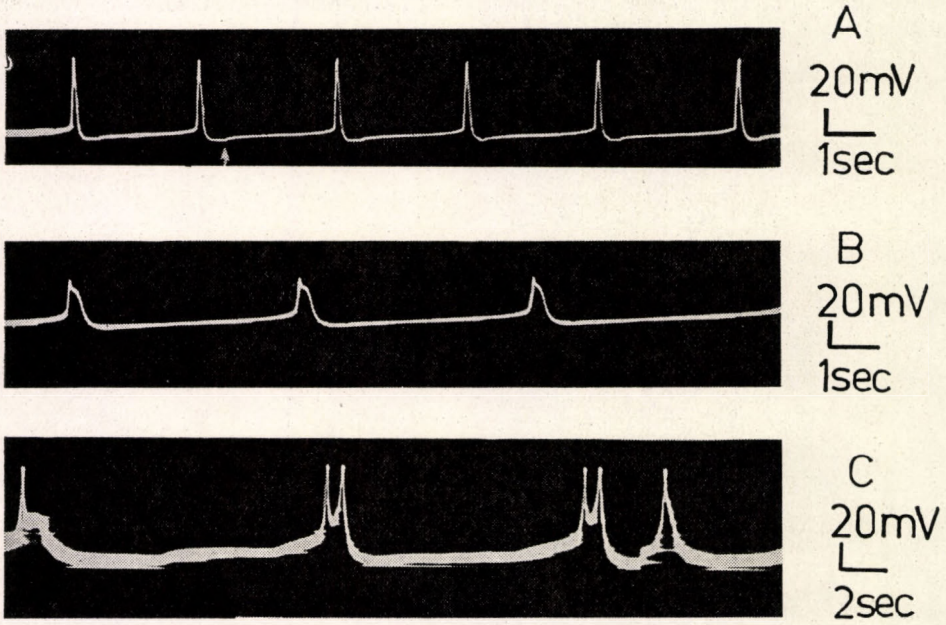

Fig. 2. Effect of lidocain and RG-1812 on the shape of the action potential *a)* arrow marks the application of 10^{-3} g/ml lidocain; *b)* 7th min. after the application of lidocain; *c)* effect of 10^{-3} g/ml RG-1812 showing the plateau formation very well

1. There is a 2—3-fold gradual increase in the spike duration *(Fig. 2)*. This increase appears to be the sum of two factors: an increased time course of the rising phase and a delayed repolarization. The latter tends to be similar to the falling phase of the action potential of heart muscle cells, in some cases a pronounced plateau is formed or during the delayed repolarization the membrane comes to be excited again resulting in a second or third peak *(Fig. 2.c)*.

2. The spike amplitude decreases. At a given concentration this reduction is proportional to the time, but the time-dependence is not linear, following abolition of the overshoot the curve becomes steeper *(Fig. 3)*. The most pronounced inflexion can be found in the case of RG-1812 of 5×10^{-4} g/ml concentration.

3. Hiperpolarizing afterpotentials tend to be reduced, at the same time, generally the rate of pacemaker depolarization decreases *(Fig. 4.b)*. After the action potential ceased, sometimes local potentials remain for a long time *(Fig. 4.c)*.

4. The spontaneous firing rate does not show any unequivocal change. Generally the effect of both drugs resulted in an increased frequency in the early period lasting for several minutes, but after 3—4 minutes there was a restoration of the control value, or a decrease to 50% of control level. In the latter case there is neither any excessive concentration-dependence nor pronounced difference between the two drugs *(Fig. 5)*. A complete abolition of spontaneous activity is caused in 5—21 min. by lidocain and in 5.7—27 min. by RG-1812. *(Fig. 6)*.

It must be noticed, that the standard deviation of data is large enough, which may be attributed to the existence of two groups of cell having different sensitivity to the drugs. The most sensitive neurones whose action potential

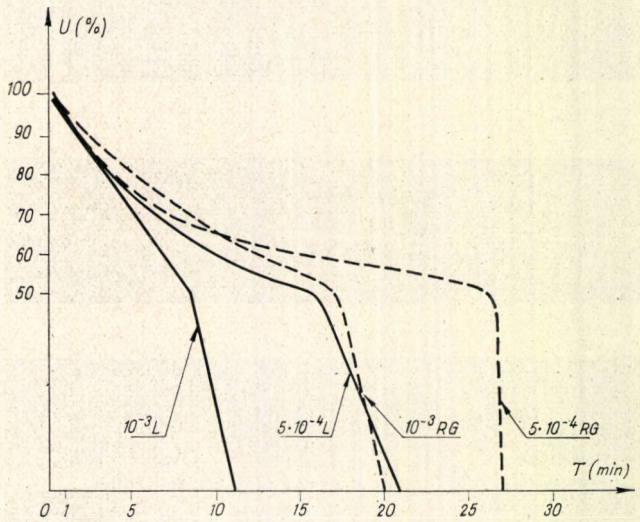

Fig. 3. Time dependence of the reduction of spike amplitude after the application of different concentrations of lidocain and RG-1812. Ordinate: diminished amplitudes as per cent of the control

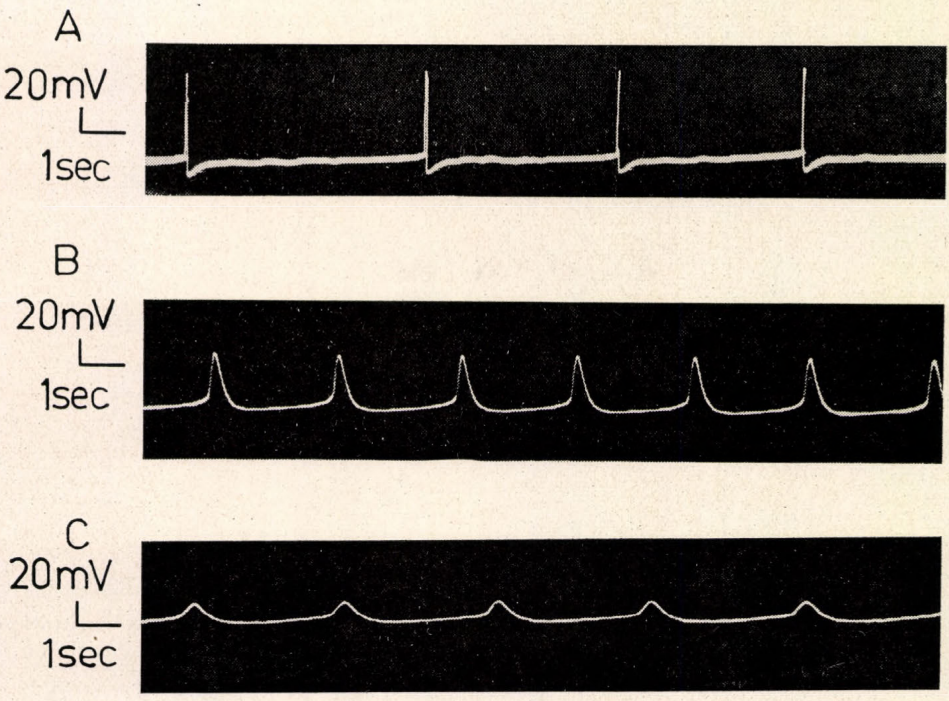

Fig. 4. Marked drug effect (RG-1812, 10^{-3} g/ml) causing a strong damage of the generation of action potential
a) control; *b)* 15th min. after the application of lidocain; *c)* in 20th min. only local potentials remain

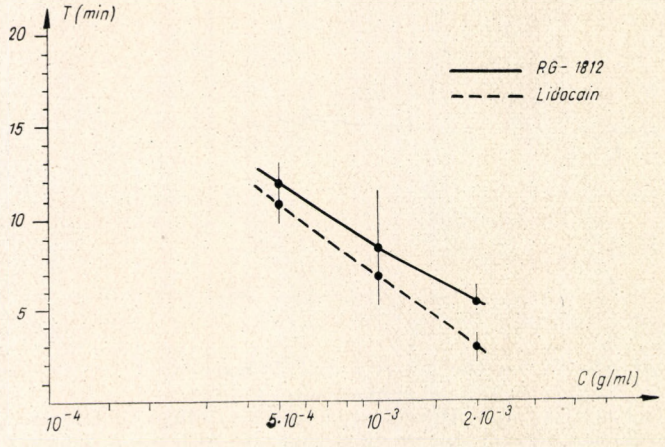

Fig. 5. Decrease in the firing rate of spontaneous activity vs. drug concentration

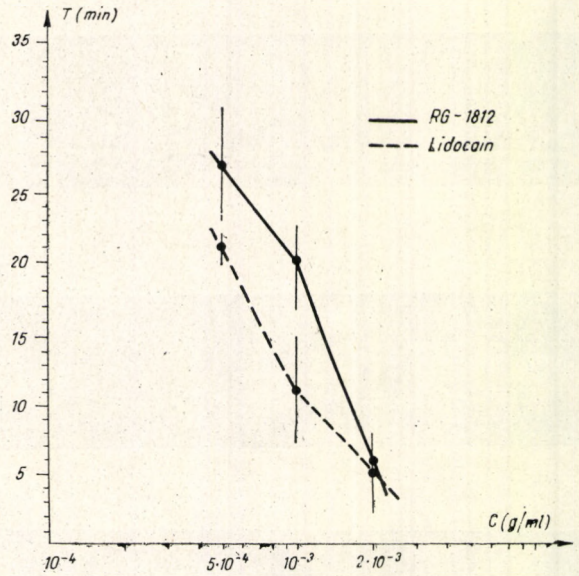

Fig. 6. Time of the complete inhibition of spontaneous activity vs. drug concentration

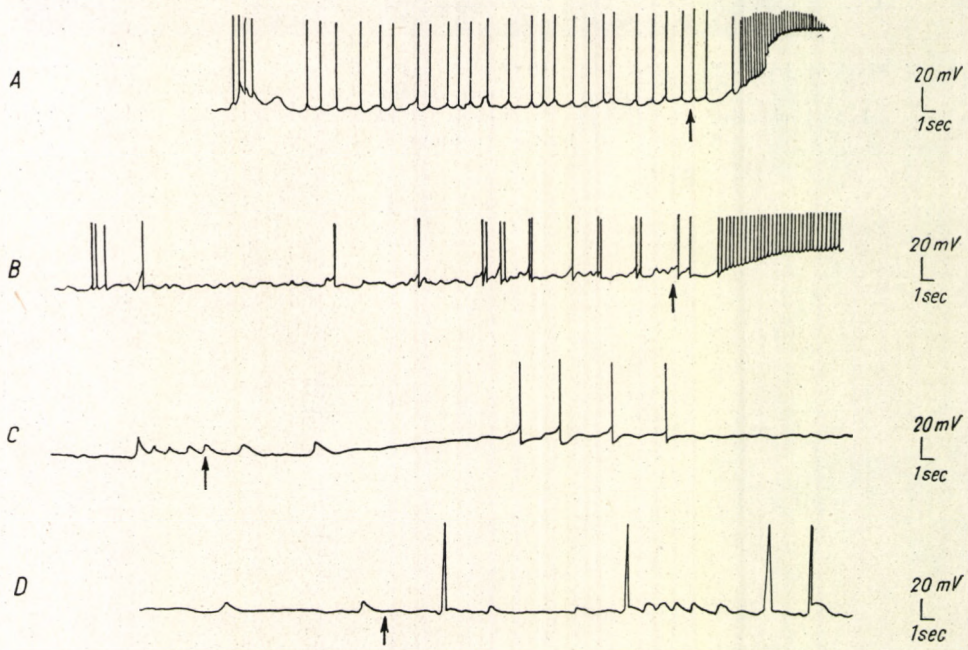

Fig. 7. Effect of lidocain on the activity and ACh-sensitivity of the neurons driven synaptically.
a) and *c)* control activity and ACh-effect; *b)* and *d)* effect of lidocain during which EPSP-s remain unchanged and the reactions given to ACh are influenced by the drugs in different ways. Arrow marks the application of ACh

can be most rapidly blocked appear to belong to the pure pacemaker cells discharging most regularly.

For making a comparison the behaviour of several synaptically driven neurones was examined. After the action potential was blocked by the drugs EPSP-s were found to remain unchanged *(Fig. 7)*.

Influence on the chemical sensitivity

For these examinations 10^{-3} and 5×10^{-4} g/ml lidocain and RG-1812 solutions were used.

a) Inhibition of the ACh-effect

The excitatory effect of ACh on D-cells is reduced by both drugs and it is completely prevented within 10 min. The effectiveness of lidocain and RG-1812 has been compared in two ways. On the one hand, the degree of inhibition has been calculated taking the increase in the firing rate caused by ACh under normal conditions as 100%. This degree is given by the mean value of data obtained within the first 9 min. following the drug application. 10^{-3} g/ml lidocain caused 56%, while the same concentration of RG-1812 96% inhibition.

On the other hand, the mean times required to prevent completely the excitatory effect of ACh by each of the drugs have been compared. In the case of RG-1812 this time is 8—9 min. *(Fig. 8 c, d)*, at the same time lidocain does not yet cause a complete disappearance of sensitivity to ACh *(Fig. 8 a, b)*.

As it has been mentioned, simultaneously with the inhibition of the spontaneous activity the examined drugs does not affect the EPSP-s. Consequently, the changes in the ACh-sensitivity cannot be so unequivocal on the driven neurones as they can be on the pacemaker ones. *Figure 7* shows two different effects of drugs on the neurones affected by ACh mainly through the postsynaptic membrane. In the "a—b" case there was a high frequency EPSP input to the postsynaptic neurone in the control state, and there is no significant reduction in the excitatory effect of ACh when lidocain is present. The figures "c—d" demonstrate the activity of a cell having a low frequency EPSP input in the control state. Following the application of lidocain the excitatory reaction of this cell given to ACh is greatly reduced.

b) Effect of 5HT in the presence of local anaesthetics

Until the 9th min. after the application of the examined drugs there is no significant change in the sensitivity to 5HT *(Fig. 9.)*. However, from the 9—10th min. 5HT is ineffective on the preparations incubated either in lidocain or in RG-1812 solution.

With respect to the influence on the serotonin-sensitivity there is no significant difference between the two drugs.

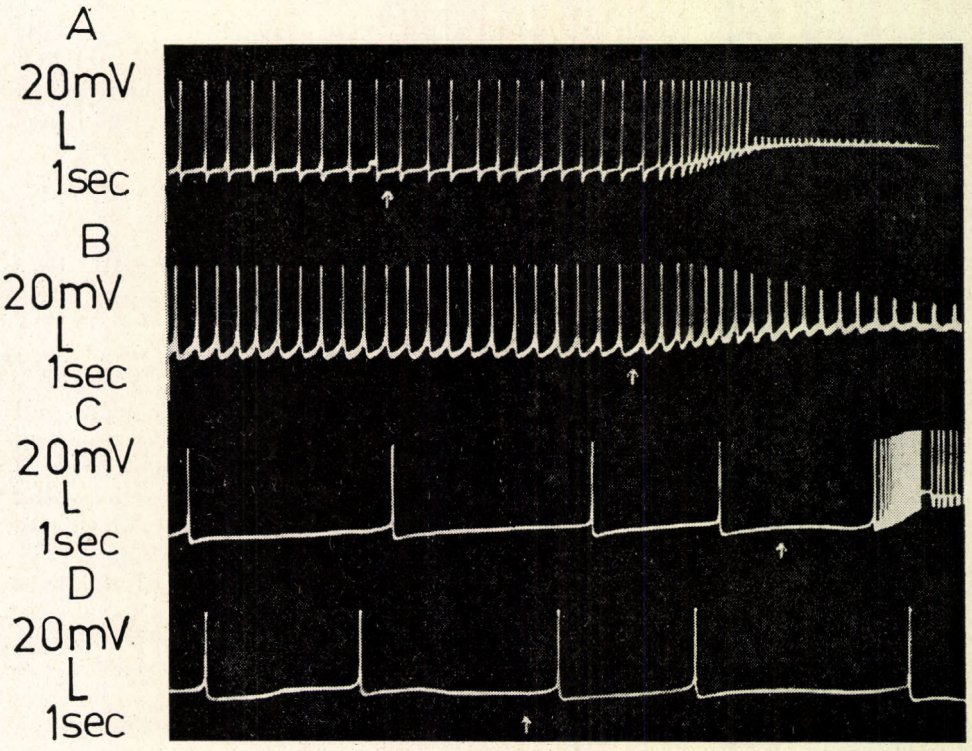

Fig. 8. Effects of drugs on the ACh-sensitivity of pacemaker neurones. *a)* and *c)* control activity and ACh-effect; *b)* in 8th min. after the application lidocain causes a decrease in the excitatory effect of ACh, but cannot completely prevent it; *d)* at the same time and concentration RG-1812 prevents completely the effect of ACh. Arrow marks the application of ACh

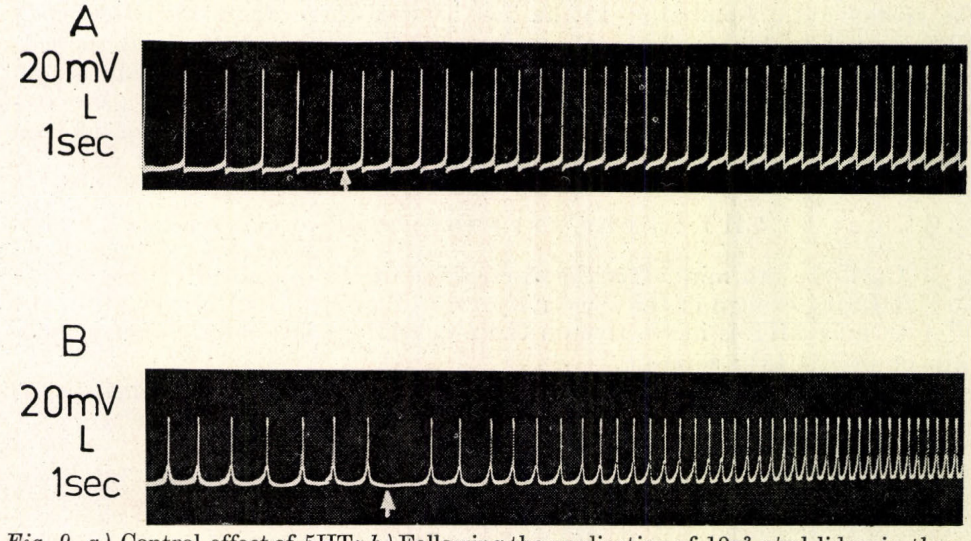

Fig. 9. a) Control effect of 5HT; *b)* Following the application of 10^{-3} g/ml lidocain there is no significant change in the effect of 5HT

Discussion

Our results show that the spontaneous firing of giant neurones reacts to lidocain and RG-1812 similarly to other excitable structures: a blockade of action potential and of the ACh-receptors of membrane is induced. There is a reason to suppose that inhibition of the spontaneous activity is resulted by a direct influence on the soma membrane, as if an inhibition of the axonal pacemaker locus occurred the somatic spike would not fall gradually, but it would be expected to disappear according to the "all or nothing" law. It is presumable again that the effect is not specific to the fundamental processes of pacemaker generation of potential as the spikes evoked synaptically are abolished too, on the other hand, the firing rate — a basic parameter of pacemaker activity — remains uneffected in a number of cases, as long as activity lasts. The oscillation in membrane potential underlining the spontaneous activity may also be maintained quite a long time after the cessation of the spike generation. It became clear that an inhibition both of the fast rising and the falling phase of an action potential occured. The decrease in amplitude as well as in steepness of the rising phase and the prolongation of repolarization take place at the same time, which indicate a simultaneous blockade of Na^+ and K^+ channels. This conclusion is in good agreement with the observations of MAENO et al. (1971) concerning lidocain.

In the present work the spontaneous activity of the soma was investigated, which was affected by ACh mainly through the receptors located on the soma membrane. Besides — as ACh was not applied by microiontophoresis — evidently the effects on the postsynaptic membrane may also be considered, but in the present case it is not the primary process, which becomes clear making a comparison with the cells driven synaptically — beside some other consideration. Namely on these driven cells one can continue to demonstrate EPSP-s of cholinergic origin after cessation of the spontaneous activity and ACh can cause an excitation similar to that obtained in the control state in spite of the presence of lidocain. This case is demonstrated in *Fig. 7a—b*, while in Fig. *7c—d* the difference between the control ACh-effect and that diminished by lidocain may be explained by a selective blockade of the soma receptors. Persistence of the synaptic input after the inhibition of spike generation can be explained by different pharmacological sensitivity of the somatic and synaptic regions. It is not surprising when one makes a comparison with some data in literature, which suggest that the Na^+-requirement for the production of axonal and somatic action potential is different (MAGURA et al., 1970), as well as that there are pharmacologically different ACh-receptors on the same individual neurone (KEHOE, 1972). In the investigations on some other objects RIKER and KOSAY (1970), CURTIS and PHILLIS (1961) and USUBIAGA and STANDAERT (1967) also found that the synaptic transmission sustained a loss later in comparison to the action potential.

If it is accepted that under the given experimental conditions the site of the action of drugs is located on the soma, the question at issue here is whether there is a common locus or there are separated loci for inhibition of the action potential and the ACh-sensitivity. Our results have demonstrated a reduction of ACh-effect, furthermore, even its complete prevention by RG-1812 occured when there was only an insignificant damage to the spike generation. These data showing the two kinds of effects to be realised at different

times support the idea of the existence of separate loci although does not verify it in se. It is rather confirmed by the results that the blockade of spontaneous activity by the drugs was more considerable at a concentration of 10^{-3} g/ml than at 5×10^{-4} g/ml, while the above difference has been failed to demonstrate for the inhibition of the ACh-sensitivity. Thus, an earlier appearance of the reduction of ACh-sensitivity might be explained as follows: After the application of the drugs more and more molecules interact with the given sites in the course of time. The ACh-receptors are sensitive even to a lower concentration, while for the receptors involved in the generation of action potential a higher concentration being reached later is required to cause a considerable inhibition. RIKER and KOSAY (1970) suggested a similar interpretation of two separated sites of lidocain action, however in their experiments the blockade of the ACh-receptors required a higher lidocain concentration because these investigations dealt with the synaptic transmission.

The prevention of 5HT-effect in 9-10th min. after the application of drugs cannot be unequivocally attributed to a specific blockade of the serotonine-receptors, since before it there was no significant inhibition, on the other hand, the spike generation is likewise considerably damaged by this time. Nevertheless, some kind of the drog-receptor interaction cannot be disregarded even so it is lower in extent compared to the inhibition of ACh. To clear up the question further investigations are required.

Comparing the effects of two examined drugs it can be established that the inhibitory effect of RG-1812 on the spike generation differs only quantitatively from that of lidocain which is shown by the dosage-effect curves, but there is qualitatively no difference, so this drug appears to be similar rather to lidocain, than to procain, regarded by MAENO et al. (1971) as a matter blocking the K^+-channel selectively.

The comparison has led to a surprising result. Lidocain, which has proved to be a stronger inhibitor of the spontaneous spike generation, is less effective on the ACh-sensitivity compared with RG-1812 having less influence on the spike generation. This fact can also be regarded, as an indirect evidence of the existence of separate sites of the action on the spontaneous activity and ACh sensitivity. Besides it is of interest to make a comparison with VARANKA's unpublished data showing on the CVC of *Anodonta* that the RG-1812 has the stronger anaesthetic effect.

Summary

Effects of lidocain and RG-1812 on the spontaneous activity and chemical sensitivity of the giant neurones of *Lymnaea stagnalis* were studied. Both drugs were found to inhibit the generation of action potential as well as to prevent the excitatory effect of ACh. It may be supposed that these two kinds of effect occur on separate receptors as their time- and concentration-dependence were not similar.

During the inhibition of action potential the rising phase and the repolarization were simultaneously damaged, which indicated a blockade of both Na^+ and K^+-channel.

The effect of RG-1812 differs from that of lidocain only quantitatively, it causes a smaller inhibition of the spontaneous spike generation, but has a more considerable effect on the ACh-sensitivity.

The effects of the drugs on the sensitivity to serotonin have not proved to be unequivocally specific.

REFERENCES

ALVING, B. O. (1968): Spontaneous activity in isolated somata of *Aplysia* pacemaker neurons. — *J. Gen. Physiol.* **51,** 29—45.

ARIENS, E. J., A. M. SIMONIS, J. M. VAN ROSSUM (1964): Chemical and physical properties of drugs with local anaesthetic action in: *Molecular pharmacology*, ed. ARIENS, E. J. *Acad. Press New York, London* pp. 352—371.

BIGGER, J. T., Jr. W. J. MANDEL (1970): Effect of lidocaine on the electrophysiological properties of ventricular muscle and Purkinje fibers. — *J. Clin. Invest.* **49,** 63—77.

BLAUSTEIN, M. P., D. E. GOLDMAN (1966): Competitive action of calcium and procain on lobster axon. A study of the mechanism of action of certain local anaesthetics. — *J. Gen. Physiol.* **49,** 1043—1063.

BRYANT, S. H. (1958): Transmission in Squid giant synapses. — *J. Gen. Physiol.* **41,** 473—484.

CURTIS, D. R., J. W. PHILLIS (1961): The action of procaine and atropine on spinal neurones. — *J. Physiol.* **153,** 17—34.

JULLIEN, A., J. RIPPLINGER (1948): Sur l'automatisme du ventricule isolé du coeur de Limnée. — *C. R. Acad. Sci. (Paris)* **226,** 1396.

KÁRPÁTI, E., L. SZPORNY (1971): Pharmacological investigation of a new local anaesthetic. — *Ist Congress of Hungarian Pharmacological Society (Budapest).*

KEHOE, J. (1972): Three acetylcholine receptors in *Aplysia* neurones. — *J. Physiol.* **225,** 115—147.

KISS, I., J. SALÁNKI (1971): The heterogenic chemical sensitivity of the central neurones of *Lymnaea stagnalis* L. — *Annal. Biol. Tihany* **38,** 39—52.

MAGURA, I. S., I. KISS, O. A. KRYSHTAL (1970): Current-voltage relations of the giant neurone soma membrane of *Lymnaea stagnalis.* — *Acta Physiol. Acad. Sci. Hung.* **40,** 221—228.

MAENO, T., O. EDWARDS, S. HASHIMURA (1971): Difference in effects on endplate potentials between procaine and lidocaine as revealed by voltage clamp experiments. — *J. Neurophysiol.* **34,** 32—46.

MAUTNER, H. G., D. D. DEXTER, B. W. LOW (1972): Conformational requirements of ACh-analogues and local anaesthetics. — *Nature* **238,** 87—88.

NACHMANSOHN, D. (1961): Chemical factors controlling nerve activity. — *Science* **134,** 1962—1968.

NARAHASHI, T., N. C. ANDERSON, J. W. MOORE (1967): Comparison of tetrodotoxin and procaine in internally perfused squid giant axons. — *J. Gen. Physiol.* **50,** 1413—1428.

RIKER, W. K., S. KOSAY (1970): Drog induction and suppression of stimulus-bound repetition in sympathetic ganglia. — *J. Pharmacol. Exp. Therap.* **173,** 284—292.

SALÁNKI, J., I. KISS (1969): Identified cells in the central nervous system of *Lymnaea stagnalis* L. — *Annal. Biol. Tihany* **36,** 63—75.

SHANES, A. M., W. H. FREYGANG, H. GRUNDFEST, E. AMATNIEK (1959): Anaesthetic and calcium action in the voltage clamped giant axon. — *J. Gen. Physiol.* **42,** 793—802.

SZPORNY, L., E. KÁRPÁTI (1972): Investigations of a new infiltration and conduction anaesthetic. — *Vth Intern. Congr. on Pharmacology (San Francisco, Abstr. No.* 1363.)

USUBIAGA, J. E., F. STANDAERT (1967): The effects of local anaesthetics on motor nerve terminals. — *J. Pharm. Exp. Therap.* **159,** 353—361.

VÉRÓ, M. (1971): Negative capacitance amplifier for microelectrode investigations. — *Annal. Biol. Tihany* **38,** 107—115.

WAZIRI, R. W., W. FRAZIER, E. R. KANDEL (1965): Analysis of "pacemaker" activity in an identifiable burst generating neuron in *Aplysia.* — *Physiologist* **8,** 300—310.

WOOD, J. D. (1972): Excitation of intestinal muscle by atropine, TTX and xylocaine. — *Am. J. Physiol.* **222,** 118—125.

HELYI ÉRZÉSTELENÍTŐK HATÁSA AZ ÓRIÁS NEURONOK AKTIVITÁSGENERÁLÁSÁRA ÉS KÉMIAI ÉRZÉKENYSÉGÉRE

Kiss István és Vadász István

Összefoglalás

A lidocain és az RG-1812 jelzésű anyag hatását vizsgálták *Lymnaea stagnalis* óriás neuronjainak spontán aktivitására és kémiai érzékenységére. Mindkét drogra jellemző, hogy gátolja az akciós potenciál generálását és kivédi az ACh serkentő hatását. Feltételezhető, hogy a kétféle hatás különálló receptorokon érvényesül, mivel idő és koncentrációfüggésük nem egyezik meg.

Az akciós potenciál blokkolása folyamán a fel- és leszálló szár egyaránt sérül, amely a Na- és K-csatorna egyidejű blokkjára enged következtetni.

Az RG-1812 jelzésű anyag hatása csak mennyiségileg tér el a lidocainétól: gyengébben gátolja a spontán potenciál képzést, ugyanakkor erősebben hat az ACh-érzékenységre.

A drogok hatása a szerotonin-érzékenységre nem egyértelműen specifikus.

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FLUORESCENCE MICROSCOPY AND MICROSPECTROFLUORIMETRY OF THE MONOAMINES IN THE BRAIN OF *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. F. (INSECTA, ORTHOPTERA) WITH SPECIAL REGARD TO THE PROTOCEREBRUM

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Among the neurotransmitters of insects the presence of biogenic monoamines was first investigated in the whole body extracts where dopamine, noradrenaline as well as adrenaline were detected (ÖSTLUND, 1953; v. EULER, 1961).

Using the fluorescence histochemical method described by FALCK and HILLARP, numerous papers have been published concerning the localization of the biogenic monoamines in the central nervous system of insects (FRONTALI and NORBERG, 1966; PLOTNIKOVA, 1967; FRONTALI, 1968; KLEMM, 1968; 1971; MANCINI and FRONTALI, 1970; ČECH and KNOZ, 1970; ELOFSSON and KLEMM, 1972; SCHÜRMANN and KLEMM, 1973). KLEMM (1971 a) investigated with that method the distribution of biogenic monoamines even in the stomatogastric nervous system and in the corpora cardiaca of *Schistocerca gregaria*.

Serotonin, noradrenaline and dopamine have been demonstrated in body fragments containing the central nervous system of various insects (GERSCH et al., 1961; FRONTALI and HÄGGENDAL, 1969; HIRIPI and S.-RÓZSA, 1973).

Microspectrofluorimetric measurements have been reported only recently (BJÖRKLUND et al., 1970; KLEMM and BJÖRKLUND 1971; ELOFSSON and KLEMM, 1972; SCHÜRMANN and KLEMM, 1973). Mainly dopamine and less frequently noradrenaline have been detected. Serotonin was also found in the optic lobe of some insects (ELOFSSON and KLEMM, 1972).

The distribution of biogenic monoamines is not uniform in every respect in the central nervous system of insects even in closely related species. In certain parts of the brain there is a specific fluorescence in all species investigated, but in other ones it varies. The data regarding the presence of serotonin are contradictory. In spite of the fact that serotonin had been demonstrated for example in *Periplaneta* extract containing the brain as well as the corpora cardiaca and corpora allata (GERSCH et al., 1961), FRONTALI and NORBERG (1966) failed to find any yellow fluorescence using the fluorescence histochemical method, corresponding to the fluorophore of serotonin. Serotonin was not detected in *Anabolia nervosa* (Trichoptera) either in the extract of the whole head (KLEMM and BJÖRKLUND, 1971) or during the fluorescence histochemical investigation of supra- and suboesophageal ganglia (KLEMM, 1968; 1971).

In the central nervous system of *Locusta migratoria migratorioides* R. F. only some parts of the ventral cord and the tritocerebrum of the brain have been investigated by means of the fluorescence histochemical method (PLOTNIKOVA and GOVYRIN, 1966; PLOTNIKOVA, 1967).

The present work was intended partly at comparing the distribution of nerve elements containing biogenic monoamines in various parts of the brain (proto-, deuto- and tritocerebrum, except the optic lobe), and partly at investigating microspectrofluorimetrically the presence of serotonin the relatively great amounts of which were found during biochemical analyses of locusts (HIRIPI and S.-RÓZSA, 1973).

Material and methods

Adult specimens of *Locusta migratoria migratorioides* R. F. of both sexes were used for investigations. We had to modify in part the fluorescence histochemical method described by FALCK and OWMAN (1965), since the parameters of reaction elaborated on vertebrates failed to give acceptable results. KLEMM (1968) also reported on methodical difficulties. Our modifications concerned the conditions of reaction.

The brains without the optic lobes were quenched in isopentane cooled by liquid nitrogen and freeze-dried in an apparatus type HVG 1 (Ilmeneau, GDR). The cooling agent was dry ice-aceton mixture and the end vacuum reached 10^{-5} Torr. The formaldehyde treatment was carried out with paraformaldehyde of 80% relative humidity for 30 min at 80° C (during experiments durations from 15 min. till 3 hours this time proved to be optimal). After treatment the material was kept in a closed vessel over phosphorus pentoxide for 1 hour at room temperature in darkness. Embedding in paraffin lasted 1 hour at 60° C in vacuo. The specificity of fluorescence reaction was tested on a material, heated (30 min at 80° C) without formaldehyde gas. Serial sections of about 10 microns were either uncovered or covered with Entellan (Merck) + 10% xylene, however, the majority of micrographs were taken from uncovered slides. Zeiss NU2 microscope was used as a fluorescence microscope with BG12 excitation and OG1 ocular filters. The light source was HBO 200 mercury-vapour lamp.

Microspectrofluorimetric analysis was carried out from the protocerebral lobe containing fluorescence fibres and from the central body. Leitz-MPV cytophotometer attached to Leitz Ortholux II microscope was used with a photomultiplier type RCA 1P-21. Suitable interference filters were applied for taking the excitation and emission spectra. The correction of curves and the further details are described elsewhere (ZS.-NAGY and DEÁK, 1973). For chemical identification of fluorophores, the deparaffinized sections were treated with HCl-vapour according to the method of BJÖRKLUND et al. (1968).

Results

We found only green fluorescence in the parts of the locust-brain (proto-, deuto- and tritocerebrum, except the optic lobe). According to the controls, the fluorescence was the result of formaldehyde treatment, i.e. specific to the

biogenic monoamines. The most intensive fluorescence reaction occurred in the central body and in some parts of the corpora pedunculata. The characteristic features of parts of the brain termed according to BULLOCK and HORRIDGE (1965) is now described in detail.

I. *Protocerebrum*

1. Corpora pedunculata: The perikaryon layer and the calyx neuropile contained no fluorescing elements. The parts of the pedunculus adjacent to the calyx displayed intensely fluorescing varicose fibres of rather short diameter, running nearly parallel to the long axis of the pedunculus *(Fig. 1)*. In oblique or longitudinal sections of the pedunculus, a narrow fluorescence-free stripe could be observed running centrally over whole pedunculus.

The fluorescence reaction was more intense in the alpha and beta lobes than that of the pedunculus. In the sections of given thickness single fibres were not separated *(Fig. 2)*. The fluorescence showed some layering perpendicular to the longitudinal axis in both lobes but mainly in the beta lobe.

2. Central body: It showed very intense fluorescence in the centre of the brain consisting of two parts. Fluorescence was more intense in the dorsal part showing a septate shape like a fan *(Fig. 3)*. The ventral part was of paler fluorescence and of more homogeneous in appearance. The pictures give the impression that the fluorescing fibre bundles of the dorsal part pass over into the ventral one at some places *(Fig. 3)*.

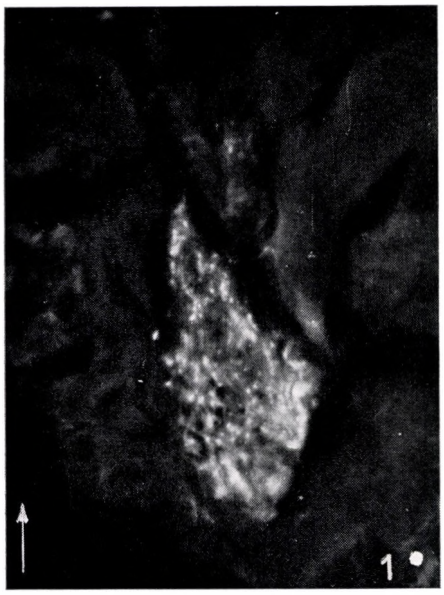

Fig. 1. Beginning of the pedunculus of mushroom body. Frontal section, ×470. (Arrow indicates the dorsal direction in each micrograph.)

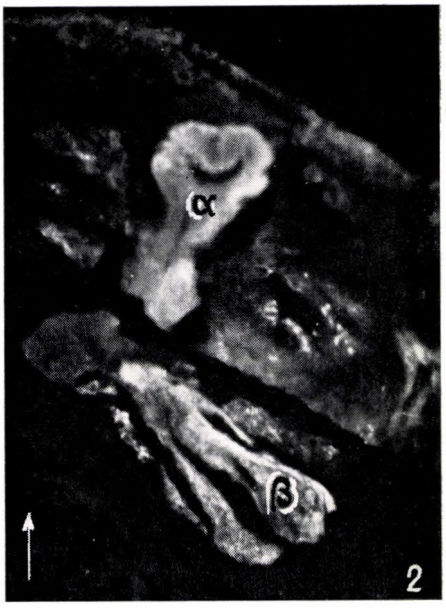

Fig. 2. The alpha *(α)* and the beta *(β)* lobes of mushroom body. Frontal section, ×190

3. Other protocerebral parts: The definite structures mentioned above are surrounded by the protocerebral neuropile. Scattered varicose, green fluorescing fibres were found even in this neuropile. These fibres formed groups in some parts *(Fig. 5)*, while there were only few of them elsewhere *(Fig. 4)*. Especially numerous fluorescing fibres were seen around the alpha lobe and in the vicinity of the frontal part of the central body, as well as in the fibre bundles running toward the optic lobe *(Fig. 5)*.

In the protocerebrum green fluorescing perikarya were also visible. They were solitary or grouped in the pars intercerebralis *(Fig. 6* and *7)*. Further investigations will reveal the topography and connections of those cells.

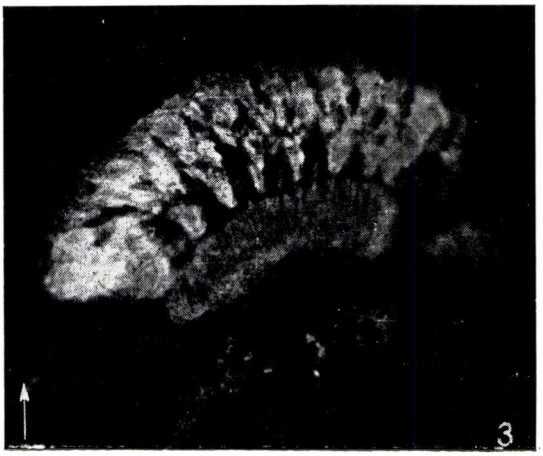

Fig. 3. Central body. The dorsal and ventral portions are well separated. Frontal section, ×190

Fig. 4. Scattered varicose fibres in the protocerebral lobe. Frontal section, ×470.

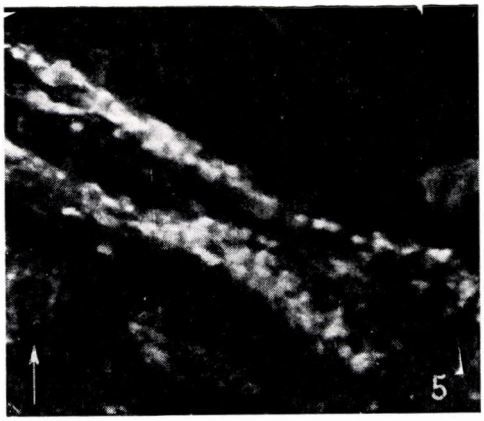

Fig. 5. Detail from the protocerebral lobe. The fluorescing varicose fibres are concentrated here and run toward the optice lobe. Frontal section, × 250.

Fig. 6. Solitary fluorescing perikaryon in the pars intercerebralis. Frontal section, ×470

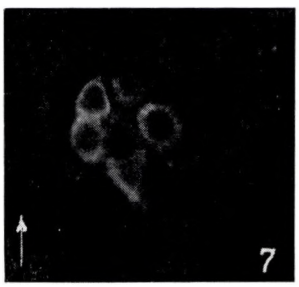

Fig. 7. Grouped fluorescing perikarya in the pars intercerebralis. Frontal section, ×470

II. *Deutocerebrum*

We failed to observe any specific fluorescence in this part of the brain. It should be noted that the structural preservation of this region was always worse than that of the other parts, therefore the absence of specific fluorescence should be not intergreted as an absence of biogenic monoamines.

III. *Tritocerebrum*

The neuropile contained uniformly distributed green fluorescin gfibres. In some places the varicose fibres were concentrated according to the description of PLOTNIKOVA and GOVYRIN (1966) concerning this area of the brain.

Microspectrofluorimetry:

1. Excitation spectra: The excitation spectra were registered in 3 quadrangular regions of $20 \times 20\ \mu^2$ size of both the central body and the fluorescing fibres of the neuropile of the protocerebral lobe. The averages of these measurements are shown in Figs 8 and 9. The excitation maximum is at 425 nm corresponding to that of the fluorophore originating from the catecholamines.

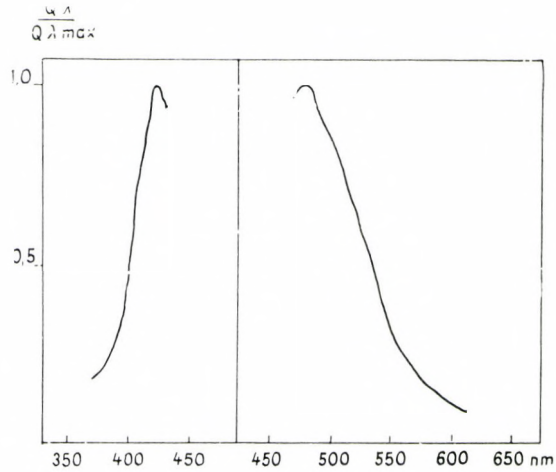

Fig. 8. Excitation (left) and emission (right) spectra of the fluorophore in the protocerebral neuropile. The spectra are expressed as relative quanta versus wavelength Corrected curves

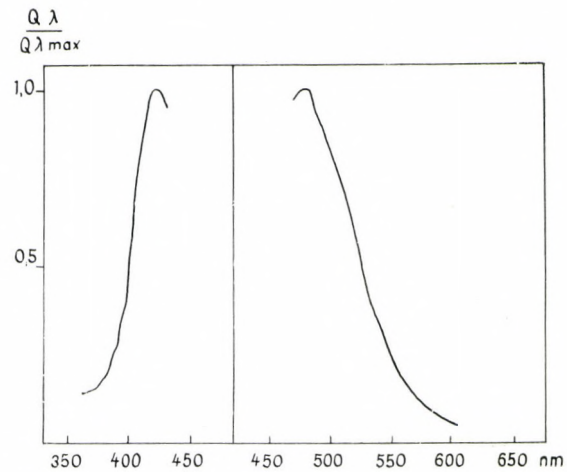

Fig. 9. Excitation (left) and emission (right) spectra of the fluorophore of central body The spectra are expressed as relative quanta versus wavelength. Corrected curves

2. Emission spectra: The emission spectra were recorded at an excitation wave-length of 410 nm. Under given methodical conditions (Zs.-Nagy and Deák, 1973) the recording of the emission spectrum was possible from 470 nm. Areas of 10×40 μ^2 containing at least 2—3 varicose fibres were measured in order to achieve the necessary intensities. The corrected curves are shown in *Figs 8* and *9*. The emission maximum is at 482 nm and the curve is characteristic of recordings obtained from vertebrate nerve elements containing only catecholamines (Björklund et al., 1968). There are no other maxima or plateau on the curves indicating the simultaneous presence of catecholamines and serotonin (Möllmann et al., 1972) in the parts of the brain under investigation.

Results of exposure to HCl-vapour: The excitation spectrum was altered already after very short treatments of 20—30 seconds in so far as the maximum shifted to shorter wave-lengths of about 390 nm. Extending the time of treatment to 2 minutes the maximum shifted further to 380 nm. Further increase of the exposure time to 4—5 min induced no change of the maximum. After exposures longer than 4 min strong background fluorescence appeared, therefore, the measurements became impossible. These results show that the fluorophore present in the neurons originates mainly from dopamine (BJÖRKLUND et al., 1968).

Using the fluorescence microscope with BG12 excitation filter, we failed to observe any changes after HCl-treatment. This is in accordance with the facts mentioned above.

Discussion

Our results partly agree, partly disagree with the data obtained on other Orthoptera and Trichoptera species. Apart from the pedunculus of mushroom body specific fluorescence had been detected in every part of the brain of other species where we observed too (FRONTALI and NORBERG, 1966; FRONTALI, 1968; PLOTNIKOVA and GOVYRIN, 1966; PLOTNIKOVA, 1967; KLEMM, 1968; 1971; MANCINI and FRONTALI, 1970; KLEMM and BJÖRKLUND, 1971). As regards the pedunculus, the literary data are contradictory. Specific fluorescence could not been detected in the pedunculus of *Periplaneta americana* (Orthoptera) nor in that of several Trichoptera species (FRONTALI and NORBERG, 1966; KLEMM, 1968; 1971), whereas in the brain of *Acheta domesticus* (also Orthoptera) the pedunculus proved to be rich in fluorescing fibres (SCHÜRMANN and KLEMM, 1973). Our observations agree with those of the latter authors who are on the opinion that the globuli cells of the mushroom body are of catecholaminergic character in spite of the fact that catecholamines are found only in the nerve fibres and not in the soma. Those authors explained the exclusive axonal occurrence of catecholamines with the absence of synapses around the somas, hence the catecholamines required for the synaptic functions are produced and stored only in the axons, while the soma contains catecholamines only below the threshold of demonstrable amount. The question is further complicated by the fact that the fibres of the globuli cells are also present in the calyx glomeruli (VOWLES, 1955) where fluorescence characteristic of catecholamines was not found. Since the globuli cells are considered to be unipolar, it has to be supposed that the branches of the same nerve can chemically be differentiated in so far as one of them entering the calyx neuropile does not contain detectable amount of catecholamines, whereas the other one reaching other parts of the mushroom body does. The chemical differentiation may be related to the postsynaptic, dendritic character of the intrinsic fibres present in the calyx neuropile (ZS.-NAGY and B.-MUSKÓ, 1972) while in the pedunculus and in both lobes the intrinsic fibres are presynaptic, axonic in character.

A further question is what kind of mediation realizes the transmission of impulses in species where the pedunculus contains no catecholamines? since synaptic structures were encountered in the pedunculus of all species investig-

ated so far electron microscopically (SCHÜRMANN, 1970; ZS.-NAGY and B.-MUSKÓ, 1972; SCHÜRMANN and KLEMM, 1973).

The microspectrofluorimetric analyses show that the fluorophore originates only from catecholamines in the regions investigated. In cells where catecholamines occur together with serotonin, e.g. in the glomus caroticum of rabbit, the emission curve recorded by microspectrofluorimetry after FALCK—HILLARP method is characteristically of a "two-humped" appearance (MÖLLMANN et al., 1972). It has been shown that this curve represents mathematical summation of emissions of catecholamines and serotonin. On the basis of our results we have to deny the assumption that the fluorophores of catecholamine and serotonin would be present together in the fluorescing regions only, the green fluorescence of the former covers the yellow one of the latter. Such simultaneous presence has been assumed among others by SCHÜRMANN and KLEMM (1973) in the central body of the *Acheta* brain. The questions of localization of relatively great amount of serotonin revealed by biochemical analyses of homogenates of locust ganglions (HIRIPI and S.-RÓZSA, 1973) remains yet to be answered. Since the latter authors analysed homogenates containing the whole cerebral ganglion including even the optic lobes as well as the suboesophageal ganglia, it is possible that serotonin is bound to the parts have not been investigated by us. In other species some neuropile regions of the optic lobe have shown yellow fluorescence characteristic of serotonin (ELOFSSON and KLEMM, 1972). It cannot, however, be excluded that the serotonin present in the cerebral ganglion fails to react to the formadehyde treatment for some special reason, hence fluorescing products do not appear. The fluorescence intensity of serotonin fluorophore is about three times lower than that of catecholamines of the same concentration (JONSSON, 1971), nevertheless, owing to that in *locust* the concentration of serotonin is about 50 percent higher than the concentrations of noradrenaline and dopamine together (HIRIPI and S.-RÓZSA, 1973), it should be expected to find areas containing either only yellow fluorescence at some places or emission curves indicating the presence of catecholamine-serotonin mixture. The question is further complicated by the findings that in Trichoptera species serotonin was not found in the head (KLEMM and BJÖRKLUND, 1971) and in the supra- and suboesophageal ganglia (KLEMM, 1968; 1971) either biochemically or histochemically. In the central nervous system of *Periplaneta americana* serotonin was detected by GERSCH et al. (1961), while FRONTALI and NORBERG (1966) failed to observe serotonin in the supra- and suboesophageal ganglion using the fluorescence histochemical method. Due to the above contradictions one can draw no definitive conclusion regarding the cellular localization of serotonin on the basis of data available.

The green fluorescence observed in the locust brain behaved upon HCl-treatment similarly to dopamine in model experiments (BJÖRKLUND et al., 1968). Therefore, one can state that the great majority of the fluorophore originate from dopamine, and only small portion may do so in noradrenaline. According to biochemical measurements (HIRIPI and S.-RÓZSA, 1973), the quantity of noradrenaline occurring in the locust is 6 times less than that of dopamine. Even in other species dopamine proved to be predominant among the catecholamines in both biochemical and microspectrofluorimetric as well as electron histochemical examinations (FRONTALI and HÄGGENDAL, 1969; KLEMM and BJÖRKLUND, 1971; MANCINI and FRONTALI, 1970).

Summary

The cerebral ganglion of the *Locusta migratoria migratorioides* was investigated except the optic lobe. Only green fuorescence was found after FALCK—HILLARP formaldehyde reaction in the regions investigated. In the mushroom body green fluorescence was observed in the pedunculus, alpha and beta lobes, but was not in the globuli cell layer and the calyx neuropile. The most intense fluorescence was detected in the central body. Varicose, fluorescing fibres are present now and then in the protocerebral neuropile as well as in the tritocerebrum, in some places they are grouped. We failed to observe any fluorescing structures in the deutocerebrum.

The microspectrofluorimetric analysis of the fluorophore revealed that the fluorescence originates only in catecholamines, fluorophores of catecholamines and serotonin do not occur simultaneously in the regions investigated. The exposure to HCl-vapours proved that the catecholamines consist mainly of dopamine, in accordance with other species.

REFERENCES

BJÖRKLUND, A., B. EHINGER, B. FALCK (1968): A method for differentiating dopamine from noradrenaline in tissue sections by microspectrofluorimetry. — *J. Histochem. Cytochem.* **16,** 262—270.

BJÖRKLUND, A., B. FALCK, N. KLEMM (1970): Microspetərofluorimetric and chemical investigation of catecholamine-containing structures in the thoracic ganglia of Trichoptera. — *J. Insect Physiol.* **16,** 1147—1154.

BULLOCK, T. H., G. A. HORRIDGE (1965): Structure and function in the nervous systems of invertebrates. — *Freeman and Co. San Francisco and London.*

ČECH, S., J. KNOZ (1970): Monoamine-containing structures in the nerve cord of some representatives of Diptera. — *Experientia (Basel)* **26,** 1125—1126.

ELOFSSON, R., N. KLEMM (1972): Monoamine-containing neurons in the optic ganglia of Crustaceans and Insects. — *Z. Zellforsch.* **133,** 475—499.

EULER, U. S. v. (1961): Occurrence of catecholamines in acrania and invertebrates. — *Nature (Lond.)* **190,** 170—171.

FALCK, B., CH. OWMAN (1965): A detailed methodological description of the fluorescence method for cellular demonstration of biogenic amines. — *Acta Univ. Lundens., Sect. II.* **7,** 1—23.

FRONTALI, N. (1968): Histochemical localization of catecholamines in the brain of normal and drog-treated cockroaches. — *J. Insect Physiol.* **14,** 881—886.

FRONTALI, N., J. HÄGGENDAL (1969): Noradrenaline and dopamine content in brain of the cockroach *Periplaneta americana.* — *Brain Res.* **14,** 540—542.

FRONTALI, N., K.-A. NORBERG (1966): Catecholamine containing neurons in the cockroach brain. — *Acta Physiol. Scand.* **66,** 243—244.

GERSCH, M., F. FISCHER, H. UNGER, W. KABITZA (1961): Vorkommen von Serotonin im Nervensystem von *Periplaneta americana* L. (Insecta). — *Z. Naturforsch.* **16b,** 351—352.

HIRIPI, L., K. S.-RÓZSA (1973): Fluorimetric determination of 5-hydroxytryptamine and catecholamines in the central nervous system and heart of the *Locusta migratoria migratorioides* R. F. — *J. Insect Physiol.* **19,** 1481—1485.

JONSSON, G. (1971): Quantitation and differentiation of biogenic monoamines demonstrated with the formaldehyde fluorescence method. — *Progress in Brain Res.* **34,** 53—61.

KLEMM, N. (1968): Monoaminhaltige Strukturen im Zentralnervensystem der Trichoptera (Insecta). Teil I. — *Z. Zellforsch.* **92,** 487—502.

KLEMM, N. (1971): Monoaminhaltige Strukturen im Zentralnervensystem der Trichoptera (Insecta). Teil II. — *Z. Zellforsch.* **117,** 537—558.

KLEMM, N. (1971a): Monoaminhaltige Zellelemente im stomatogastrischen Nervensystem in den corpora cardiaca von *Schistocerca gregaria* FORSK. (Insecta, Orthoptera). — *Z. Naturforsch.* **26,** 1085—1086.

KLEMM, N., A. BJÖRKLUND (1971): Identification of dopamine and noradrenaline in nervous structures of the insect brain. — *Brain Res.* **26,** 459—464.

MANCINI, G., N. FRONTALI (1970): On the structural localization of catecholamines in the beta lobes (corpora pedunculata) of *Periplaneta americana.* — *Z. Zellforsch.* **103,** 341—350.

MÖLLMANN, H., D. H. NIEMEYER, H. ALFES, H. KNOCHE (1972): Mikrospektrofluorimetrische Untersuchungen der biogenen Amine im Glomus caroticum des Kaninchens nach Reserpin- und PCPA-Applikation. — *Z. Zellforsch.* **126,** 104—115.

ÖSTLUND, E. (1953): Adrenaline, noradrenaline and hydroxytyramine in extracts from Insects. — *Nature (Lond.)* **172,** 1042—1043.

PLOTNIKOVA, S. I. (1967): The structure of the sympathetic nervous system of Insects. — In: *Symp. on Neurobiol. of Invertebrates.* (Ed.: J. SALÁNKI) *Publ. House Hungarian Acad. Sci.* 59—68.

PLOTNIKOVA, S. I., V. A. GOVYRIN (1966): С. И. Плотникова, В. А. Говырин (1966): Распределение нервных элементов, содержащих катехоламины, у некоторых представителей кишечнополостных и первичноротых. — *Архив Анатомии, Гистологии и Эмбриологии* **50,** 79—87.

SCHÜRMANN, F. W. (1970): Über die Struktur der Pilzkörper des Insectenhirns. I. Synapsen im Pedunculus. — *Z. Zellforsch.* **103,** 365—381.

SCHÜRMANN, F. W., N. KLEMM (1973): Zur Monoaminverteilung in den Corpora pedunculata des Gehirns von *Acheta domesticus* L. (Orthoptera, Insecta). Histochemische Untersuchungen, mit Vergleichen zur Struktur und Ultrastruktur. — *Z. Zellforsch.* **136,** 393—414.

VOWLES, D. M. (1955): The structure and connexions of the corpora pedunculata in bees and ants. — *Quart. J. Micr. Sci.* **96,** 239—255.

ZS.-NAGY, I., GY. DEÁK (1973): Characteristics of catecholamine fluorophores in the ganglia of the bivalve *Anodonta cygnea* L. as revealed by a single method of microspectrofluorimetry. In preparation.

ZS.-NAGY, I., I. B.-MUSKÓ (1972): The fine structure of corpora pedunculata in *Gryllotalpa gryllotalpa* L. (Insecta, Orthoptera). — *Ann. Biol. Tihany* **39,** 81—87.

A *LOCUSTA MIGRATORIA MIGRATORIOIDES* R. F. (INSECTA, ORTHOPTERA) AGYA MONOAMIN TARTALMÁNAK FLUORESZCENS MIKROSZKÓPOS ÉS MIKROSPEKTROFLUORIMETRIÁS VIZSGÁLATA KÜLÖNÖS TEKINTETTEL A PROTOCEREBRUMRA

B.-Muskó Ilona, Zs.-Nagy Imre és Deák György

Összefoglalás

A *Locusta migratoria* cerebrális ganglionja képezte vizsgálat tárgyát, kivéve az optikus-lebenyt. A vizsgált területeken csak zöld fluoreszcencia fordult elő a FALCK—HILLARP formaldehid reakció után. A zöld fluoreszcencia a gombatest részei közül megfigyelhető volt a pedunculusban, az alfa- és béta-lebenyben, de hiányzott a globulussejtek rétegében és a kalyxban. A legintenzívebben a corpus centrale fluoreszkált. Elszórt, varikózus, fluoreszkáló axonok láthatók a protocerebrális neuropilben, valamint a tritocerebrumban, helyenként tömörülve. A deutocerebrumban nem sikerült fluoreszkáló területeket megfigyelni.

A fluoreszcencia mikrospektrofluorimetriás vizsgálata azt mutatta, hogy csak katecholaminoktól származik, szerotonin és katecholaminok fluoroforja keverten nem fordult elő a vizsgált területeken. A HCl-kezelés azt bizonyította, hogy megegyezően más rovarfajokkal, a katecholaminok túlnyomó többségét dopamin képezi.

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RESPONSES OF CENTRAL NEURONES TO THE STIMULATION OF HEART CHEMORECEPTORS IN THE SNAIL, *HELIX POMATIA* L.

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It has been described that beside miogeneicity the heart of the snail *Helix pomatia* is controlled also by extracardial innervation (KRIJGSMAN and DIVARIS, 1955). On the other hand, well defined impulses evoked by the tactile, pressure, osmotic and chemical stimulation of the heart are running to the CNS through the intestinal nerve (S.-RÓZSA, 1972). We found that tactile stimulation of the heart can modify the activity of some central neurones by causing acceleration in some cases while inhibition in others, and also the efferent connection between some of the modulated neurones and the heart has been proved (S.-RÓZSA and SALÁNKI, 1973).

In our present work we wanted to elucidate whether there are special neurones in the central nervous system responding to chemical stimuli applied to the heart or not, and further, if they are present, what is their reaction at different modes of stimulation. Also the character of the respond evoked by the chemical stimulation of the heart was determined, and the reactions of neurones belonging to the heart-heart reflex were investigated.

Material and methods

Experiments were carried out on brain-heart preparation of *Helix pomatia* L. Snails kept in hibernation during winter were waken and activated by keeping them at room temperature and at increased humidity while they were fed. The heart rate of the animals activated by this way was regular and could be kept functioning in half-isolated conditions for long time (24 hours).

The shell of the animals was removed and the central nervous system was exposed. The circumoesophageal ganglionic ring was separated from the surroundings, and with the exception of the intestinal nerve all of the neural connections of the ganglia were cut. The intestinal nerve was cleared from the connective tissue and the blood vessels and side branches were transsected only the fine nerve running to the heart remained intact *(Fig. 1)*.

The heart was freed by opening the pericard. Cannula were inserted into the pulmonal vein and into the aorta near to the ventricle for perfusion. The conditions of the perfusion, assuring constant pressure, were described in details previously (S.-RÓZSA and GRAUL, 1964).

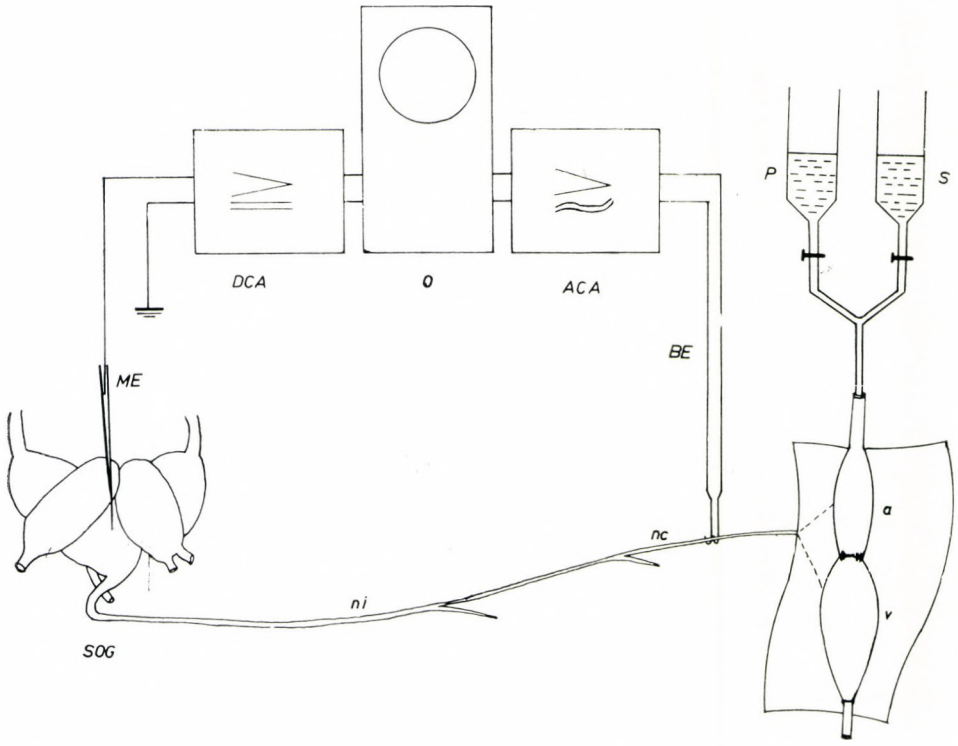

Fig. 1. Experimental arrangement. SOG — suboesophageal ganglionic mass; ni — n. intestinalis; nc — heart nerve; a — atrium; v — ventricle; ME — microelectrode; DCA — DC amplifier; BE — bipolar electrodes; ACA — AC amplifier; O — oscilloscope; p — perfusion chamber for physiological solution; S — chamber for substance (5HT)

The preparation consisting of the brain, intestinal nerve and heart was placed in a special chamber and the connection between the ganglia and the heart was maintained only through the nerve. The distance from the brain to the heart measured about 2 cm. Both heart and brain was protected from drying by physiological saline. For keeping the tissues wet and for solving substances MENG-solution (MENG, 1958) was used. The preparation and the experimental circumstances are given in *Fig. 1.*

For stimulating the heart chemoreceptors 5-hydroxytryptamine (5HT) was used. 5HT was perfused in 10^{-6}—10^{-3} mol concentration intracaridally with a pressure identical to the normal perfusion, using a turning cock. Tactile stimulation was performed at the atrio-ventricular area, with a fine brush.

The electrical activity of the intestinal nerve was recorded by bipolar Ag-AgCl electrodes, extracellularly. After heart stimulation the impulsation increases both in frequency and amplitude (S.-RÓZSA, 1972). The membrane and action potentials of the central neurones were recorded with conventional glass microelectrodes filled with KCl, and having resistance from 5 to 15 MOhms. In the experiments high input impedance amplifier (VÉRÓ, 1971) was used, and polarization could be performed with appropriate bridge circuit. For amplification and recording the potentials ALVAR instruments were used·

Neurones responding to the stimulation of the chemoreceptors of the heart were mapped using the method worked out previously on *Helix* brain (SAKHAROV and SALÁNKI, 1969). Part of these neurones were identical with cells responding to tactile stimulation (S.-RÓZSA and SALÁNKI, 1973).

Experiments were performed in autumn and winter at room temperature (20°—22 °C).

Results

Altogether 31 neurones from the visceral and right parietal ganglia of 19 preparations were investigated. Some of the cells could be identified in different preparations due to their constant localization. The numbering and location of the different neurones responding to the stimulation of the heart chemoreceptors with 5HT are shown in *Fig. 2.*

Most of the cells performed spontaneous activity during experiments, being the generation of action potentials endogenous (pacemaker cells) or evoked by synaptic potentials (driven cells). Several "silent neurones" were also investigated.

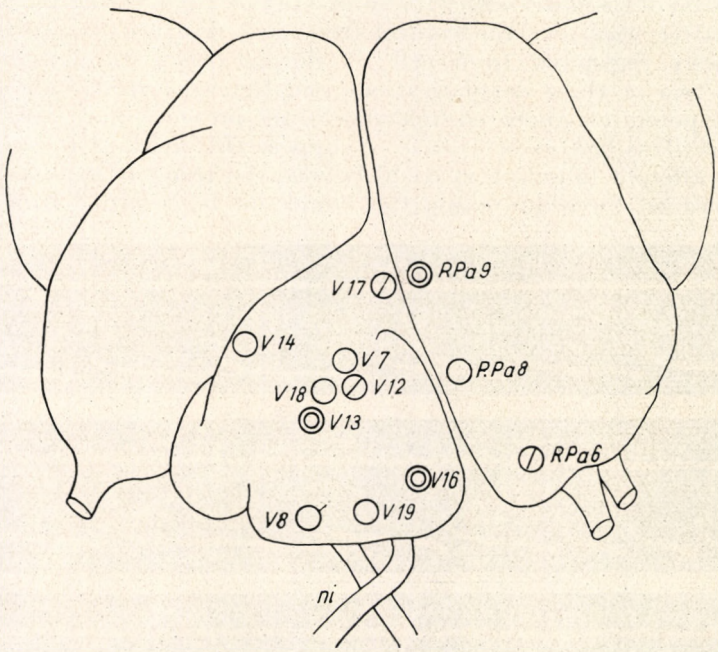

Fig. 2. Localization and reaction type of neurones responding to the stimulation of the heart with 5HT.

◎ — increase of activity,

○ — decrease of activity,

⊘ — biphasical reaction,

○ — no reaction

The characteristics of the afferent impulsation running from the heart to the CNS through the intestinal nerve were described previously (S.-Rózsa, 1972). In the present experiments both the stimulation and the recorded extracellular potentials were identical to that what was found earlier. All the neurones responding to the chemical stimulation of the heart were tested whether they give a response to the tactile stimulation of the heart or not.

1. *Central neurones responding to the stimulation of the heart chemoreceptors*

a) Neurones responding with the decrease of the activity

Five neurones have been found whose activity decreased due to the intracardially administered 5HT. These were V7, V14, V18, V19 and RPa8 neurones. The increased afferent impulsation recorded from the intestinal nerve was followed by the frequency decrease and further by elimination of the spike generation of the neurone recorded with intracellular electrode. As a rule, IPSP-s could not be recorded from the cell, and release from inhibition was not followed by increased activity. As it is shown in *Fig. 3* the perfusion of the heart with 5HT caused inhibition in the activity of neurone V7, and after wash out the control frequency returned only gradually. No IPSP-s were registered from cell V7 during heart stimulation, however, following two or three depolarizations and hyperpolarizations characteristic inhibitory potentials appeared, referring to the presence of synaptic influences on the cell. This fact gives a basis to suppose that inhibition occurring after increased afferent impulzation could be also the result of synaptic bombardment, however, for some reason this could not be recorded from the soma.

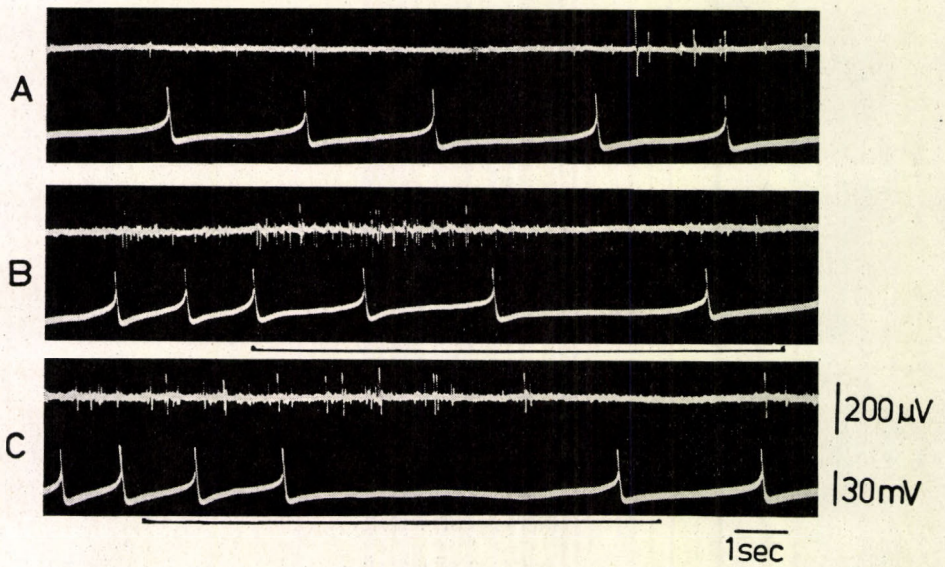

Fig. 3. Reaction of neurone V7 to the chemical stimulation of the heart. A — control; B and C — effect of stimulation. Here and in the following Figs: upper — extracellular recording from the heart nerve; lower: intracellular recording from the soma of the neurone

b) Neurones responding with the increase of the activity

Due to 5HT stimulation of the heart the activity of the neurones V13, V16 and RPa9 increased. This was preceded by the increased afferent impulsation recorded from the intestinal nerve *(Fig. 4)*. The increased activity of the neurones was observed until the elimination of the serotonin from the heart and the returning of the control activity of the intestinal nerve.

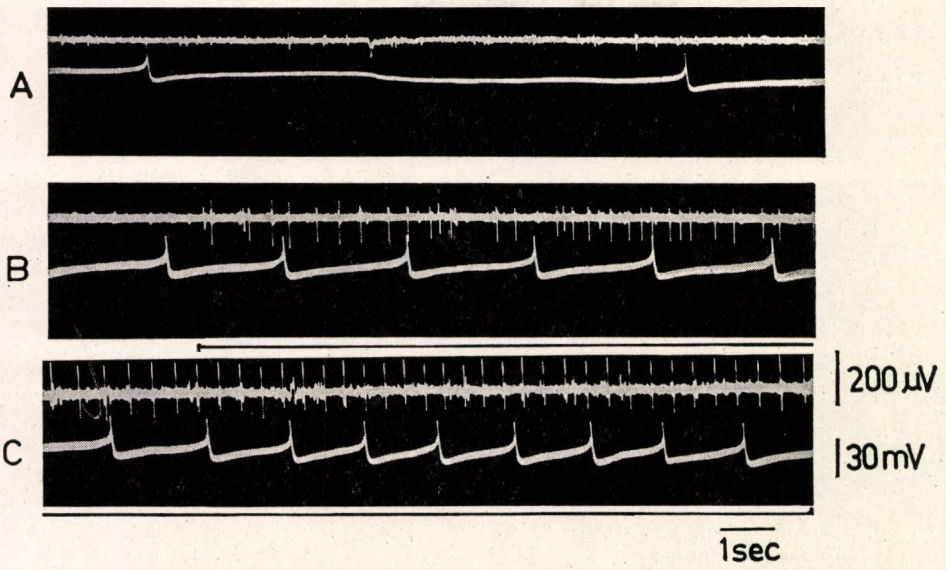

Fig. 4. Response of neurone V16 to the chemical stimulation of the heart. A — control; B and C — effect of continuous stimulation

Furthermore the activity of cell V13 was in close correlation with one of the components recorded from the heart nerve *(Fig. 5)*. As the intracellular spikes preceded the extracellular potentials, these latter can be considered as efferent impulses originating from the neurone and running towards the heart. This supposition is supported by the findings that the close correlation of the two signals could be observed also at the depolarization of the neurone *(Fig. 6)*.

Figure 5 shows that the chemoreceptors of the heart become active to 5HT not instantaneously, but there is at the beginning only a week signalization *(Fig. 5B)*. Also the elimination of the effect of the 5HT and the decrease of the activity of the intestinal nerve take place gradually. The activity of cell V13 remains enhanced until the activity of the intestinal nerve is higher than that of the control *(Fig. 5E, F)*.

No EPSP-s could be registered from neurones responding to the heart stimulation with the increase of the spike generation.

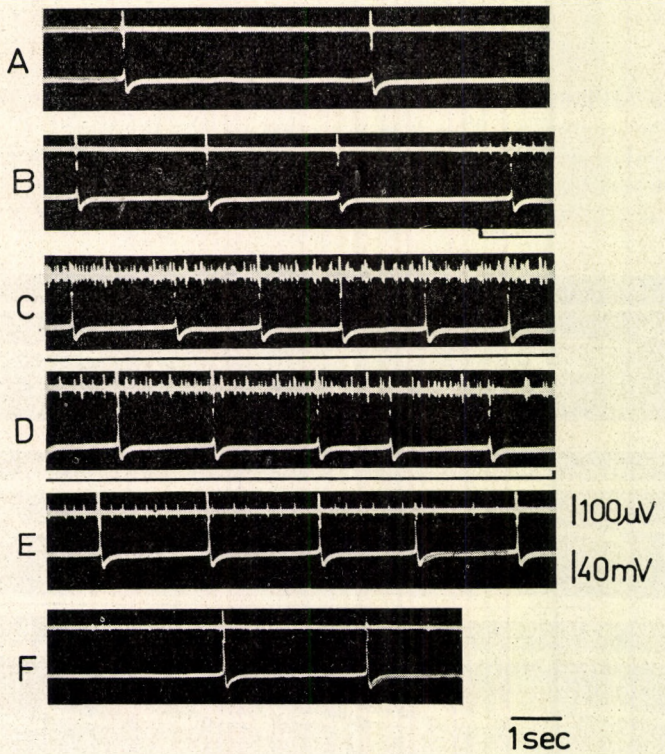

Fig. 5. Response of cell V13 to the chemical stimulation of the heart. A and B — control and beginning of stimulation; C — and D — during stimulation after 1 min; E and F — end of wash out after 3 min. It can be seen that the intracellular spike is followed by an extracellular one in each case

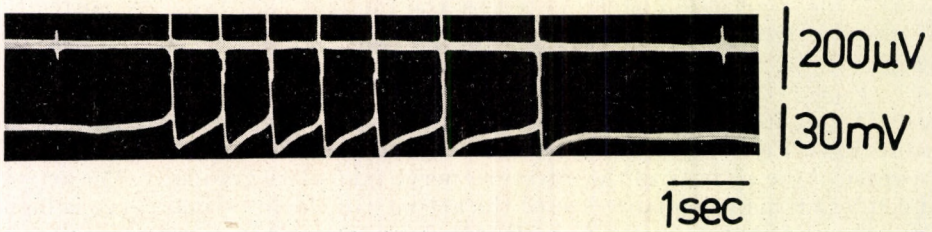

Fig. 6. Response of cell 13 to depolarization of the soma membrane. Note the correlation between the intra- and extracellular components

c) Neurones with biphasical response

There were cells responding biphasically to the chemo-stimulation of the heart. The frequency of the activity of cells V12, V17 and RPa6 increased at the beginning, than it decreased. The reaction of neurone V17 is demonstrated in *Fig. 7*. It can be seen that after perfusing the heart with 5HT both the amplitude and the frequency of the afferent impulsation increased and following this, the frequency of the action potentials recorded from neurone V17 was nearly doubled *(Fig. 7B)*. However, after 2 min. the activity of the

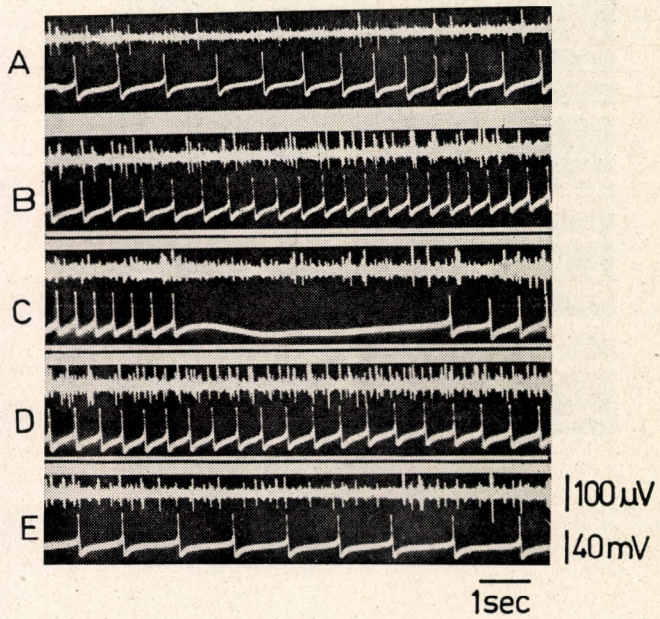

Fig. 7. Response of cell V17 to the stimulation of the heart with 5HT. A — control; B, C and D — continuous stimulation of the chemoreceptors; E — after washing

neurone was inhibited while first depolarization, than hyperpolarization occurred, and at the same time the frequency of the extracellular potentials with high amplitude was decreased *(Fig. 7C)*. Recovering from the phase of inhibition the cell is more active than was in the control state *(Fig. 7D)*, while after elimination of the 5HT it produced a lower activity compared to the control *(Fig. 7E)*. This type of reaction was very characteristic to this neurone and could be evoked repeatedly with very similar time curves in each case.

Cell V12 responds similarly to V17 *(Fig. 8)*, however, in this case the increase of the activity is only of short duration after heart stimulation *(Fig. 8B)*, and alteration of frequency increase and decrease was observed. This type of potential generation, resembling to a bimodal, bursting activity was observed also during the wash out of serotonin *(Fig. 8E,F)*. In some cases during the inhibitory phase IPSP-s were observed causing probably the in-

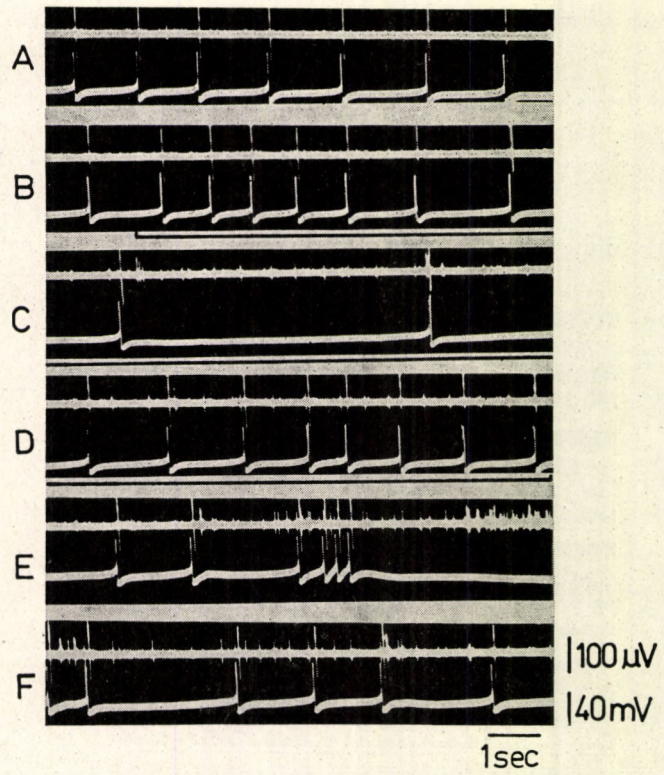

Fig. 8. Response of neurone V12 to heart stimulation. A — control; B, C and D — effect of stimulation; E — during wash out after 2 min; F — after 5 min. Note the synchronous extracellular potential following the intracellular spikes

hibition of the spike generation. In case of cell V12 also a close correlation was found between the intracellular spikes and a given extracellular potential type.

The initial increase of activity was followed by inhibition of the potential generation in case of the RPa6 neurone, too. The spikes of the cell could not be recorded in the extracellular registration from the heart nerve, nevertheless, the general impulzation of this nerve was lowered during the phase of inhibiton of the cell activity. This means that the RPa6 neurone is connected not to a particular axon in the heart nerve, but with several other neurones, sending their axon into this nerve. The generation of potentials in this neurone was clearly of synaptic origin driven by EPSP-s.

d) Effect of tactile stimulation of the heart on neurones giving reaction to chemo-stimulation

The reactions of neurones to chemical and tactile stimulation of the heart were compared in order to clear up the specificity of the central representation of the effect evoked with stimulation of different types. *Table I* summarizes the results.

TABLE I

Response of identified central neurones to the chemical and tactile stimulation of the heart

Neurone	Reaction to chemical stimulus	Reaction to tactile stimulus
V7	−	−
V8	0	−
V12	+,−	+
V13	+	−
V14	−	0
V16	+	+
V17	+,−	+,−
V18	−	+
V19	−	−
RPa6	+,−	−
RPa8	−	−
RPa9	+	−

+: increase of activity
−: decrease of activity
0: no effect

As can be seen, there were only two neurones responding only to one sort of stimulation. Cell V8 gave reaction only to tactile, while cell V14 to chemical stimulation of the heart. Cells V13, V18 and RPa9 gave antagonistic reaction to the chemical and tactile stimulation of the heart: V13 and RPa9 neurones became stimulated at chemical, and were inhibited at tactile stimulation. Cell V18 gave reversed response. Biphasic reaction was observed in the case of cell V17 to both types of stimulation, while cell V12 and RPa6 responded biphasically to chemo-stimulation of the heart. In these cases acceleration was the first phase, followed by a decrease and again by an increase of the spike generation.

Tactile stimulation caused on cell V12 only stimulation while on cell RPa6 only inhibition, however, one must take into consideration that the duration of the tactile stimulus is comparatively short, while chemical stimulation is long. Cells V7, V19 and RPa8 were inhibited and cell V16 was stimulated both at tactile and chemical stimulation of the heart.

e) Postsynaptic potentials on the central neurones at the stimulation of heart receptors

From the soma of neurones connected with heart receptors postsynaptic potentials could be registered only rarely. Cell RPa6 was the single neurone where EPSP-s were recorded in control conditions and from cell V7 could be recorded IPSP-s after repeated de- and hyperpolarization.

In two cells, however, where the effects of tactile and chemical stimulation were antagonistic, PSP-s occurred, and the effect was realized clearly through PSP-s. In cell V12 after chemo-stimulation of the heart depolarization took place at the beginning, without noticeable PSP-s. However, in the second phase the inhibition took place with the appearance of IPSP-s *(Fig. 9)*. On

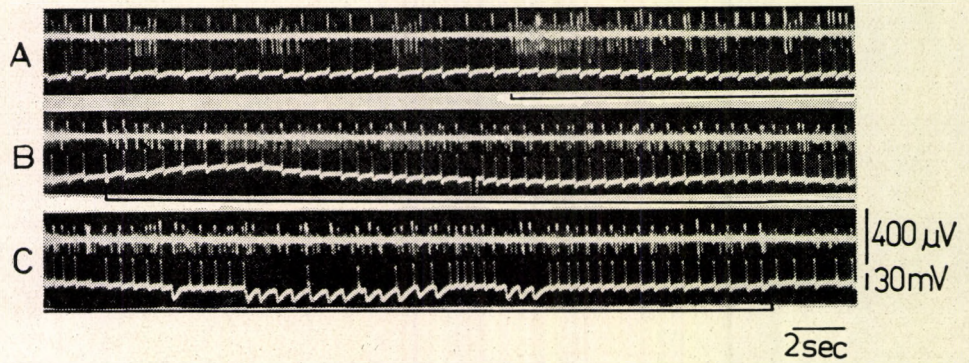

Fig. 9. Two phases of the response of neurone V12 in a case, when after the chemical stimulation of the heart ISPS-s were recorded. A — control and the effect of tactile stimulation of the heart; B — excitatory phase following the chemical stimulation of the heart; C — inhibitory phase during stimulation

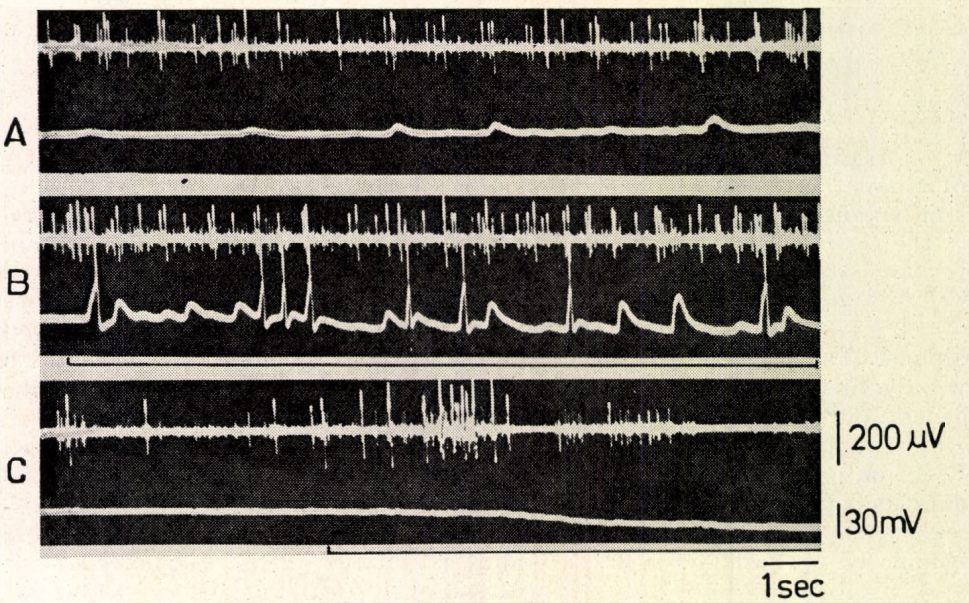

Fig. 10. Response of cell V18 to heart stimulation. A — control; B — effect of tactile stimulation of the heart. Note the increase of EPSP-s. C — effect of chemical stimulation of the heart

cell V12 the increase of activity appeared without EPSP-s also in case of tactile stimulation. On cell V18 the tactile stimulation of the heart resulted in the increase of EPSP-s and enhanced spike activity. On this cell the inhibition evoked by chemo-stimulation of the heart appeared without IPSP-s, however, the amplitude of the EPSP-s was drastically reduced, and membrane hyperpolarization was observed *(Fig. 10)*. In contrary to the fact that PSP-s were recorded only rarely these cases show that signalization arriving from the

heart can cause strong synaptic influence on the neurones. It seems probable that the modification of the cell activity occurs much more frequently through synaptic potential than we could observed, however, being the synapses far from the soma when recording with intracellular electrodes, these potentials do not become visible.

Discussion

It was shown earlier that the tactile stimulation of the heart modifies the activity of a number of central neurones (S.-Rózsa and Salánki, 1973). The present results give evidence that the chemoreceptors of the heart also have a similar central representation at cellular level. Our experiments were conducted on the visceral and right parietal ganglia, and these neurones were found not in groups, but in dispersed localization. It can be supposed, that neurones connected with heart chemoreceptors could be found in other parts of the ganglionic mass too.

According to these data neurones receiving signals from the heart at different modes of stimulation are mixed in the snail brain similarly to the central neurones of heart chemo- and baroreceptors in the brainstem of vertebrates (Miura and Reis, 1972; Spyer, 1972). Our earlier histological data also proved that the neurones innervating the heart of Gastropoda are in the ganglia scattered and do not form any definite group (Gubicza and S.-Rózsa, 1969).

Examining the neurones whether they can be influenced both from the chemoreceptors and tactile receptors we found that there is only a few of them responding only to one of the stimulus types. There were a few more cells responding antagonistically to the two different stimuli. The identical representation of different stimuli show that primary sensory neurones responding directly to stimulation can be present among the investigated cells only in a limited number. Such primary sensory neurones are the mechano- and stretch receptors of crabs, the chordotonal organ of arthropods, the muscle proprioceptors, while the central light sensisitive system of the arthropods and most of other sensory systems are composed of secondary sensory neurones (Grundfest, 1971). Most probably such secondary sensory neurones take part in the central representation of sensory areas of the *Helix* heart. The sensory character could explain that postsynaptic potentials were not registered from most of the neurones both at rest and after chemical or tactile stimulation of the heart. However, to explain the lack of PSP-s one can suppose too that the place of the synaptic influence and the location of the recording electrode are situated to a distance, and so the synaptic potentials can influence the spike generation, but cannot be recorded from the soma. If this supposition is true, one could record a more violent synaptic bombardment from the synaptic region than from the soma.

In case of biphasical responses sometimes PSP-s appeared clearly. This type of activity is based probably in each case upon synaptic influences, when the competition of excitatory and inhibitory inputs cause alteration in the cell activity. It can be supposed that the occurrence of biphasical responses would be more frequent after tactile stimulation if we apply this stimulus for minutes instead of seconds.

The occurrence of neurones responding differently to different modes of stimulation is an indirect proof showing that among receptors special chemo- and tactile receptors must exist, however, we are lack of any information about their morphological structure. As both type of stimulus cause an increase in the electrical activity of the intestinal nerve, the different neuronal reactions refer to the fact that specificity must exist also at neuronal level according to the sensory area. It cannot be excluded that this specificity is manifested in the form of the presence of primary sensory neurones. It can be supposed too that neurones influenced by heart receptors form a coupled system similarly to that described for the touch receptors of *Hirudo* (BAYLER and NICHOLLS, 1969). Such a coupling can give a basis not only for differentiation of various signals, but also for integration of different inputs and for the discrimination of the place of stimulus.

The functional role of some giant neurones is well known in the control of centrally triggered motor functions (WILLOWS and HOYLE, 1968) and in some reflex responses (KUPFERMANN and KANDEL, 1969; PERETZ, 1969; WEEVERS, 1971) in Gastropoda. In the visceral ganglion of *Limax* a neurone was described causing increase in the heart rate (MCKAY and GELPERIN, 1972). We have reported also about some neurones in the CNS of *Helix* taking part in the heart-heart reflex evoked by tactile heart stimulation (S.-RÓZSA and SALÁNKI, 1973). According to our present results the reflex responds occurring at the stimulation of chemo- and tactile receptors are very similar, and in some cases they are realized with the participation of the same neurones (cells V12 and V13). Similar common representation was described for the central neurones of the chemo- and baroreceptors in vertebrates (MIURA and REIS, 1972). Obviously, central neurones influenced by heart stimulation can take part in the functioning of other reflex pathways, too.

Our results give evidence for the central representation of the heart chemo-receptors at cellular level and prove on its basis the existence of such a heart-heart reflex which can take part in the extracardial regulation of the heart. The fact, that the activity of most of the neurones can be modulated by different types of stimuli calls the attention to the existence and functioning of regulatory systems distributed diffusely in the ganglia.

Summary

The responses of central neurones to chemical stimulation of the heart were examined in the visceral and right parietal ganglia of the snail *Helix pomatia* L. These responses were compared with reactions evoked by stimulation of the mechanoreceptors of the heart. The following results were obtained:

Twelve neurones were identified in the visceral and parietal ganglia responding to the stimulation of the heart with 5HT. The response was either an increase or a decrease in activity.

Four neurones, three from the visceral (V12, V13 and V17) and one from the right parietal ganglion (RPa9) gave biphasic reaction at chemical stimulation of the heart: the initial excitatory phase was followed by the inhibition of spike generation. In case of long lasting stimulation the alteration of the two phases was observed.

Cells V12 and V13 belong definitely to central neurones of the heart reflex. At stimulation of the chemoreceptors a close correlation was found between the spikes of these neurones and a particular component of the heart nerve activity. These neurones can be considered as central neurones of the extracardial heart regulation.

Most of the neurones investigated responded both to the chemical and tactile stimulation of the heart, however, in some cases the reaction was different to different modes of stimulation. Neurones, responding only to one type of stimulus can be considered as primary sensory neurones.

REFERENCES

BAYLOR, D. A., J. G. NICHOLLS (1969): Chemical and electrical synaptic connections between cutaneous mechanoreceptor neurones in the central nervous system of the leech. — *J. Physiol.* **203,** 591—609.

GRUNDFEST, H. (1971): The general electrophysiology of input membrane in electrogenic excitable cells. — *Handbook of sensory physiology Vol.* **1.** *Principles of receptor physiology.* Ed. W. R. LOEWENSTEIN, *Springer-Verlag, Berlin, Chapter 4,* 136—165.

GUBICZA, A., K. S.-RÓZSA (1969): Identification of central neurons innervating the heart of *Lymnaea stagnalis* L. (Gastropoda). — *Annal. Biol. Tihany* **36,** 3—10.

KRIJGSMAN, B. J., G. A. DIVARIS (1955): Contractile and pacemaker mechanisms of the heart of Molluscs. — *Biol. Rev.* **30,** 1—39.

KUPFERMAN, I., E. R. KANDEL (1969): Neuronal controls of a behavioural response mediated by the abdominal ganglion of *Aplysia.* — *Science* **164,** 847—850.

MCKAY, A. R., A. GELPERIN (1972): Pharmacology and reflex responsiveness of the heart in the giant garden slug, *Limax maximus.* — *Comp. Biochem. Physiol.* **43A,** 877—896.

MENG, K. (1958): 5-hydroxytryptamine und Acetylcholine als Wirkungsantagonisten beim *Helix*-Herzen. — *Naturwiss.* **19,** 470—481.

MIURA, M., D. J. REIS (1972): The role of the solitary and paramedian reticular nuclei in mediating cardiovascular reflex responses from carotid baro- and chemoreceptors. — *J. Physiol.* **223,** 525—548.

PERETZ, B. (1969): Central neurons inhibition of periodic gill movements. — *Science* **166,** 1167—1172.

S.-RÓZSA, K. (1972): Characterization of the feed-back system in the heart of *Helix pomatia* L. — *Annal. Biol. Tihany* **39,** 29—38.

S.-RÓZSA, K., C. GRAUL (1964): Is serotonin responsible for the stimulative effect of the extracardiac nerve in *Helix pomatia?* — *Annal. Biol. Tihany* **31,** 85—96.

S.-RÓZSA, K., J. SALÁNKI (1973): Single neurone responses to tactile stimulation of the heart in the snail, *Helix pomatia* L. — *J. Comp. Physiol.* **84,** 267—279.

SAKHAROV, D. A., J. SALÁNKI (1969): Physiological identification of neurons in the central nervous system of *Helix pomatia* L. — *Acta Physiol. Acad. Sci. hung.* **35,** 19—30.

SPYER, K. M. (1972): Baroreceptor sensitive neurones in the anterior hypothalamus of the cat. — *J. Physiol.* **224,** 245—257.

VÉRÓ, M. (1971): Negative capacitance amplifier for microelectrode investigatiors. — *Annal. Biol. Tihany* **38,** 107—115.

WEEVERS, R. G. (1971): A preparation of *Aplysia fascinata* for intrasomatic recording and stimulation of single neurons during locomotor movements. — *J. Exper. Biol.* **54,** 659—676.

WILLOWS, A. O. D., G. HOYLE (1968): Correlation of behaviour with the activity of single identifiable neurons in the brain of *Tritonia.* — p. 443—461. *Neurobiology of Invertebrates,* Ed. J. SALÁNKI. *Plenum Press, New York and Akadémiai Kiadó, Budapest.*

KÖZPONTI IDEGRENDSZERI NEURONOK VÁLASZREAKCIÓI A SZÍV KEMORECEPTORAINAK INGERLÉSÉRE *HELIX POMATIÁN*

S.-Rózsa Katalin és Salánki János

Összefoglalás

Tanulmányozták a központi neuronok válaszreakcióit a szív kemoreceptorainak ingerlésére *Helix pomatia* L. viscerális és perietális ganglionjaiban. A kemoreceptorok ingerlésekor megfigyelt központi effektusokat összevetették a szív mechanoreceptorainak ingerlésekor leírt reakciótípusokkal. Az eredmények szerint:

1. A szív kemoreceptorainak ingerlésére 12 sejt reagált a viscerális és jobb parietális ganglionokban; a válaszreakció a spontán aktív sejteken gátlás vagy serkentés volt.

2. A viscerális ganglion több sejtje (V12, V13, V17), valamint a jobb parietális ganglion egyik sejtje (RPa9) kettősen reagált a szív kemoreceptorainak ingerlésére: a kezdeti serkentő hatást gátló fázis követte. Tartós ingerlés esetén a két fázis váltakozhat is.

3. A vizsgált sejtek közül a V12 és V13 bizonyítottan szív-szívreflex központi neuronja, mivel a kemoreceptorok ingerlésekor szoros korreláció mutatható ki a sejt aktivitása, valamint a szívidegről elvezetett aktivitás komponensei között. E korreláció törvényszerűen minden preparátumon kimutatható. E sejtek a szív extrakardiális szabályozásának központi neuronjai.

4. A vizsgált központi neuronok többsége egyaránt reagált a szív taktilis és kémiai ingerlésére, azonban az esetek egy részében a válasz a két ingerféleségre ellentétes volt. A kizárólag egyik ingerre reagáló sejtek elsődleges érző neuronok lehetnek.

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HORIZONTAL PULLER FOR THE PREPARATION OF GLASS MICROELECTRODES

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The mechanical and electrical parameters of microelectrodes applied for electrophysiological investigation of cells are determined by the differing requirements of intracellular connections used for various objects. The most important requirement is the elimination of destruction which would interfere with the normal operation of the cell (NASTUK, 1963). In order to satisfy this requirement, the tip diameter of the microelectrode should be within 0.1 to 1 μm (LAVALLÉE et al., 1969). The problems associated with the extremely high resistance and capacitance of the low-diameter tip can be minimized by using a high input impedance, negative capacitance amplifier (SCHANNE et al., 1968; VÉRÓ, 1971).

For the preparation of glass microelectrodes, pullers with horizontal or vertical arrangements are in use (DONALDSON, 1958). Considering merits and demerits of these arrangements, a horizontal type puller has been developed in our Institute which has a more straightforward pulling force control as compared to the ALEXANDER—NASTUK type generally used (ALEXANDER and NASTUK, 1953).

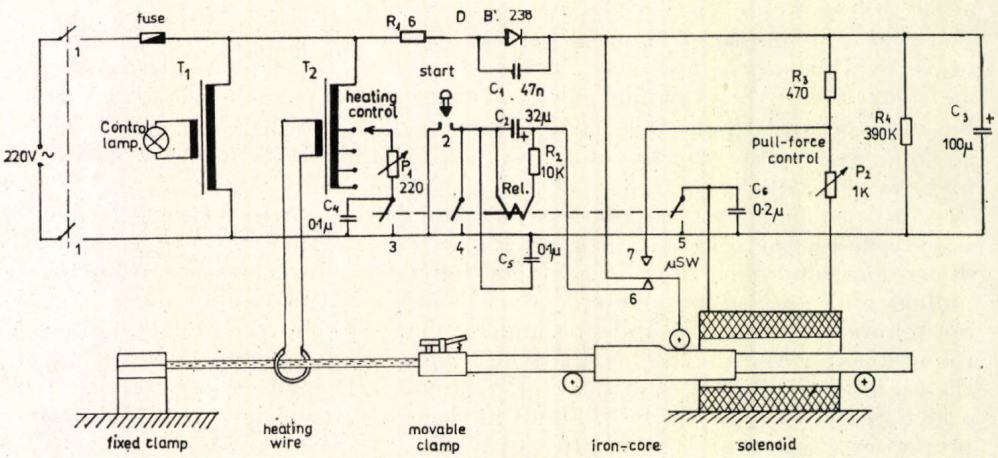
By utilizing glass capillars with diameters of 0.5 to 3 mm, the device is suitable for the preparation of microelectrodes having suitable geometrical dimensions and electrical parameters for intracellular investigations. The pulling and heating parameters are adjustable within wide ranges, so the optimum electrode shape and tip diameter may be well suited for the different requirements. With glass capillars of unchanging diameter and material, the stability of pulling and heating parameters is high enough to result in a high degree of reproducibility, both in electrode dimensions and in electrical properties (BAKER and YORK, 1971).

The operation of the electrode puller is shown by the circuit diagram given in Fig. 1 which also gives the operation phases. The circuit is operated from a 220 volts mains supply. Switching on will generate 300 volts DC to supply the solenoid and relay currents. Operating the START button, the relay REL will operate and close the solenoid circuit. Simultaneously, transformer T_2 is energized giving the heating power. In the first phase, pulling force is F_1, and this is adjustable by the PULL FORCE CONTROL potentiometer. As soon as the glass capillar reaches the plastic stage, it will be stretched by the pulling force F_1, and after reaching a certain length, the microswitch SW,

controlled by a movable iron core, will switch the solenoid to a maximum voltage. In this phase, the maximum pulling force F_2 is established which has the effect to tear the glass and thus form the suitable electrode tip diameter. The starting of the pulling phase with pulling force F_2 is adjustable by the setting of microswitch SW. 3 seconds after the switching on of pulling force F_2, pulling force and the heating are both switched off, thus terminating one pulling period. A definite termination time is assured by the time constant in the operating circuit of relay REL.

In *Fig. 1*, the layout of the solenoid supplying the pulling force and the movable iron core with bearings, and their relation to the electronic circuit are illustrated. The movable clamp fixing one end of the glass capillar is mounted on the movable core axis; the other end is connected to a fixed clamp.

The electrode puller actually built is shown in *Fig. 2*. The solenoid supplying the pulling force, the movable iron core with bearings at both ends, and a suitable profile at the iron core side for controlling the microswitch SW, are all mounted on the top cover of the cabinet. Fine adjustment of the microswitch Sw along the iron core axis is possible, resulting in an accurate switch-on time of the pulling force F_2. The two ends of the glass capillar are held by the movable and fixed clamps shown in the *Figure*. The electrode is heated by the heating filament at the middle part of the capillar. The filament has a width of 4 mm and a thickness of 0.2 mm, rolled from Kanthal, with a resistance of 0.1 ohm and a maximum heating power of 40 watts.



OPERATING PHASES	1	2	3	4	5	6	7
STAND-BY: mains on, F_1 pulling force energized with clamping of capillar.	■					■	
PULLING: F_1 pulling force and heating started by pushing START button.	■		▩	■	■		■
BREAKAGE: F_2 pulling force and heating switched on. Starting time of F_2 is adjustable.	■		▩	■	■	□	■
FINISH: F_2 pulling force and heating switched off 3 seconds after F_2 switch-on time	■		□	□	□	■	□

Switch conditions : ■ closed normal switch ▩ closed snap switch □ open switch

Fig. 1. Circuit diagram and operating phases of the glass microelectrode puller.

According to *Fig. 2*, the operation of the electrode puller has following phases:

1 — The glass capillar is mounted between the movable and fixed clamps and the mains is switched on. This brings the pulling force F_1 into the stand-by stage.

2 — The starting time of the pulling force F_2 and the pulling and heating parameters are adjusted. Operating the START button will start the pulling of the electrode.

3 — After completion of the pulling procedure, another capillar may be clamped in, bringing the puller into a fresh stand-be stage.

Fig. 3 shows the electronic circuit mounted on a printed circuit board. This makes simple adjustment and easy control possible.

The resistance and tip potential of the manufactured electrodes, filled with an electrolyte 3M KCl, is checked by a FET-input amplifier developed in our Institute and used for intracellular connections (VÉRÓ, 1971). The arrangement for resistance measurements is shown in *Fig. 4a*. The Wheatstone-bridge at the amplifier input has a potentiometer calibrated in megohm values and is suitable for measuring electrode resistances in the range of 1 to 50 megohms. An electrode with a resistance lower than 1 megohm usually pertains to a tip diameter higher than 1 μm, which may cause destruction of the cell membrane, and thus will not be suitable. An electrode of high tip potential is equally unsuitable as it will polarize the cell when connected intracellularly, and thus interfere with cell operation, or in extreme cases may even completely prevent cell operation. The tip potential value is controlled as shown in *Fig. 4b*. In high precision measurements, even a low or changing tip potential may cause troubles. In this case, a special measuring bridge suitable for tip potential compensation should be associated with the amplifier (KISS et al., 1972) as

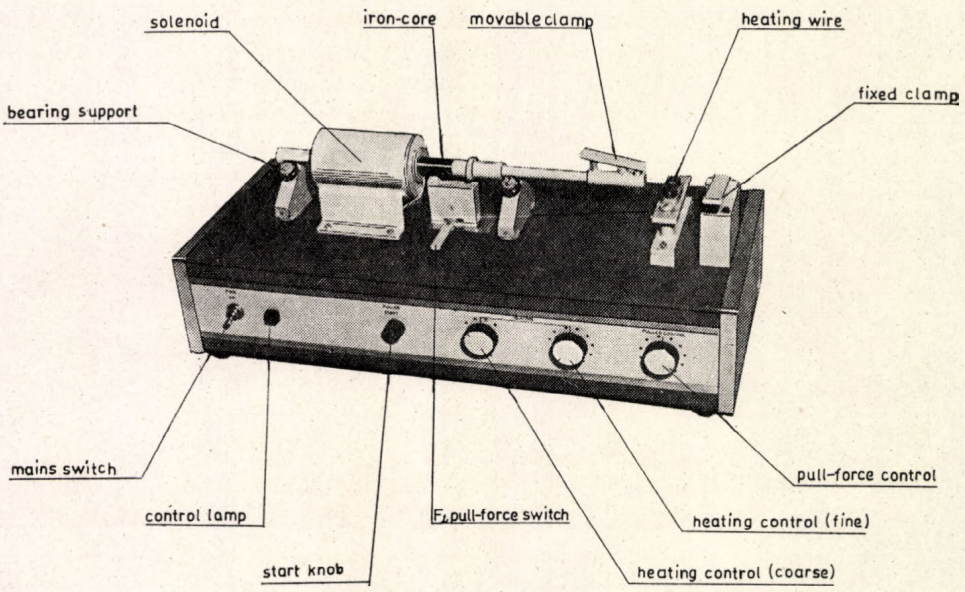


Fig. 2. Mechanical layout of the electrode puller

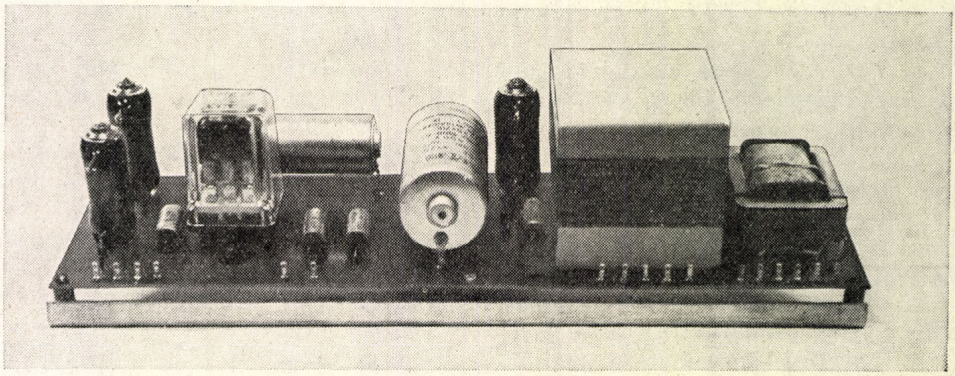


Fig. 3. Printed circuit board arrangement of the electronic assembly

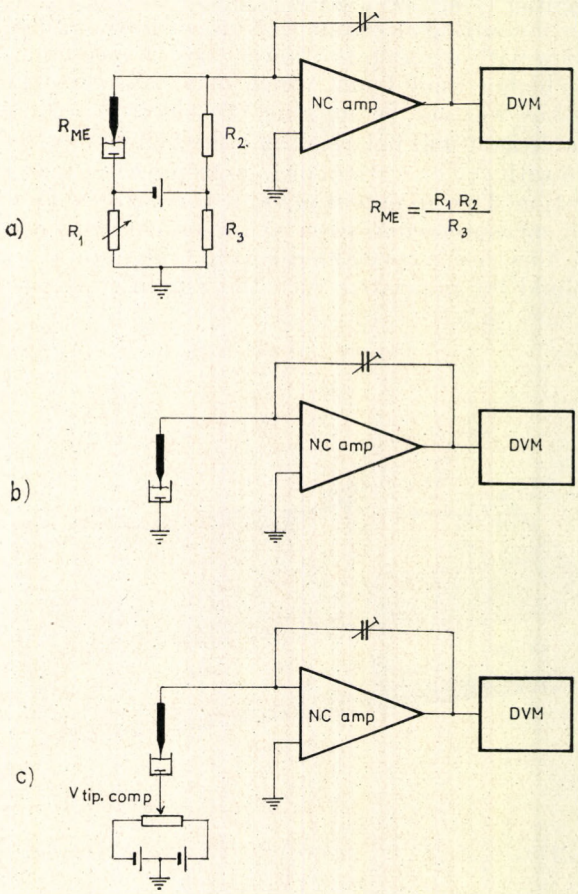


Fig. 4. Control of microelectrode resistance and tip potential: *a)* resistance measurement, *b)* tip potential measurement, *c)* tip potential compensation

shown in simplified form in *Fig. 4c*. Microelectrodes with stable tip potentials not changing with time are to be preferred as the compensation adjustment during operation is difficult and sometimes impossible. Stable tip potential requires very low impurity in the glass material and in the solutions during the whole electrode pulling process.

Summary

The puller is used to prepare microelectrodes suitable for intracellular connection from glass capillars having diameters of 0.5 to 3 mm. The puller has a horizontal arrangement, pulling and heating parameters are adjustable within a wide range, so the electrode shape and tip diameter suitable for diverse requirements are easily realizable. The stability of pulling and heating parameters will result in a high degree of reproducibility of the electrical and geometrical electrode data. Operation of the puller and relations between electronic and mechanical parts are described in detail and shown by figures. A bridge input electrometer amplifier is used for control of the most important electrical parameters of the electrodes prepared.

REFERENCES

Alexander, J. T., Nastuk, W. L. (1953): An instrument for the production of microelectrodes used in electrophysiological studies. — *Review of Sci. Instr.* **24,** 528.

Baker, F. L., York, D. H. (1971): A reliable technique for making glass microelectrodes of known resistance. — *Proc. of the IEEE*, **59,** p. 1711.

Donaldson, P. E. K. (1958): Electronic apparatus for biological research. — *Butterworths Scientific Publication.*

Kiss, I., Salánki, J., Véró, M. (1972): Dependence of reaction to ACh on the membrane potential of neurones *Lymnaea stagnalis.* — *Annal. Biol. Tihany* **39,** 21—27.

Lavallée, M., Schanne, O. F., Hébert, N. C. (1969): Glass microelectrodes. — *John Wiley.*

Nastuk, W. L. (1963): Physical techniques in biological research. — *Acad. Press London.*

Schanne, O. F., Lavallée, M., Laprade, R., Gagué, S. (1968): Electrical properties of glass microelectrodes. — *Proc. of the IEEE* **56,** 1072—1082.

Véró, M. (1971): Negative capacitance amplifier for microelectrode investigations. — *Annal. Biol. Tihany* **38,** 107—115.

HORIZONTÁLIS HÚZÓKÉSZÜLÉK ÜVEGMIKROELEKTRÓDÁK KÉSZÍTÉSÉHEZ

Véró Mihály

Összefoglalás

A készülékkel 0,5 mm-től 3 mm átmérőig terjedő üvegkapillárisokból intracelluláris elvezetésre alkalmas mikroelektródák készíthetők. Az elektródahúzó horizontális elrendezésű, húzási és fűtési paraméterei széles határok között változtathatók, így a különböző elvezetési követelményeknek megfelelő optimális elektróda forma és hegyátmérő nagy biztonsággal kialakítható. A készülék húzási és izzítási paramétereinek stabilitása biztosítja az elektródák elektromos és geometriai adatainak nagyfokú reprodukálhatóságát. Az elektródahúzó működését, elektronikai és mechanikai részeinek kapcsolatát, az ábrák alapján részletes leírás ismerteti. Az elkészült elektródák legfontosabb elektromos paramétereinek ellenőrzése mérőhíd bemenetű elektrométer erősítővel történik.

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MULTICHANNEL ANALYZER CLASSIFYING CIRCUIT FOR THE DETERMINATION OF ACTION POTENTIAL INTERVALS AND DURATIONS

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Activity patterns of spontaneous active (pacemaker) nerve cells are frequently different, and the individual patterns are closely related to the nerve cell functions. Spike generation frequency and duration of the single spikes are both different (BULLOCK and HORRIDGE, 1965). Even a single cell may show different activity patterns during operation as a result of an afferent input (S.-RÓZSA and SALÁNKI, 1973), and the operating frequency of stimulated cells is highly dependent on the temperature, ion content etc. of the physiological solution surrounding the cell (CARPENTER, 1967). These parameters which are primarily of external origin will have an effect not only on the operating frequency of the cell but also on the duration of the action potentials. Investigations classifying cell responses on external parameter changes are extremely useful.

The above considerations led to experiments in our Institute concerning the operation of Br type cells of *Helix pomatia* L. as a function of temperature. (SALÁNKI et al., 1973). This cell will show at normal room temperature (22 deg C) a specific activity pattern *(Fig. 1b)*. Increasing or decreasing the temperature of the physiological solution surrounding the cell will change the activity pattern, i.e. the length of the intervals and the duration of the action potentials *(Figs. 1a* and *1c)*. For detailed investigation of this phenomenon, pulse trains pertaining to different temperatures are needed, and these can only be evaluated economically by electronic methods.

To solve the above problem, the 1024-channel analyzer type NTA-512B, developed by the Central Research Institute for Physics "KFKI", Budapest, has been applied. Suitable additional circuits and modifications have been used in order to realize the high precision and rapid data analysis needed.

The principle of data analysis is shown by the block diagram of *Fig. 2*. In order to achieve off-line type data analysis, the action potentials are amplified by a FET input preamplifier (VÉRÓ, 1971) and are stored on an FM type analog magnetic data recorder. From the recorder, data are transferred through a FET input amplifier on an operational amplifier which will limit the signals at a hight corresponding to half of the action potentials. The next circuit is a squarer which generates square pulses having widths equal to the action potential duration. Finally, short pulses coinciding with the leading

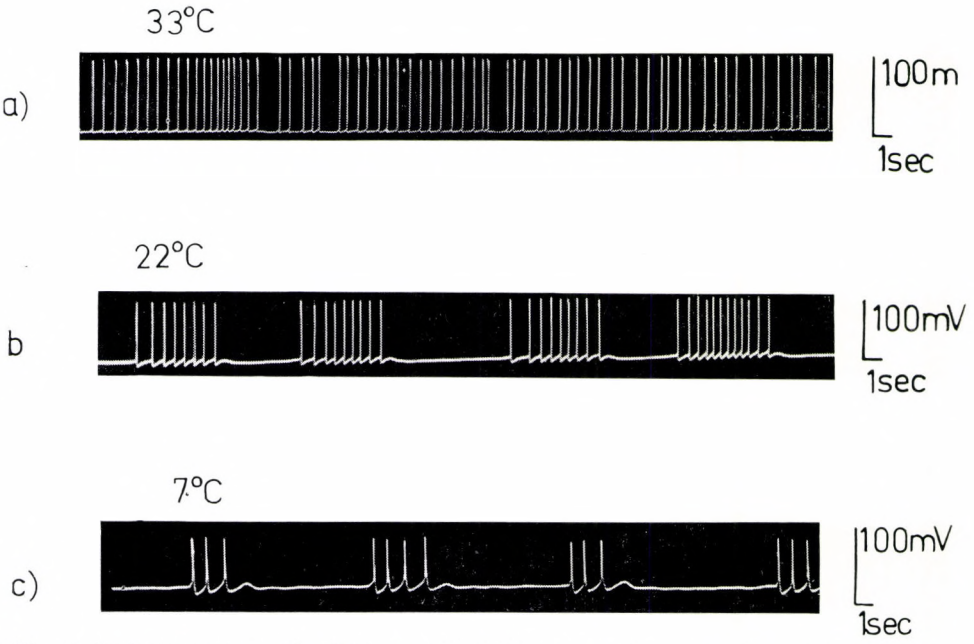

Fig. 1. Activity pattern of a Br type cell of *Helix pomatia* L. at different temperatures

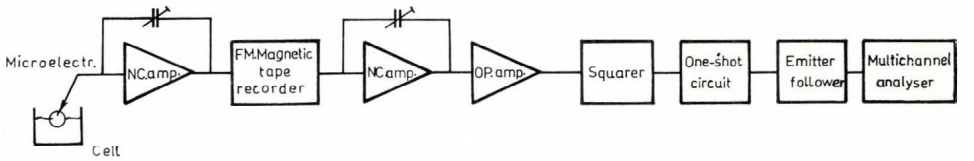

Fig. 2. Block diagram of the data evaluation system

and trailing edges of the square pulses are generated, thus producing trigger signals to be analyzed by the analyzer (D'ALTON and RYAN, 1972).

The special classifying task has necessitated the modification of some of the analyzer circuits as given in the original Instructions Manual for the NTA-512B Multichannel Analyzer System, prepared by the Central Research Institute for Physics. First, the address increase function by the internal clock generator had to be substituted by an address increase by the pulses generated from the action potentials. Charging of the channels is given by the clock generator which is not used for address increase any more, and this will satisfy the accuracy requirements, as the counting time can be adjusted in the range of 20 μsec to 10^3 sec. The block diagram of the analyzer thus modified is shown in *Fig. 3.*

The circuit for generating the analyzer trigger from the recorded analog signal is shown in *Fig. 4.* The action potential is routed through a FET input preamplifier to the inverting input of an operational amplifier. This amplifies the signal and introduces squaring at a definite level (STARR, 1953). Two methods may be used for exact adjustment of squaring level. Either the

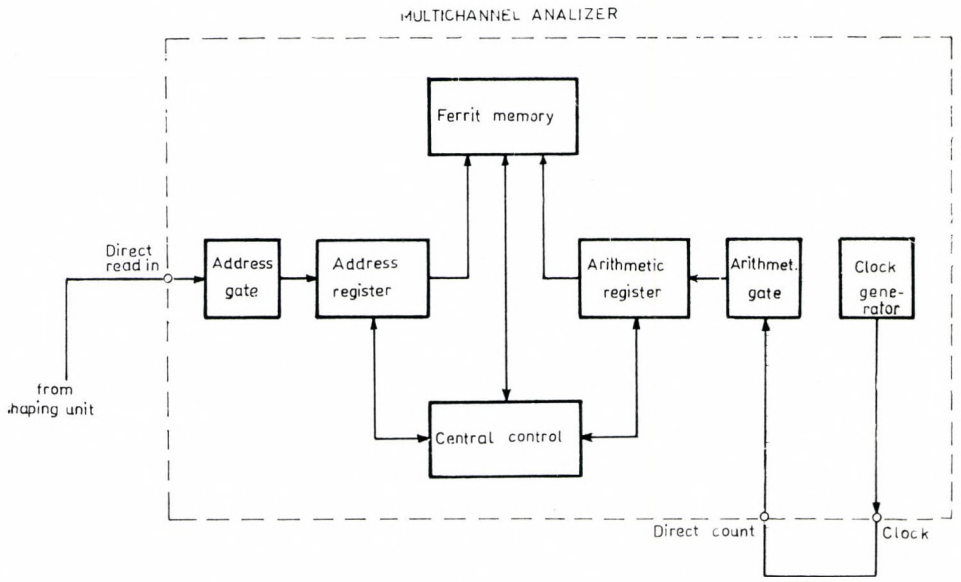

Fig. 3. Block diagram of the modified analyzer read-in operating mode

amplification of the operational amplifier or the amplification of the FET input preamplifier may be adjusted according to the square level required. The squared signal at the operational amplifier output is given to the base of the OC 1070 transistor operating in the switching mode. Thus the collector signal will have leading and trailing edges coinciding with the instants when the input signal passes through the squaring level. The leading edge of this signal will trigger the base of the emitter coupled one-shot multivibrator which has a flip-flop time of 0.2 msec. This mode of operation is used for measuring the interval T_s between action potentials.

An other mode of operation is used for measuring the duration of action potentials. In this mode, the trailing edges of the square pulses at the OC 1070 output will again operate the one-shot multivibrator on the collector. Thus, the assessment of the action potential duration is simplified to the measurement of time-durations between two 0.2 msec pulses. The pulses appearing at the one-shot multivibrator collector are used to drive an emitter follower which supplies an output signal corresponding to the analyzer logic level at the low impedance required (MALVINO and LEACH, 1967; DAKIN and COOKE, 1968). The trigger signals are given on the address gate system input (DIRECT READ IN on the analyzer type used).

Analog presentation of the analyzed data in the activity pattern shown in *Fig. 1b* is given in *Fig. 5b*. The address numbers along the horizontal axis correspond to the successive action potentials. The address content, i.e. the time duration pertaining to the addresses are plotted along the vertical axis. In *Fig. 5b*, evaluation of the interval times T is shown, whereas in *Fig. 6b*, the action potential durations τ' and the $\tau'' = T_s - \tau'$ values are shown together. Thus, the interval times $T_s = \tau' + \tau''$ and $T_b = T_b' + \tau'$ can be calculated by simple addition from *Fig. 6b*.

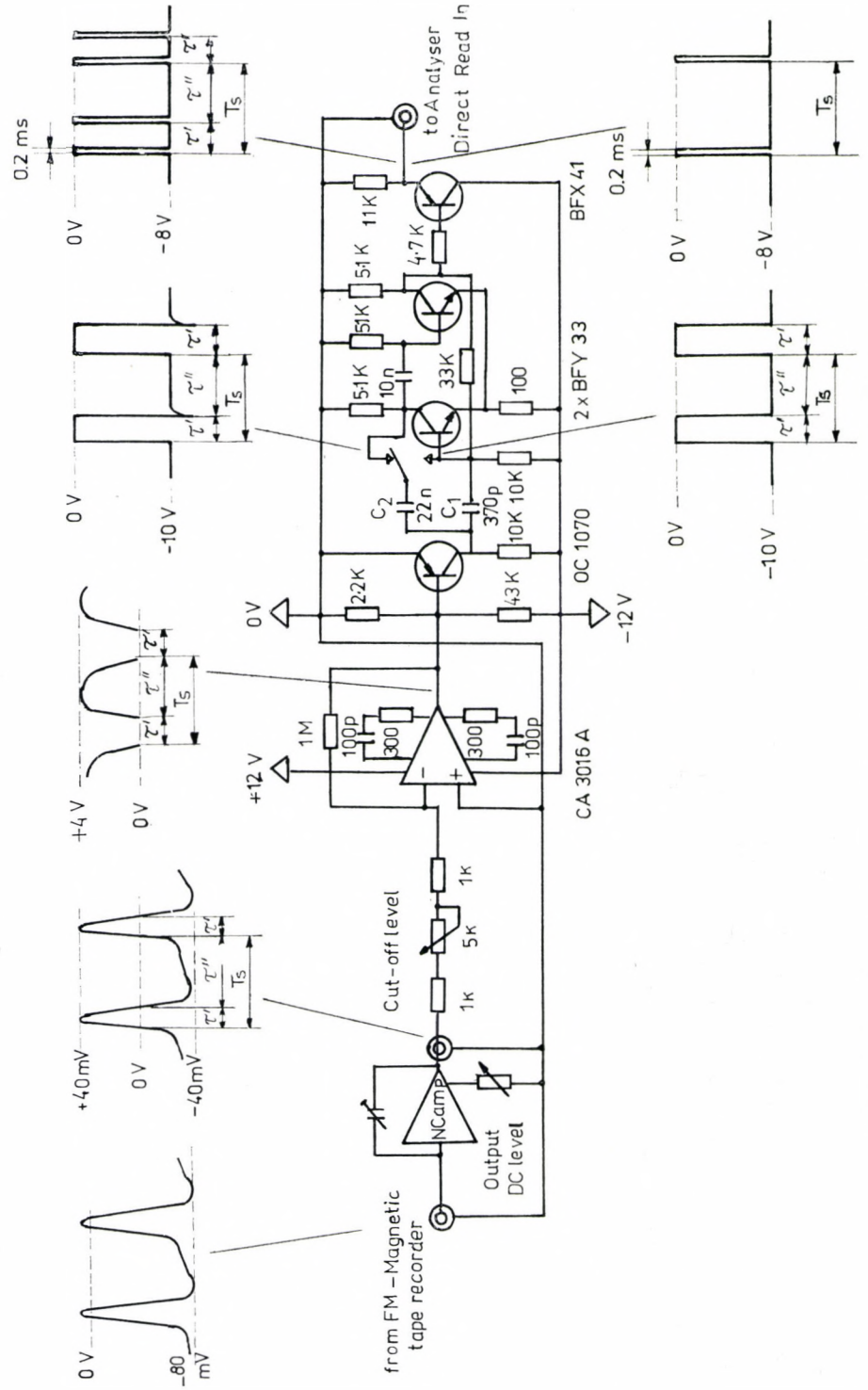

Fig. 4. Circuit and relevant waveforms of the analyzer trigger signal arrangement

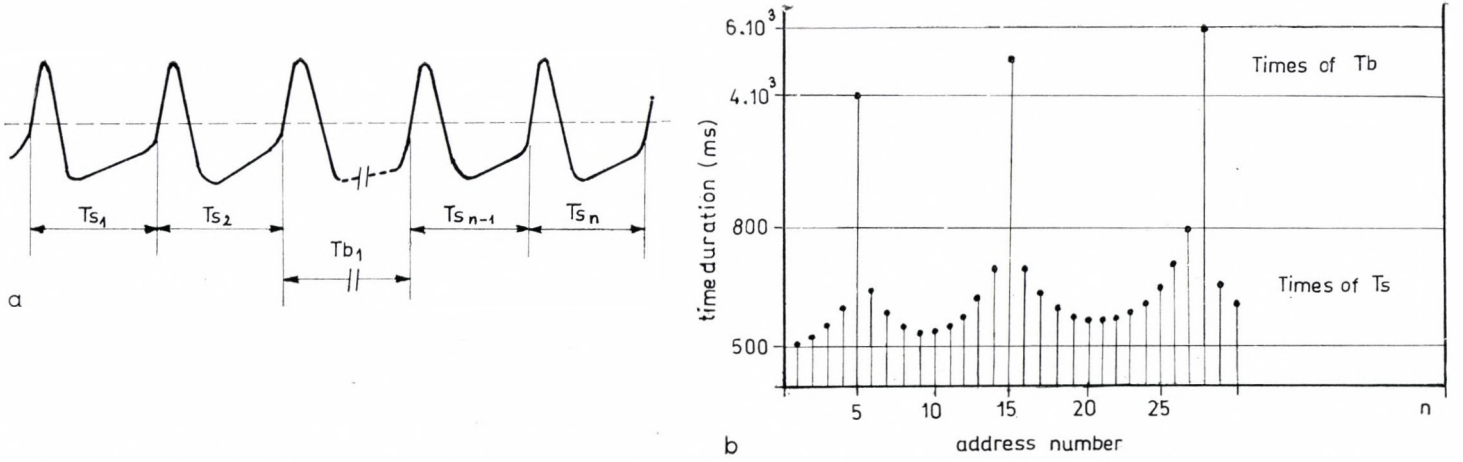

Fig. 5. a) Enlarged waveform of the action potential shown in *Fig. 1b; b)* Interval evaluation and the results presented by the analyzer. T_s — interspike interval, T_b — interburst interval

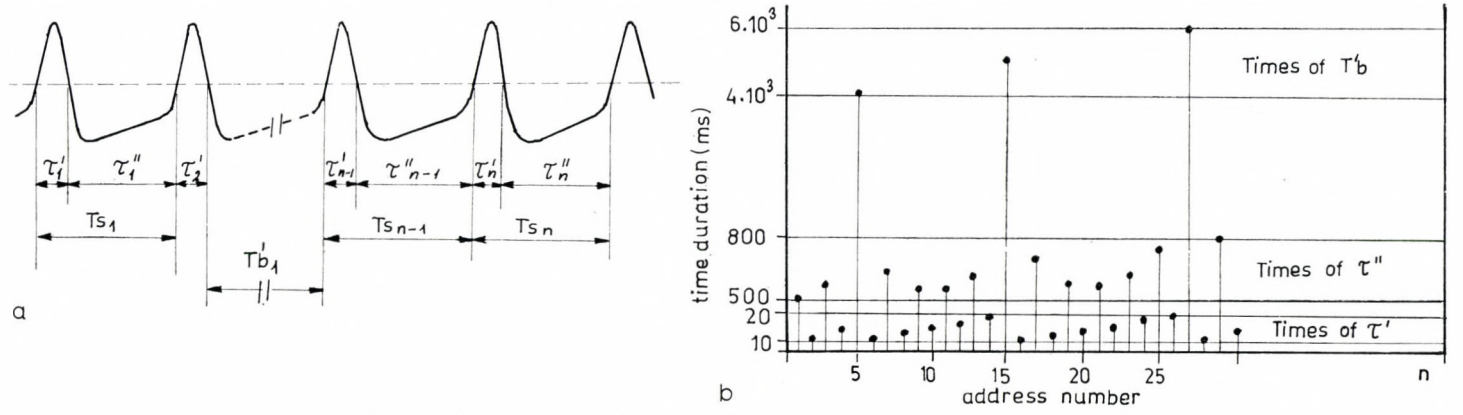

Fig. 6. a) Enlarged waveform of the action potential shown in *Fig. 1b; b)* Pulse width and interval evaluation, and the results presented by the analyzer. τ = pulse width, $\tau_n'' = T_{sn} - \tau_n'$, T_s = interspike interval ($T_{sn} = \tau_n' + \tau_n''$), T_b = interburst interval ($T_{bn} = T_{bn}' + \tau_{n-1}'$)

Summary

A multichannel analyzer classifying circuit suitable for rapid and accurate assessment of intervals between action potentials and durations of the individual potentials is presented. A detailed description of the circuit used for generating the analyzer trigger signal from the analog signal and the modifications carried out on the applied 1024-channel analyzer type NTA-512B are given. A display is presented showing the interval and width evaluation of a nerve cell activity sample.

REFERENCES

BULLOCK, T. H., HORRIDGE, G. A. (1965): Structure and function in the nervous systems of invertebrates. — *Vol.* **1,**, W. H. FREEMAN and Co., *San Francisco, California.*

CARPENTER, D. O. (1967): Temperature effects on pacemaker generation, membrane potential and critical firing threshold in *Aplysia* neurons. — *J. Gen. Physiol.* **50,** 1469—1484.

DAKIN, C. J., COOKE, C. E. G. (1968): Circuits for digital equipment. — *Illife Books Ltd., London* 66—77.

D'ALTON, L. G., RYAN, P. J. (1972): Practical technic for measuring probability density. — *El. Eng.* **44,** 54—56.

MALVINO, A. P., LEACH, D. P. (1967): Digital principles and applications. — *McGraw-Hill Book Co., New York,* 11—20.

NTA-512B Multichannel Analyzer System. — *Instruction Manual, Central Res. Inst. for Physics, Budapest.*

S.-RÓZSA, K., SALÁNKI, J. (1973): Single neurone responses to tactile stimulation of the heart in the snail, *Helix pomatia* L. — *J. Comp. Physiol.* (in press)

STARR, A. T. (1953): Radio and Radar Technique. — *Pitman, London,* 544—548.

SALÁNKI, J., VADÁSZ, I., VÉRÓ, M. (1973): Temperature dependence of the activity pattern in the Br-type cell of the snail *Helix pomatia* L. — *Acta phys. Acad. Sci. hung.* (in press)

VÉRÓ, M. (1971): Negative capacitance amplifier for microelectrode investigations. — *Annal. Biol. Tihany* **38,** 107—115.

ÁRAMKÖR SOKCSATORNÁS ANALIZÁTORHOZ AKCIÓSPOTENCIÁLOK INTERVALLUMAINAK ÉS SZÉLESSÉGEINEK ÉRTÉKELÉSÉRE

Véró Mihály

Összefoglalás

A szerző idegsejtek aktivitás mintáinak analizátoros feldolgozásához alkalmas áramkört ismertet, amely lehetővé teszi az elvezetett akcióspotenciálok közötti intervallumok, és az egyes potenciálok szélességének pontos és gyors kiértékelését. A cikk részletesen ismerteti azt az áramköri megoldást, amely az analóg jelből az analizátor indításához szükséges jelet előállítja, és ugyancsak ismerteti az alkalmazott NTA-512B típusú 1024 csatornás analizátor szükséges módosításait. Végezetül példa szemléleteti, a kiértékelésnél kapott eredmények analóg display-en történő adatábrázolását.

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THE DISTRIBUTION OF CYTOSOMES IN THE NON NERVOUS TISSUES OF *ANODONTA CYGNEA* L. (MOLLUSCA, PELECYPODA)

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A great number of yellow pigment granules called cytosomes by NOLTE et al. (1965) occur in the nerve cells of many species of bivalves and snails. Although these granules display some similarity to the lysosomes suggested even by the name cytosome (DEDUVE and WATTIAUX, 1966) functions differing from that of the lysosomes should also be attributed to them. Namely, activities of certain respiratory enzymes could be detected in the cytosomes of the nerve cells of *Anodonta cygnea* (ZS.-NAGY, 1967; ZS.-NAGY and KERPEL-FRONIUS, 1970a). Furthermore, it has been proved that the cytosomes possess an energy production of oxidative character even under anoxic conditions (ZS.-NAGY and KERPEL-FRONIUS, 1970b; ZS.-NAGY, 1971a; KERPEL-FRONIUS and ZS.-NAGY, 1973).

Considering the results mentioned above, the necessity emerged to investigate the occurrence and distribution of cytosomes in the extraneuronal tissues in order to decide the question whether the results obtained on the nervous tissue can be generalized. The present work was aimed at investigating this problem.

Material and methods

The investigations were carried out on the adult specimens of 14—18 cm body length of *Anodonta cygnea* L. Only completely intact, normal animals were used which had been kept durably in aquaria supplied with the water of Lake Balaton and sufficient areation. The following organs were investigated: oral palps; siphon; mantle; heart ventricle; central part of the intestine; gonads; kidney (Bojanus organ); statocyst; epithelium, musculature and excretory glands of the foot; hepatopancreas; adductor muscles and osphradium. The investigations were made in the months of May and June.

Parts of the above organs were fixed in 2.5 percent glutaraldehyde diluted from a 25 percent stock solution by tap water, at 4° C for 16 hr, then washed and postfixed in osmic acid for 30 min (2 percent OsO_4 buffered with 0.1 M s-collidine) at 0 °C and 10 min at room temperature. Dehydration took place by ethanol and propylene oxide, then the blocks were embedded into araldite. In other cases only a simple osmium fixation was performed

just as described above. The sections were cut on LKB Ultrotome III, contrasted by uranyl acetate and lead citrate. Micrographs were taken on a TESLA BS 413A electron microscope.

Results

The ultrastructure of cytosomes found in the organs investigated is identical with that of the neuronal cytosomes (Zs.-Nagy, 1967; 1968; Zs.-Nagy and Borovyagin, 1972), therefore the main characteristics of the cytosomes will only be described briefly.

The cytosomes are composed of three basic components: *a)* membranes, *b)* fine granulated matrix and *c)* lipid droplets of various, mainly high density.

a) Two types of membranes can be distinguished: the outer limiting membrane and the internal ones. The limiting membrane is of about 80—85 Å thickness and displays a regular unit membrane structure. It is of somewhat higher density than the internal membranes. The latter are embedded in the fine granulated matrix inside the cytosomes. In case of double fixation they often occur in the form of five-layered complexes of membranes showing a thickness of 135—140 Å. In case of simple osmium fixation even the internal membranes are mainly of unit membrane composition. Membranes are always present as a rule at the periphery of the cytosomes and one can often observe a direct continuation of the membranes into the lipid droplets.

b) The fine granulated matrix is of moderate electron density. It consists of granules much smaller than the ribosomes, filling out the space between the membranes and lipid droplets within the cytosomes. It is composed probably of substances of protein nature.

c) The lipid droplets represent always a considerable mass in the normal cytosomes. Their density is not uniform, it is very high at some places corresponding most probably to phospholipids. Elsewhere there is a lower density which may indicate the presence of neutral lipids.

The components related above may form extremely variable structures. More or less cytosomes are present in all basic types of tissues. Their size varies between 1—5 μ.

Most of the cytosomes were seen in the intestinal and kidney epithelium among the epithelial tissues investigated. There is a single ciliated cylindric epithelium in the intestine. The apical part of this epithelium is occupied by numerous ciliary rootlets and the cytosomes are localized in the middle zone of it. These cytosomes are as a rule of irregular or elongated shape *(Fig. 1)*. It is especially characteristic for the cytosomes of the intestinal epithelium that the matrix is rather poor and instead of the matrix, one can find many small vesicles of various content *(Fig. 1)*. The cytosomes of the kidney epithelium are of similar character, however, they are more abundant in membranes *(Fig. 2)*. Other epithelial tissues also contain cytosomes, although they occur less frequently elsewhere. There are cytosomes in the epithelium of the statocyst, the siphon, some channels of gonads, the foot and the mantle. The cells of the foot glands and the hepatopancreas also contain cytosomes. The cytosomes of the hepatopancreas are poor in internal membranes and are filled in with a mainly granulated, apparently homogeneous substance *(Fig. 3)*.

The connective tissue of molluscs is rather poor in cells, it consists of mainly atypic, thin collagen fibres. The cells found in the connective tissue

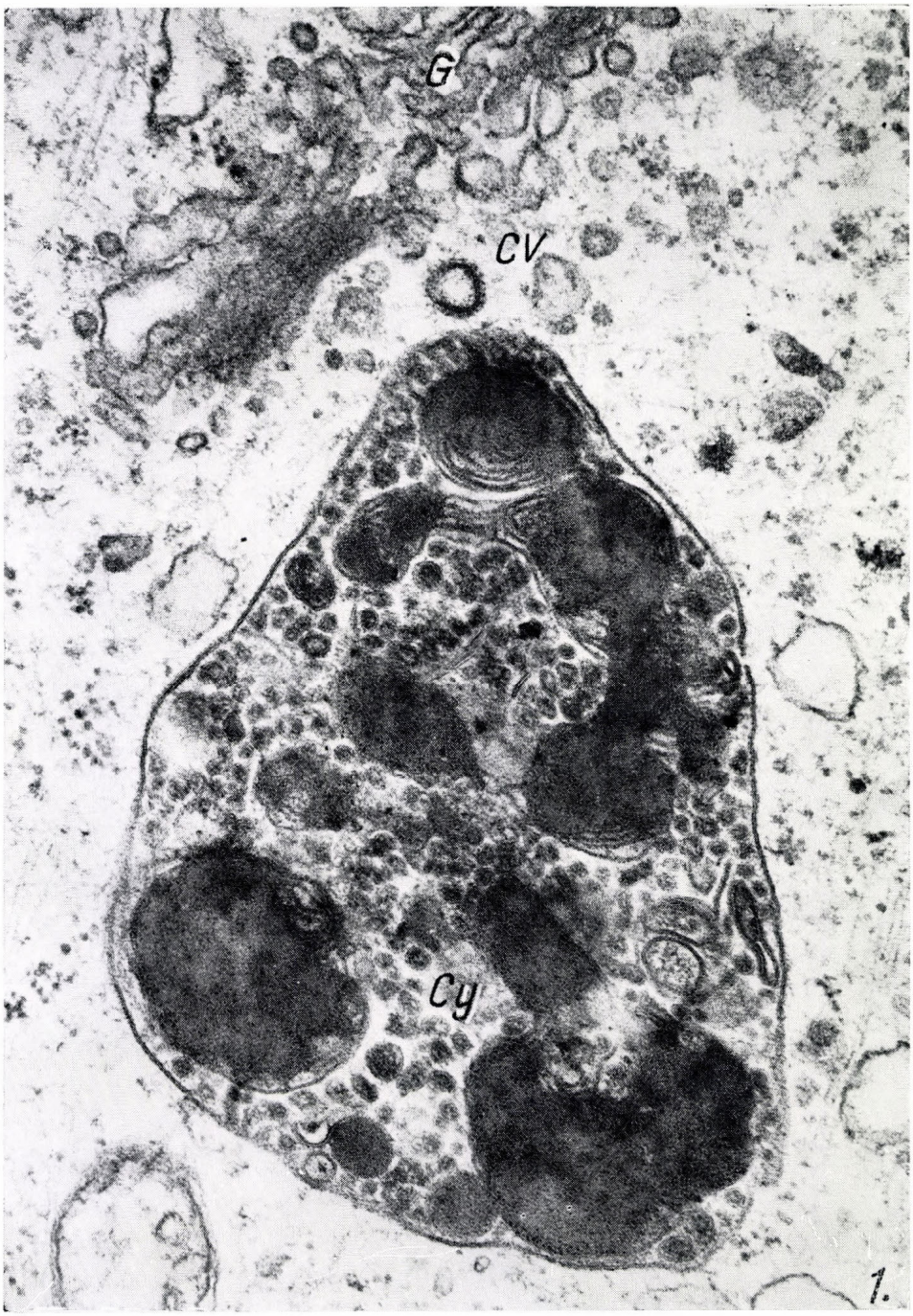

Fig. 1. Detail of an epithelial cell from the central part of the intestine. G — Golgi; Cy — cytosome; CV — coated vesicle; ×58 500

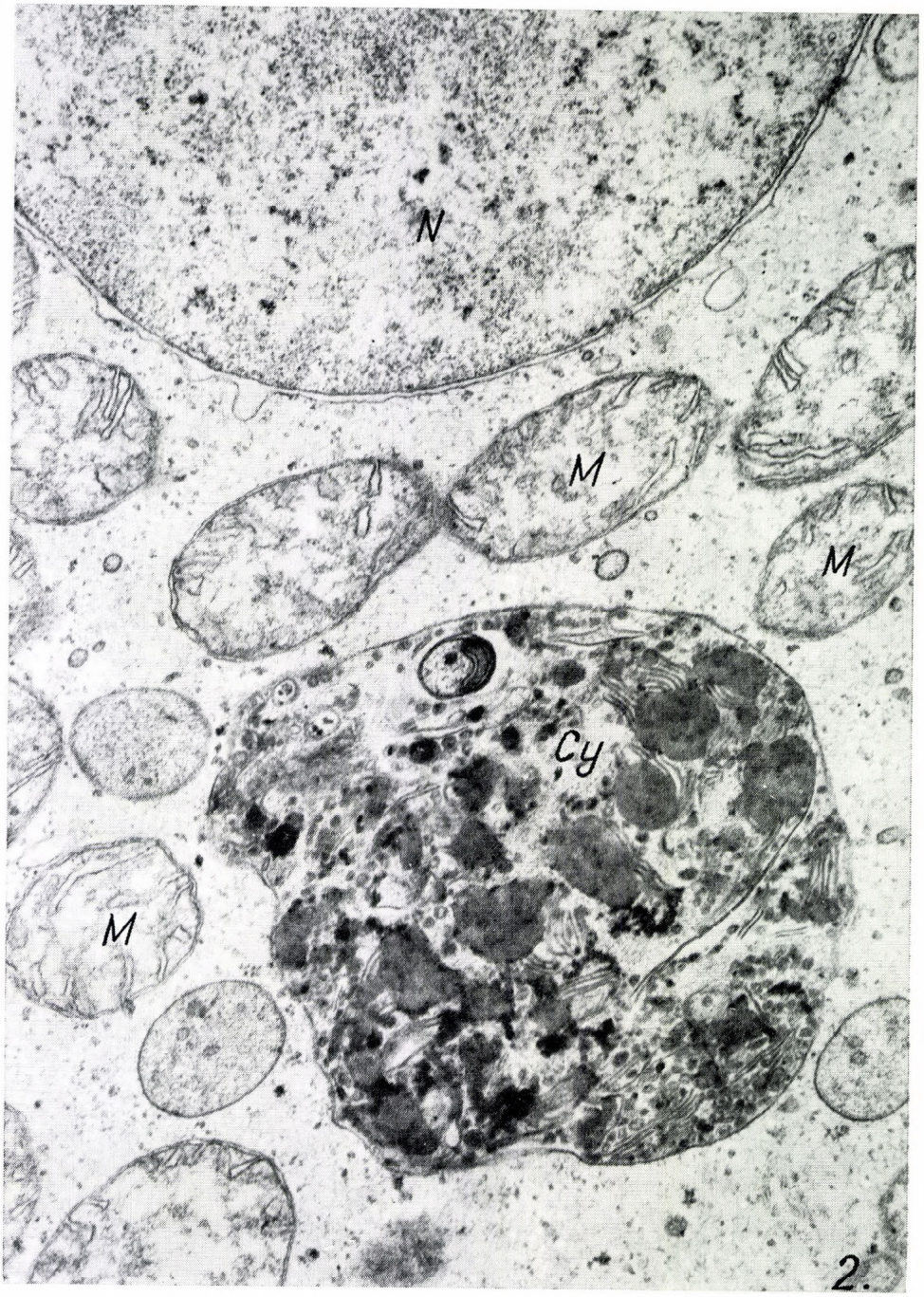

Fig. 2. Detail of an epithelial cell from the kidney. N — nucleus; M — mitochondrium; Cy — cytosome. ×30 000

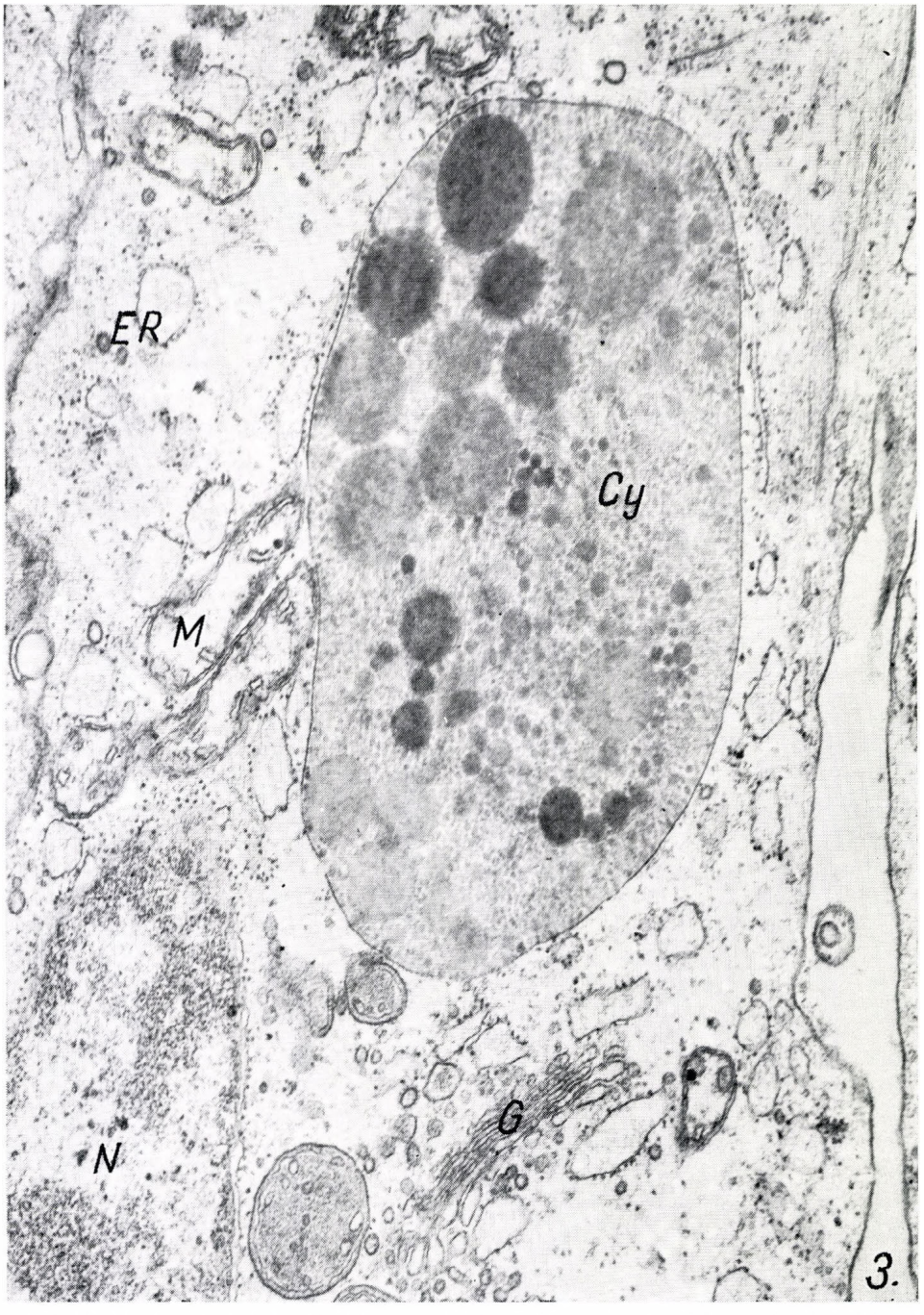

Fig. 3. Detail of a cell from the hepatopancreas. N — nucleus; M — mitochondrium; Cy — cytosome; G — Golgi; ER — endoplasmic reticulum. ×30 000

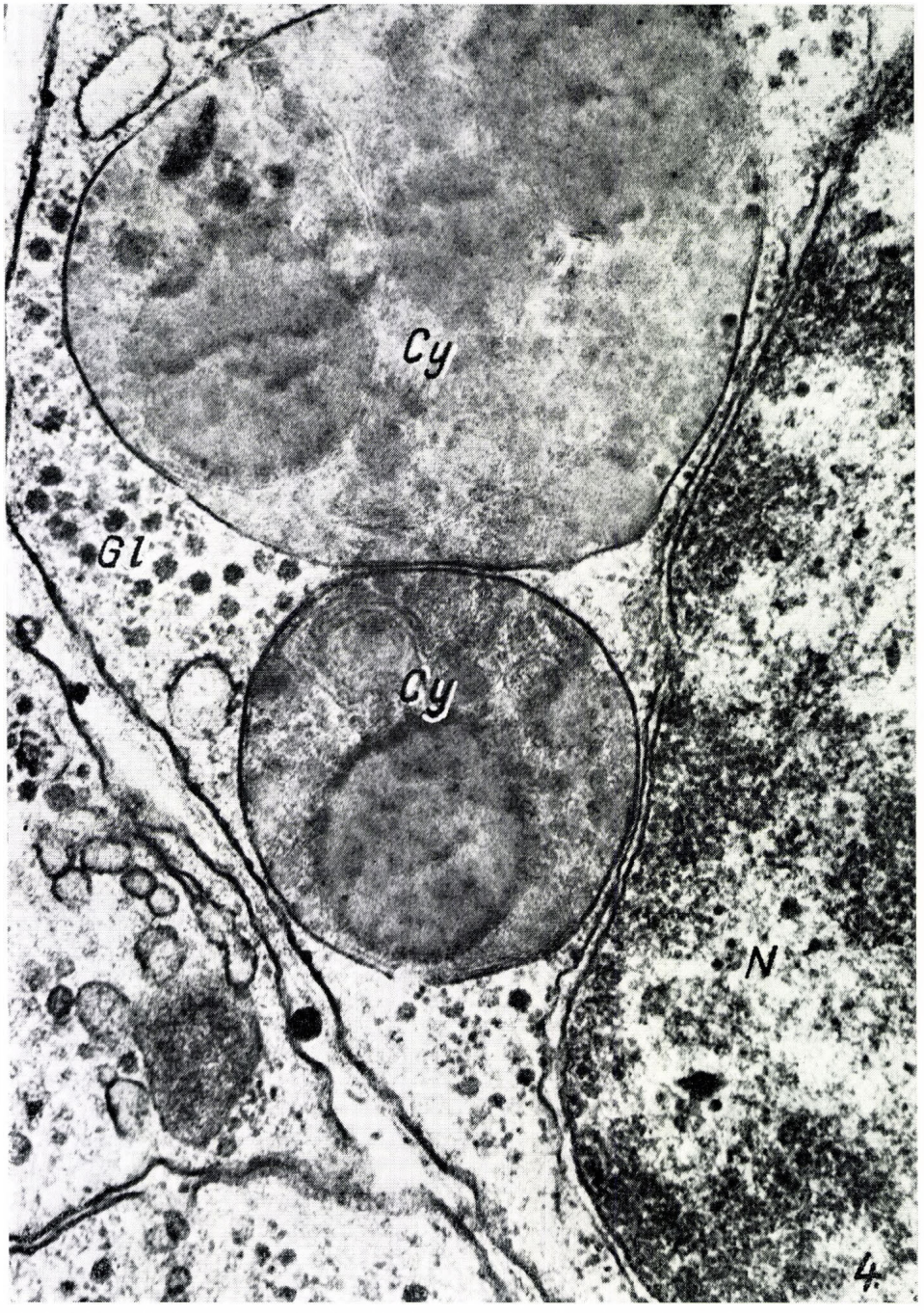

Fig. 4. Detail of an interstitial connective tissue cell of the heart musculature. N — nucleus; Gl — glycogen; Cy — cytosome. ×58 500

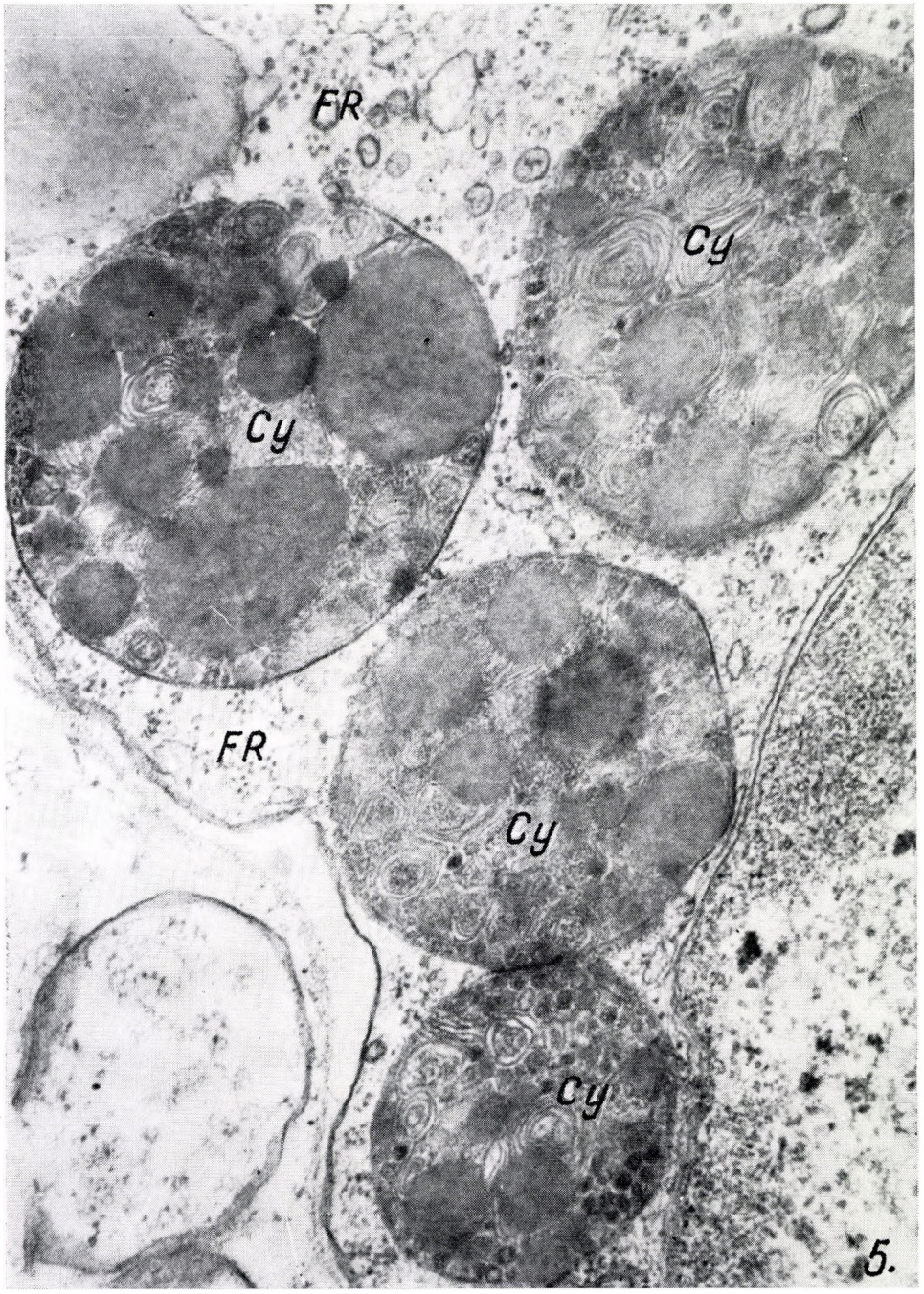

Fig. 5. Detail of a fibrocyte from the subepithelial connective tissue of the foot. N — nucleus; Cy — cytosome; FR — free ribosomes. ×48 500

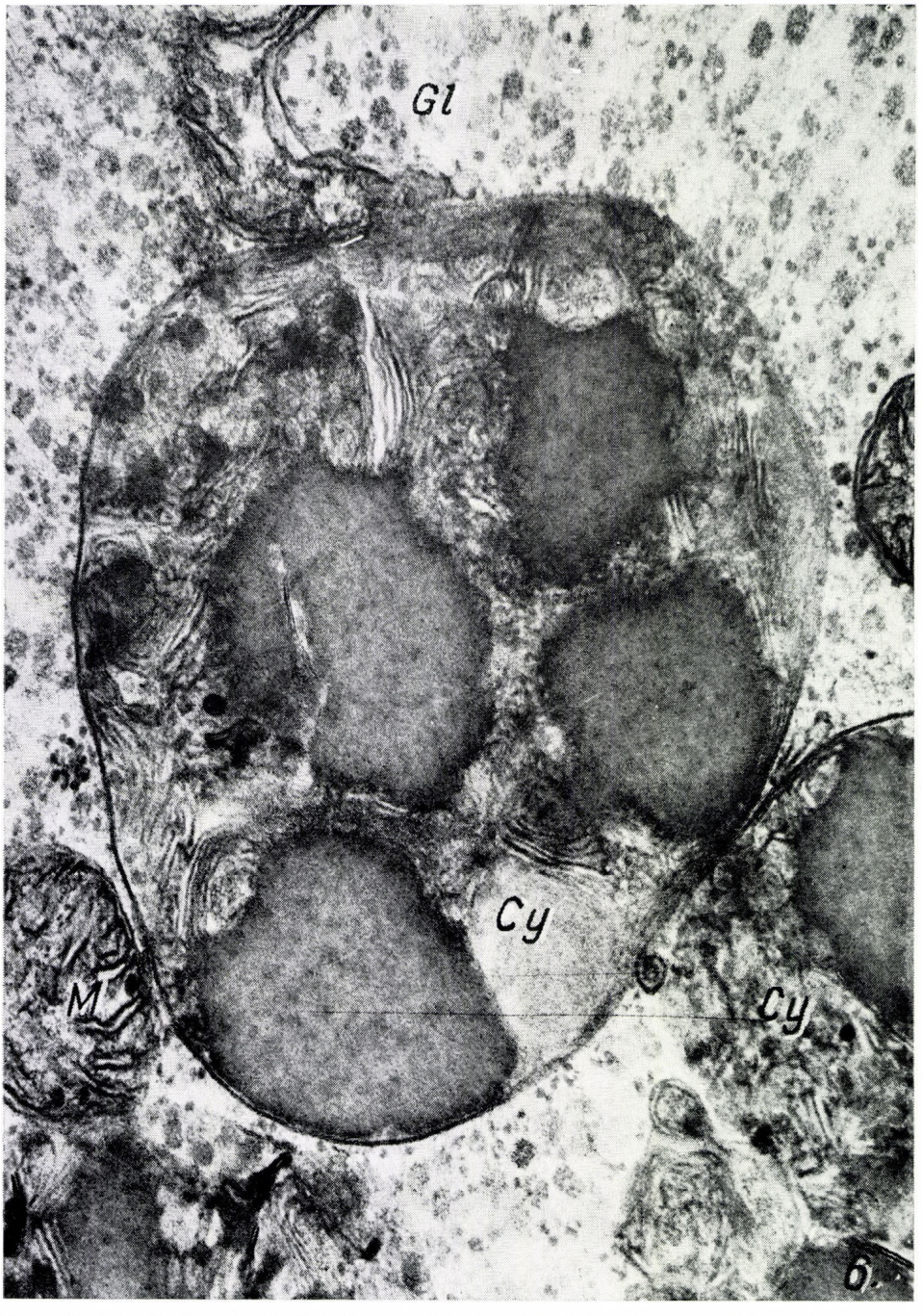

Fig. 6. Detail of a heart muscle cell from the ventricle. Gl — glycogen; M — Mitochondrium; Cy — cytosome. ×58 500

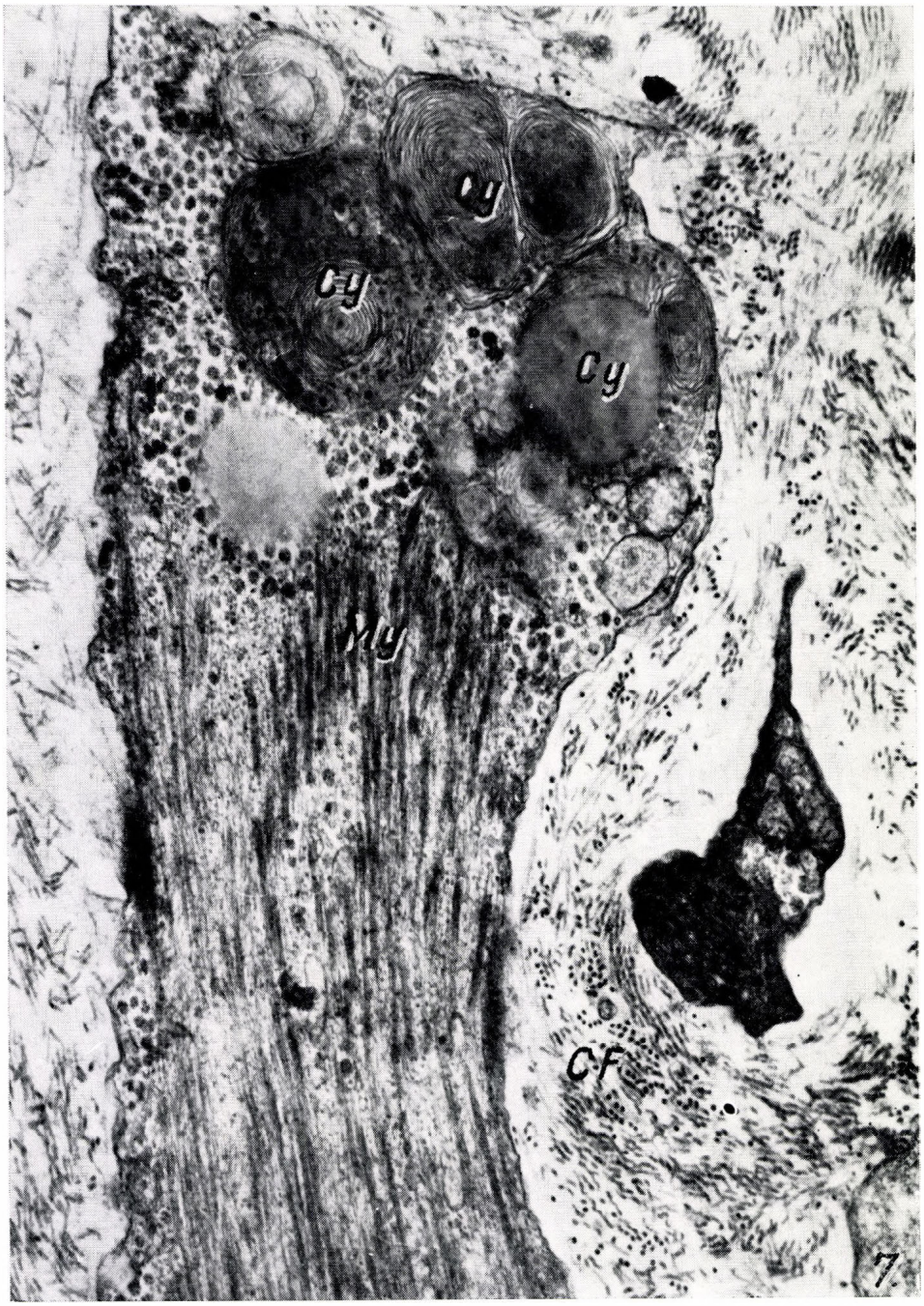

Fig. 7. Detail of the subepithelial layer of the oral palp containing collagen and scattered muscle cells. My — myofilaments in the muscle cell; Cy — cytosomes; Gl — glycogen; CF — collagen fibres. ×30 000

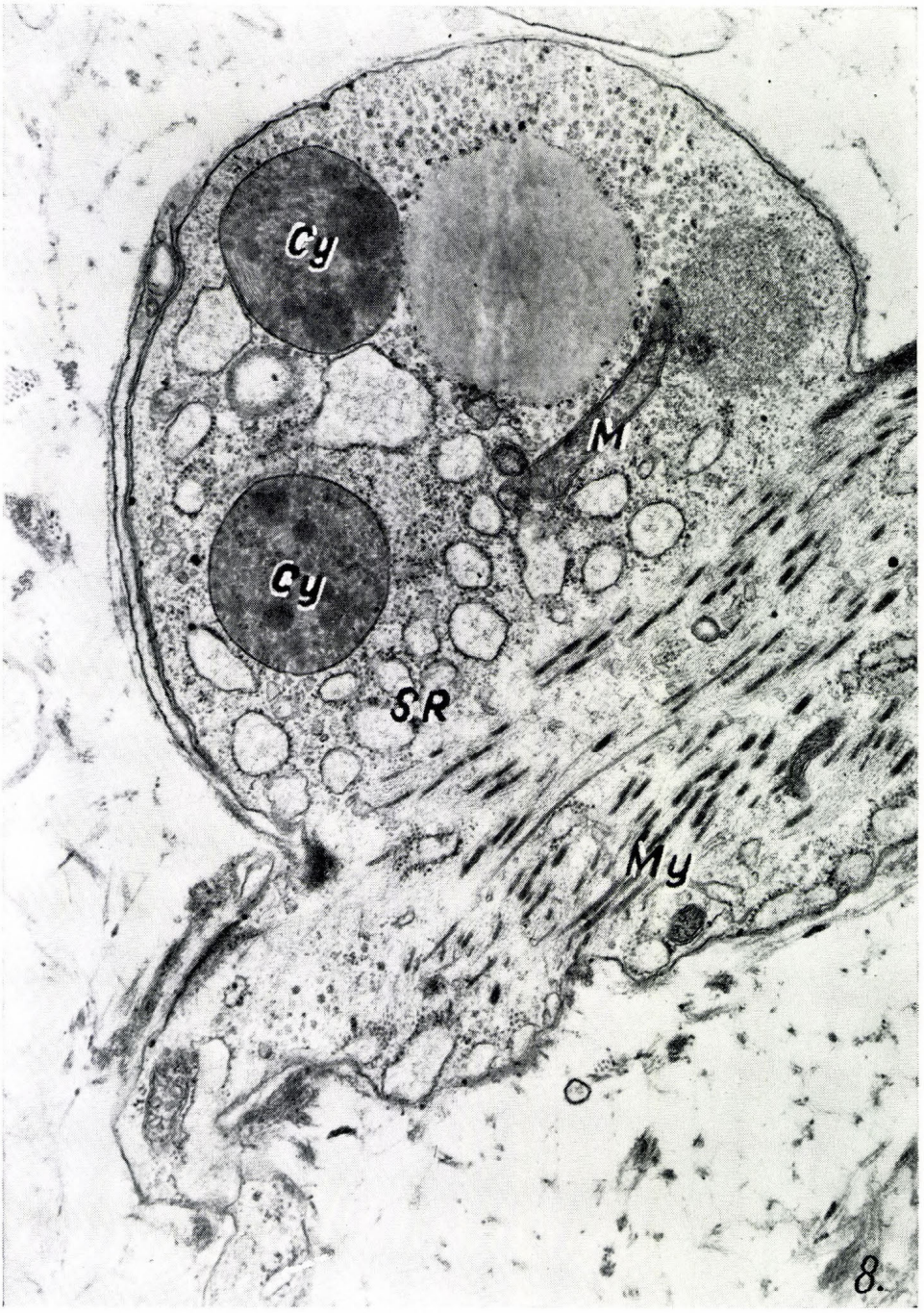

Fig. 8. Detail of a muscle cell from the intestinal wall. My — myofilaments; SR — sarcoplasmic reticulum; Cy — cytosome; M — mitochondrium. ×25 000

contained cytosomes in every organ investigated. The cytosomes of fibrocyte-like cells are presented from the heart muscle interstitium *(Fig. 4)* and from the subepithelial connective tissue of the foot *(Fig. 5)*.

Among the muscle tissues, the largest number of cytosomes were found in the heart ventricle. In the heart muscle and every other muscle, the cytosomes appear in groups either in the vicinity of the nucleus or in other regions, mixed with other sarcoplasmatic elements and numerous glycogen granules *(Fig. 6)*. The contractile elements are missing at these places. Cytosomes often occur in the muscle cells of smooth character of the oral palp *(Fig. 7)*, the intestinal wall *(Fig. 8)*, the mantle, the siphon and the foot. At the same time, the adductor muscles contain only very few cytosomes, and in relatively large areas cytosomes could not be observed at all.

Discussion

Our investigations show unanimously that the cytosomes generally occur in many tissues of *Anodonta cygnea*. Apart from some variability, they can be regarded as cell particles of uniform composition, therefore we have a sufficient basis to consider them as cell organelles, like the mitochondria or other ones.

Nevertheless, the cytosomes cannot be classified into any known categories of cell organelles. They show some functional relations to the lysosomes, since certain cytosomes displayed acid phosphatase activity under certain circumstances (Meek and Lane, 1964; Lane, 1966; Zs.-Nagy and Borovyagin, 1972). At the same time, it should be noted that the morphological appearance as well as the high lipid content of cytosomes strongly differs from those of usual lysosomes (DeDuve and Wattiaux, 1966). Similarly, one has to interpret as a difference that respiratory enzymes have not been described in the lysosomes (Gahan, 1967). It is a question what can be the function of the acid phosphatase in the cytosomes the activation of which was observed even by us during anoxia (Zs.-Nagy and Borovyagin, 1972). Since this enzyme is known generally as a lysosomal marker, it seems to be reliable that lytic processes also take place during certain stages of cytosomal development, however, the total cytosomal function is more than the lytic activity of the common lysosomes.

The frequency of cytosomes is undoubtedly the highest in the nerve cells, however, they can be found practically in all basic types of tissues. Their functional significance is indicated by the observation (Zs.-Nagy, 1971a) that the molluscan species the nervous systems of which contain no cytosomes, are not able to survive anoxic conditions at all, whereas those having pigmented ganglion cells display a significant anoxic tolerance being more or less proportional to the degree of pigmentation.

The practically ubiquitous occurrence of cytosomes in the tissues of *Anodonta cygnea* admits a generalization. Namely, the respiratory enzyme activities observed in the cytosomes of the nerve cells, as well as the cytosomal, anoxic, energy dependent Sr^{++}-accumulation (Zs.-Nagy, 1967; 1971a; Zs.-Nagy, and Kerpel-Fronius, 1970a; 1970b; Kerpel-Fronius and Zs.-Nagy, 1973) may take part in the anoxic metabolic processes even in the non nervous tissues. On the other hand, the frequency of occurrence of cyto-

somes just indicates that the highest number of cytosomes are present in the nervous system and the heart musculature representing the energetically most exigent tissues, whereas less of them occur in the other types of tissues.

The almost complete absence of cytosomes in the adductor muscles may be connected with the fact that the tonic contraction of the adductors is brought about practically without fatigue, without energy expenditure. This phenomenon has been related to the increased crystallization of paramyosin (see for ref. Zs.-Nagy and Salánki, 1973). The low number of mitochondria in the adductors may have the same explanation. The main source of energy of the adductors for the phasic contractions is most probably the anaerobic glycolysis or glycogenolysis taking place without organelles in the cytosol. In the elimination of the glycolytic products perhaps other organs take part, connected with the adductors by the circulation of the hemolymph.

After all, our investigations offered a sufficient morphological basis for the amplification of the mechanism called "anoxic endogenous oxidation" having been attributed so far to the neuronal cytosomes (Zs.-Nagy and Ermini, 1972b; Zs.-Nagy, 1971a; 1973) to the total body of the animals.

On the basis of cytosomal properties mentioned above, one can assume an analogy between them and the polymelanosomes of the higher animals (Van Woert et al., 1967). The polymelanosomes have mostly been investigated in the liver of amphibia. They consist of melanin granules and a matrix surrounded by a membrane and possess also internal membranes. Mitochondrial enzymes have been detected in them and they accumulate Mn^{++}-ions similarly as the mitochondria (Van Woert et al., 1967; Prasad et al., 1965). Because of its semiquinon free radicals, the melanin is known as an extremely good electron acceptor (Pullman and Pullman, 1961). The polymelanosomes of amphibia showed no phosphorylation in isolated state in the presence of oxygen (Van Woert et al., 1967), whereas the polymelanosomes obtained from mouse melanomas phosphorylated as intensely as the well prepared mitochondria (Dorner and Reich, 1961). The cytosomes of molluscs differ from the polymelanosomes in so far as melanin could not be detected in them by means of the Lillie's method (Pearse, 1964) (Own, unpublished observation). Nevertheless, the cytosomes also contain some kind of internal electron acceptor (Zs.-Nagy, 1971b) belonging to the group of lipochrome pigments, being hence soluble in organic solvents such as ethanol and propanol (Zs.-Nagy and Ermini, 1972a). Apart from this difference, there exists an essential morphological and functional resemblance between the cytosomes and polymelanosomes.

Summary

The following organs of *Anodonta cygnea* were investigated: oral palps; siphon; mantle; heart ventricle; central part of the intestine; gonads; kidney (Bojanus organ); statocyst; epithelium, musculature and excretory glands of the foot; hepatopancreas; adductor muscles and osphradium. It has been established that all basic types of tissues contain more or less cytosomes the structural composition of which is principially identical with those having been described and studied in detail within the nervous system. The cytosomes should be considered as generally occurring cell organelles in the tissues of

Anodonta cygnea. A role is attributed to the cytosomes in the processes of anoxic energy production. The cytosomes show morphological and functional resemblance in many respects to the polymelanosomes of higher animals, although they contain no melanin but a lipochrome pigment having electron acceptor character.

REFERENCES

DORNER, M., REICH, E. (1961): Oxidative phosphorylation and some related phenomena in pigment granules of mouse melanomas. — *Biochim. Biophys. Acta* **48,** 534—546.

DEDUVE, C., WATTIAUX, R. (1966): Functions of lysosomes. — *Ann. Rev. Physiol.* **28,** 435—492.

GAHAN, P. B. (1967): Histochemistry of lysosomes. — *Intern. Rev. Cytol.* **21,** 1—57.

KERPEL-FRONIUS, S., ZS.-NAGY, I. (1973): Electron microscopic demonstration of energy production in molluscan neurons. — *Acta biol. Acad. Sci. hung.* (in press)

LANE, N. J. (1966): The fine-structural localization of phosphatases in neurosecretory cells within the ganglia of certain gastropod snails. — *Am. Zoologist.* **6,** 139—157.

MEEK, G. A., LANE, N. J. (1964): The ultrastructural localization of phosphatase in the neurones of the snail, *Helix aspersa*. — *J. Roy. Micr. Soc.* **82,** 193—204.

NOLTE, A., BREUCKER, H., KUHLMANN, D. (1965): Cytosomale Einschlüsse und Neurosekret im Nervengewebe von Gastropoden. — *Z. Zellforsch.* **68,** 1—27.

PEARSE, A. G. E. (1964): Histochemistry, Theoretical and Applied. — *Churchill, London.*

PRASAD, K. N., JOHNSON, H. A., COTZIAS, G. C. (1965): A cytoplasmic organelle of melanocytes. — *Nature* **205,** 525—526.

PULLMAN, A., PULLMAN, B. (1961): The band structure of melanins. — *Biochim. Biophys. Acta* **54,** 384—385.

VAN WOERT, M. H., NICHOLSON, A., COTZIAS, G. C. (1967): Mitochondrial functions of polymelanosomes. — *Comp. Biochem. Physiol.* **22,** 477—485.

ZS.-NAGY, I. (1967): Histological, histochemical and electron microscopical studies on the cytosomes of the nerve cells in *Anodonta cygnea* L. (Mollusca, Lamellibranchiata). — *Annal. Biol. Tihany* **34,** 25—39.

ZS.-NAGY, I. (1968): Fine structural analysis of the neurons of *Anodonta cygnea* L. (Pelecypoda). — *Annal. Biol. Tihany* **35,** 35—59.

ZS.-NAGY, I. (1971 a): Pigmentation and energy dependent Sr^{++}-accumulation of molluscan neurons under anaerobic conditions. — *Annal. Biol. Tihany* **38,** 117—129.

ZS.-NAGY, I. (1971 b): The lipochrome pigment of molluscan neurons as a specific electronacceptor. — *Comp. Biochem. Physiol.* **40A,** 595—602.

ZS.-NAGY, I. (1973): Carbohydrate consumption and ATP production in the tissues of *Andonta cygnea* L. (Mollusca, Pelecypoda) under normal and anoxic conditions. *Acta biochim. biophys. Acad. Sci. hung. (in press).*

ZS.-NAGY, I., BOROVYAGIN, V. L. (1972): Organization of the cytosomal membranes of molluscan neurons under normal and anaerobic conditions as revealed by electron microscopy. — *Tissue and Cell* **4,** 73—84.

ZS.-NAGY, I., ERMINI, M. (1972 a): Oxidation of $NADH_2$ by the lipochrome pigment of the tissues of the bivalve *Mytilus galloprovincialis* (Mollusca, Pelecypoda). — *Comp. Biochem. Physiol.* **43B,** 39—46.

ZS.-NAGY, I., ERMINI, M. (1972 b): ATP production in the tissues of the bivalve *Mytilus galloprovincialis* (Pelecypoda) under normal and anoxic conditions. — *Comp. Biochem. Physiol.* **43B,** 593—600.

ZS.-NAGY, I., KERPEL-FRONIUS, S. (1970 a): The ultrastructural localization of succinic dehydrogenase activity in the nervous system of *Anodonta cygnea* L. (Mollusca, Pelecypoda). — *Acta biol. Acad. Sci. hung.* **21,** 105—113.

ZS.-NAGY, I., KERPEL-FRONIUS, S. (1970 b): Electron microscopic histochemical investigations on the energy production in the neurones of Pelecypoda (Mollusca). — *Septième Congrès International de Microscopie Electronique, Grenoble, Vol.* **3**. pp. 155—156.

ZS.-NAGY, I., SALÁNKI, J. (1973): Polarization and electron microscopic alterations in the white and yellow adductors of *Anodonta cygnea* L. in different phases of periodic activity. — In: *Neurobiology of Invertebrates. Mechanisms of rhythm regulation.* (Ed.: J. SALÁNKI) *Akadémiai Kiadó, Budapest,* pp. 327—337.

CITOSZÓMÁK EXTRANEURONÁLIS ELŐFORDULÁSA AZ *ANODONTA CYGNEA* L. (MOLLUSCA, PELECYPODA) KÜLÖNBÖZŐ SZÖVETEIBEN

Zs.-Nagy Imre

Összefoglalás

Vizsgáltuk az *Anodonta cygnea* következő szerveit: szájvitorla, szifótájék, köpeny, szívkamra, bélcsatorna középső szakasza, ivarmirigyek, Bojanus-szerv (vese), statocysta, láb fedőhámja, izomzata és mirigyei, hepatopancreas, záróizmok, valamint az osphrádium. Megállapítást nyert, hogy valamennyi alapszövetféleség tartalmaz többkevesebb citoszómát, amelyek szerkezeti felépítése elvileg azonos a korábban az idegrendszerben leírt és részletesen tanulmányozott citoszómákéval. A citoszómákat általánosan előforduló sejtorganellumnak kell tekintenünk az *Anodonta cygnea* szöveteiben, amelyeknek szerepet tulajdonítunk az anoxiás energiatermelő folyamatok véghezvitelében. A citoszómák számos morfológiai és funkcionális vonatkozásban hasonlóságot mutatnak a magasabbrendűek polymelanosomáihoz, jóllehet bennük nem melanintípusú, hanem lipochrom természetű elektronakceptor pigmentanyag fordul elő.

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NEMATODES OF LAKE BALATON. IV. SEASONAL QUALITATIVE AND QUANTITATIVE CHANGES

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Nematodes are difficult to study. Only a few researches work on them in our country although these animals gain an increasing significance today. The members of this group counting several ten-thousands of species are partly human parasites, partly animal and plant parasites causing severe damages, while another part they are free-living and contribute to the mineralization of organic substances. Their individual number can be very high, e.g. in 1 hectare of forest soil it may reach 70 thousand millions per year, their total weight amounts to 90 kg, although the majority of those species weigh a mere few tenths of microgram. They play a very important role in the sediments of clean waters, in the purification of sewage-waters, e.g. in the dropping bodies and even in the life of the waste-stabilization ponds.

The nematodes living in the sediment of Lake Balaton have been studied by DADAY (1897), BIRÓ (1968; 1969; 1972), BIRÓ et al. (1968), PONYI et al. (1971), whereas those living in the coating of reed have been described by MESCHKAT (1934).

The "Research of Balaton" program of the Biological Research Institute of Tihany gave an opportunity for the present series of investigation. The work was intended at establishing the quantitative seasonal changes of Nematoda fauna.

Places of samplings, material and methods

Samplings were carried out from three points of each five transversal sections of Lake Balaton once a month *(Fig. 1)* from April till November during 1966–1968, as well as from under the ice in January and February from the point of sampling in front of Tihany (point "A_0") in 1968. The places of sampling were as follows:

"M" section: between Gyenesdiás and the mouth of river Zala.
"K" section: between Szigliget and Balatonmária.
"G" section: between Balatonakali and Balatonszemes.
"A" section: between Balatonfüred and Zamárdi (Tihany).
"E" section: Balatonalmádi–Balatonvilágos line.

Some data concerning the collections are summarized in *Table I*. The temperature data refer to the water. Samples from the soft bottom were collected by the modified Craib's bottom-dredge (PONYI et al., 1967) from under

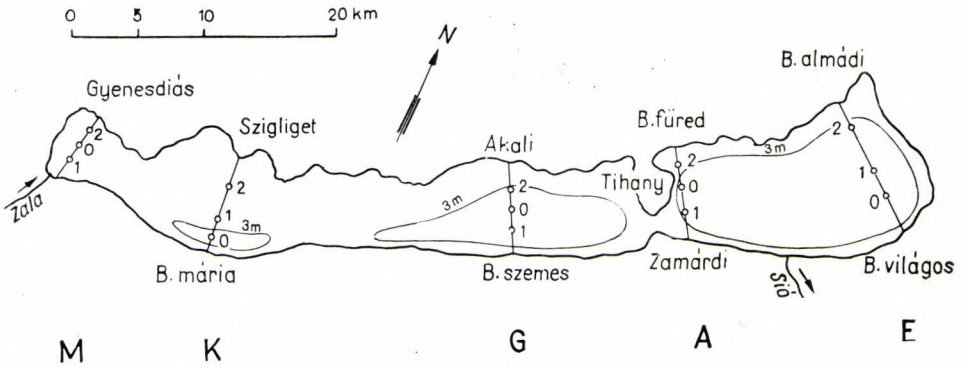

Fig. 1. The collecting places. "M", "K", "G", "A" and "E" indicate the sections, 0, 1 and 2 the points of sampling. The sign 0 indicates the deepest point of the sections

TABLE I

Data of water temperatures and depths

Places of samplings Date	M_0		K_0		G_0		A_0		E_0	
	cm	°C	cm	°C	cm	°C	cm	°C	cm	°C
1966 V	275	19	368	19	428	19	368	20	480	20
VI	292	23	419	23	443	23	410	22	410	22
VII	300	19	400	20	420	20	395	21	445	22
VIII	285	22	420	22	440	23	360	22	450	22
IX	271	17	298	17	421	19	398	18	460	18
X	280	16	385	16	420	16	380	17	420	17
XI	264	4	378	6	420	6	380	6	460	6
1967 IV	276	12	375	12	423	11	372	12	450	12
V	268	19	405	19	430	19	410	19	457	19
VI	271	20	390	19	405	20	385	24	441	25
VII	268	20	370	22	410	22	359	24	457	23
VIII	244	20	367	20	410	21	370	21	432	21
IX	255	16	369	17	392	18	400	17	418	18
X	266	16	370	16	397	17	373	13	420	14
XI							353	2	354	2
1968 I							375	0.2*		
II							383	0.7*		
IV	264	14	378	14	395	13	435	11	380	12
V	269	16	368	15	388	15	380	18	428	17
VI	272	20	372	20	395	20	385	20	432	20
VII	269	21	364	21	375	21	353	21	416	21
VIII	261	20	351	20	367	20	345	20	400	20
IX	257	18	346	18	366	18	352	18	394	18
X	255	15	355	15	370	15	372	16	382	16
XI	288	8	375	8	390	8	378	7	392	8

* thickness of the ice 25 cm

water in 240—480 cm depth. Each sample was about 3 cm thick and was taken from a surface of 13 cm^2. Three parallel samples were united at each place and fixed in formalin. The separated nematodes were investigated in glycerinized preparations. The calculation of the biomass was carried out by using the method of ANDRÁSSY (1956).

Occurrence of species at different regions

About 20 thousand of individuals belonging to 31 species were collected. The frequent nematodes were found everywhere in the mud of the lake *(Paraplectonema pedunculatum* S., *Paraphanolaimus behningi* M., *Ironus tenuicaudatus* dM., *Theristus setosus* B., *Monhystera paludicola* dM.). From the species of rarer occurrence *Hemicycliophora aquatica*, *Prismatolaimus dolichurus*, *Plectus tenuis* were encountered only in the north-eastern basin, whereas *Chromadorina bercziki*, *Neochromadora izhorica* and *Punctodora dudichi* were collected only in the south-west *Monhystera stagnalis* and *Tripyla papillata* were also more frequent in the south-western basin, however, one specimen of each was found in the other basin, too.

Table II lists the species found in Lake Balaton and compared to the data of DADAY (1897). Some of the species described by the latter author proved to be synonyms as well as uncertain species, therefore, only 26 of his list of species could be identified with certainty. The present paper uses the nomenclature of ANDRÁSSY (1972).

In the fauna of Hungary and Lake Balaton, *Paraphanolaimus anisitsi* (DADAY, 1905; ANDRÁSSY, 1968) is of rare occurrence. During the recent years it was recovered from Paraguay (ANDRÁSSY, 1968), Columbia (RIEMANN, 1971) and in Europe from the Lake Leman (JUGET, 1969), and Lake Balaton (BIRÓ, 1972). Although the specimen found by JUGET was identified to be *Paraphanolaimus behningi* MIC. 1923, on the basis of his drawing and description it is *Paraphanolaimus anisitsi* (D.) A. A male *Paraphanolaimus behningi* M. was found in Lake Balaton (BIRÓ, 1968) and also in the Soviet-Union (GAGARIN, 1970). It can well be observed on this species that the spiculum is thin and its length is about 4—5 times larger than the anal width of the body, whereas in the case of *P. anisitsi* the spiculum is bulky and its length hardly reaches one and a half times the anal width of the body *(Figs. 2* and *3)*.

Paraplectonema pedunculatum (H.) S. *(Fig. 4)* occurs sporadically in Europe. However, in the open water sediment of Lake Balaton and Lake Fertő it is (SCHIEMER et al. 1969) the most frequent Nematoda. This species seems to prefer the detritus-rich mud-layers of 2—5 cm thickness under the shallow waters of pH 8.3.

The quantity of nematodes

Among the nematodes found during 1966—68, the most frequent species was *Paraplectonema pedunculatum:*

Paraplectonema pedunculatum S.	20.3%
Paraphanolaimus behningi M.	20.0%
Ironus tenuicaudatus dM.	18.9%
Theristus setosus M.	16.6%
Monhystera paludicola dM.	15.1%
Other 26 species	9.1%

TABLE II

The distribution of nematodes found in the five sections of Lake Balaton during 1966—68

	1897 Daday	1966—68					%
		M	K	G	A	E	
Achromadora terricola (dM.) M.		+		+	+		0.02
Acrobeloides emarginatus (dM.) T.	+						
Aphanolaimus aquaticus D.	+		+	+	+	+	2.13
Aporcelaimellus obtusicaudatus (B.) A.	+						
Campydora balatonica (D.) A.	+						
Chromadorina bercziki A.		+					0.04
Chromadorina bioculata (S.) W.	+						
Diplogaster rivalis (L.) B.	+						
Dorylaimus helveticus (S.) A.				+	+		0.09
Dorylaimus stagnalis D.	+	+		+	+	+	0.11
Ethmolaimus pratensis dM.			+	+	+	+	0.84
Eudorylaimus bryophilus (dM.) A.	+						
Hemicycliophpora aquatica (M.) L.					+	+	0.04
Heterocephalobus elongatus (dM.) A.	+						
Ironus colourus S.		+				+	0.02
Ironus tenuicaudatus DM.	+	+	+	+	+	+	18.94
Laimydorus flavomaculatus (L.) S.	+						
Mesodorylaimus bastiani (B.) A.	+						
Mermis sp.	+			+	+	+	0.03
Microlaimus globiceps dM.				+	+		0.02
Monhystera andrassyi B.		+	+	+	+	+	0.14
Monhystera dispar B.				+			0.01
Monhystera gerlachi M.		+					0.02
Monhystera macramphis F.		+	+	+	+	+	0.33
Monhystera paludicola dM.		+	+	+	+	+	15.10
Monhystera stagnalis B.	+	+	+	+	+	+	0.19
Monhystera vulgaris dM.			+	+			0.02
Mononchus truncatus B.	+						
Neochromadora izhorica (F.) S.		+					0.12
Paractinolaimus macrolaimus (dM.) A.	+						
Paradorylaimus filiformis (B.) A.	+						
Paraphanolaimus anisitsi (D.) A.		+	+	+	+	+	0.82
Paraphanolaimus behningi M.		+	+	+	+	+	20.06
Paraplectonema pedunculatum (H.) S.		+	+	+	+	+	20 30
Plectus cirratus B.	+						
Plectus parvus B.	+						
Plectus tenuis B.	+				+		0.01
Prismatolaimus dolichurus dM.	+				+	+	0.04
Punctodora dudichi A.		+					0.02
Punctodora ratzeburgensis (L.) F.	+			+		+	0.06
Theristus setosus (B.) M.	+	+	+	+	+	+	16.60
Tobrilus gracilis (B.) A.	+	+	+	+	+	+	3.32
Tobrilus helveticus (H.) A.			+	+	+	+	0.22
Tobrilus longus (L.) A.					+		0.04
Tobrilus pellucidus (B.) A.	+						
Tripyla glomerans B.	+	+	+	+	+	+	0.30

Note: the percentage indicates the properties as compared to the total number of individuals encountered. "M", "K", "G", "A" and "E" are the places of collecting.

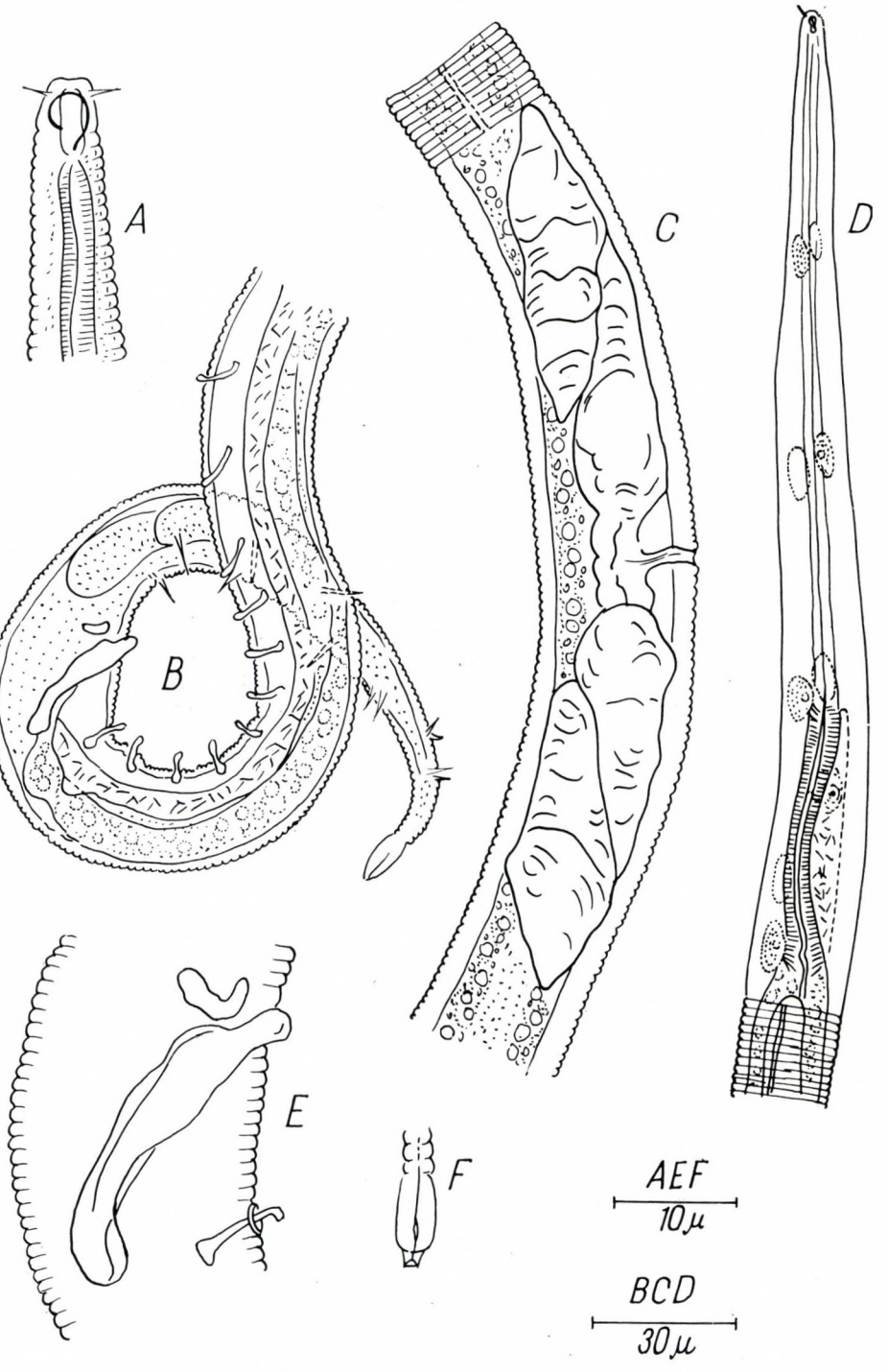

Fig. 2. Paraphanolaimus anisitsi (Daday, 1905) Andrássy, 1968. A = oral end of the animal; b = caudal end of the animal; C = female genital organs; D = oral part of the animal with the oesophagus; E = spiculum; gubernaculum and one of the preanal papillae; F = caudal end

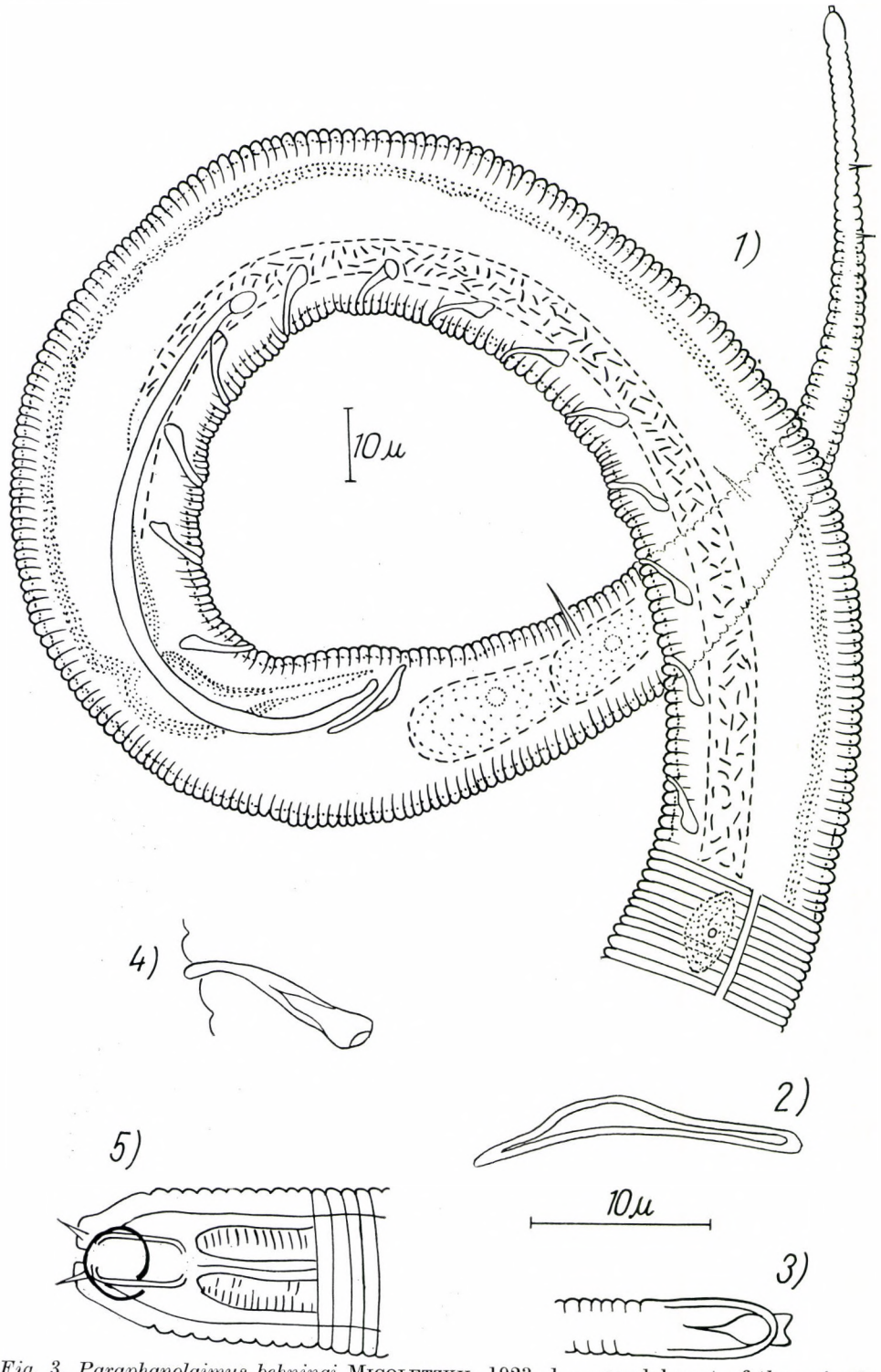

Fig. 3. Paraphanolaimus behningi Micoletzky, 1923. 1 — caudal part of the animal; 2 — gubernaculum; 3 — caudal end; 4 — preanal papilla; 5 — oral end of the animal

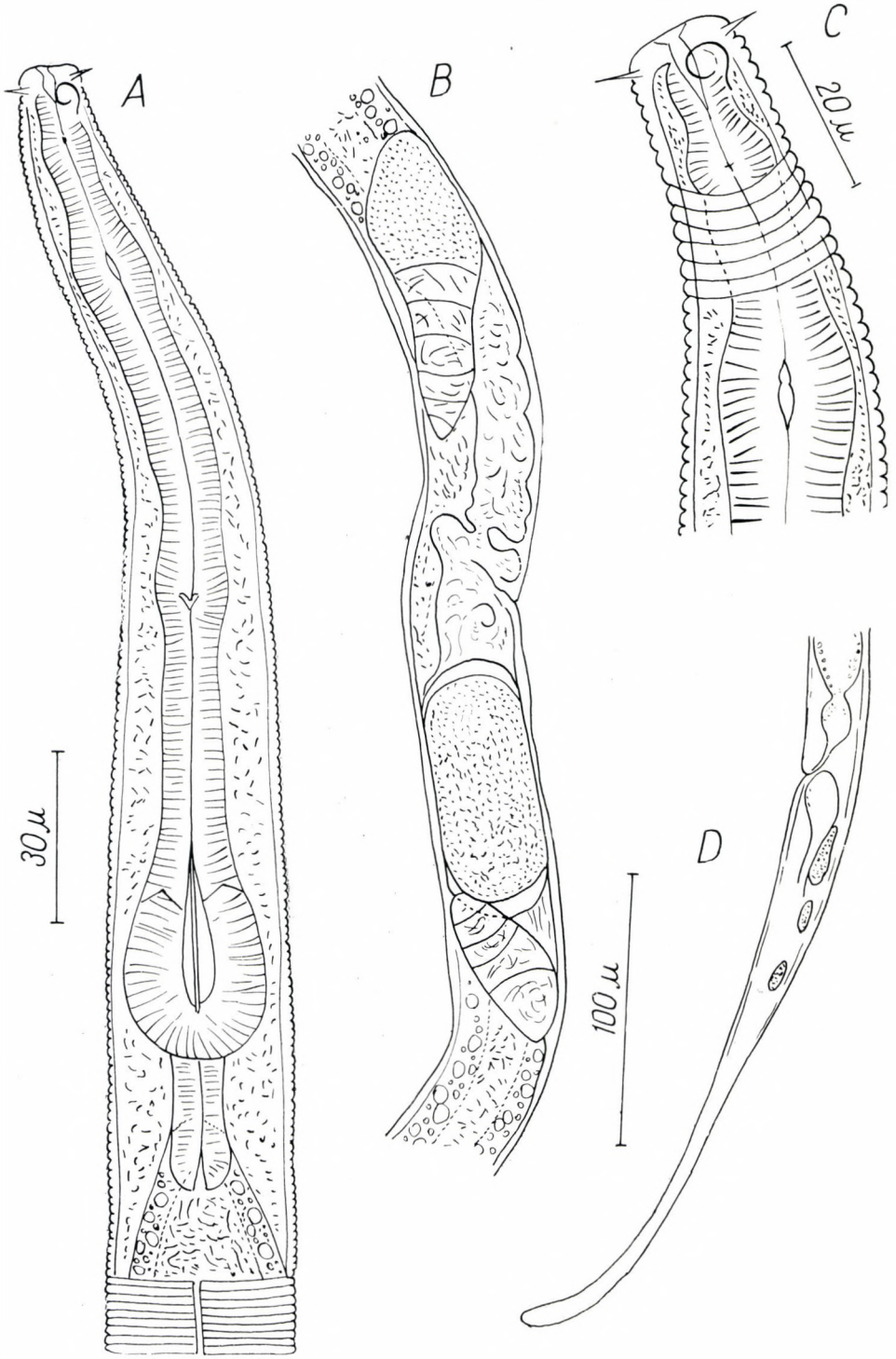

Fig. 4. Paraplectonema pedunculatum (Hofmänner, 1913) Strand, 1934. A = anterior part of the animal with the oesophagus; B = female genital organs; C = oral end of the animal; D = caudal end of the body

A seasonal change was observed in the proportions of species. When the water temperature was below 12 °C, the proportions of species were different from those of the warmer periods:

	Periods	
	"cold" below 12°C	"warm" above 12°C
Paraplectonema pedunculatum S.	13.7%	25.8%
Paraphanolaimus behningi M.	6.7%	24.2%
Ironus tenuicaudatus dM.	17.1%	19.4%
Theristus setosus M.	32.1%	11.6%
Monhystera paludicola dM.	20.4%	10.7%
Other nematodes	10.0%	8.3%

An almost identical order of magnitude of frequency was observed in cases of fine species during three years in average (20, 20, 17, 19, 15 percent). During the cold periods, *Theristus setosus* and *Monhystera paludicola* (32 and 20 percent), during summer, *Paraplectonema pedunculatum, Paraphanolaimus behningi* and *Ironus tenuicaudatus* (25, 24 and 20 percent) predominated. During the winter season under the ice, as high as 60 percent of frequency of *Theristus setosus* was observed in the section between Zamárdi and Balatonfüred ("A_0"). *Tobrilus* species could hardly be collected in other periods of the year, nevertheless under the ice they reached even 15—20 percent of the total number of individuals.

The distribution of nematodes was not uniform at different regions of Lake Balaton. Considering the monthly averages Balatonszemes—Balatonakali ("G") was the richest section and the poorest was that of the Keszthely Bay ("M" section) *(Fig. 5)*. According to the findings, the number of nematodes was lower on identical surfaces during the summer, than during the cold season i.e. in winter it amounted only to 60—75 percent of the latter. Evaluating the collections of the past three years, one can state that the lowest number of nematodes occurs in late summer (10—15 000 individuals per m^2). As against to the other places of collection, in the Keszthely Bay the lowest number of individuals was found in early summer (10 000 i/m^2), whereas by the end of summer *Paraplectonema pedunculatum* propagated so profusely that the highest number of nematodes was found there (50 000 i/m^2). The highest number of nematodes was found during the spring season at the other regions of the south-western basin ("K" and "G") as well as in the north eastern one "A" and "E"), reaching 60—80 000 i/m^2 *(Fig. 5)*.

The Keszthely-Bay is the poorest in nematodes ("M") among all the areas of the lake. The average number of individuals was 19 000 i/m^2 in 1966 and 1967, however in 1968 it reached 35 000 i/m^2. During all the three years the lowest number (10 000 i/m^2) was found in July and the highest one (40—60 000 i/m^2) in August (first column of *Fig. 5)*. The summer increase was caused by the quick propagation of *Paraplectonema pedunculatum*. Of the five species of highest frequency occurring everywhere, the highest number of *Theristus setosus* was encountered during the cold period, that *Ironus tenuicaudatus* and *Paraplectonema pedunculatum* during the warm period.

Several species were found toke absent in other regions of the lake: *Chromadorina bercziki, Monhystera gerlachi, Neochromadora izhorica, Punctodora dudichi*. The last but one is known from the delta of river Neva, i.e. from a slowly flowing water, while the first and last are known from the mud of River Danube, i.e. also from a flowing water. Their presence can perhaps be explained by the vicinity and effect of the river Zala.

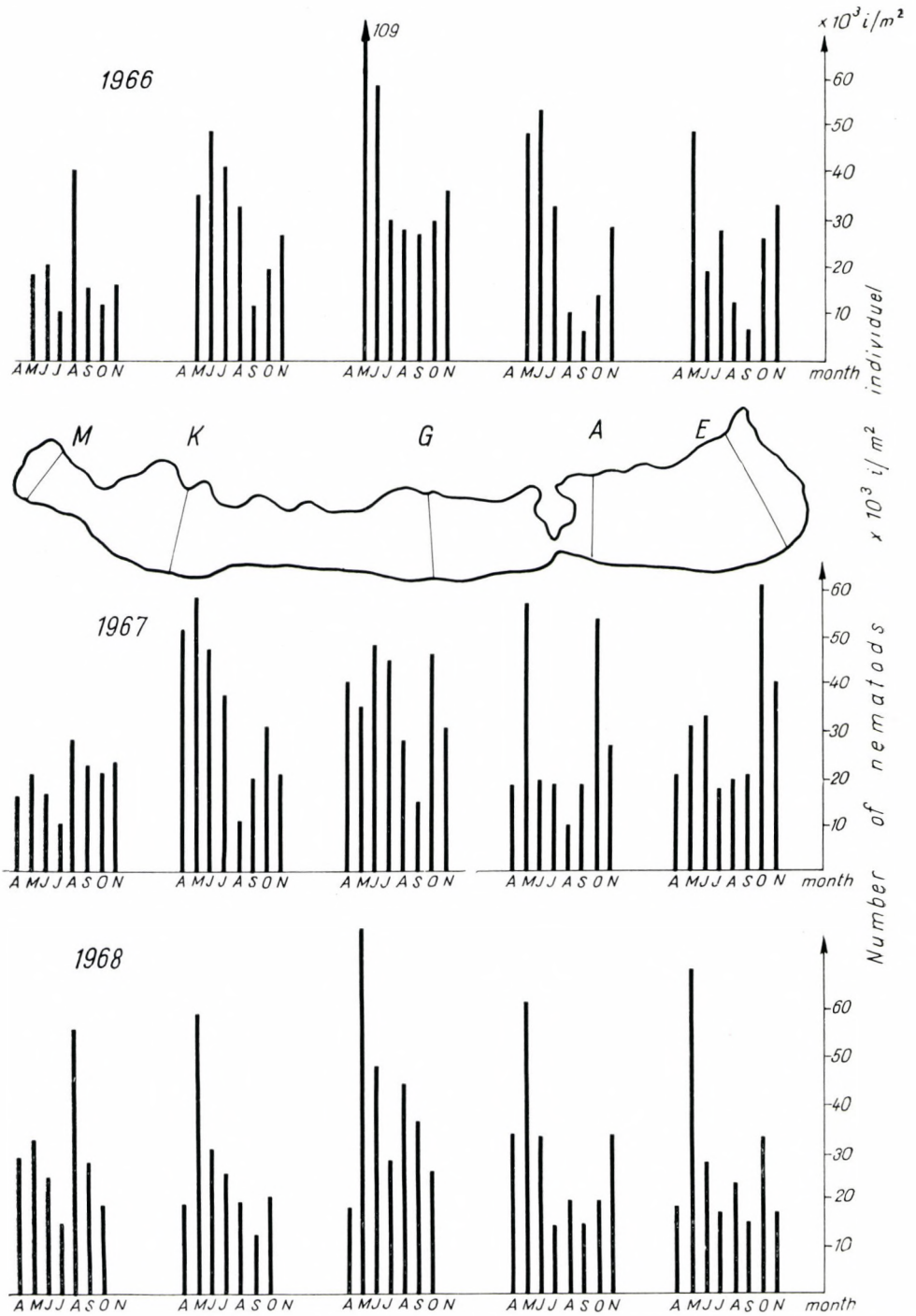

Fig. 5. The number of nematodes in the five transversal sections of Lake Balaton during 1966—68 in number of individuals per m² (i/m²). Each date represents the average of three points (e.g. M_1M_a and M_2) of each section

In the area of Szigliget Bay ("K") the average number of nematodes was 30—40 000 i/m² during all the three years. The number of individuals was the highest in early summer (55—60 000 i/m²), and it charply fell by the late summer down to 11 000 i/m² (second column of *Fig. 5)*. In the average of three years, the most frequently occurring Nematoda was *Theristus setosus* during the cold and *Paraplectonema pedunculatum* during the warm period. Among the less frequent species, *Tripyla glomerans* was found only sporadically in the Keszthely-Bay ("M"), whereas in the regions of Szigliget and Balatonszemes ("K" and "G") it was more frequently observed. A low number of individuals *Aphanolaimus aquaticus* and *Ethmolaimus pratensis* lives in the Szigliget-Bay but they were somewhat more frequent in the region of Balatonszemes, whereas they were completely absent in the Keszthely-Bay.

In the section "G" of the south-western basin the highest number of nematodes (above 100 000 i/m²) was observed during the spring season, caused mainly by the intense propagation of *Monhystera paludicola*, *Ironus tenuicaudatus* and *Paraphanolaimus behningi*. In late summer the lowest number of individuals was observed (20 000 i/m²). During the other periods of the year, the actual frequency of nematodes is roughly the same around 30 000 i/m² (third column of *Fig. 5)*. During the cold period, *Paraphanolaimus behningi* showed the highest numbers here, whereas at the other regions of the south-western basin the species *Theristus setosus* was the most frequent species. During the warm periods, mainly *Paraplectonema pedunculatum* was found in the sections "M" and "K", whereas in section "G" the *Paraphanolaimus behningi* was more frequent. Among the rarer species, *Punctodora ratzeburgensis* and *Monhystera vulgaris* were observed. The Monhysteridae proved to be the richest family here both in the number of species and individuals.

Two regions of the north-eastern basin were investigated: between Balatonfüred and Zamárdi ("A") and between Balatonalmádi and Balatonvilágos ("E").

In section "A" near the peninsula Tihany the amounts of nematodes were of an average of 60 000 i/m² in spring, 20 000 i/m² in summer and 40 000 i/m² in autumn (fourth column of *Fig. 5)*. In January 1968 the number of individuals was 60 000 i/m² in front of Tihany (point "A_0") consisting of *Theristus setosus* in 60 percent. In February of the same year the total number reached 109 000 i/m². The amount of *Theristus setosus* remained virtually unchanged (60 percent), however, its absolute number increased and the proportion of *Tobrilus* species also reached 20 percent. After thawing, the amount of nematodes decreased to 20 000 i/m². During the cold period, the most frequent Nematoda of this section was *Theristus setosus*, whereas during the warm periodi, were *Paraphanolaimus behningi* and *Ironus tenuicaudatus*. Relatively larger numbers of *Aphanolaimus aquaticus*, *Paraphanolaimus anisitsi* and *Ethmolaimus pratensis* belonging to the rarer species occurred here. *Prismatolaimus dolichorus* and *Hemicycliophora aquatica* were also found at this point living only from the north-eastern basin.

In section "E" (Balatonalmádi—Balatonvilágos) 20—30 000 i/m² was found during spring and early summer of 1966 and 1967, whereas by the spring of 1968 it was more than twice of this number: 70 000 i/m². By the end of summer of 1966 this number decreased to 18 000 i/m², and during summer of 1967 and 1968 it amounted to 20 000 i/m². The autumnal number was 40—60 000 i/m² during all the three years (fifth column of *Fig. 5)*. At this

place, *Theristus setosus* was the most frequent species during the cold while during the warm season, *Ironus tenuicaudatus* was. The highest amounts of *Tobrilus gracilis* and *Tobrilus helveticus* occurred in the area of section "E", especially during the cold period.

It can be stated in general that the number of individuals was higher in spring and autumn than in summer months.

According to the benthic investigations of STANCZYKOWSKA (1966) in Lakes Mikolajskie and Taltowisko, the highest number of nematodes was encountered in autumn: 40 000 i/m², the lowest one did in winter: 6000 i/m², and there was a gradual increase from spring. The water temperature of the Polish lakes may vary maximally around 10—12 °C. This temperature represents the transition of the cold and warm periods in Lake Balaton during spring. According to our results, the number of individuals was higher in Lake Balaton during spring, whereas in the Polish lakes during autumn. It is very likely that not the period but the suitable temperature represent the more important factor for the activity and propagation of nematodes.

The species display a seasonal variation: during the cold period, i.e. below 12 °C, everywhere the *Theristud setosus* was the most frequent species. During the warm period, i.e. above 12 °C, the following species predominated: *Paraplectonema pedunculatum* in the Keszthely-Bay ("M") and Szigliget-Bay ("K"); *Paraphanolaimus behningi* at Balatonszemes ("G"); the latter and partly *Ironus tenuicaudatus* in front of Tihany in the north-eastern basin ("A"); and the last one at Balatonalmádi ("E").

The amounts of the frequent species

Paraplectonema pedunculatum (H.) S. was the most frequent nematoda. Its distribution was rather heterogeneous in the whole area of the lake changing by seasons and even by months: its number varied between 1000 and 25 000 i/m² *(Fig. 6)*. In the north-eastern basin its number showed spring and autumnal maxima (14—16 000 and 7—10 000 i/m², respectively). Just the opposite was observed in the Keszthely-Bay ("M") where the increase of number was significant during summer, reaching 10—25 000 i/m². In the Szigliget-Bay ("K") the number was higher (25 000 i/m²) during summer than during spring and autumn, whereas at Balatonszemes ("G") a reversed state was recorded. Considering the whole lake, this species lived during summer rather more in the south-western basin and during spring and autumn rather more in the north-eastern basin. Generally its number decreased to a minimum (1000—5000 i/m²) by the early autumn and increased again by the late autumn (10 000 i/m²), then with the decrease of the water temperature it decreased again. Under the ice only 1—2000 i/m² were found.

The quantity of *Paraphanolaimus behningi* M. showed two maxima per year *(Fig. 7)*. In early spring low number was observed (3000 i/m²), and as soon as the water temperature rose above 10—12° C, it abruptly propagated to reach 10 000 i/m². During summer this number somewhat lowered (5000 i/m²), during autumn increased (15 000 i/m²) and by late autumn hardly a few thousand were present. During winter only a small number lives under the ice. It was collected always in low numbers in the Keszthely-Bay ("M") (2—5000 i/m²), it was the most frequent in the sections "K" and "G" (8—12 000 i/m²), whereas in the north-eastern basin moderate quantities of roughly identical

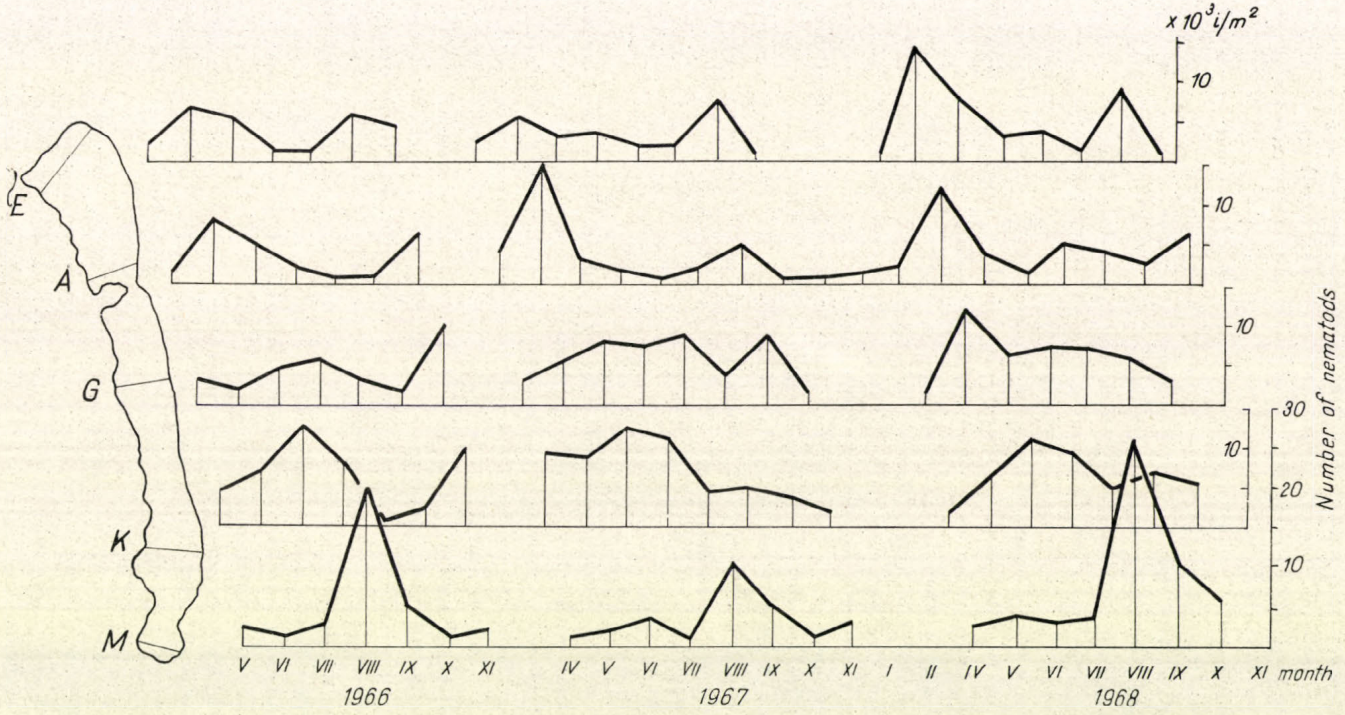

Fig. 6. Paraplectonema pedunculatum (H.) S. The change in the number of individuals in the five transversal sections of Lake Balaton during 1966—68. Each date represents the average of three points of each segment

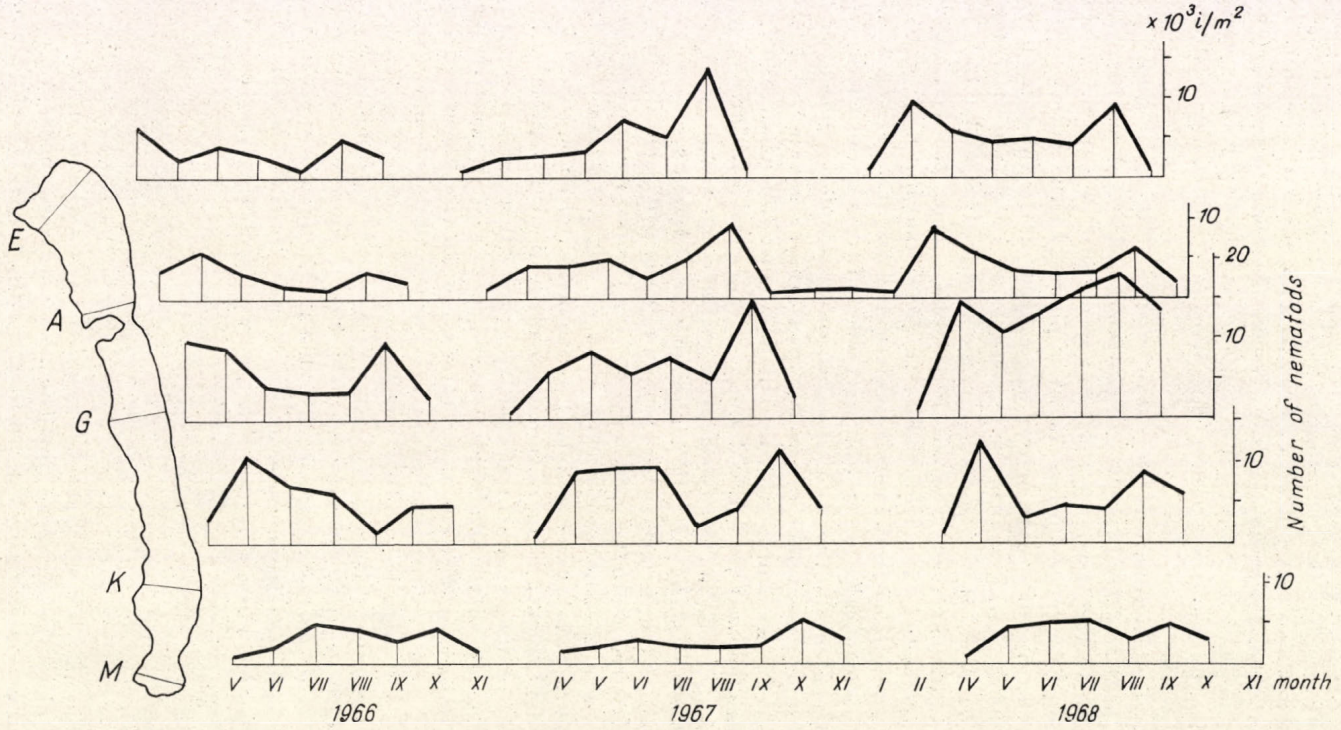

Fig. 7. The monthly change in the number of individuals of *Paraphanolaimus behningi* M. in the five transversal sections of Lake Balaton during 1966—68. Each date represents the average of three points of each segment

distribution were found. The change in the number of individuals showed a maximum in the autumn of 1967 as well as a spring and an autumnal maxima in 1966 and 1968 in the north-eastern basin. At Balatonszemes ("G") there was rather an autumnal maximum, but at Szigliget ("K") a spring one was observed. In the Keszthely-Bay ("M") its amount was identical and varied practically at the same level.

The highest number of *Ironus tenuicaudatus* dM. was found in the centre of the lake in the spring of 1966 at Balatonszemes ("G") reaching 15—25 000 i/m² *(Fig. 8)*. In the spring of 1967 and 1968 only 10 000 i/m² were observed at the same place and even less at other places. In the middle of summer it was present in 5—8000 i/m² in the whole area of the lake, no increase was observed during autumn either. Generally one can state that the highest number of *Ironus*, 10 000 i/m² in average occurred in Lake Balaton during the autumn, this number gradually decreased during the year, nevertheless it could be collected even from under the ice (1000 i/m²).

Theristus setosus B. definitely prefers cold water *(Fig. 9)*. It was found to be frequent especially in the samples collected from under the ice, representing 50—60 percent of the total number of nematodes reaching 55 000 i/m². During summer its number decreased to a minimum value of 1—3000 i/m², at some places it was completely absent in August. The change in the number of individuals showed the same tendency during all the three years. It was more frequent in the north-eastern than in the other basin.

Monhystera paludicola dM., similarly to *Theristus setosus*, was found in higher amounts in spring and autumn *(Fig. 10)*. During winter under the ice it occurred in 15 000 i/m² not reaching the number of *Theristus setosus*. Its number was 4—6000 i/m² during early spring, it increased to 15 000 i/m² by May and at Balatonszemes ("G") it reached even 65 000 i/m² in 1966. But it decreased to 2000 i/m² or at some places even to 0, especially in 1967, as soon as the prolonged warm period appeared. It propagated again during autumn, at early winter it already showed 5000 i/m². It was frequent everywhere in the spring of 1966 and 1967 except at the Keszthely-Bay ("M"), it displayed a uniform distribution in summer and autumn during all the three years, although its number was low.

Tobrilus gracilis B. was found in the samples representing hardly 3 percent of all the nematodes. Its number was negligibly in summer, whereas during the cold season it reached 2—3000 i/m², and was even more frequent in samples collected from under the ice. More individuals were collected in the north-eastern basin ("A" and "E") as well as in the Keszthely-Bay ("M") than in the other regions of the lake.

The biomass and its changes

The biomass is roughly proportional to the number of individuals present in the samples. When calculating the biomass, first the average weights of the species were determined according to ANDRÁSSY (1956). The weight of the animals (G) was calculated with the following equation

$$G = \frac{a^2 \times b}{1\,600\,000},$$

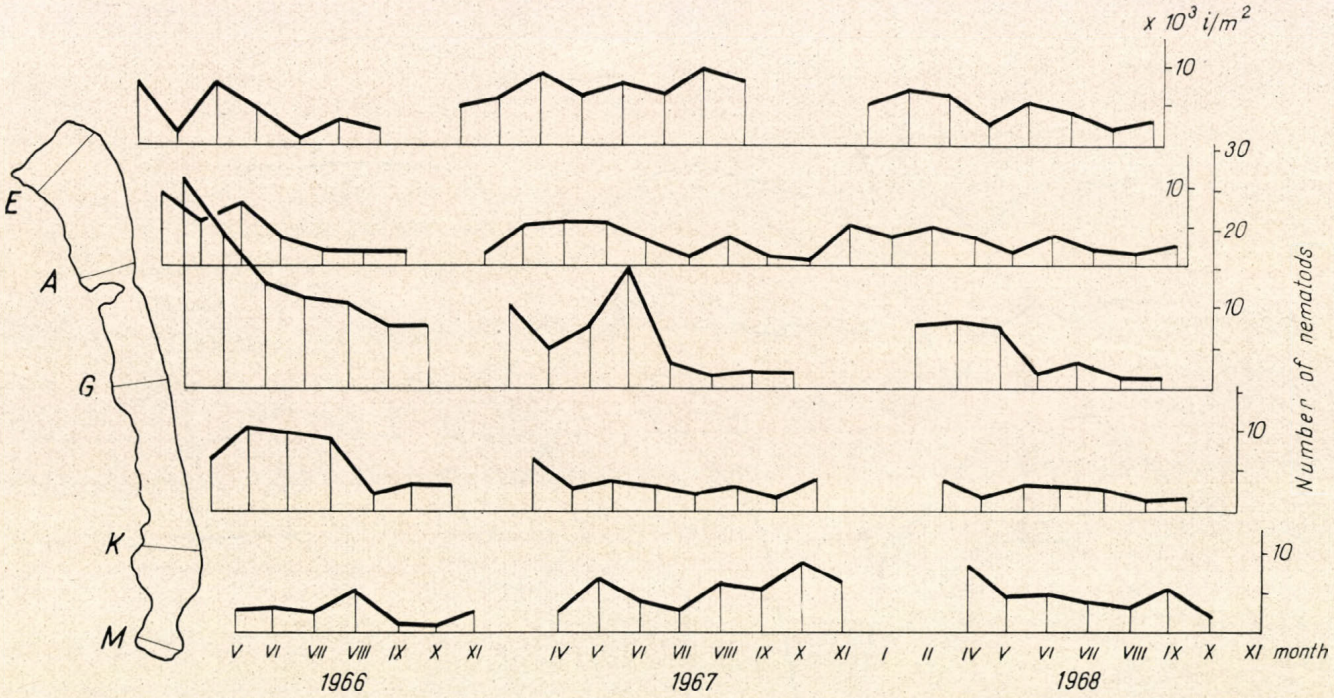

Fig. 8. The monthly change in the number of individuals of *Ironus tenuicaudatus* in the five transversal sections of Lake Balaton during 1966—68. Each date represents the average of three points of each section

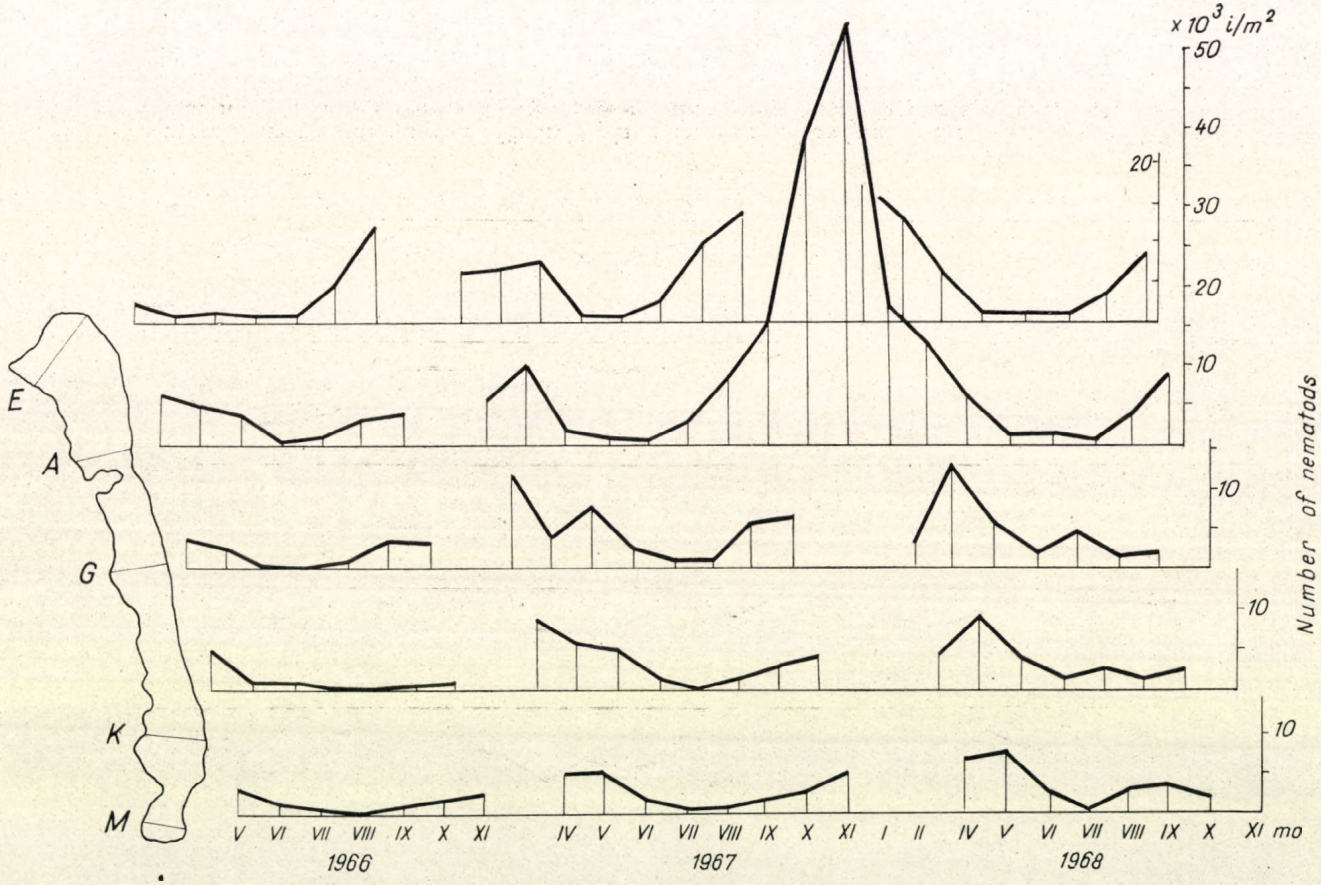

Fig. 9. The monthly change in the number of individuals of *Theristus setosus* (B.) F. in the five transversal sections of Lake Balaton during 1966—68. Each date represents the average of three points of each section

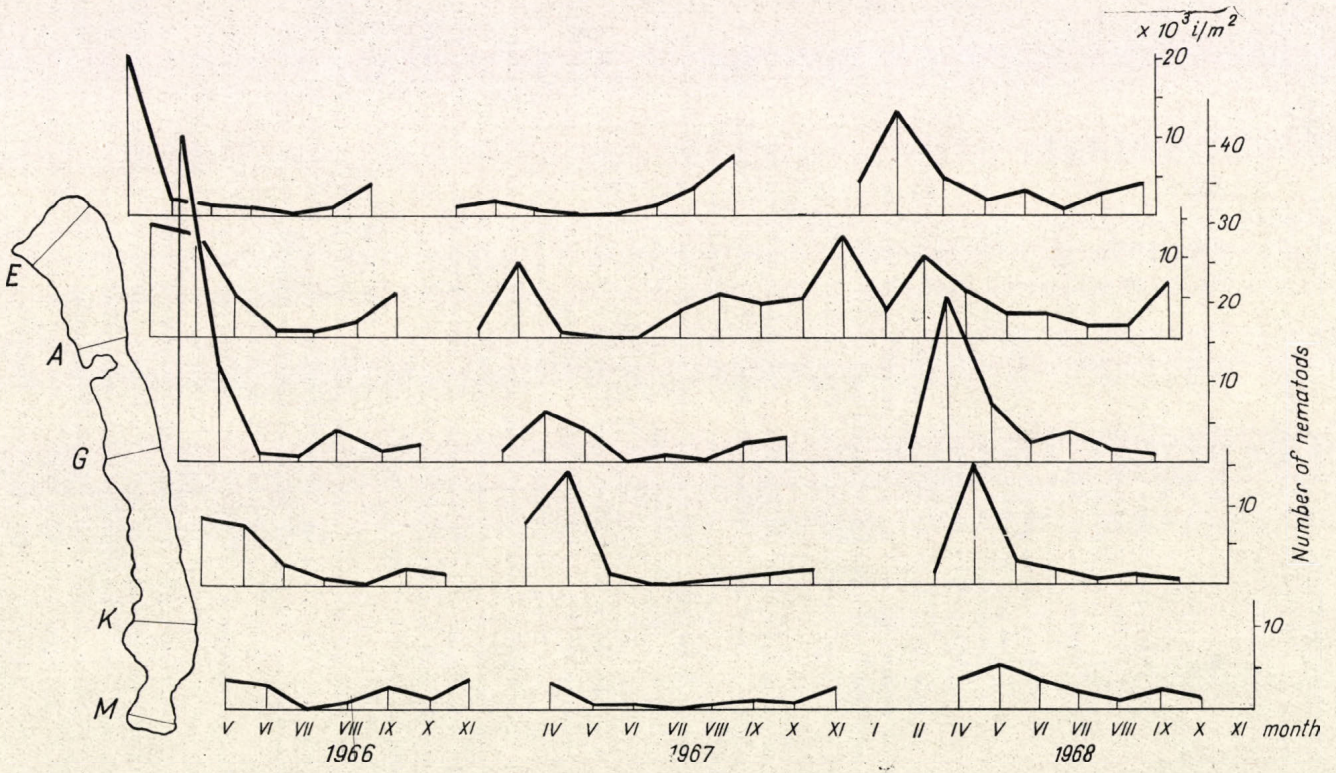

Fig. 10. The monthly change in the number of individuals of *Monhystera paludicola* dM. in the five transversal sections of Lake Balaton during 1966—68. Each data represents the average of three points of each section

where a is the largest diameter of the body and b is the length of the body, both in microns, and G is in μg units. The weihgt of the most frequent nematodes reached 1 μg only rarely:

Ironus tenuicaudatus	1.26 μg
Paraplectonema pedunculatum	0.06 μg
Paraphanolaimus behningi	0.08 μg
Theristus setosus	0.10 μg
Monhystera paludicola	0.07 μg
Tobrilus gracilis	0.76 μg
Aphanolaimus aquaticus	0.05 μg
Ethmolaimus pratensis	0.09 μg
Monhystera macramphis	0.08 μg
Tripyla glomerans	0.91 μg
Dorylaimus stagnalis	2.49 μg
Monhystera stagnalis	0.05 μg

Four of the five most frequent species do not reach even 0.1 μg, only the weight of *Ironus* is to 1.2 μg. The weight of *Dorylaimus stagnalis* (2.5 μg) could increase the biomass of the lake to a significant extent, however, it lives in the open-water sediment in such a small number that it effect is undetectable.

The μg values were multiplied by the number of individuals and at each species were calculated for 1 m^2 separatedly *(Fig. 13)*. Considering the whole lake, the biomass of nematodes varies between 4 and 20 mg/m^2. This value is nearly twice as high (16—20 mg/m^2) in spring than in summer the lowest value (4—6 mg/m^2) usually was observed in early autumn then it increased slowly.

The biomass of nematodes varied in the Keszthely-Bay ("M") during 1966 (first column of *Fig. 11)*. In August it reached a maximum of 13 mg/m^2, then decreased to 2 mg/m^2 during autumn, representing the lowest value which had ever been observed during the investigations. The tendencies in the change of biomass were identical even during 1967 and 1968. From the level of 14—16 mg/m^2 observed in spring, it decreased by the end of summer to 5—8 mg/m^2, increased again to 14—15 mg/m^2 during early autumn and dropped again within several months.

In the Szigliget-Bay ("K") (second column of *Fig. 11)* the biomass value was 20 mg/m^2 during the whole summer of 1966, it decreased below 6 mg/m^2 only during autumn then increased again. In 1967 and 1968 the spring value was 8—10 mg/m^2, it decreased gradually below 4 mg/m^2 and remained at 4—6 mg/m^2 during the whole autumn.

At Balatonszemes ("G") (third column of *Fig. 11)* an extreme value of 40 mg/m^2 was observed in May 1966 caused by the mass appearance of *Monhystera paludicola* and *Ironus tenuicaudatus*. Such high values were not obtained in 1967, although a high number of nematodes was present in the early spring samples. The changes of biomass values displayed a similar tendency during all the three years. The spring value of 21—40 mg/m^2 decreased to its half or third parts by the early autumn then increased again by winter.

Samples could be taken in every periods of the year from the section "A" between Balatonfüred and Zamárdi. The changes in the number of individuals and the biomass showed two maxima during one year: the first one in to February under the ice, the second one appeared in May. According to the observations, the biomass was nearly one third higher under the ice

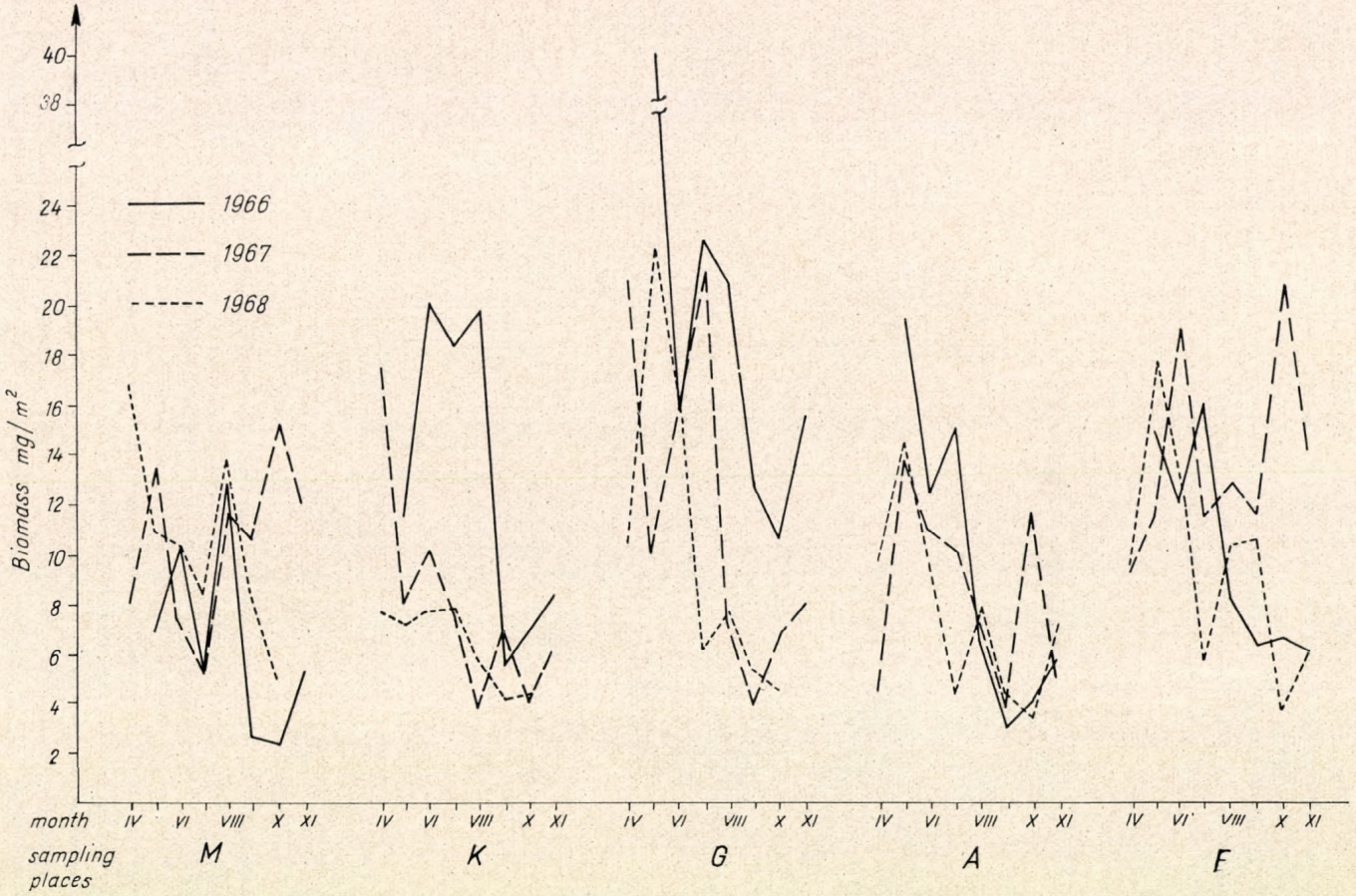

Fig. 11. The monthly change of nematoda biomass in the five transversal sections of Lake Balaton during 1966—68. Each date represents the average of three points of each section

(24 mg/m²) than the value of May (16 mg/m²), and about six-times higher than the value of late summer (4 mg/m²) (fourth columns of *Fig. 11* and *12)*.

The analysis of biomass values was not so simple at Balatonalmádi ("E"). In 1966 the general tendency was similar to that observed in the other regions, namely, the higher biomass value, 16—17 mg/m², of spring and early summer reached a minimum by the early autumn (4—6 mg/m²). On the other

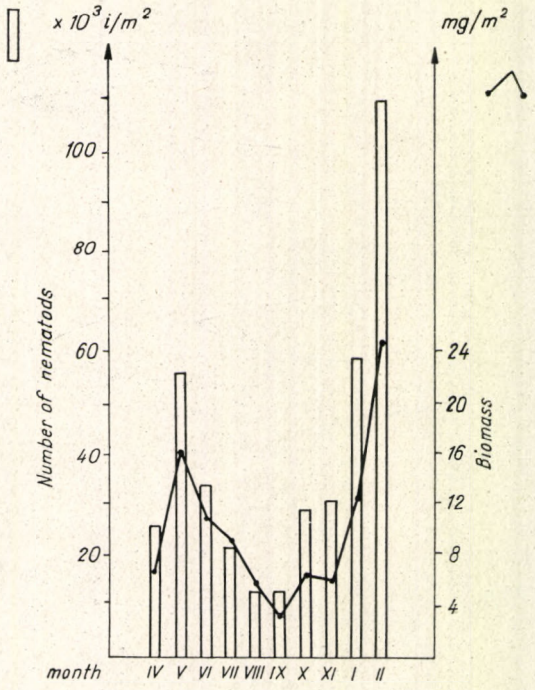

Fig. 12. The change in the number (i/m²) and biomass of nematodes at the point "A_0" at Tihany in the section Balatonfüred—Zamárdi in the average of three years

hand, in 1968 two maxima were observed, one in June (19 mg/m²) and an other in October (21 mg/m²), whereas in the other months of the year the biomass was nearly identical, about 12 mg/m² (fifth column of *Fig. 11)*.

The yearly change of the nematode biomass has been estimated. The biomass values of months, species, sections were considered at that estimation in the average of three years. According to the calculations, the biomass of nematodes amounts to 13 mg/m² at early spring in the western and 9 mg/m² in the eastern basin of the lake. In the north-eastern basin and east to it up to Balatonszemes ("G"), the biomass increases to 16—20 mg/m² during May and after that period there is a gradual decrease over the whole area of the lake to 6—8 mg/m² until the end of September, however, parallel with the cooling down of temperature, a reincrease can again be observed *(Fig. 13)*.

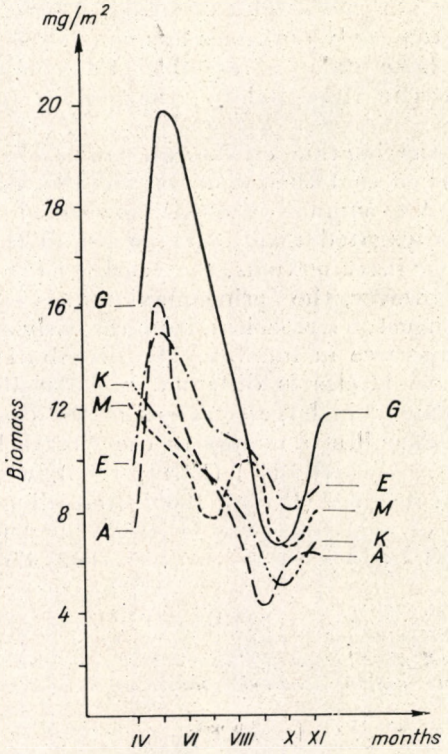

Fig. 13. The change of nematoda biomass in the five sections of Lake Balaton in the average of three years

Discussion

SCHIEMER (1969) reported on the nematoda fauna of Lake Fertő (Neusiedler See). That lake is similar to Lake Balaton in so far as it is shallow, with a flattened bottom having a sediment mixed with detritus and water of about pH 8. One part of the species found there occurred even in Lake Balaton. The most frequent ones are: *Paraplectonema pedunculatum* (about 70 percent), *Tobrilus gracilis* (about 15 percent) and *Monhystera paludicola* (about 5 percent). SCHIEMER disclosed a relation between the quality of sediment and the distribution of species, namely the *Paraplectonema pedunculatum* did not prefer the detritic mud of 5—10 cm thickness and neither did the roughly granulated sand. The occurrence of this species in Lake Balaton further supports that observation. In the Keszthely-Bay ("M") where the sediment particles of less than 2 μ size represent 25—30 percent (MÜLLER, 1969), much more *Paraplectonema pedunculatum* occurred during summer than in the Szigliget-Bay ("K") where 40 percent and than at the sandy shore of south where 10—15 percent was the proportion of sediment particles of less than 2 μ size. In Lake Fertő SCHIEMER described to be directly proportional the number of individuals of *Tobrilus gracilis* and *Monhystera paludicola* to the thickness of mud. This could not be observed in Lake Balaton. In the soft mud of

Lake Fertő 10^5—10^6 i/m² nematodes occurred, whereas in the places having a more compact bottom, only ten times less nematodes were found. The open-water sediment of Lake Balaton resembles the compact sediment of Lake Fertő. At such places in Lake Balaton the number of nematodes was 10—20 000 i/m².

Investigating the benthic crustacean fauna of Lake Balaton, PONYI (1966; 1969) established that their number was the highest at early summer (June), the lowest at late summer (August), however, by early winter (November) the crustacea propagated again reaching almost the values of early summer. According to our investigations, the yearly change of nematodes follows also that rhythm, however, the spring maximum is earlier, already in May, the minimum is in August, nevertheless, from the early autumn till the thawing of ice, there is an increase in number. The distribution of small crabs, the Chironomidae and nematodes is different in Lake Balaton. The larvae of Chironomidae were found in largest masses in the centre of the lake ("G") in May 1965, and in smallest amounts in the Keszthely-Bay ("M") and the north-eastern basin ("A" and "E") (ENTZ, 1965), whereas the distribution of Crustacea was just reversed (PONYI, 1969). According to the present results, the highest number of nematodes was in the central part of the lake ("G") during 1966—68 and the lowest in the Keszthely-Bay ("M") *(Table III)*.

TABLE III

The amount and distribution of "microcrustacea", larvae of Chironomus plumosus MEIG. *as well as nematodes living in the sediment of Lake Balaton*

	Keszthely "M"	Szigliget "K"	B. Szemes "G"	Tihany "A"	Fűzfő "E"
	i/m²				
"Microcrustacea" (PONYI, 1969)	14 800	4 300	11 800	14 800	15 800
Larvae of Chironomus plumosus (ENTZ, 1965)	24	148	356	5	5
Nematoda 1966—68 average	23 000	31 000	44 000	28 000	28 000

It became clear on the basis of investigations of the benthic animals that the open-water sediment of a great extent, i.e. Lake Balaton can be divided into three large parts:

1. North-eastern basin ("A" and "E").
2. Keszthely-Bay ("M").
3. Transitional regions between the former two ("K" and "G").

The Szigliget-Bay is mainly of outstanding character, however, often resembles the Keszthely-Bay. In the central part of Lake Balaton, in the region of section "G" predominate the properties of the north-eastern basin, nevertheless often some altered characteristics are realized.

Summary

In the open-water sediment of Lake Balaton the following nematodes predominated during 1966—68: *Paraplectonema pedunculatum* S. (20 percent), *Paraphanolaimus behningi* M. (20 percent), *Ironus tenuicaudatus* dM. (19 percent), *Theristus setosus* (B.) M. (17 percent), *Monhystera paludicola* dM. 15 percent), Their occurrence varies by seasons: during winter (between 4 and 12 °C) *T. setosus* and *M. paludicola* are the most frequent species, while during summer they are absent at some places. A rare species, *Paraphanolaimus anisitsi* (D.) ANDRÁSSY 1968 was also observed in the sediment of the lake.

The highest number of nematodes was found in spring (May), 60—80 000 i/m², there was a gradual decrease during summer 10—20 000 i/m², then from the middle of autumn (October) there was an increase again. During winter 100 000 i/m² nematodes were observed under the ice at Tihany ("A_0").

The regional distribution of nematodes was also variable: the highest number was at Balatonszemes ("G") averaging 44 000 i/m², the lowest one in the Keszthely-Bay ("M") averaging 23 000 i/m².

The biomass of nematodes varied between 4—20 mg/m². Generally this value is nearly twice as high in spring than in summer, it is the lowest usually in early autumn (4—6 mg/m²) and then slowly increases. The highest value of biomass was found at Balatonszemes ("G").

On the basis of the benthic fauna, the Lake Balaton can be divided into three regions: the north-eastern basin ("A" and "E"), the Keszthely-Bay ("M") and the central part of the lake ("K" and "G").

Acknowledgments: Author expresses his thanks to Prof. Dr. I. ANDRÁSSY, Eötvös Loránd University, Budapest for his kind help, in identification of nematodes, as well as to the leaders in the identification of nematodes, as well as to the leaders their aid in the performance of the work.

REFERENCES

ANDRÁSSY, I. (1956): Die Rauminhalts- und Gewichtsbestimmung der Fadenwürmer *(Nematoden). Acta Zool. Acad. Sci. hung.* **2,** 1—15.

ANDRÁSSY, I. (1968): Fauna Paraguayensis. 2. Nematoden aus der Galerie-Wäldern des Acaray-Flusses. — *Opusc. Zool. Budapest* **8,** 167—351.

ANDRÁSSY, I. (1972): Verzeichnis der in Ungarn bisher nachgewiesenen freilebenden Fadenwürmer *(Nematoda).* — *Állatt. Közlem.* **59,** 161—171.

BIRÓ, K. (1968): The nematodes of Lake Balaton. II. The nematodes of open water mud in the Keszthely Bay. — *Annal. Biol. Tihany* **35,** 109—116.

BIRÓ, K. (1969): Eine neue Monhystera-Art (Nematoda) aus dem Balaton, Ungarn. — *Opusc. Zool. Budapest* **9,** 255—257.

BIRÓ, K. (1972): Nematodes of Lake Balaton. III. The fauna in late-summer. — *Annal. Biol. Tihany* **39,** 89—100.

BIRÓ, K., J. PONYI, P. N. ZÁNKAI (1968): Die Nematoden im Schlamm des offenen Wassers des Plattensees. I. Die horizontale Ausbreitung der Fadenwürmer im Frühjahr 1966. — *Állatt. Közlem.* **55,** 33—35. (in Hungarian with German summary)

DADAY, J. (1897): Fadenwürmer *(Nematoda).* In: Entz, G.: Die Fauna des Balatonsees. — *Resultate der wiss. Erforschung des Balatonsees* **2,** IV, pp. 75—109.

ENTZ, B. (1965): Untersuchungen an Larven von Chironomus plumosus Meig. im Benthos des Balatonsees in den Jahren 1964—1965. — *Annal. Biol. Tihany* **32,** 129—139.

GAGARIN, V. G. (1971): New and rare species of nematodes from the Uchinsky and Dubossarsky water reservoirs. — *Zool. Zsurn.* **50,** 474—482. (in Russian with English summary)

JUGET, J. (1969): Description de quelques formes rares ou nouvelles de Nématodes libres du bassin du Léman. — *Bulletin de la Société vaudoise des Sciences naturelles* **70,** 141—173.

MESCHKAT, A. (1934): Der Bewuchs in den Röhrichten des Plattensees. — *Arch. Hydrobiol.* **27,** 436—517.

MÜLLER, G. (1969): Sedimentbildung im Plattensee/Ungarn. — *Naturwissenschaften* **56,** 606—615.

PONYI, J. (1966): Orientierende Untersuchungen über die qualitativen und quantitativen Verhältnisse der schlammbewohnenden Krebse im offenen Wasser des Balaton. — *Annal. Biol. Tihany* **33,** 177—192. (in Hungarian with German summary)

PONYI, J. (1969): Quantitative investigations on mud-living crustaceans in the open water of Lake Balaton. — *Annal. Biol. Tihany* **35,** 213—222.

PONYI, J., K. BIRÓ, P. N. ZÁNKAI (1967): Die Sammeltechnik der schlammbewohnenden Tiere des Balaton und ihre Probleme. — *Állatt. Közlem.* **54,** 129—134. (in Hungarian with German summary)

PONYI, J., J. OLÁH, P. BIRÓ, K. BIRÓ (1971): Comparative investigations on the benthic fauna at two sewage inflows of Lake Balaton. — *Annal. Biol. Tihany* **38,** 199—226.

RIEMANN, R. (1971): Freilebende Nematoden aus dem Grenzbereich Meer-Süss-Wasser in Kolumbien, Südamerika. — *Veröff. Inst. Meeresforschung, Bremerhaven* **12,** 365—412.

SCHIEMER, F., H. LÖFFLER, H. DOLLFUSS (1969): The benthic communities of Neusiedlersee (Austria). — *Verh. Internat. Verein Limnol.* **17,** 201—208.

STANCZYKOWSKA, A., M. PRZYTOCKA-JUSIAK (1968): Variations in abundance and biomass of microbenthos in three Mazurian lakes. — *Ekol. Pol. Seria A* **16,** 539—559.

A BALATON NEMATÓDÁI.
IV. A FAUNA ÉVSZAKOS VÁLTOZÁSA

Biró Kálmán

Összefoglalás

A Balaton nyíltvízi üledékében 1966—68-ban a *Paraplectonema pedunculatum*, *Paraphanolaimus behningi*, *Ironus tenuicaudatus*, *Theristus setosus* és *Monhystera paludicola* a leggyakoribb fonálféreg *(2. táblázat)*. Évszakonként az egyes fajok mennyisége változik: télen (+4 és +12 C° vízhőmérséklet között) és a jég alatt *Theristus setosus* és *Monhystera paludicola* a leggyakoribb, de ezek nyáron hiányozhatnak is.

A fonálférgek mennyisége a tó különböző területein változó *(5. ábra)*. A legtöbb tavasszal (május) 60—80 000 i/m², a nyár folyamán fokozatos csökkenés következett (10—20 000 i/m²), majd az ősz közepétől (október) ismét számbeli növekedés volt (20—30 000 i/m²). Télen a jég alatt Tihanynál („A_0") 100 000 i/m² mennyiséget észleltem. Legtöbb fonálféreg B.-szemesnél („G"), átlag 44 000 i/m², legkevesebb a Keszthelyi öbölben („M"), átlag 23 000 i/m² volt.

Három éven keresztül a tó különböző területein megfigyeltem a leggyakoribb fajok egyedszám változását *(6., 7., 8., 9., 10. ábrák)*.

A fonálféreg biomassza 4—20 mg/m² között változott. Tavasszal közel kétszer akkora (16—20 mg/m²) ez az érték, mint nyáron, ősz elején volt a legkisebb (4—6 mg/m²) és ezután lassú emelkedést tapasztaltam *(11. ábra)*. A legtöbb biomasszát B.-szemesnél („G") mértem.

Tihany előtt („A_0") februárban a jég alatt a biomassza (és egyedszám) csaknem kétszerese a tavaszi (május) értéknek és hatszorosa a nyári (szeptember) minimumnak *(12. ábra.)*

A Keszthelyi („M") és a Szigligeti öbölben („K") a koratavaszi (április) biomassza maximumtól fokozatos csökkenés figyelhető meg az ősz végéig (október) 12 mg/m²-ről 6 mg/m²-re, addig B.-szemestől Fűzfőig („G", „A", „E") áprilisi alacsony biomassza májusban majdnem megduplázódik, és csak ez után következik a biomassza fokozatos csökkenése az őszvégi minimumra és a víz lehűltével mindenütt fokozatosan emelkedik *(13. ábra.)*

Az üledékfauna alapján a Balaton 3 területre tagolható: ÉK-i medence („A" és „E"), Keszthelyi öböl („M") és a tó középső része („K" és „G").

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THE FOOD OF PIKE-PERCH (*LUCIOPERCA LUCIOPERCA* L.) IN LAKE BALATON

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Continuing our previous studies, the food of pike-perch was investigated in Lake Balaton during 1970 and 1971, in order to complete the data available. Now we have a sufficient basis for giving a general idea of the nutritional-ecological role played by the 3—5-year-old specimens representing the majority of the pike-perch population in the lake, as well as for comparison of qualitative and quantitative characteristics of the food for several years back. Such a comparison may lead to valuable conclusions from the point of view of biological processes accompanying the rapid eutrophication of Lake Balaton observed during the recent years. Thus e.g. it is very likely that the decrease in rate of growth of pike-perch as compared to the data obtained some 40 years ago is a result of changes taking place during the last decades in the ecosystem of this shallow lake (UNGER, 1931; BIRÓ, 1970).

This fact can be brought into direct connection with the quantitative insufficiency of the food of pike-perch already interpreted by WOYNÁROVICH (1959). Similar conclusions were drawn by our earlier papers evidencing the low food turnover of pike-perch in Lake Balaton (BIRÓ and ELEK, 1969; BIRÓ, 1969). The slow and uneven growth appearing in consequence of undernourishment during fry stage (BIRÓ, 1972b) which is of critical significance, decisively influences the further development of pike-perch.

SEBESTYÉN (1967) has pointed out that the role of fishes played in the ecosystem is the easiest to approach from the side of nutrition and growth. Accordingly, we attempt to analyze the nutritional biology and role of pike-perch in the lake. Therefore, the present paper was intended at comparing the food of the pike-perch for several years back. Furthermore, an answer was searched for the question of the effect of pike-perch on the food-fish populations of Lake Balaton and of the interpretation of this niche in the ecosystem of the lake.

Material and methods

Our material was collected during 1970 and 1971 partly by means of a special stomach-pump (WOYNÁROVICH, 1958) partly by preparing the internal organs of pike-perches, carried out by fishermen of Fish-farms of

Balaton. We investigated the stomach content of fish of 300—500 g body weight group representing the majority of the annual pike-perch catch. They were fixed in 4 percent formalin separately until the analysis. The stomach contents were analyzed in a Petri dish under stereomicroscope after dilution with water.

Conclusions were drawn for the qualitative change of the food from the seasonal distribution of the species. As possibility offered we determined the degree of digestion of the food (FORTUNATOVA, 1950), then the prey-fishes found in the stomach content were identified in the cases of Cyprinids on the basis of pharyngeal teeth (VÁSÁRHELYI, 1956; BERINKEY, 1966) or sometimes on the structure of scales (DYK, 1956). In the cases of Percids the species could be distinguished depending on the degree of digestion, on the basis of morphology of the operculum, preoperculum, dentale, scales and the stomach, of number of pyloric appendices as well as on the composition of the stomach-content (BERINKEY, 1958; 1966; DYK, 1956; WOYNÁROVICH, 1959).

The quantitative evaluation of the food was carried out on the basis of number of fishes found in the stomach as well as for their measured or reconstructed weights. The stomach contents were divided into six groups according to their weights and the pike-perches were evaluated according to their percentual distribution within those groups. The sum of the original body weight of the food-fishes was estimated, the group averages were calculated with the values of standard deviation and variation coefficients. The food coefficient was calculated from the connection of the actual weight of the stomach-content and the body weight of the pike-perch. For the reconstructions of the weights, the allometric equations of length-weight relationships determined for different fish species were used. The determination of the time of digestion belonging to different temperature ranges was carried out by means of the method of MOLNÁR et al. (1967), allowing us to draw conclusions on the intensity of nutrition. The daily and monthly quantities of the food as well as the daily and monthly rations of food are given using the methods of BAJKOV (1935) and FORTUNATOVA (1950).

The stomach contents of altogether 3347 pike-perches were analyzed during 1970 and 1971.

Results

The quality of food of pike-perch

More or less digested food was found in the stomach of 909 from 1118 pike-perches in 1970, containing 1806 food-fishes, 1472 of which could be identified, whereas 334 could not because of the advanced stage of digestion *(Table I, Fig. 1)*. The food was included the following fish species in a sequence of decreasing frequency: ruff *(Acerina cernua)*, bleak *(Alburnus alburnus)*, pike-perch fry *(Lucioperca lucioperca* L.) and bream *(Abramis brama)*. Altogether 10 species were found in the stomachs, among them the bleak was predominating except in August when the number of fry of the most common Percids (ruff, pike-perch) suddenly increased in the food. The significance of the other species is lower in the food, they represent only 0.3—4.5 percent.

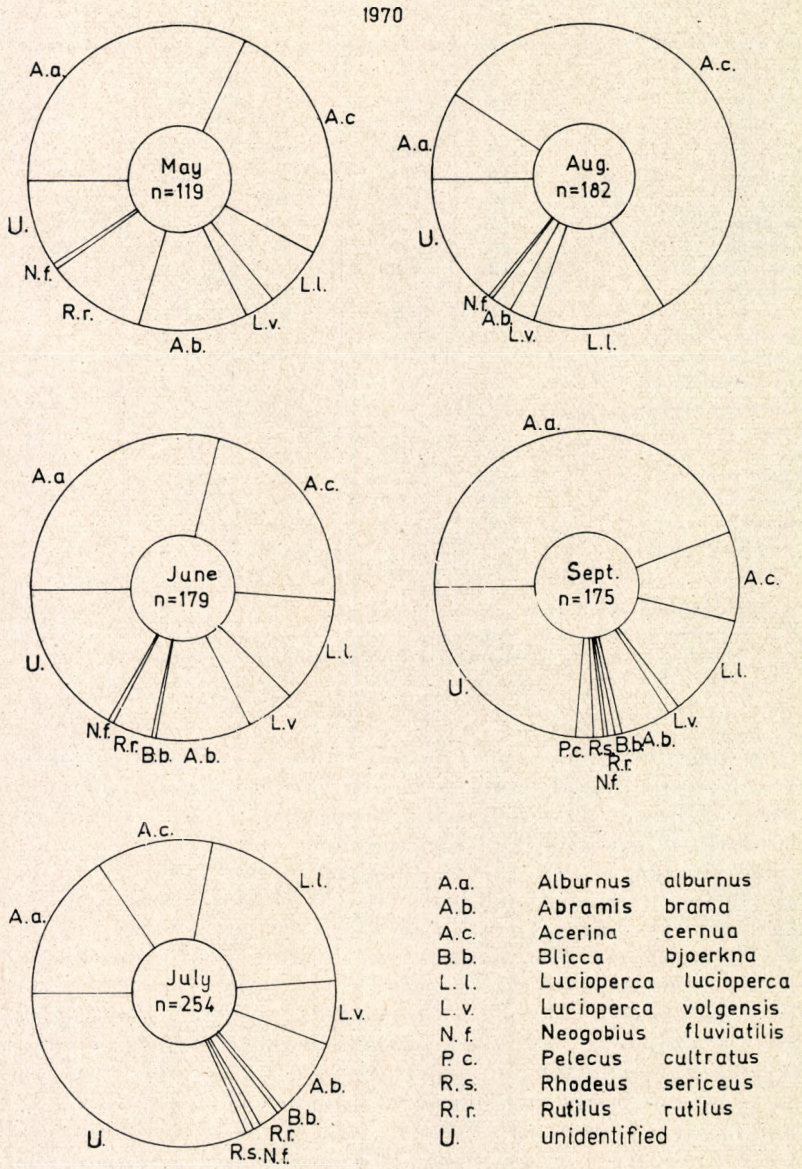

Fig. 1. The food spectrum of pike-perches in Lake Balaton of 300—500 g body weight during 1970. The percentual distribution of food-fishes. n = the number of pike-perch stomachs containing food

TABLE I

The distribution of fish-remains found in the pike-perch stomachs during 1970

	May	June	July	Aug.	Sept.	Total
N	177	226	294	187	234	1118
n	119	179	254	182	175	909
e	58	47	40	5	59	209
Alburnus alburnus	57	74	52	72	116	371
Acerina cernua	46	57	41	447	25	616
Lucioperca lucioperca	12	29	69	112	29	251
Lucioperca volgensis	6	13	22	22	3	66
Abramis brama	21	26	28	18	14	107
Blicca bjoerkna	—	—	1	2	2	5
Rutilus rutilus	19	11	7	—	2	39
Neogobius fluviatilis	1	1	2	2	1	7
Rhodeus sericeus amarus	—	—	2	—	3	5
Pelecus cultratus	—	—	—	—	5	5
Identified fish	162	212	225	673	200	1472
Unidentified fish	16	42	103	110	63	334
Total number of fish	178	254	328	783	263	1806
Dreissena polymorpha	2	2	1	—	—	5
Water-weed fragments	—	1	2	3	1	7

N = Total number of stomachs investigated
n = Number of stomachs containing food
e = Number of empty stomachs (including some pulpy content)

Lucioperca volgensis and roach *(Rutilus rutilus)* are qualified as occasional food. The former occured more frequently during the summer months and the latter did during May. A recently propagated Ponto-Caspian goby *(Neogobius fluviatilis)* could be found in the stomachs in every month, although in a small number of individuals. This species has been living in the lake probably for quite some time (Biró, 1972a). *Blicca bjoerkna*, the bitterling *(Rhodeus sericeus amarus)* and *Pelecus cultratus* occurred in the certain months. The latter may play a more considerable role during autumn. Apart from fishes, sporadically mussels *(Dreissena polymorpha)* and water-weed fragments were also found in the stomachs.

Food was found in 982 (44.1 percent) of the 2229 pike-perches investigated during 1971, while the stomach of 1247 (55.9 percent) was empty. Altogether 1916 prey-fishes were found, we succeeded in identifying 1626 specimens but failed in 290 cases *(Table II, Fig. 2)*. The food contained mainly bleak *(Alburnus alburnus)* and ruff *(Acerina cernua)*, pike-perch fry *(Lucioperca lucioperca)* as well as bream *(Abramis brama)* also occurred. Altogether 13 fish species were found in the stomachs during that year. *Leucaspius delineatus*, eel *(Anguilla anguilla)* and crucian carp *(Carassius carassius)* were first observed in the stomachs, however, because of their low number, they are of less significance. Apart from the main four prey-fishes, the increasing rate of *Blicca bjoerkna* and *Neogobius fluviatilis* was characteristic, whereas the amount of roach was the same as in 1970. *Dreissena* was also observed and several small specimens of *Unio* and *Anodonta* of 1—2 cm shell length were encountered, too.

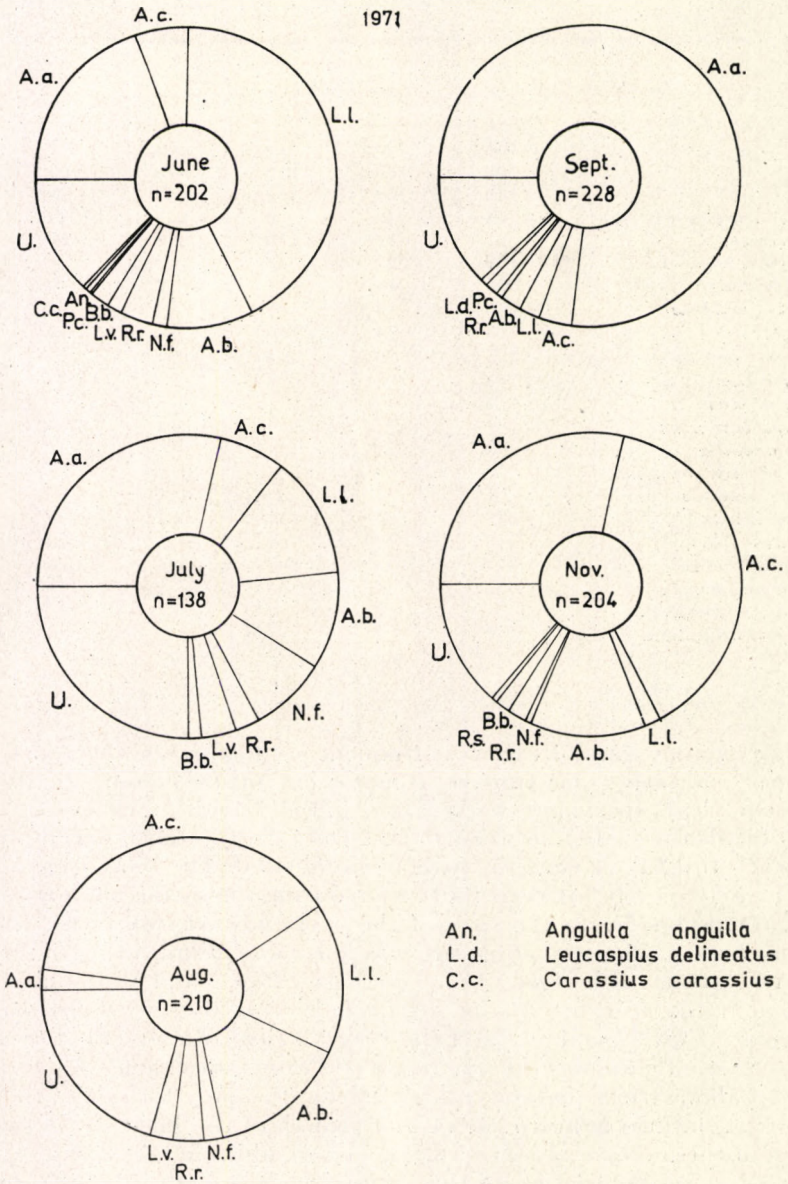

Fig. 2. The food spectrum of pike-perches of 300—500 g body weight in Lake Balaton during 1971. (Explanation as in *Fig. 1)*

TABLE II

The distribution of fish-remains found in the pike-perch stomachs during 1971

	June	July	Aug.	Sept.	Nov.	Total
N	451	400	618	468	292	2 229
n	202	138	210	228	204	982
e	249	262	408	240	88	1 247
Alburnus alburnus	74	48	6	444	144	716
Acerina cernua	21	12	116	20	196	365
Lucioperca lucioperca	159	22	48	12	8	249
Abramis brama	35	18	44	12	60	169
Neogobius fluviatilis	6	14	6	4	2	32
Rutilus rutilus	13	4	10	—	10	37
Lucioperca volgensis	7	6	8	—	—	21
Blicca björkna	8	2	—	—	8	18
Pelecus cultratus	1	—	—	8	—	9
Leucaspius delineatus	—	—	—	4	—	4
Anguilla anguilla	3	—	—	—	—	3
Rhodeus sericeus amarus	—	—	—	—	2	2
Carassius carassius	1	—	—	—	—	1
Identified fish	328	126	238	504	430	1 626
Unidentified fish	46	42	60	72	70	290
Total number of fish	374	168	298	576	500	1 916
Dreissena polymorpha	3	—	2	—	—	5
Unio and *Anodonta* sp.	—	2	2	—	—	4
Water-weed fragments	1	4	—	—	6	11

The size of prey-fish

The average sizes of the most frequent six food species changed parallel with the qualitative-quantitative composition of the food. Mainly of the 5.5—7 cm bleak specimens were eaten *(Figs 3* and *4)*, whereas the body length of ruffs fluctuated between 4 and 6 cm *(Figs 5* and *6)* except in August 1970, when the 3.5—4 cm specimens predominated. The body length of pike-perch fry were mostly between 7 and 8 cm during the spring and early summer season, corresponding to the sizes of the overwintered specimens. In August 1970 and June 1971 the average size was 2.5—5.5 cm indicating an increasing role of fry masses in the food of pike-perches *(Figs 7* and *8)*. Similar changes appeared in the size distribution of *Lucioperca volgensis* *(Figs 9* and *10)*, the bream *(Figs 11* and *12)* and the roach *(Figs 13* and *14)*. The maximal length of prey-fishes was 14 cm (several *Pelecus* specimens).

The allometric equations calculated for the prey-fishes i.e. the length-weight relationships indicate the rate of growth of the linear body size in the function of the increase of body weight, and in addition, allow us to carry out a more exact reconstruction of the original body weight at the quantitative evaluation of the food. The calculated equations for the different fish species are as follows:

Bleak: $\log W = -4.6731 + 2.8865 \cdot \log L$
Ruff: $\log W = -4.8993 + 3.0855 \cdot \log L$
Pike-perch fry: $\log W = -4.6088 + 2.8379 \cdot \log L$
Bream: $\log W = -4.8146 + 3.0644 \cdot \log L$
Roach: $\log W = -4.9698 + 3.1441 \cdot \log L$
Goby: $\log W = -5.0386 + 3.1398 \cdot \log L$

where W is the body weight in grams and L is the standard length in mm.

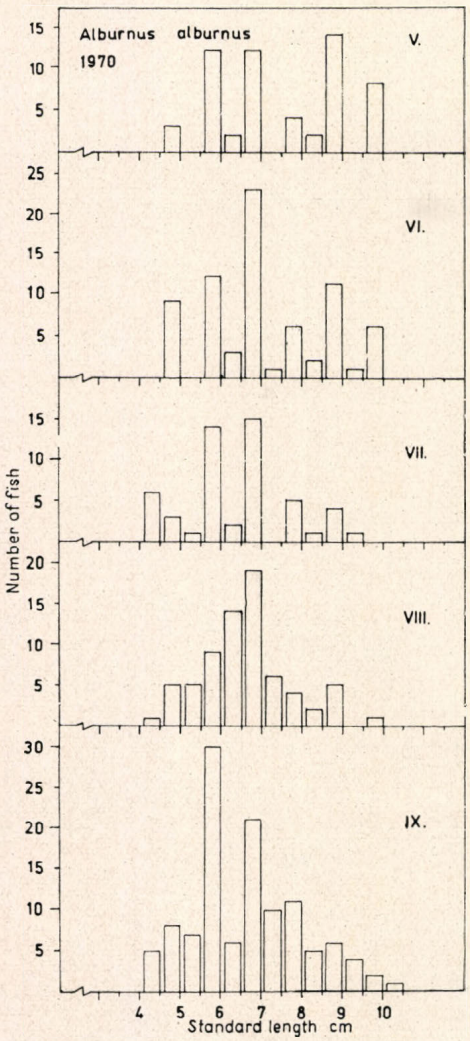

Fig. 3. The size distribution of bleak *(Alburnus alburnus)* in the food of pike-perch in 1970

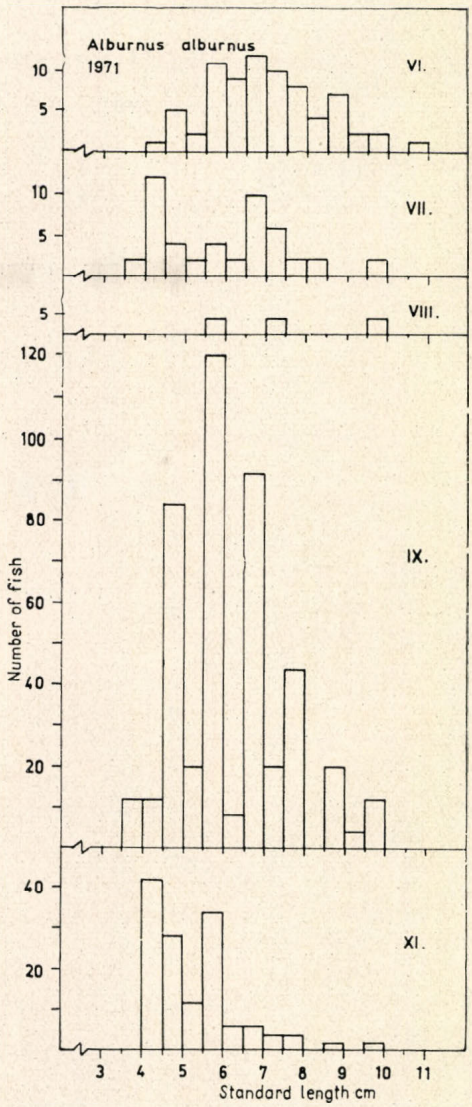

Fig. 4. The size distribution of bleak *(Alburnus alburnus)* in the food of pike-perch in 1971

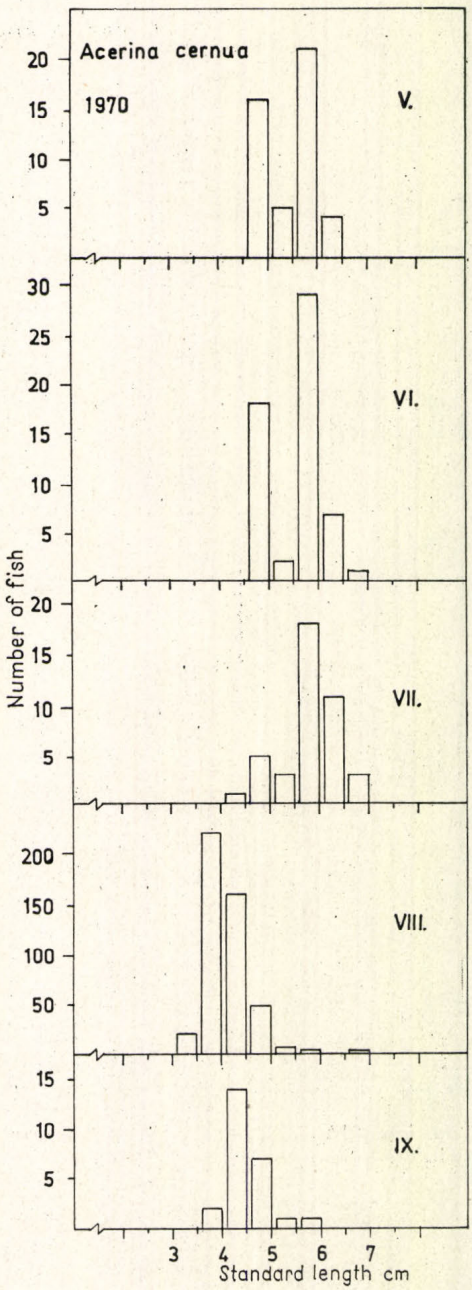

Fig. 5. The size distribution of ruff *(Acerina cernua)* in the food of pike-perch in 1970

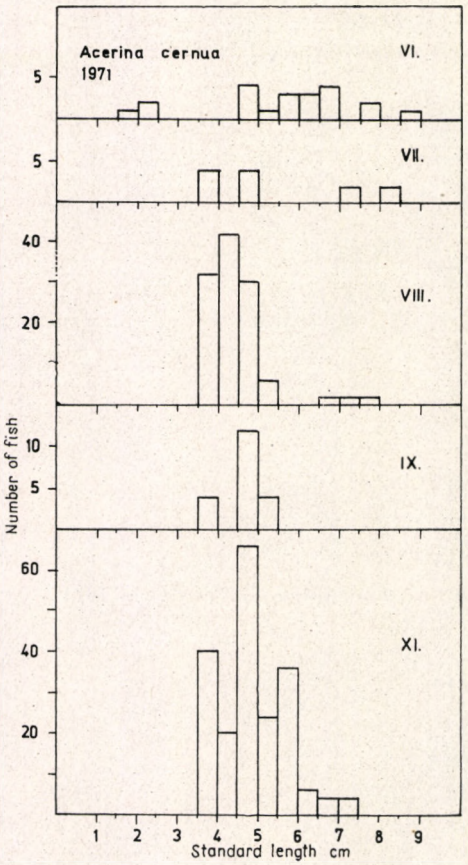

Fig. 6. The size distribution of ruff *(Acerina cernua)* in the food of pike-perch in 1971

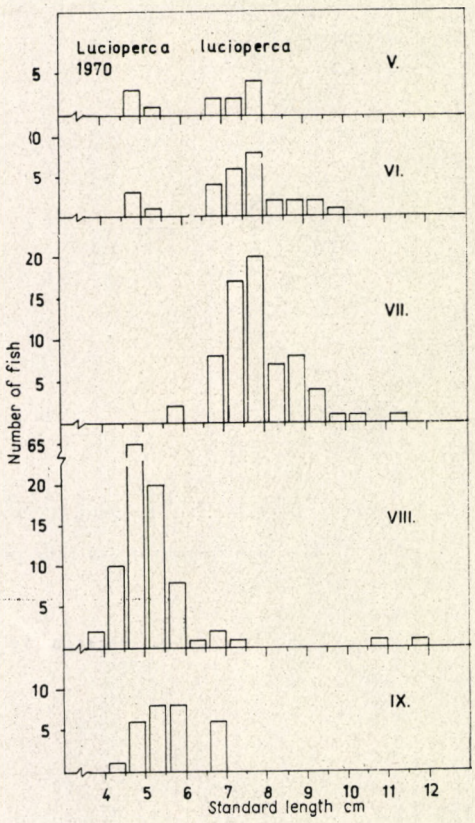

Fig. 7. The size distribution of pike-perch fry *(Lucioperca lucioperca)* in the food of older pike-perches during different months of 1970

The number of consumed prey-fishes

The majority of the pike-perch stomachs (56 percent) contained 1—2 fish in 1970, whereas those containing 2—3 fish were less frequent. Only in August could be observed an abrupt increase of prey-fishes maximally up to 15 per stomach because of the small sizes of the fry *(Fig. 15)*. In June 1971 the maximal number of fish consumed by one pike-perch was 28 and in November was 25 *(Fig. 16)*. According to our experience, the number of consumed fish depends on the mass appearance of the fry of a species of suitable size. The population density of food-fishes and their occurrence in the stomachs of pike-perches are in direct connection, indicating obviously the seasonal character of nutrition of pike-perches in Lake Balaton.

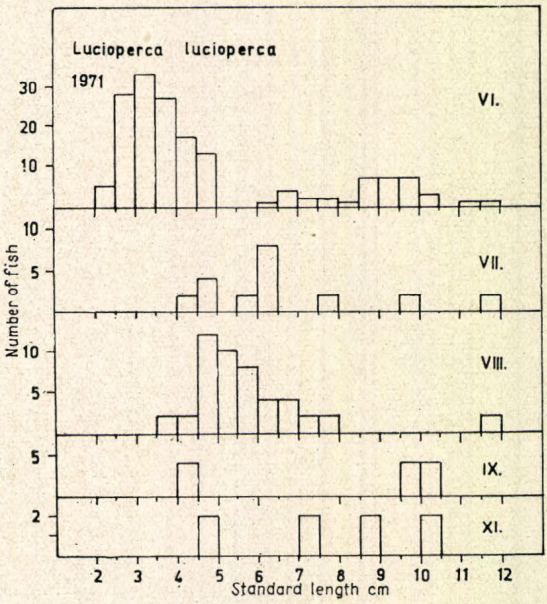

Fig. 8. The size histogram of pike-perch fry *(Lucioperca lucioperca)* in 1971

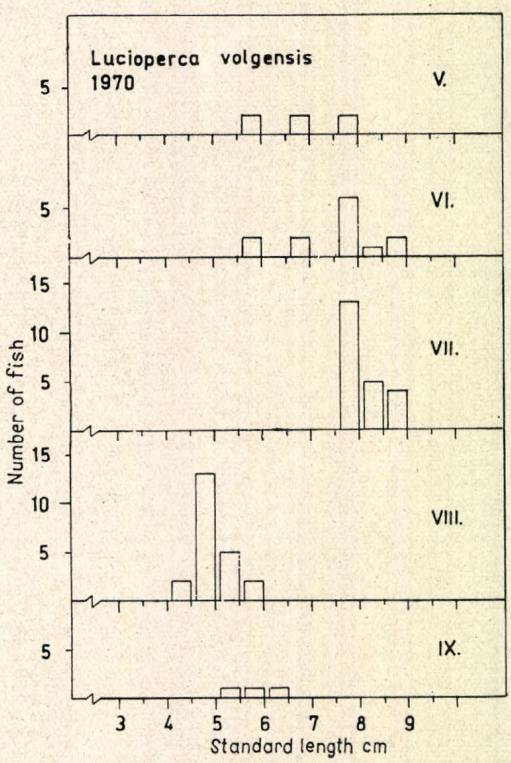

Fig. 9. The size distribution of *Lucioperca volgensis* in the stomach-content of pike-perches in 1970

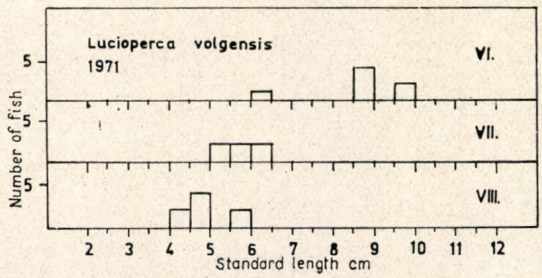

Fig. 10. The change of sizes of *Lucioperca volgensis* in the stomach-content of pike-perches during 1971

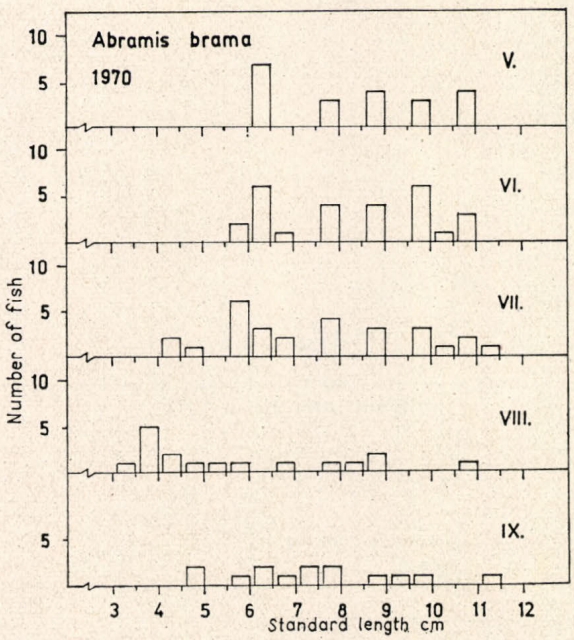

Fig. 11. The change of sizes of bream *(Abramis brama)* in the food of pike-perches during different months in 1970

The observed weight of stomach-content

In 1970 the average food per pike-perch was 6.3 g, and the average per one stomach containing food was 7.8 g *(Fig. 17)*. In 1971 these data were 4 and 8.3 g, respectively *(Fig. 18)*. Those data give only some orientation, since they are not in connection with the digestion rate.

The distribution of the number of individuals as well as the percentage of pike-perches within the stomach-content weight-groups showed a pattern similar to the distribution of the number of food-fishes: the mass of stomach-contents was 0.1—5.0 g in nearly 46 percent, 5.1—10 g in 30 percent, and larger weights of stomach-contents were found only in a less number of pike-

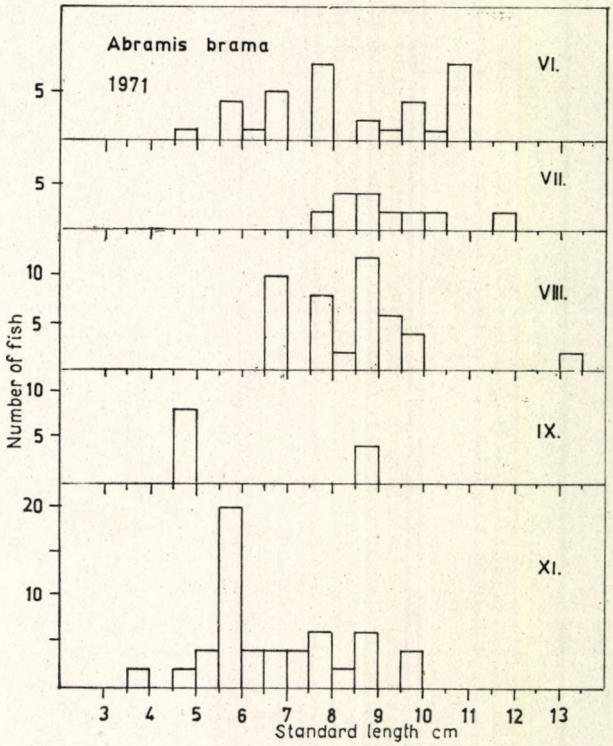

Fig. 12. The change of sizes of bream *(Abramis brama)* in the food of pike-perches during different months in 1971

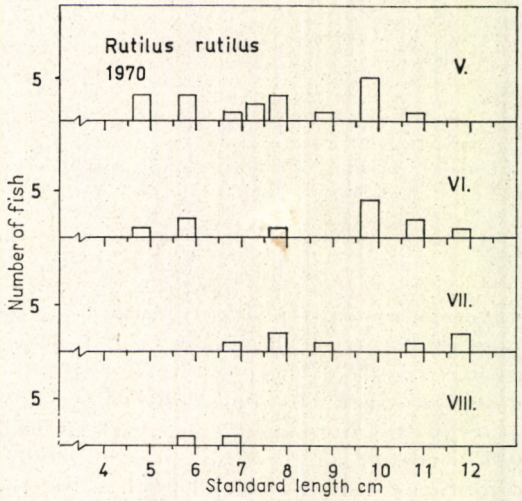

Fig. 13. The size distribution of roach *(Rutilus rutilus)* in the food of pike-perches during 1970

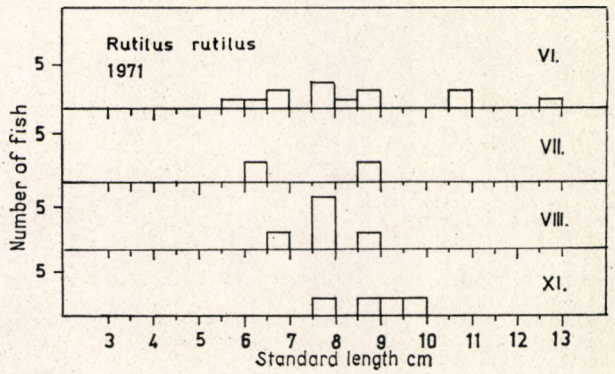

Fig. 14. The size distribution of roach *(Rutilus rutilus)* in the food of pike-perches during different months in 1971

perches *(Figs. 19* and *20)*. The distribution of the number of individuals within the stomach-content weight-groups, the averages of the groups, the numerical values of deviation and variation coefficient are near to each other showing a similar trend during the different months. The values in 1971 were rather the same, however, the number of pike-perches eating 10—20 g increased. Nevertheless, the deviation from the average in every weight-group of stomach-contents was a much lower value as compared to that of the previous year. With the increase of actual weight of the stomach-contents the number of pike-perches decreases. Only a few pike-perch may have opportunity to consume an amount of fishes reaching 10 percent of the average body weight for a prolonged time because of the seasonal changes of the population density of the food-fishes.

Digestion rate and feeding intensity

The increase of the average temperature of the water results in a more rapid digestion, accompanied by an increase of the intensity of nutrition and more frequently repeated incorporation of food *(Table III)*. The rate of digestion (the time necessary for the emptying of the stomach) and the average, monthly number of food incorporations are closely, significantly correlated ($P < 0.001$).

Food consumption

The food consumption estimated on the basis of method of BAJKOV (1935) and FORTUNATOVA (1950), related to the rate of digestion, indicates a low level of food turnover in the weight group investigated. The daily and monthly food consumption depending on the temperature and rate of digestion are low, in 1970 they amounted to 3.2—3.9 and 52—61.4 g, respectively, in average. The amount of food consumed during the period of investigations was 307 and 260 g per one pike-perch *(Fig. 21)*. An increased food consumption was observed during June and August, whereas during May, July and September food consumption decreased. The results obtained during 1971 strongly differ from those of the previous year in so far as the daily and monthly food

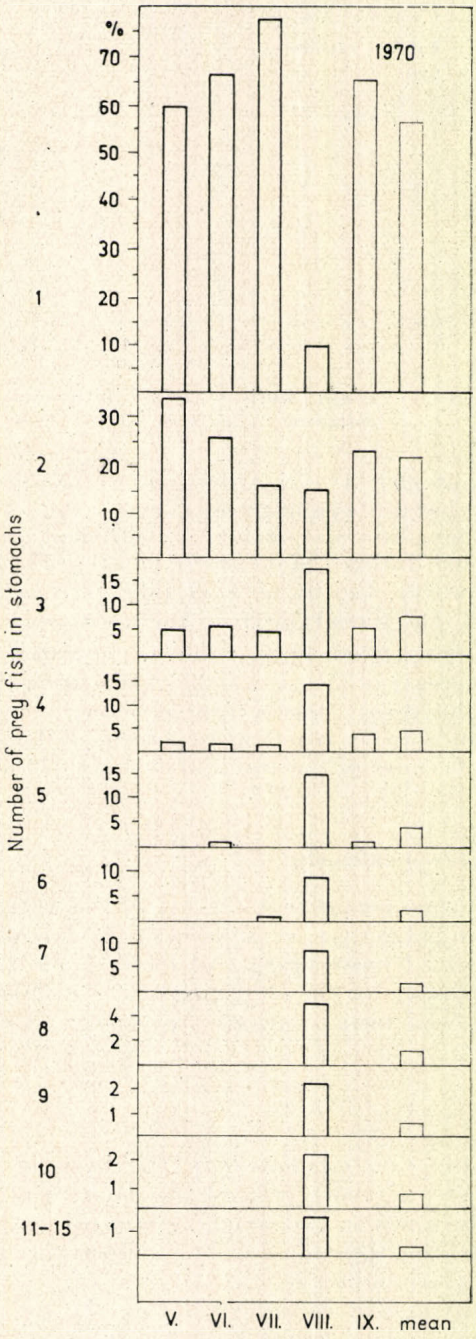

Fig. 15. The percentual distribution of pike-perches of 300—500 g body weight according to the number of fish-remains found in their stomachs during different months in 1970

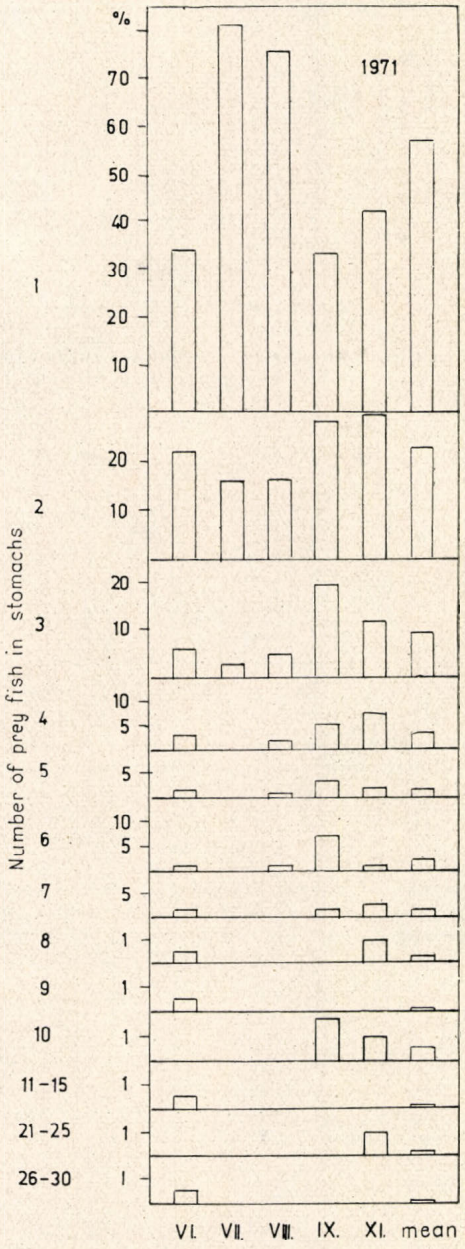

Fig. 16. The percentual distribution of pike-perches of 300—500 g body weight according to the number of fish-remains found in their stomachs during different months in 1971

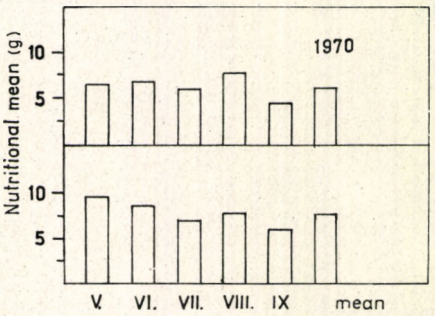

Fig. 17. The amount of food per one pike-perch stomach (upper part) and per one stomach containing food (lower part, in g) during 1970

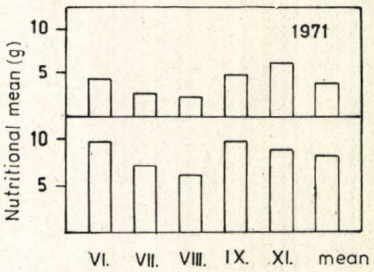

Fig. 18. The amount of food in g per one pike-perch stomach (upper part) and per one food-containing stomach (lower part) in 1971

TABLE III

The rate of digestion depending on the monthly average temperature of Lake Balaton as well as the average frequency of food uptakes during different months

Year	Month	T	V	y	i
1970	May	15.5	2.75	66.2	11.2
	June	20.7	1.85	44.5	16.2
	July	21.1	1.79	43.0	17.3
	August	22.7	1.63	39.2	19.0
	September	18.4	2.17	52.3	13.8
1971	June	20.3	1.90	45.7	15.8
	July	21.9	1.72	41.2	18.0
	August	23.7	1.54	36.9	19.5
	September	15.8	2.69	64.6	11.5
	November	8.1	6.74	161.8	4.6

T = average water temperature (°C); V = time of digestion in days; y = time of digestion in hours (= duration of emptying of the stomach); i = number of food uptakes per month. The data are calculated on the basis of equation describing the logarithmic relationship of temperature and digestion according to MOLNÁR et al. (1967):

$$y = -1.3759 \cdot \log T + 3.4589$$

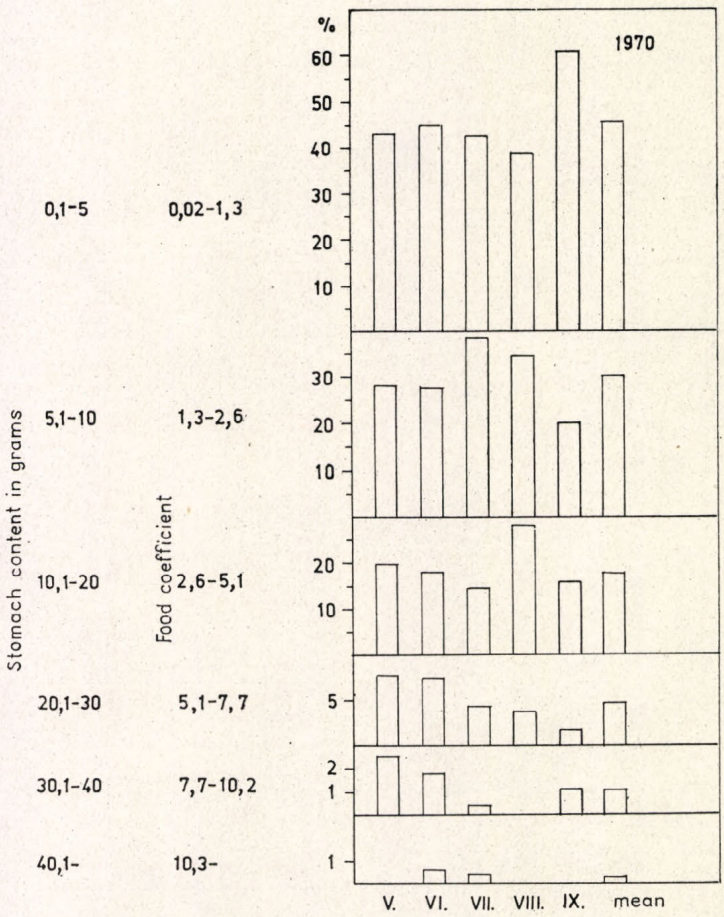

Fig. 19. The percentual distribution of pike-perches of 300—500 g body weight according to the total weight of the stomach-contents as well as to the value of food-coefficient in 1970

consumptions displayed an almost continuous decrease from June till November *(Fig. 22)*. The daily consumption reached 2.2—3.6 g, the monthly 28.7—56.5 g, thus, the amount of food consumed by one pike-perch during the five months of the investigations can be estimated as 144—283 g. There is also a close, significant correlation between the temperature of water and the amount of food consumed. The reason of decreased food consumption observed in 1971 is not known, presumably some environmental changes were responsible.

Daily and monthly rations

The daily and monthly food rations are low. The evaluations by the two methods resulted in 0.82—0.99 percent and 13.34—15.74 percent, respectively for 1970. Their dependence on the temperature is highly significant. Nearly

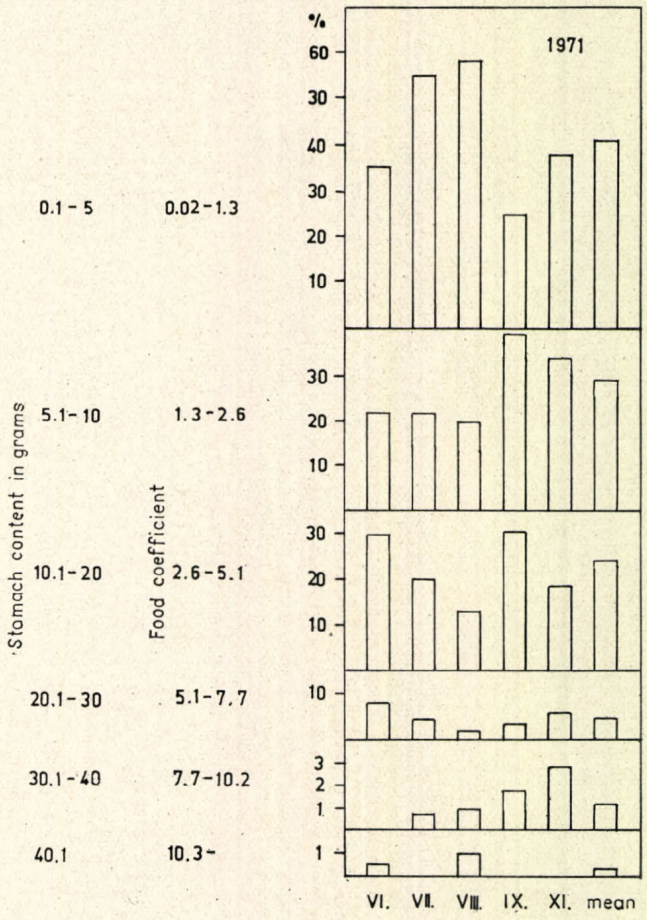

Fig. 20. The percentual distribution of pike-perches of 300—500 g body weight according to the total weight of the stomach-contents as well as to the values of food-coefficient in 1971

identical but low values were calculated during May and September, July showed intermediary values, then higher but almost identical ones were found in July and August *(Fig. 23)*. During the five months of investigations in 1970, the amount of food consumed by one pike-perch reached only 67—79 percent of the average body weight.

In 1971 the decreasing tendency of daily and monthly food rations were characteristic similarly to the food consumption. They were 0.4—0.9 percent daily and 7.35—14.49 percent monthly in average, and obviously are extremely low, since one pike-perch could consume only 37—72 percent of its own body weight during the period of June—November (except October) *(Fig. 24)*. The estimated food consumption and its rate to the average body weight evidence that pike-perches of 300—500 g weight group representing the majority

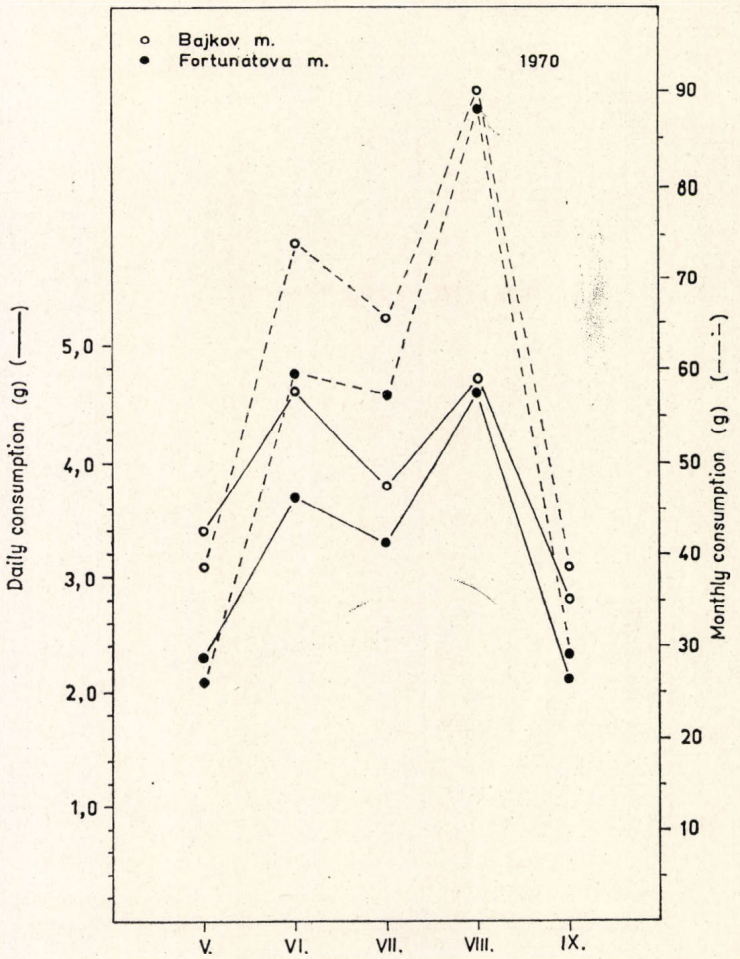

Fig. 21. The daily and monthly food consumption of a pike-perch of 390 g average body weight, in g during 1970, calculated on the basis of rate of digestion using the method of BAJKOV (1935) and FORTUNATOVA (1950)

of the catcheable portion of the population in the lake are underfed. This part of the pike-perch stock is usually represented by 3—4 or ofter 5-year-old specimens.

Discussion

Summing up the results of a series of investigations spread over several years, one can establish that the food of pike-perch in Lake Balaton is reprezented of 10—15 species of fish varying according to season. In the stomachs 2—3 species appear in masses. Among the latter the significance of bleak gradually increased against ruff forming an almost decisive majority in the food during the former years (WOYNÁROVICH, 1959). Occasionally extensive cannibalism

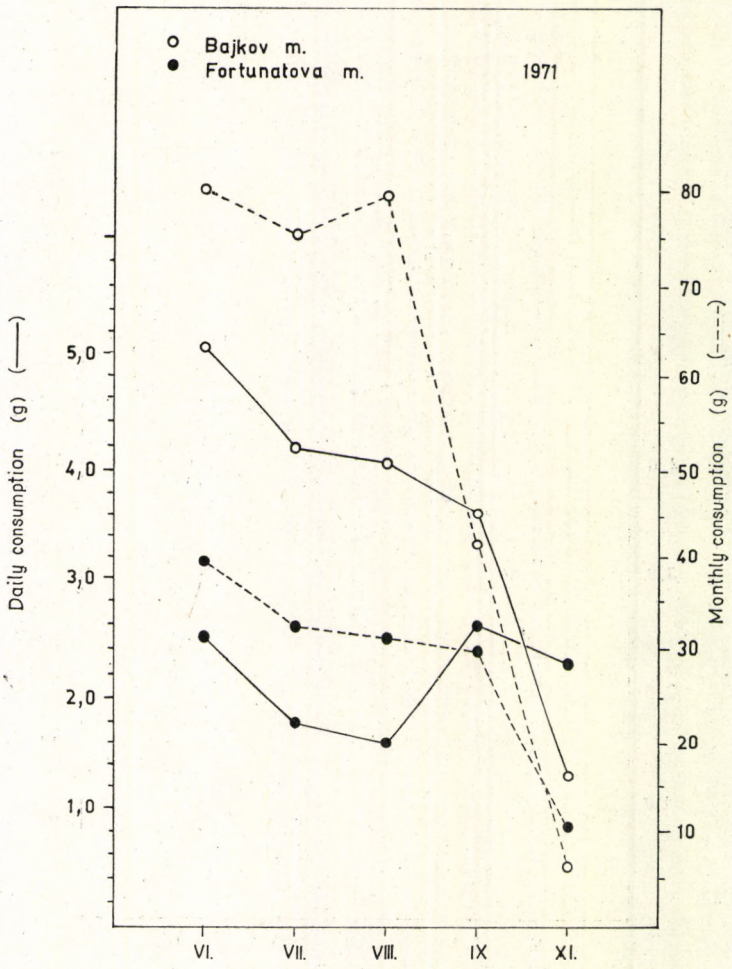

Fig. 22. The daily and monthly food consumption of a pike-perch of 390 g average body weight, in g during 1971, calculated on the basis of rate of digestion using the method of BAJKOV (1935) and FORTUNATOVA (1950)

had been observed among the pike-perches belonging to the size groups investigated, the reason of which can be searched in the decrease of the population density of fish species living near the bottom and the pike-perch fry living in the same region are available for the older predators. The increase of the food-fish species also indicates that the population density of the food-fishes decreased and the pike-perches take food from the littoral zone, the upper and deeper areas of the open water and from the areas of water-weed, too. The appearance of five new species in the food cannot be neglected. They are: *Leucaspius delineatus*, *Tinca tinca*, *Carassius carassius*, *Anguilla anguilla* and *Neogobius fluviatilis*. The last one abruptly propagated in Lake Balaton in 1970 (BIRÓ, 1972a) and gradually became significant for pike-perch. The remains of mussels and water-weed fragments frequently found in the stomachs

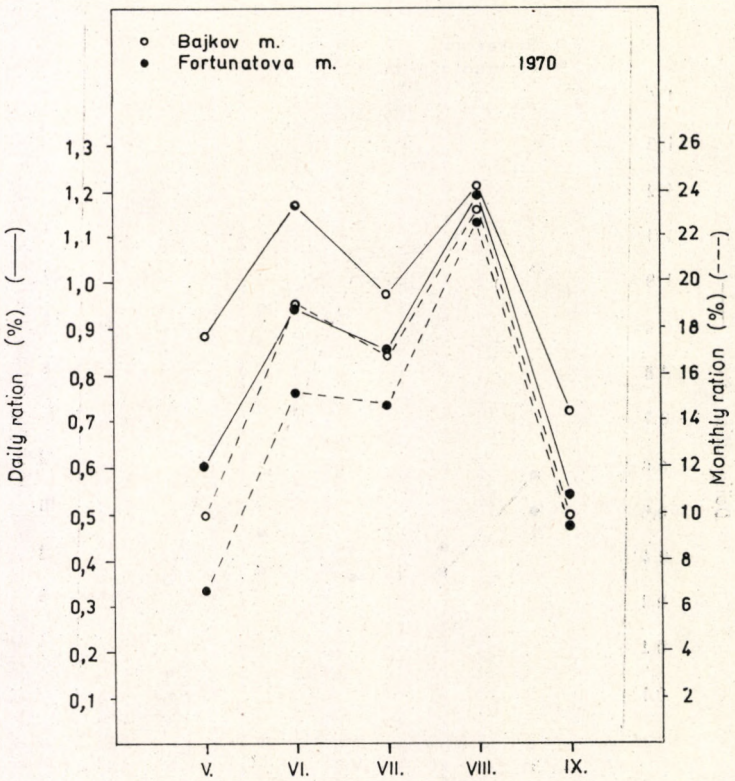

Fig. 23. The daily and monthly rates of food of a pike-perch of 390 g average body weight during different months in 1970

could passively have got in during predation. The increase of the number of Cyprinids living in the littoral zone and near the surface in the food as well as the yearly changing water-level can be brought into close relation with each other.

Surveying the papers analyzing the food of pike-perch in Lake Balaton, one can see that the highest frequency was reached by the ruff *(Acerina cernua)* and the pike-perch fry *(Lucioperca lucioperca)*, furthermore the rates of bleak *(Alburnus alburnus)* as well as sichel *(Pelecus cultratus)* were also considerable (LUKÁCS, 1932a; 1932b; ENTZ and LUKACSOVICS, 1957; WOYNÁROVICH, 1959). The quality of the food strongly changed after 1965, since beside the Percids the bleak became of an ever increasing significance (BIRÓ and ELEK, 1969) further supported now by the investigations of the years 1970—71.

The seasonal changes of the composition of food are induced first of all by the availability and density of fry of the food-fishes. Sometimes extensive cannibalism can be observed among the pike-perches of Lake Balaton which may indicate the absence of other forms of food, and the high mortality rate observed among the fry in the first summer (BIRÓ, 1972b) can be some kind of an explanation to it. The pike-perch population maintains a self-regulation

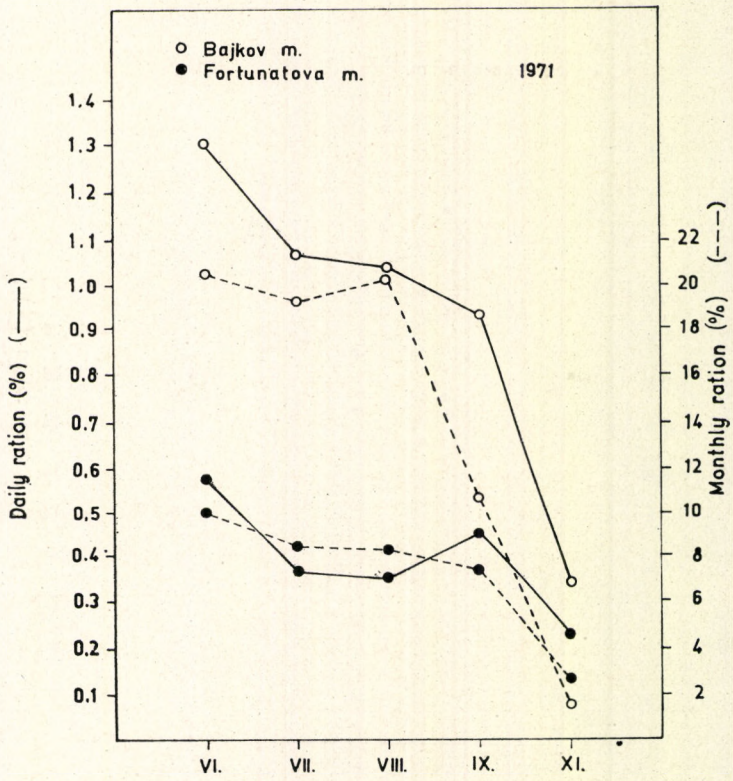

Fig. 24. The daily and monthly rates of food of a pike-perch of 390 g average body weight during different months in 1971

by consuming their own fry and regulates the other prey-fish populations to a variable extent by selecting the groups of sizes preferred (FORTUNATOVA, 1957). The niche of pike-perch within the ecosystem of Lake Balaton can be interpreted principally through this effect.

The change in the rates of pike-perches with empty stomach and with food must be analyzed with reservations, since the actually empty stomach in itself does not wholly indicate an insufficiency of the food. It is more likely a physiological necessity, since the digestion depends on the temperature of the water and the latter determines the feeding intensity even in the case of good food supply. LUKÁCS (1932a) found the stomach to be empty in 27 percent of the animals, ENTZ and LUKACSOVICS (1957) observed the same in 22—75 percent, whereas WOYNÁROVICH (1959) reported on a yearly average of 37 percent. Extensively variable rates were observed between 1965 and 1971 by us, the rate of pike-perches with actually empty stomach varied between 19 and 59 percent (c.f. BIRÓ and ELEK, 1969). WOYNÁROVICH (1959) concluded on the basis of the high number of empty stomachs that pike-perches starve and if we neglect the physiological significance of the empty stomach, even our data indicate the insufficiency of the food to a greater extent than a decade before.

Apart from the quality of the food, the quantitative comparisons revealed a more disadvantageous direction of changes. The daily food consumed depending on the rate of digestion reaches as a rule 1 percent of the body weight of the pike-perch, and what is more in 1971 it was below this level. The daily food amount of pike-perch varied between 1—4 g in the majority of cases, representing an insufficient quantity. The slow growth is a consequence of the undernourishment, observed both in fry and in older fish (BIRÓ, 1970; 1972b).

The investigations on young pike-perches of 0.19—13.3 g body weight (POLTAVCHUK, 1965) revealed a food coefficient 3.2—3.9 (average: 3.5) remaining unchanged between 11 and 26° C. ZAMOJSKA (cit. BACKIEL, 1971) investigated the age group 0+ kept in cages in temperated fish-ponds of 23—28° C and found a coefficient of 2.8—5.9 without temperature dependence. IVANOVA (1968) also studied pike-perches using the method of FORTUNATOVA and found the following values: 1.4 at age groups 2+ and 4+; 5.1 at age groups 5+ and 7+. The value of the food coefficient changes with the age however, it is also bears relation with the body size (BACKIEL, 1971). In our investigations the daily amount of food was 1—4 g. The time of digestion of that amount depends also on the quality of the food (WINDELL, 1966; 1967; POPOVA, 1967; BARRINGTON, 1957; HUNT, 1960).

Considering either the daily or the monthly values of food consumed by the pike-perch of Lake Balaton, one has to conclude that they are underfed. Comparing this fact with the eutrophication of the lake, it is difficult to explain why the food of pike-perches has decreased since the investigations of WOYNÁROVICH (1959). The explanation lies in the drop of the populations of food-fishes already described (BIRÓ and ELEK, 1969). The insufficiency of miscellaneous food is indicated by the second-third place occupied by the pike-perch fry in the stomach-contens of older pike-perches. Thus, the food-competition becomes sometimes of high significance. Competition for the pike-perch fry with other fish-species become sharper in the regions of 100—300 m width near the shore. On the basis of all these considerations, it is justifiable to assume that the density of food diminished owing to pollution and toxic substances further decreases and an increasing interspecific competition is at issue. Our observations regarding the food of pike-perches support the earlier findings related to the growth of this species (BIRÓ, 1970).

Summary

The stomach-contents of 3347 pike-perches of 300—500 g body weight were analyzed during 1970 and 1971. The food is composed of 10 fish species to a seasonally variable extent. Among them only 2—3 play a decisive role owing to their frequency of occurrence. They are the bleak *(Alburnus alburnus)*, the ruff *(Acerina cernua)* and the pike-perch fry *(Lucioperca lucioperca)*. When the fry appear, the food consists almost exclusively of them. The most commonly fed size-groups of each food-fish species can clearly be determined from the sizes of fishes found in the stomachs.

The stomachs of pike-perches investigated as a rule contained 1—2 fish, apart from those having an actually empty stomach. The number of fish consumed depends among others on the mass appearance of fry of suitable sizes.

The value of food coefficient in the majority of cases was 0.02—2.6 (76 percent), and values between 2.6 and 5.1 were encountered in about 17 percent of the predators. The food consumption is insufficient, since the daily amount of food is only 1—4 g. Accordingly, also the food rate is very low, hardly reaching the value of 1 percent of body weight during both years.

Five new species of fish were found in the food of the pike-perch of Lake Balaton which were not consumed formerly. Among them the *Neogobius fluviatilis* may later achieve greater significance because of its mass appearance and propagation observed during recent years.

REFERENCES

Backiel, T. (1971): Production and food consumption of predatory fish in the Vistula River. — *J. Fish. Biol.* **3,** 369—405.

Bajkov, A. D. (1935): How to estimate the daily food consumption of fish under natural conditions. — *Trans. Amer. Fish. Soc.* **65,** 288—289.

Barrington, C. J. W. (1957): The alimentary canal and digestion, pp. 109—161. — In: Brown, M. E. (Ed.) *The Physiology of Fishes, Vol.* **I.** *New York, Acad. Press Inc.*

Berinkey, L. (1958): The osteology of *Lucioperca lucioperca* and *Lucioperca volgensis.* — *Annal. Hist.-Natur. Mus. Nat. Hung. Ser. nov.* **9,** 313—329.

Berinkey L. (1966): Halak — Pisces. — *Fauna Hung.* **79.** *Akadémiai Kiadó, Budapest.*

Biró, P., Elek, L. (1969): The spring and summer nutrition of the 300—500 g pike-perch *(Lucioperca lucioperca* L.) in Lake Balaton in 1968. I. Data bearing relation to the nutritional conditions succeeding the destruction of fish in 1965. — *Annal. Biol. Tihany* **36,** 135—149.

Biró, P. (1969): The spring and summer nutrition of the 300—500 g pike-perch *(Lucioperca lucioperca* L.) in Lake Balaton in 1968. II. The calculation of the consumption, daily and monthly rations. — *Annal. Biol. Tihany* **36,** 151—162.

Biró, P. (1970): Investigation of growth of pike-perch *(Lucioperca lucioperca* L.) in Lake Balaton. — *Annal. Biol. Tihany* **37,** 145—164.

Biró, P. (1972a): *Neogobius fluviatilis* in Lake Balaton — a Ponto-Caspian goby new to the fauna of central Europe. — *J. Fish. Biol.* **4,** 249—255.

Biró, P. (1972b): First summer growth of pike-perch *(Lucioperca lucioperca* L.) in Lake Balaton. — *Annal. Biol. Tihany* **39,** 101—113.

Dyk, V. (1956): Naše ryby. — *Ceskoslovenska Akad. Věd. Praha.*

Entz, B., Lukacsovics, F. (1957): Vizsgálatok a téli félévben néhány balatoni hal táplálkozási viszonyainak megismerésére. — *Annal. Biol. Tihany* **24,** 71—86.

Fortunatova, K. R. (1950): Фортунатова К. Р.: Биология питания Scorpaena porcus L. — *Тр. севастопол. биол. Ста.* **7,** 193—235.

Fortunatova, K. R. (1957): Фортунатова К. Р.: Некоторые данные о влиянии хищников на размерный состав популяции рыб. — *Зоол. журнал* **36:** 575—586.

Hunt, B. (1960): Digestion rate and food consumption of Florida gar, warmouth and largemouth bass. — *Trans. Amer. Fish. Soc.* **89,** 206—210.

Ivanova, M. N. (1968): Иванова М. Н.: Пищевые рационы и кормовые коэффициенты хищных рыб в Рыбинском водохранилище. — *АН СССР, Инст. Биол. Внутр. Вод, Труды* **17 (20):** 180—198. *Сборник выд.* Б. С. Кузин, *Изд. «Наука»-Ленинград.*

Lukács K. (1932 a): A balatoni fogasról. — *Term. Tud. Közl.* **64,** 3.

Lukács K. (1932 b): A Balaton halainak gyakoriságáról. — *Magy. Biol. Kut. Munk.* **5,** 17—27.

Molnár, Gy., Tamássy, E., Tölg, I. (1967): The gastric digestion of living predatory fish, pp. 135—149. — In: Gerking, S. D. (Ed.) *The Biological Basis of Freshwater Fish Production, Blackwell Sci. Publ. Oxford.*

Poltavchuk, M. A. (1965): Полтавчук М. А.: Биология и разведение днепровского судака в замкнутых водоемах. — *АН СССР, «Наукова Думка», Киев,* 1—257.

Popova, O. A. (1967): The predator-prey relationship among fish, pp. 359—376. In: Gerking, S. D. (Ed.) *The Biological Basis of Freshwater Fish Production, Blackwell Sci. Publ. Oxford.*

SEBESTYÉN O. (1967): A kemizáció kihatása vízi ekoszisztémákban. — *MTA V. Oszt. Közl.* **18,** 389—391.
UNGER, E. (1931): Alter und Wachstum der zwei Zanderarten des Balaton-Sees. — *Verh. d. Int. Ver. für Limnologie* **5,** 315—430.
VÁSÁRHELYI I. (1956): Adatok a pontyfélék torokfoggal való meghatározásához. — *Borsodi Szemle* **2,** 3—16.
WINDELL, J. T. (1966): Rates of digestion in bluegill sunfish. — *Invest. Indiana Lakes Streams* **7,** 185—214.
WINDELL, J. T. (1967): Rates of digestion in fishes, pp. 151—173. — In: GERKING, S. D. (Ed.) *The Biological Basis of Freshwater Fish Production, Blackwell Sci. Publ. Oxford.*
WOYNÁROVICH, E. (1958): Ein Gerät zur quantitative Prüfung des Mageninhaltes von Raubfischen. — *Z. für Fischerei* **7,** 549—553.
WOYNÁROVICH E. (1959): A 300—500 g súlyú (IV. osztályú) süllő *(Lucioperca sandra* CUV. et VAL.) táplálkozása a Balatonban. — *Annal. Biol. Tihany* **26,** 101—120.

A FOGASSÜLLŐ *(LUCIOPERCA LUCIOPERCA* L.) TÁPLÁLÉKA A BALATONBAN

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Összefoglalás

1970—71 években 3347 db, 300—500 g súlyú fogassüllő gyomortartalmát analizáltuk. A táplálékot szezonálisan változó mértékben kb. 10 halfaj alkotja, ezek közül 2—3 fajnak van gyakorisága alapján döntő jelentősége. Ezek a küsz *(Alburnus alburnus)* vágódurbincs *(Acerina cernua)* és a fogassüllő-ivadék *(Lucioperca lucioperca).* Az ivadékhalak megjelenésével a táplálék csaknem kizárólag ezekből áll. A gyomrokban talált táplálékhalak méreteiből világosan megállapítható, hogy melyek a legáltalánosabban fogyasztott méretcsoportok a különböző fajoknál.

A tanulmányozott méretcsoportú süllők gyomra általában 1—2 db halat tartalmazott, leszámítva a pillanatnyilag üres gyomrú halakat. Az elfogyasztott halak száma — többek között — egy-egy faj alkalmas méretű ivadékainak tömeges megjelenésétől függ. A táplálék együttható értéke az esetek többségénél 0,02—2,6 közötti (kb. 76%-nál), 2,6—5,1 közötti értéket a süllőknek kb. 17%-ánál találtunk. A táplálékfogyasztás nem kielégítő mértékű, mert a megevett halak tömege naponta 1—4 g. Ennek megfelelően a táplálék arány is igen alacsony, amelynek napi értéke mindkét vizsgálati évben alig érte el a ragadozó testsúlyának 1%-át.

A balatoni fogassüllő táplálékából 5 újabb halfaj került elő, amelyeket korábban nem fogyasztott, s ezek közül nagyobb jelentőségűvé, az utóbbi években észlelt tömeges elszaporodása miatt a *Neogobius fluviatilis* válhat.

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SEASONAL CHANGE OF THE ORGANIC CARBON CONTENT OF LAKE BALATON DURING 1972

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The distribution of the organic carbon content in the upper layer of the sediment of the open water of Lake Balaton has roughly been outlined in our previous papers (PONYI et al., 1972; FRANKÓ and PONYI, 1973). It has been established that Keszthely Bay and its surrounding significantly differ from the mud of other regions of the lake. Recently the seasonal change of the organic content of the mud has been investigated. However, the method of "dry combustion" having been applied so far did not prove to be suitable for this purpose, mainly because of its high time-consumption. Among the methods of "wet combustion" we found to be the best described in No. 16 of the IBP Handbook, the application of which allows us to compare our results in the future with those obtained during the examination of other lakes.

Therefore the present paper has two aims: to present the results of organic carbon analyses carried out mainly in Keszthely Bay, and on other hand, to compare the formerly used and the new methods on the mud samples of Balaton according to necessity.

Dates, places and method of collecting

Mud samples were taken monthly from the middle of May till the middle of November 1972 from 5 points of each of the 5 standard transversal sections of the lake by means of an Ekman-Birge dredge *(Fig. 1)*. Aliquotes were taken

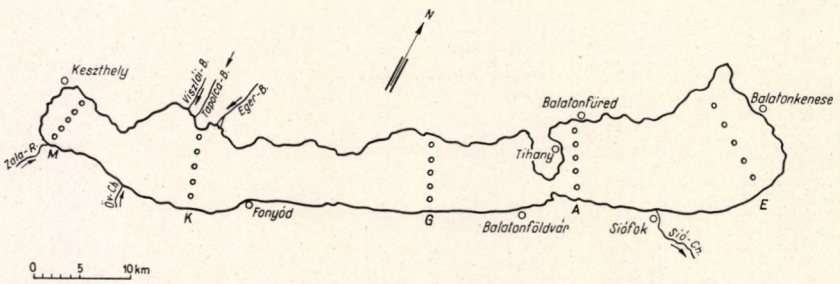

Fig. 1. Collecting places in Lake Balaton

from the upper layer of 5 cm thickness, dried at 40—50° C in a ventillated exsiccator, then powderized. The organic carbon content was determined using the method of WALKLEY and BLACK (1934) (cit. by HOLME and MCINTYRE, 1971).

Results

Mud samples taken during September 1971 were analyzed by both methods of determination of organic carbon content *(Table I)*. No essential difference was found between the two series of results, apart from the samples of Keszthely Bay being richer and more heterogeneous in organic substances, where the differences were larger. The standard errors of the means obtained from the 5 places of the transversal section originate not only in the inaccuracy of the method but also in the different structure within the section.

TABLE I

Comparison of organic carbon determinations by means of the wet combustion (WALKLEY *and* BLACK, *1934) and the dry one* (ENTZ *et al. 1963) on the samples taken from different regions of Lake Balaton during September 1971*

Section	% of organic carbon, value of chromic acid oxidation	% of organic carbon, value of dry oxidation
M (Keszthely)	2.23±0.05	4.61±1.90
K	1.83±0.03	2.00±0.04
G	1.71±0.02	1.83±0.34
A	1.52±0.08	1.70±0.33
E (Bfűzfő)	1.53±0.34	1.60±0.01

The results of a spring and autumn series of samples from 1972 clearly indicate the separation of Keszthely Bay from the other regions of the lake as regards the organic carbon content *(Table II)*. A strongly significant difference was found between section M (Keszthely) and section A (in front of the Biological Institute) ($P < 0.01$).

TABLE II

The percentual occurrence of organic carbon in the mud of 5 sections of Lake Balaton in May and September 1972

Section	May	September
M (Keszthely)	1.98±0.05	2.01±0.05
K	1.66±0.04	1.78±0.01
G	1.61±0.03	1.52±0.03
A	1.59±0.05	1.60±0.03
E (Bfűzfő)	1.52±0.02	1.54±0.01

TABLE III

Change of the organic carbon content in the transversal section of Keszthely Bay (M) during 1972

Month	% of organic carbon
May	1.98±0.05
June	1.85±0.04
July	1.84±0.03
August	1.83±0.04
September	2.01±0.05
October	1.96±0.07
November	2.27±0.09

The monthly analyses carried out in Keszthely Bay display a significant change of the organic carbon content *(Table III)*. During the warmer months (June, July and August) it varied between 1.83—1.85 percent, whereas during the colder spring and autumn months, values between 1.96—2.27 percent were found.

Discussion

The results obtained using the method of "wet combustion" for the determination of organic carbon content support the earlier findings (Ponyi et al., 1972), namely that the organic carbon content of the mud of the open water of Lake Balaton is low, furthermore, the mud of Keszthely Bay differs from other regions of the lake because of its higher organic matter content.

Essentially similar results were obtained using the former and the present method, differences occur only in the section of Keszthely Bay. Considerably wide variations were found between the 5 points of this section by means of the method of "dry combustion". The question may arise in connection with that method that the duration (15 min) of destruction by the mixture of chrome and sulphuric acids may perhaps be short for the mud samples being richer in organic substances (cf. *Table I)*. However, the treatments of the same samples of the section M for 15 and 60 min resulted in the same figures (2.21 percent after 15 and 2.30 percent after 60 min). On this basis one can assume that above 2 percent organic substance content, the results obtained by the method of "wet combustion" are more reliable than those of the "dry" method.

Apart from Keszthely Bay, the distribution of the organic substance of the mud of the open water is relatively homogeneous which can obviously be explained by the effects of wind and waves. One can mention as a characteristic instance for that the percentual frequency of distribution of the organic carbon content. Comparing the data of 5 points of each of the 20 sections dividing the points into groups being nearer to the south and the north shoreline, one can find that in a ratio of fifty : fifty either the southern or the northern values are higher. (The 20 sections used for this comparison involve all the standard sections in May and September and only section M during the other months.)

The decrease of organic carbon content observed in the mud of Keszthely Bay during the summer months and its increase during the colder period is undoubtedly connected with the amount and activity of bacteria (PONYI et al., 1972). The carbon content of the richest alga biomass in the water (TAMÁS, 1972) is negligibly low as compared to a difference of 0.2 percent of the organic matter content observed by us. The reed-grass vegetation amounts to 281 tons wet weight per year (KÁRPÁTI and VARGA, 1970) in Keszthely Bay. This and the reed detritus having not been calculated may be sufficient for the seasonal change of the organic matter content. This indicates the importance of the macrovegetation in our lake.

Summary

1. Determinations of organic carbon content obtained by means of the "dry" (ENTZ et al., 1963) and "wet" (WALKLEY and BLACK, 1934) methods of oxidation are to be used well on the mud of the open water of Lake Balaton. The former method gives higher values above 2 percent organic carbon content than the latter one.

2. Apart from the Keszthely Bay, the organic carbon content in the mud of the open water of Lake Balaton shows a relatively uniform distribution, it varies between 1.52—1.78 percent.

3. The amount of the organic carbon changes seasonally in the transversal section through the Keszthely Bay. It was 1.83—1.85 percent during summer (June—August) and 1.96—2.27 percent during the spring and autumn. The autumnal increase represents a consequence of the destruction of the macrovegetation.

REFERENCES

BUCHANAN, J. B., KAIN, J. M. (1971): Measurement of the physiological and chemical environment. — In: N. A. HOLME, A. D. MCINTYRE: Methods for the study of marine benthos, *IBP Handbook No.* **16,** 30—58.

ENTZ, B., PONYI, J. E., TAMÁS, G. (1963): Sedimentuntersuchungen im südwestlichsten Teile des Balaton, in der Bucht von Keszthely in 1962. — *Annal. Biol. Tihany* **30,** 103—125.

FRANKÓ, A., PONYI, J. E. (1973): A szén és nitrogén arányának változása a Balaton felső iszaprétegében. — *Hidrológiai Közl.* **2,** 81—84 (in Hungarian with English summary).

KÁRPÁTI, I., VARGA, GY. (1970): Results of studying the reed-grass vegetation of Keszthely-Bay. — *Publ. Agricult. Coll. Keszthely, Hungary* **12,** 67. (in Hungarian with English, German and Russian summary).

PONYI, J. E., OLÁH, J., FRANKÓ, A. (1972): Distribution of organic matter and bacteria in the upper layer of bottom deposit in the open water of Lake Balaton. — *Annal. Biol. Tihany* **39,** 144—148.

TAMÁS, G. (1972): Horizontal phytoplankton studies in Lake Balaton based on scoped samples and filtrates taken in 1967. — *Ibid.* **39,** 151—188.

VITUKI Report (1966): Interagency researches on sedimentation of Lake Balaton in 1963—64. — Ed.: SZESZTAY, K. pp. 88. *(Lithogr., in Hungarian.)*

WALKLEY, A., BLACK, I. A. (1934): An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. — *Soil. Sci.* **37,** 29—38. *(cit. ap.* J. B. BUCHANAN, J. M. KAIN, 1971).

A SZERVES SZÉN MENNYISÉGÉNEK ÉVSZAKOS VÁLTOZÁSA A BALATONBAN 1972-BEN

Frankó András és Ponyi Jenő

Összefoglalás

1. A száraz (Entz et al., 1963) és nedves oxidálás (Walkley és Black, 1934) módszerével kapott szerves szén meghatározások a Balaton nyíltvízi iszapján jól használhatók. A száraz oxidációval 2% organikus szén felett magasabb értékeket kapunk, mint nedves oxidációval.

2. A Keszthelyi öböltől eltekintve a Balaton nyíltvízi iszapjának szerves szén tartalma viszonylag egyenletes megoszlást mutat, és 1,52—1,78% között változik.

3. A Keszthelyi öbölben vizsgált keresztszelvényen a szerves szén mennyisége évszakosan változik. Nyáron (június—augusztus) 1,83—1,85, őszi és tavaszi időszakban pedig 1,96—2,27% között ingadozik.

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INCORPORATION OF 1-^{14}C STEARIC ACID AND 1-^{14}C LINOLENIC ACID INTO THE LIVER LIPIDS OF THE CARP (*CYPRINUS CARPIO* L.)

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In earlier in vitro and in vivo experiments the incorporation of 1-^{14}C palmitic acid was studied in tissues of carp (HERODEK, 1966; 1969a). Now tissue slices, prepared from carp liver were incubated with 1-^{14}C stearic and 1-^{14}C linolenic acids to investigate their incorporation into different lipids, and the effect of temperature on this process.

In rats a considerable amount of fatty acids is incorporated into diglycerides of long turnover time. (HERODEK, 1967; 1968; 1972). To see, whether such diglycerides are formed also in fish, a part of the liver slices incubated with labeled stearic acid was reincubated in inactive medium.

Materials and methods

Carps *(Cyprinus carpio* L.) weighing 1.5—2.0 kg, netted from Lake Balaton on the day of the experiment (18th November, 1971) were used. One gram of liver slices, prepared by rasor blade was incubated in 20 ml medium. This medium consisted of a Krebs—Ringer phosphate buffer, with a view to the lower osmotic pressure of the fish blood containing instead of 0.9 only 0.7 percent NaCl. To this solution 5 percent bovine serum albumine was added. Part of the solution was used in this form henceforth referred to as inactive medium. The other part was divided into two portions, and labeled stearic or linolenic acid was added to them. The concentration of fatty acids was 2 μmole/ml in both solutions.

The specific activity of the 1-^{14}C stearic acid (REANAL, Budapest) was 1, 379 mCi/mmole. The original specific activity of 1-^{14}C linolenic acid (UVVVR, Prague) was 450.0 mCi/mmole. This was diluted by inactive linolenic acid (Applied Science Laboratories) to obtain the needed amount of fatty acids. This way the end concentration of linolenic acid was 0.6 mCi/mmole. The fatty acids were saponified with a small excess of NaOH, and added to the preheated inactive medium. The solution was vigorously shaken and filtered.

Liver slices of the first four fish were divided into three groups. The first was incubated with labeled stearic acid for 30 min at 28° C. The second was incubated with labeled stearic acid for 30 min at 8° C. The third was incubated

with labeled stearic acid for 30 min at 28° C, then the slices were rinsed in inactive medium and reincubated in pure inactive medium at 28° C for two hours. Liver slices of the other four fish were incubated with labeled linolenic acid for 30 min at 28° C and 8° C respectively. During incubation the samples were gently shaken.

After incubation the slices were quickly rinsed in pure inactive medium then in Krebs—Ringer solution, weighed and homogenized in chloroform-methanol 2 : 1.

Lipids were extracted according to FOLCH et al. (1957). The extract was evaporated in Rotadeszt (KUTESZ, Budapest) apparatus under CO_2 atmosphere. Lipid classes were separated by thin layer chromatography, and eluted from the silica gel as described earlier (HERODEK, 1968). Lipids were dissolved in 10 ml toluene containing 4 percent PPO and 0.1 percent POPOP. The radioactivity was measured by USB-2 liquid scintillation detector (Biuro Urzadzen Technici Jadrovej).

Results and discussion

Labeled stearic and linolenic acids exhibited rather similar distribution in the lipid classes *(Table I)*. The only significant difference was found in the cholesterol esters, where the stearic acid incorporation was rather low. Temperature had no demonstrable effect on the distribution of fatty acids in lipid classes. On the contrary, the absolute amount of incorporated fatty acids depends on the type of the acid and on the temperature. Liver slices incorporated significantly ($P < 0.5$) more linolenic than stearic acid at both temperatures, and from both acids significantly ($P < 0.05$) more was incorporated at 28° C than at 8° C.

The effect of temperature on the fatty acid composition was demonstrated in microorganisms (PEARSON and RAPER, 1937; GAUGHRAN, 1947; CHRISTO-

TABLE I

The amount of 1-^{14}C stearic acid and 1-^{14}C linolenic acid in the lipid classes of liver slices after 30 min incubation at 28° C and at 8° C. (10^{-9} mole/g liver)

Labelled fatty acid	Stearic	Stearic	Stearic	Linolenic	Linolenic
Temperature °C	28	8	28	28	8
Note			reincubated in inactive medium for two hours		
Number of animals	4	4	3	4	4
Cholesterol esters	0.7±0.2	0.8±0.5	1.7±0.6	10.7±0.8	5.4±3.8
Triglycerides	33.4±7.1	18.1±9.4	27.4±7.2	45.7±4.7	27.3±3.0
Diglycerides	22.6±3.1	17.4±5.7	10.5±0.9	35.2±3.5	37.5±4.9
Phospholipids	26.2±5.4	14.9±4.9	47.7±16.1	21.4±1.6	16.1±1.5
Total esterified fatty acids	82.9±13.2	51.2±6.4	87.3±21.6	113.0±8.6	86.3±9.3
Free fatty acids	145.0±28.4	153.7±20.5	14.0±0.8	126.2±14.6	133.8±14.6
Total fatty acids	227.9±34.3	204.9±26.5	101.3±21.8	239.2±21.0	220.1±6.1

Mean ± standard error of the mean.

PHERSEN and KAUFMANN, 1955) plants (IVANOV, 1922), vertebrate and invertebrate animals (HENRIQUES and HANSEN, 1901; FAWCETT and LYMAN, 1954; THIELE, 1960). Among water organisms planktonic crustaceans, the most important natural food of fishes exhibited a rather expressed response on the effect of temperature (FARKAS and HERODEK, 1964; HERODEK, 1969 b). In these animals the amount of polyunsaturated acids, primarily that of the most unsaturated docosahexaenoic acid increased by decreasing and decreased by increasing temperature ensuring this way the steadily optimal physical state of the depot fat throughout the whole year. Of the fishes *Lebistes reticulatus*, *Salmo gairdneri*, *Gambusia affinis* and *Carassius auratus* when kept in colder aquarium contained more unsaturated fat, than under warmer conditions (KAYAMA et al., 1963; KNIPPRATH and MEAD, 1966 a; 1966 b; 1968). It was also demonstrated that in *Carassius auratus* the rate of biosynthesis of unsaturated fatty acids related to that of saturated acids increased at lower temperature (KNIPPRATH and MEAD, 1968). It is possible, that the temperature exerts its effect mainly in this way on the fatty acid composition.

However, there was an additional possibility if the temperature influenced to different degrees the rate of incorporation of saturated and unsaturated acids. Were the incorporation of stearic acid into the lipids more retarded at low temperature, than that of linolenic acid, it would lead to the accumulation of linolenic acid and its derivates, as the docosahexaenoic acid in the animals. By dividing the quantity of fatty acids esterified at 28° C by that esterified at 8° C in liver slices of the same fish, the mean value of the four fish and its standard error were 1.66 ± 0.21 in the case of stearic and 1.35 ± 0.15 in the case of linolenic acids. According to the means, lower temperature decreased more the incorporation of the saturated than that of the unsaturated acid, however owing to the high standard errors of the means more parallels were necessary to decide whether the fatty acid pattern is in fact influenced by the temperature in this way.

During the two-hour reincubation in inactive medium of tissue slices previously incubated with labeled stearic acid the radioactivity of diglycerides fell only to its half. Diglycerides are generally regarded as intermediates in triglyceride synthesis. They are formed from phosphatidic acid and completed by a third fatty acid to triglyceride. It was, however, found in rat tissues, that if they were incubated with labeled palmitic acid, then reincubated in inactive medium, during this second incubation the radioactivity of diglycerides fell only after one—two hours to its half value (HERODEK, 1967; 1968). Diglycerides of such long life are formed from endogenous fatty acids too, synthesized intracellularly from ^{14}C-acetate or ^{14}C glucose (HERODEK, 1972). Triglycerides must be synthesized through diglycerides of much shorter turnover time, it can therefore be supposed that two pools of diglycerides exist, in one the molecules are immediately transformed to triglycerides, in the other they persist for a longer period. The possible role of this second pool of diglycerides was discussed elsewhere (HERODEK, 1972). That they are to be found in carps similarly as in rats suggests the quite general nature of this phenomenon.

More than half of the radioactivity taken up by tissue slices was detected in the free fatty acids. While the amount of esterified fatty acids depended on the type of the acid and on the temperature, liver slices bound practically the same amount of free fatty acids from both stearic and linolenic acids at both temperatures.

In rat liver slices, incubated with labeled palmitic acid the amount of free fatty acids increased rapidly in the first 10 min, but changed little thereafter (VAVRECKA et al., 1966). Comparing the results of the present experiment, where incubation lasted for 30 min with results of an earlier experiment (HERODEK, 1966), where liver slices of carp were incubated for 10 min with labeled palmitic acid, it can be seen that the radioactivity of esterified lipids is about 3 times higher after 30 min incubation than after 10 min incubation the radioactivity of free fatty acids on the other hand increased but very little. About the same quantity of free fatty acids was bound by one gram rat and one gram carp liver. The exact binding site is not known. Free fatty acids were not removed by rapid rinsing, but during the two hour reincubation in inactive medium their radioactivity fell to a rather low level without a corresponding increase in the radioactivity of esterified lipids. It is therefore probable that the bulk of free fatty acids was released into the medium. This indicates rather extracellular than intracellular binding.

Summary

Liver slices of carps were incubated at 28° C and 8° C with 1-^{14}C stearic and 1-^{14}C linolenic acids for 30 min. From stearic acid at 28° C 82.9 $\pm$ 13.2, at 8° C 51.2 $\pm$ 6.4, from linolenic acid at 28° C 113.0 $\pm$ 8.6 at 8° C 86.3 $\pm$ 9.3 nannomole fatty acid was esterified by one gram liver. Temperature had no detectable effect on the distribution of fatty acids in the lipid classes.

Radioactivity of diglycerides in liver slices first incubated with labeled stearic acid decreased to its half only after a two-hour reincubation in inactive medium. This indicates some other role of diglycerides in addition to the participation in the rapid process of triglyceride synthesis.

The amount of free fatty acids bound by liver slices was independent of the type of fatty acid given and of the temperature, but decreased to a very low level during reincubation in inactive medium.

REFERENCES

CHRISTOPHERSEN, J., KAUFMANN, W. (1955): Spektraloptische und papierchromatographische Untersuchungen an Lipoiden und Fetten von Hefezellen bei verschiedenen Züchtungstemperaturen. — *Kieler milchwirtsch. Forschungsber.* **7,** 323—335.

FARKAS, T., HERODEK, S. (1964): The effect of environmental temperature on the fatty acid composition of crustacean plankton. — *J. Lipid Res.* **5,** 369—373.

FAWCETT, D. W., LYMAN, C. P. (1954): The effect of low environmental temperature on the composition of depot fat in relation to hibernation. — *J. Physiol.* **126,** 235—247.

FOLCH, J., LEES, M., SLOANE-STANLEY, G. H. (1957): A simple method for the isolation and purification of total lipids from animal tissues. — *J. Biol. Chem.* **226,** 497—509.

GAUGHRAN, E. R. L. (1947): Saturation of bacterial lipids as a function of temperature. — *J. Bact.* **55,** 506—507.

HENRIQUES, V., HANSEN, C. (1901): Vergleichende Untersuchungen über die chemische Zusammensetzung des tierischen Fettes. — *Skand. Arch. Physiol.* **11,** 151—155.

HERODEK, S. (1966): Comparison of the triglyceride synthesis of carp and rat in adipose tissue and liver slices incubated with 1-^{14}C-palmitic acid. — *Annal. Biol. Tihany* **33,** 151—158.

HERODEK, S. (1967): The distribution of labelled palmitic acid into the diglycerides and triglycerides of rat adipose tissues. — *Lipids* **2,** 299—302.

HERODEK, S. (1968): Temporal changes in the distribution of labelled palmitic acid in the different lipids of rat's adipose tissue, liver and diaphragm. — *Acta Biochim. et Biophys. Acad. Sci. hung.* **3,** 227—237.
HERODEK, S. (1969 a): The metabolism of intracardially injected 1-^{14}C palmitic acid in the carp *(Cyprinus carpio* L.). — *Annal. Biol. Tihany* **36,** 179—184.
HERODEK, S. (1969 b): Gas chromatographic studies on the seasonal changes in the fatty acid composition of the copepod (Crustacea) plankton. — *Annal. Biol Tihany* **36,** 173—177.
HERODEK, S. (1972): Formation of diglycerides of long turnover time form labelled acetate and glucose in rat tissues. — *Lipids* **7,** 572—575.
IVANOV, S. L. (1922): The influence of climatic factors on the physiologic-chemical characters of the plants. — *Trud. Prikl. Bot.* **13,** 483—491.
KAYAMA, M., TSUCHIYA, Y., MEAD, J. F. (1963): A model experiment of aquatic food chain with special significance in fatty acid conversion. — *Bull. Jap. Soc. Sci. Fish.* **29,** 452—458.
KNIPPRATH, W. G., MEAD, J. F. (1966 a): Influence of temperature on the fatty acid pattern of muscle and organ lipids of the rainbow trout *(Salmo gairdneri).* — *Fish. Ind. Res.* **3,** 23—27.
KNIPPRATH, W. G., MEAD, J. F. (1966 b): Influence of temperature on the fatty acid pattern of mosquitofish *(Gambusia affinis)* and guppies *(Lebistes reticulatus).* — *Lipids* **1,** 113—117.
KNIPPRATH, W. G., MEAD, J. F. (1968): The effect of the environmental temperature on the fatty acid composition and on the in vivo incorporation of 1-^{14}C acetate in goldfish *(Carassius auratus* L.). — *Lipids* **3,** 121—128.
PEARSON, L. K., RAPER, H. S. (1937): The influence of temperature on the nature of the fat formed by living organisms. — *Biochem. J.* **21,** 875—879.
THIELE, O. W. (1960): Die Lipide der Weinbergschnecke *(Helix pomatia).* Über die Neutralfette und Steroide. — *Z. Physiol. Chem., Hoppe-Seiler's* **321,** 29—37.
VAVRECKA, M., POLEDNE, R., PETRÁSEK, P. (1966): Kinetics of (1-^{14}C) palmitate uptake and incorporation into lipid fractions of liver slices. — *Biochem. Biophys. Acta* **125,** 176—178.

AZ 1-^{14}C SZTEARINSAV ÉS 1-^{14}C LINOLÉNSAV BEÉPÜLÉSE A PONTY *(CYPRINUS CARPIO* L.) MÁJÁNAK LIPIDJEIBE

Herodek Sándor

Összefoglalás

A pontyok májából készített metszeteket 1-^{14}C sztearinsavval, illetve 1-^{14}C linolénsavval inkubáltuk 8 és 28 °C-on 30 percig. Egy gramm máj sztearinsavból 28 °C-on 82,9 ± 13,2; 8 °C-on 51,2 ± 6,4; linolénsavból 28 °C-on 113,0 ± 8,6; 8 °C-on 86,3 ± 9,3 mμmol-t észterezett. A hőmérséklet nem befolyásolta a zsírsavak egyes lipid csoportok közötti megoszlásának arányát.

Ha az 1-^{14}C sztearinsavval inkubált májszeleteket inaktív közegben tovább inkubáltuk, a digliceridek radioaktivitása két óra múlva csökkent csak a felére, ami azt mutatja, hogy ennek a vegyületnek más szerepe is van, mint a gyors triglicerid szintézisben való részvétel.

A májszeletek által megkötött szabad zsírsavak mennyisége nem függött sem a zsírsav fajtájától, sem a hőmérséklettől, de nagyon csökkent az inaktív közegben való reinkubálás során.

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PRIMARY PRODUCTION IN THE FROZEN LAKE BALATON

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The characteristics of the aquatic environment are deeply changed by the ice cover, isolating the water body from the athmosphere. It prevents the formation of waves, the exchange of gases and alters the light conditions. The changed light conditions exert an influence in the first place on the primary production.

In shallow lakes besides that of the planktonic algae the production of benthic algae is also of importance (HARGRAVE, 1969; HUNDING, 1971; HICKMAN, 1971). According to ENTZ (1954), FELFÖLDY (1963) and OLÁH (1972) considerable algal biomass and chlorophyll are to be found at the bottom of Lake Balaton. This mass is probably the highest in winter, when the mud surface is covered by a brownish algal carpet visible to the naked eye. On the basis of the investigations in the early fifties ENTZ (1954) estimated the mass of microphytobenthos in winter to be 10 g/m^2. On the production of algae under the ice cover only a single information is available, in January—March 1956 in six weeks 4.6 mg/litre O_2 increase was detected in the water under the ice-cover by ENTZ and LUKACSOVICS (1957).

This paper offers data, collected in the winter of 1972—1973 on the light conditions, chlorophyll content and primary production of phytoplankton and phytobenthos and on the oxigen content of water under ice.

Methods

The investigations were carried at 500 m eastwards from the Institute in the pelagic zone of the lake. Light was measured by Gemware submarine photometer. The vertical distribution of the μ-algae was determined by the method of DENOYELLES (1968). The chlorophyll was measured according to STRICKLAND and PARSONS (1968).

Production of the phytoplankton. To measure the primary production of the phytoplankton 100 ml samples were taken from 25, 100, 200, and 300 cm depths. After adding 20 μCi $Na_2{}^{14}CO_3$, they were in situ exposed from 10^h to 14^h. The bottles, lowered to different depths were tied to the end of a Z shaped iron rod. The lower, horizontal part of this rod reached as far as 150 cm under the intact ice cover. The small hole cut in the ice was covered

by snow. This way the samples got into natural light conditions. Further handling of samples, radioactivity measurement and calculations were carried out as described earlier (HERODEK and TAMÁS, 1973).

Production of the phytobenthos. It was measured both by ^{14}C and O_2 techniques. Mud was brought to the surface by a HARGRAVE's (1969) sampler. The mud in its original structure was covered with water from the bottom.

By ^{14}C technique glass tubes fitted with 19 mm normal grounds on both ends were used. Their diameter was 17 mm, the length between the normal grounds 100 mm. The tubes were stuck into the mud in Hargrave's sampler to get a 2 cm thick core. The water filled the tubes to the rim. The tubes were stoppered from below, and some water was drawn off just to make room for the 1 ml water, with which the isotope was introduced. Into each tube 20 μCi Na_2CO_3 was injected in a way that the label should be mixed with water above the mud as evenly as possible. The tubes were now stoppered also from above, and the glass stoppers were fixed by thin elastic tapes. The tubes serving as dark parallels were covered with aluminium sheets. The tubes fitted on stand were lowered to the bottom through a small hole which was then masked by ice. The tubes were exposed from 11^h to 14^h. After exposal the water and the upper 3 mm mud layer were separated from the bulk of the mud, which would hinder the radioactivity measurement. The water containing the upper mud layer, the labeled hydrocarbonate and the algae was filled up with inactive Balaton-water to 50 ml, and vigorously shaken, then 5 ml aliquot of it was filtered through a membrane filter. After the samples 50 ml, previously filtered inactive Balaton-water was passed through the filters, then they were exposed to the fumes of concentrated HCl for 4 min, in order to remove the contaminating labeled hydrocarbonate. The filters were dissolved in BRAY solution, and the remaining thin mud-alga film was homogenized in POTTER—ELVEHJEM apparatus. Radioactivity was measured by liquid scintillation. Sedimentation was prevented by adding 4 percent Cab-O-Sil to the scintillation liquid. The carbon uptake was calculated from the total carbonic acid content of the water and the radioactivity of algae.

In experiments by O_2 technique glass cylinders of 50 mm diameter and 200 mm length were used. The cylinders were stuck 5 cm deep into mud, obtained by HARGRAVE's sampler. The tubes containing the intact sediment core and the water above it were stoppered bubble free by rubber corks. The upper cork was provided with spill-way. Dark controls were covered by aluminium sheets. The glass cylinders fitted on stands were in situ exposed. After exposal the water was drawn off from the cylinders avoiding contact with air. The oxygen concentration was determined by the original WINKLER method and by its Na-azide modification. The same result was obtained by both methods proving that by the applied procedure reduced materials had no disturbing effect.

Ice conditions

The lake was overfrozen on the 28^{th} December, 1973. The ice was glass-like transparent except on places of drift-ice accumulation. Snowing started on the 16^{th} January and the 25 cm thick ice was covered by a 4 cm thick snow-blanket. One week later the snow thawed and on the 31^{st} January the 18 cm thick ice opaque from small holes and cracks was covered by cca.

1 cm water. Three days later the water disappeared, the ice became opaque. On the 6th February only the upper 2 cm of the ice was of continuous structure, the lower layer was in the state of desintegration. On the 8th February the ice-cover went to pieces, and on the 12th February large areas were already ice-free.

Results

Light (Table I). On the 28th December light was measured under the thin ice lamellae formed in the freezing water. The high extinction was due to the sediment, swirled up by the strong wind of the previous day. Light conditions were similar to those generally found in Lake Balaton at windy weather (FELFÖLDY and KALKÓ, 1958; ENTZ and FILLINGER, 1961; HERODEK and TAMÁS 1973). Less than 1 percent of surface light penetrated to 1 m depth. On the 17th January the 25 cm thick ice was covered by 4 cm fresh snow and 9 percent of the light got through the snow and ice. ENTZ and FILLIGER (1962) found 7 percent of the surface light intensity under the ice covered by 8—10 cm thick fresh snow. This way, in spite of the clear water, the illumination of the deeper layers was very low. The snow-free ice transmitted the half of the light and as in the rather clear water under the ice the light intensity decreased only to its one fifth, about 1/10 of the surface illumination was measured at the bottom. In the period April—September only in two cases of the 13 measurements was found as much light at the bottom as here, while in 10 cases the illumination at the bottom was less than 1 percent (HERODEK and TAMÁS, 1973). In Lake Balaton the light conditions under the snow-covered ice are worse, under snow-free ice much better than in the open lake.

TABLE I

Illumination in the ice-covered lake (lux)

Depth, cm	28. Dec. 1972	17. Jan. 1973	18. Jan. 1973	31. Jan. 1973	5. Febr. 1973	6. Febr. 1973
0	30 600	13 500	10 500	5 500	20 500	34 500
25	21 350	1 250	1 640	2 360	11 490	21 500
100	4 190	440	800	1 650	7 320	11 380
200	940	210	650	1 140	4 620	8 050
300	260	140	450	700	2 820	5 060
360 (bottom)	140	100	350	470	2 250	4 030

Chlorophyll content of the phytoplankton and the vertical distribution of μ-algae (Fig. 1). The chlorophyll content of the water, sampled under the ice cover was much lower than in samples originating from the open lake (FELFÖLDY, 1963). In samples from 25 cm depth the chlorophyll was scarcely measurable. The chlorophyll content increased with depth. Perhaps this distribution of algae is responsible for the lack of production in the water layer nearest to the ice. The low chlorophyll content under the ice indicates a poorly developed phytoplankton. At the same time the 2—6 μ large μ-algae which are otherwise not present in considerable quantities in Lake Balaton

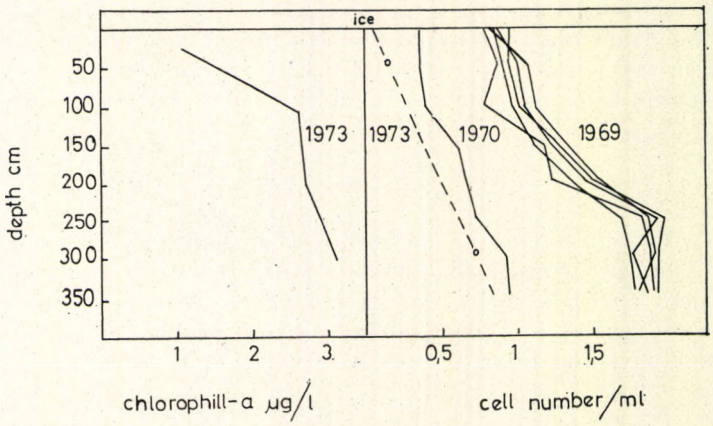

Fig. 1. Chlorophyll-a content of phytoplankton and vertical distribution of μ-algae

(OLÁH, 1970) were numerous. The surface of these tiny organisms is relatively large. They were found in the winter plankton of other lakes in great numbers (LUND, 1961; PENNAK, 1968; RODHE, 1955). Their presence indicates shortage of nutrients. The nutrients in low concentrations are better utilized by these organisms, than by larger algae. While in the unfrozen water the phytoplankton was in the average evenly distributed (HERODEK and TAMÁS, 1973) the number of μ-algae showed consequently an increase downwards during the investigations in different years.

Chlorophyll content of the phytobenthos. Only summer data were published on the chlorophyll content of the mud until now (OLÁH, 1972). In June 1972 the chlorophyll content of the 2 cm thick mud cores was 5—7 μg chlorophyll-a/g wet mud. In February 1972 the chlorophyll content was determined in mud samples from one point in front of the Institute and from three points in the Keszthely Bay *(Table II)*. Similar high values were found at all points.

TABLE II

The chlorophyll content of the microphytobenthos in February 1972
µg chlorophyll/g wet mud

	4. Febr. 1972 In front of the Institute	8. Febr. 1972 Keszthely Bay		
		point 1.	point 2.	point 3.
chlorophyll-a	46.9	40.0	43.8	38.1
chlorophyll-b	1.9	2.1	1.2	1.7
chlorophyll-c	40.9	38.5	32.4	39.6

The cores were 5 mm thick, therefore the μg/g values divided by two offer the results approximately in μg chlorophyll/cm^2. In 1973 the chlorophyll content of the mud was analyzed at two different times *(Table III)*, parallel with the measurement of the primary production of the benthos. In this case 5 mm thick cores with given surface (12 cm^2) were analyzed. This way the data

TABLE III

The chlorophyll content of the microphytobenthos in front of the Institute in the winter of 1973
$\mu g/cm^2$

	31. January		5. February	
	point 1.	point 2.	point 1.	point 2.
chlorophyll-a	8.54	6.58	15.50	15.13
chlorophyll-b	0.10	0.09	0.96	1.13
chlorophyll-c	2.64	2.22	2.38	2.55

are directly comparable with those of the primary production also related to the surface area. The chlorophyll level was high, but lower than in the previous winter. On the 5th February the chlorophyll content was higher than on the 31st January, on the other hand the parallels of the same days showed similar values. In the samples there was much chlorophyll-c, characteristic for the diatoms.

Primary production of the phytoplankton (Fig. 2) The production of phytoplankton showed different pictures at the days investigated. On the 28th December the tin ice absorbed practically no light, and in strong sunshine at 25 cm depth photo-inhibition was found. The maximum was at 1 m depth, at 2 m the production was already somewhat lower, and at 3 m it was very low owing to the insufficient illumination in the turbid water. On the 17th January in the darkness under the snow-cover the maximum was in the highest level at 25 cm, but decreased downwards. This was the lowest production per surface area found in this lake. On the 31st January, under snow-free ice, the sequence of the production of the water layers was the opposite. The highest production was observed at 3 m, it decreased at 2 m and at 1 m, and at 25 cm under the ice there was practically no production. Under the snow-free ice the production per unit of surface area was 20 mg $C/m^2/h$. It is lower, than in the warm period (HERODEK and TAMÁS, 1973) but not lower than usually in autumn.

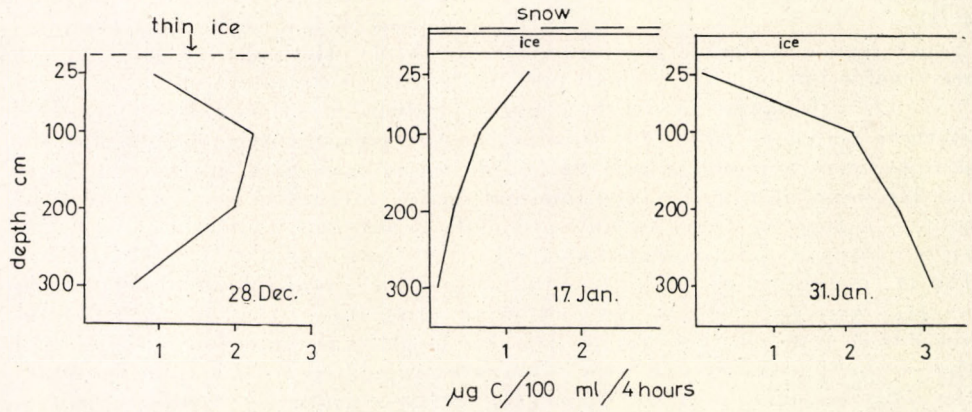

Fig. 2. Primary production of the phytoplankton

Primary production of the phytobenthos. We tried to measure the primary production of the benthos on the 26th April, and the 18th July by ^{14}C technique, and on the 13th July, 18th August and 20th September by O_2 method. No significant difference from the dark parallels was observed. Accordingly on these days there was no primary production in the benthos, or it was negligible as compared to that of the plankton. Production (463 mg O_2/m^2) was detected for the first time on the 13th October *(Fig. 3)*. Oxygen consumption of the mud per the gross oxygen production, i.e. the R/P quotient was 0.52. In the case of mud the respiration of algae amounts to the smaller part of the total oxigen consumption, the bacteria and animals are responsible for the remainder. On the 14th December the oxigen production was consider-

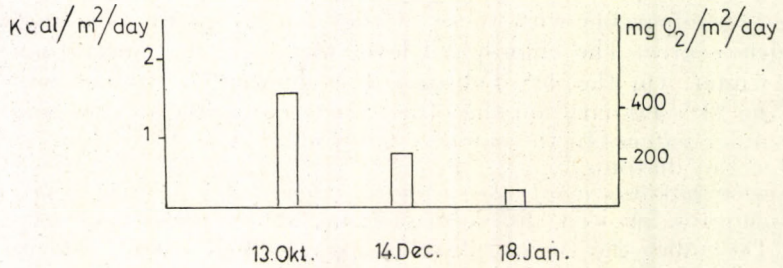

Fig. 3. Primary production of microphytobenthos in the unfrozen and the frozen, snow-covered lake. (Exposal for 24 hours)

ably lower, and already surpassed by the respiration (R/P = 1.62). After these measurements in open water the first 24 hours experiment under the ice cover was carried out on the 18th January. This day the ice was covered by 4 cm snow, and the light intensity on the bottom was 352 lux. On the previous day under similar conditions at 2 and 3 m depths there was practically no production in the phytoplankton. The gross production of the benthos, i.e. the value of the light cylinder minus the value of the dark cylinder was 66 mg O_2/m^2/day. The net production, i.e. the difference between the O_2 in the light cylinder at the beginning and at the end of the experiment was -257 mg O_2/m^2/day. It shows that by this low illumination the mud even if covered by the algal carpet decreases the oxygen content of the water. Here again algae could be responsible but only for a small part of the oxigen consumption.

After the snow thawed the primary production of the mud was measured at three occasions *(Fig. 4)*. Contrary to the previous experiments now the samples were exposed not for 24 hours, but in case of ^{14}C method for three midday hours and in case of O_2 method for five midday hours. To express the results of the two types of measurements in common units they were converted to calories supposing that 1 g C = 10 Kcal and 1 g O_2 = 3.51 Kcal. The O_2 method gave the gross production. The values obtained by the ^{14}C method were between the net and gross productions they were. 5 percent lower than the gross production. The day length was taken for 10 hours, and the production during the exposals was extrapolated to this time to obtain the daily production. The ^{14}C and O_2 methods gave rather similar results, 3.6, 4.3 and 4.0 Kcal/m^2/day on the three different days.

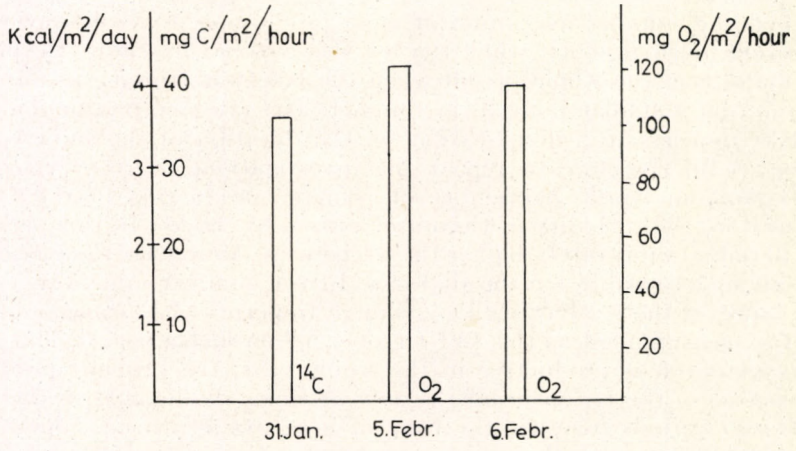

Fig. 4. Primary production of microphytobenthos in the lake covered by snow-free ice. (Exposal for the midday hours)

Oxygen content of the water. (Table IV). In the unfrozen lake the water was saturated by oxygen and became highly oversaturated when covered by ice indicating intensive photosynthesis in the frozen lake. On the 5th February the oxygen content was not higher than on the 18th January. This can be explained by the snow, covering the ice for a time in this period. In the darkness under the snow rather a decrease than an increase of oxygen content is expected.

TABLE IV

The O_2 concentration in the water of Lake Balaton

Date	Notice	Temperature	mg O_2/l
14. Dec.	no ice	5 °C	12.55
19. Dec.	no ice	4 °C	13.00
18. Jan.	ice cover	3 °C	22.59
5. Febr.	ice cover	3 °C	22.53

Discussion

The chlorophyll-a content of the phytoplankton related to surface area was 10 mg/m². This is with one order of magnitude lower than that of the benthos, where in February 1973 66—155 mg/m² was found. The primary production of the plankton and benthos, on the other hand, were similar; in case of plankton 20 mg C/m²/hour, in case of benthos 36 mg C/m²/hour. The total of the planktonic and benthic productions was 560 mg C/m²/day. In April—September the mean production of the phytoplankton was 413 mgC/m²/day, the maximal production was 588 mg C/m²/day (Herodek and Tamás, 1973). As in this warm period no primary production was found in the benthos, the total of the productions of the planktonic and benthic algae was higher in the lake covered by snow-free ice than the average in the warm months, and

approached the summer maximum of algal production. Since however there was a period, even if short, when the ice was covered by snow, the primary production during the whole period when the lake was frozen was about the same as during a similar interval in summer. The primary production of the frozen lake depends to a great extent on the duration of the snow cover, it would be useful therefore to repeat the investigations in other years, too.

The lack in April—September of primary production in the benthos is explained by the turbidity of the water caused by waves. In this period the bottom illumination is rarely higher than 1 percent. Moreover, the disturbance is by itself unfavourable for the diatoms. In the summer and even more in autumn however there are calms long enough to permit the formation of algal carpet. The measurement on the 13th October fell on such a period. Thus, while the phytoplankton is productive in the whole year, the primary production of the benthos in the unfrozen lake is only at times significant. In the frozen and unfrozen periods together the time of intensive benthic production can be estimated to roughly three months. At this time the extent of benthic primary production is similar to that of the plankton, the yearly production of microphytobenthos is therefore about one fourth of that of the phytoplankton.

The increase of oxygen concentration under the ice can serve as a measure of the production in the lake. If the 9.59 mg O_2/l increase between the measurements on the 19th December and 18th January took place after freezing of the lake on the 28th December then it corresponds to 1958 mg O_2/m^2/day or to 561 mg C/m^2/day net production. The total of the primary productions of the plankton and benthos as measured by ^{14}C technique on the 31st January was 560 mg C/m^2/day. In fact the agreement is not as tight as this since not the same "production" was measured by the two methods. The primary production measured by ^{14}C method is lower than the gross production only by the part of the newly incorporated carbon, burned by algae during the three hours of the experiment, i.e. about by 5 percent. The oxygen increase under the ice, on the other hand, was lower than the actual gross production by the oxygen consumption of all biotic and abiotic processes during the whole day.

In 3.5 m deep water the 9.59 mg O_2/l production corresponds to 33.6 g O_2/m^2 or to 11.8 g C/m^2. This carbon must exist somewhere in the form of organic material. As compared to this amount the biomass of the plankton is insignificant. Most likely the bulk of this carbon is present in the benthic algae. Dividing the 11.8 g/m^2 carbon by the 0.15 g/m^2 chlorophyll-a content of the benthic algae 78.7 is obtained. In different algae this quotient used to vary between 25 and 100. The 11.8 g C/m^2 net production corresponds to about 118 g biomass $/m^2$ which if extrapolated to the 600 km^2 surface of the lake provides 70 800 metric tons. The whole summer biomass of the lake was estimated by Entz (1954) to 30 000 metric tons. Accordingly the net increase of biomass in January 1973 was twice as high as the whole biomass in the summer of 1954. The mass of the phytoplankton and macrophytobenthos increased in the last two decades, still the biomass of the huge algal carpet, covering the bottom of the lake under favourable light conditions in winter is much larger than the sum of the masses of all planktonic algae and of the reed grasses taken all together. Owing to the algal carpet the biomass of the lake shows its maximum in winter, and this maximal value may serve as a useful index of eutophication.

Summary

Of the light one tenth was transmitted by ice covered by 4 cm snow and the half by snow-free ice. The water was rather clear under the ice, therefore in case of snow-free ice one tenth of the surface light reached the bottom. This illumination is higher than that in the unfrozen lake, where due to the turbidity caused by waves the bottom illumination is usually less than 1 percent.

The chlorophyll-a content of the water increased downwards. Related to surface it was 1 $\mu g/cm^2$. Similar vertical distribution was observed in the μ-algae, occuring in the water under the ice. The chlorophyll-a content of the bottom was 6.6—8.5 $\mu g/cm^2$ on the 31st January and 15.1—15.5 $\mu g/cm^2$ on the 5th February 1973.

When the ice was covered by snow the primary production of phytoplankton, measured by ^{14}C method, was 4 mg C/m^2/hour and decreased downwards. Under the snow-free ice it was 20 mg C/m^2/hour and increased downwards.

The production of the microphytobenthos was measured by both ^{14}C and O_2 methods. No production was found in April—September due to the turbidity of water. In the frozen lake when ice was covered by snow even the gross primary production in the benthos was very low. In case of snow-free ice intensive benthic photosynthesis was found. The primary production at three different days was 36 mg C/m^2/hour, 122 mg O_2/m^2/hour and 115 mg O_2/m^2/hour.

In the lake covered by snow-free ice the total primary production of the plankton and benthos was amounted to 560 mg C/m^2/day, which is similar to the summer maximum of planktonic production.

In the ice-covered lake the oxygen content of the water was with 9.6 mg/litre higher than before freezing, indicating the accumulation of high amounts of organic material.

REFERENCES

DeNoyelles, F. Jr. (1968): A stained-organism filter technique for concentrating phytoplankton. — *Limnol. Oceanogr.* **13,** 562—565.

Entz B. (1954): A Balaton termelésbiológiai problémái. — *MTA Biol. Orv. Tud. Oszt. Közl.* **5,** 433—461.

Entz B., Lukacsovics F. (1957): Vízi élettevékenységek tükröződése a Balaton jegében. — *Annal. Biol. Tihany* **24,** 87—91.

Entz B., Fillinger M. (1961): Adatok a Balaton fényklímájának ismeretéhez. (A víz zavarosságának okairól és kihatásairól.) — *Annal. Biol. Tihany* **28,** 49—89.

Entz B., Fillinger M. (1962): Adatok a Balaton fényklímájának ismeretéhez II. (Fényviszonyok a hóborította befagyott Balaton-vízben.) — *Annal. Biol. Tihany* **29,** 65—74.

Felföldy L. (1963): A klorofill-mérés módszertani és elvi kérdései balatoni eredményeinkkel kapcsolatban. — *Annal. Biol. Tihany* **30,** 137—165.

Felföldy L., Kalkó Zs. (1958): A víz alatti fényviszonyok és a fotoszintézis összefüggése a Balatonban, 1957 nyarán. — *Annal. Biol. Tihany* **25,** 303—329.

Hargrave, B. T. (1969): Epibenthic algal production and community respiration in the sediment of Marion Lake. — *J. Fish. Res. Bd. Canada* **26,** 2003—2026.

Herodek, S., Tamás, G. (1973): Primary production in Lake Balaton, April—September 1972. — *Annal. Biol. Tihany* **40,** 207—218.

Hickman, M. (1971): Standing crops and primary productivity of the epipelon of two small ponds in North Somerset, U.K. — *Oecologia* (Berl.) **6,** 238—253.

HUNDING, C. (1971): Production of benthic microalgae in the littoral zone of a eutrophic lake. — *Oikos* **22,** 389—397.
LUND, J. W. G. (1961): The periodicity of μ-algae in three English Lakes. — *Verh. Internat. Verein. Limnol.* **14,** 147—154.
OLÁH, J. (1970): Short periodic changes in the microbial plankton quantity of Lake Balaton. — *Annal. Biol. Tihany* **37,** 199—207.
OLÁH, J. (1972): Studies on the photosynthetic pigments and their decomposition in the sediment of Lake Balaton and Lake Belső. — *Annal. Biol. Tihany* **39,** 115—121.
PENNÁK, R. W. (1968): Field and experimental winter limnology of three Colorado mountain lakes. — *Ecology* **49,** 505—520.
RODHE, W. (1955): Can plankton production proceed during winter darkness in subarctic lakes. — *Verh. Internat. Verein. Limnol.* **12,** 117—122.
STRICKLAND, J. D. H., PARSONS, T. R. (1968): A practical handbook of seawater analysis. — *Fish. Res. Bd. Canada, Bull.* **167.**

ELSŐDLEGES TERMELÉS A BALATON JEGE ALATT

Herodek Sándor és Oláh János

Összefoglalás

A 4 cm hóval borított 25 cm vastag jégtakaró a felületére eső fény egytizedét, a hómentes jég a felét engedte át. A jég alatti víz nagyon tiszta, ezért a hómentes jég alatt az aljzat megvilágítása a jég feletti megvilágítás egytizede. Hómentes jég alatt tehát jobb az aljzat megvilágítása, mint jég nélkül, amikor a hullámok okozta zavarosság miatt az esetek többségében kevesebb mint 1% fény jut az aljzatra.

A vízoszlop klorofill tartalma 1 μg klorofill-a/cm^2 volt, és felülről lefelé nőtt. Hasonló vertikális eloszlást mutattak a jég alatti vízben megfigyelhető μ algák is.

Az aljzaton 1973 I. 31-én 6,6—8,5, I. 5-én 15,1—15,5 μg klorofill-a/cm^2-t találtunk.

Havas jég alatt a fitoplankton termelése 4 mg C/m^2/óra volt, és felülről lefelé csökkent, hómentes jég alatt 20 mg C/m^2/óra termelést mértünk, amely felülről lefelé növekedett. A fitoplankton termelését ^{14}C módszerrel mértük.

A fitobentosz termelését ^{14}C és O_2 módszerrel is mértük. Havas jég alatt a bentosz algái nem termeltek. Hómentes különböző napokon az iszap elsődleges termelése 36 mg C/m^2/óra, 122 mg és 115 mg O_2/m^2/óra volt.

Hómentes jég alatt a fitoplankton és fitobentosz együttes termelése 560 mg C/m^2/nap volt, ami hasonló a fitoplankton termelésének nyári maximumához. Nyáron a bentoszban nem tudtunk elsődleges termelést kimutatni.

A jég alatt a víz oxigén tartalma 9,6 mg/liter-rel magasabb volt, mint befagyás előtt, ami nagy tömegű szerves anyag keletkezésére utal.

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THE PRIMARY PRODUCTION OF PHYTOPLANKTON IN LAKE BALATON APRIL—SEPTEMBER 1972

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Numerous data are available on the number and biomass of planktonic algae in Lake Balaton (ENTZ et al., 1937; SEBESTYÉN et al., 1951; SEBESTYÉN, 1953; TAMÁS, 1955; 1967; 1969).

The chlorophyll content of the water (FELFÖLDY, 1963) and in the lake the photosynthesis of algae obtained from pure cultures (FELFÖLDY and KALKÓ, 1958; FELFÖLDY, 1959; 1962) were also studied. Several papers dealt with the light conditions of Lake Balaton (FELFÖLDY and KALKÓ, 1958; ENTZ and FILLINGER, 1961; 1962).

On the other hand a detailed study of the primary production of phytoplankton lagged behind. The production was too low to be measured by the O_2 technique (FELFÖLDY and KALKÓ, 1958), and our Institute was not equipped for ^{14}C measurements. Preliminary investigations by ^{14}C method were carried out in 1961 (BÖSZÖRMÉNYI et al., 1962).

We started in spring of 1972 to study the yearly cycle of the production of phytoplankton. Here the data concerning April—September are published, when the water temperature was above the mean temperature. The data of the other half-year will be published in a separate paper. The illumination, the composition, biomass and production of the phytoplankton were determined fortnightly in four depths. It is hoped that these data may contribute to the construction of the production biological model of the lake, and serve as reference point in studies on the process of eutrophication.

Materials and Methods

Investigations were carried out fortnightly irrespective of the weather. This way all meteorological factors had a probability to occur during the investigations corresponding to their frequency. The investigated point was two kilometres eastwards of Tihany. Water depth, temperature, Secchi transparency were determined at this point. The illumination was measured by Gemware Submarine Photometer (Model No. 268 WA 310) at the surface and at 25, 100, 200, 300 and 370 cm depths, the last value representing the bottom illumination. Total irradiation was measured by the Meteorological Station of Siófok. Water samples were taken in 250 ml glass flasks. Direct illumination of the water samples during further manipulations was avoided.

Of this water 100 ml was transferred into pyrex glass flasks, fitted with normal ground, made directly for this purpose by KUTESZ. They were used for the exposal. The remaining water was conserved by J_2/KJ and served algological determinations.

Algae were counted by UTERMÖHL's (1958) plankton microscope. The biomass was determined from the volume of individuals. In case of more complicated forms it was determined by modelling, while the form of other species was assumed to correspond to simple geometrical solids, and their volume was determined by calculation. Partly earlier (SEBESTYÉN, 1954; TAMÁS, 1955), partly recently determined values were used.

The methods of biomass determination were recently discussed by SCHNESEE and SCHWARTZ (1971), that of primary production measurement by HÜBEL (1971) and VOLLENWEIDER (1969).

To each sample used in primary production measurement 20 μCi $Na_2{}^{14}CO_3$ (Isotope Institute, Budapest) was added. Its specific activity was 290 μCi/mg. The samples were lowered to their original places, and in situ exposed from 10^h to 14^h. The bottles were kept horizontally (ELSTER and MOTSCH, 1966), and were suspended in such a manner that the buoy did not throw shade them. Dark parallel was always prepared. After 4 hours of exposure the samples were put in a dark box, transferred to the laboratory and filtered through a membran filter of 0.2 μ pore size (Sartorius Membranfilter GmbH).

In order to remove radioactive contamination, after the samples also 50 ml previously filtered inactive lake water was passed through the filters, then they were exposed to the fumes of concentrated HCl for four minutes. The filters were then dissolved in 10 ml Bray solution. One liter of this scintillation liquid contains in addition to dioxane 0.2 g POPOP, 4.0 g PPO, 60.0 g naphtalene, 20 ml ethylene glycol and 20 ml methanol. As the filters dissolved the algae were suspended in the liquid.

Radioactivity was measured by USB-2 liquid scintillation detector (Biuro Urzadzen Technici Jadrowej, Warszawa). Counting efficiency was determined separately for each sample by toluene-7-^{14}C internal standard (Isotope Institute, Budapest). In case of Geiger—Müller technique the samples must be dried before counting. During this procedure losses of ^{14}C content of the algae may occur up to 30% (WALLEN and GEEN, 1968). It is therefore a great advantage of the liquid scintillation technique, that it needs no dried samples.

The total carbonic acid content of the water was determined by pH measurement and by titrating three times 50 ml membrane filtered Balaton water by 0.1 N HCl against methylorange indicator. From the radioactivity of algae and the specific activity of the total carbonic acid content of the water, allowing for 5 percent isotope effect, the weight of the carbon taken up by the phytoplankton was calculated. Each value was reduced by that of the dark parallel. The results are given in this from, without further corrections. According to STEEMANN NIELSEN (1964) these values should be multiplied by 1,06 to obtain gross, and by 0.96 to obtain net production. By converting the biomass and production values to surface area the sample at 25 cm was taken as representing the water layer between 0 and 50 cm that at 1 m representing the 50—150 cm, the sample at 2 m the 150—250 cm, and the sample at 3 m the 250—350 cm layers. Accordingly, by adding the values of the lower three samples and the half value of the sample at 25 cm the bio-

mass and production per cm^2 were obtained. These values were then converted into g biomass and mg C production/m^2 respectively. The biomass of the single species in the different depths is not given separately, but for brevity's sake only the harmonic mean of the four depths is given, i.e. calculations as above, and the results divided by 3.5.

Results and discussion

Water temperature, Secchi transparency, total irradiation, surface and underwater illumination data are presented in *Table 1*. Further characteristics of experimental days:

Apr. 5. Overcast, at the beginning strong waves.
Apr. 18. Overcast, at times sunshine. Moderate wind, moderate waves.
May 5. Sunshine, calm. Barely rippling water.
May 16. Overcast, at times sunshine. Wavy water.
May 31. At first overcast, in the last two hours sunshine.
Jun. 13. Sunshine with floating clouds. Moderate wind, rippling water.
Jun. 27. Heavy storm on the previous day. Overcast, at times strong sunshine. Gently rippling surface, calm before the storm.
July 11. The night before rather heavy storm. Overcast, drizzling. Storm with very strong wind and large waves.
July 27. The day before heavy storm. Overcast. Light wind, big waves.
Aug. 10. Calm for days. Sunshine. Dead calm. Stillness. Unruffled surface.
Aug. 24. Four days storm and one day of very strong wind preceded the experiment. Sunshine, later clouds. Breeze. Unruffled surface, milky water.
Sept. 7. After a cooler period rise in temperature, calm preceded the experiment. Strong sunshine with floating clouds. Medium then soft wind. Moderate waves then unruffled surface.
Sept. 28. The day before relatively calm. Sunshine with floating clouds. Moderate wind, strong waves.

At the first experiment water temperature was 13° C, and returned to 13° C at the last one. As the mean temperature of the lake is 12° C, it can be said, that the half year above the mean temperature was investigated. This year the spring came before time, the summer was late. The water temperature attained 20° C first in June. During the heavy storm of $20-24^{th}$ August the lake cooled down very much, and even in later times its temperature did not rise above 20° C.

Lake Balaton has a large surface, thus big waves are easily formed. On the other hand the lake is shallow, therefore the mud is easily stirred up by the waves. This renders the underwater light conditions extremely unstable. For example on the 10^{th} of August, the Secchi transparency was 180 cm, and at 3 m depth the surface illumination was 20 percent, while on 11^{th} of July the Secchi transparency was only 20 cm, and at 1 m depth only 2 percent of the surface illumination could be measured. Data of other days are between these two extremes. In general, light conditions of deeper layers depend more on the turbidity, i.e. on the wind, than on the cloud cover.

Altogether 108 alga species, 5 varieties and 1 form were found in the samples, collected from four different depths at 13 different days. Their distribution between the phyla was the following: Cyanophyta 12, Euglenophyta 9, Pyrrophyta 6, Chrysophyta 50, Chlorophyta 37.

For easier survey only those 22 species are listed separately in *Table II* whose biomass attained in some days 10 mg /m³. The biomass of the other species were summed up, and indicated in the *Table* for each phylum. As *Table II* shows the plankton was dominated unequivocally by diatoms till the middle of July. In this year they were replaced only at this time by the *Ceratium hirundinella* stand. Of the diatoms *Cyclotella bodanica* was the most dominant. In the first three months investigated this single species amounted to half of the total biomass of the phytoplankton. This year *Melosira granulata* became numerous only towards the middle of summer.

Lake Balaton is rich in benthic algae, and the storm turns the lake upside down. The importance of benthic algae in the plankton compared to the euplanktonic forms has been frequently discussed. The present data show, that in clear or moderately disturbed water, i.e. in most cases the plankton is dominated by euplanktonic algae. Benthic elements do not attain one fifth of the total biomass. On the other hand during a storm this ratio suddenly increases. In a heavy storm on the 11th July the biomass of the phytoplankton was doubled by the benthic elements. Of the tychoplanktonic algae the largest biomass was given by *Surirella robusta* var. *splendida*. The biomass of Cyanophyta, Euglenophyta and Chlorophyta phyla are inferior.

The vertical distribution of the phytomass *(Table III)* was always uneven, but in the average of the phytomass values measured in the different days there are no differences by depths. Generally phytoplankton shows an even vertical distribution.

The primary production at the different depths *(Table IV)* varied much from time to time. Usually the maximal production was at 1 m, in clearer water at 2 m depths. At 3 m the production is much lower, in two third of the measurements there was scarcely any production at all. At the surface the production was usually inhibited by the excessive light. There are however significant deviations from this most frequent picture. At the 13th of June the whole water column exhibited a high production. This day yielded the highest production per surface area. The water was very clear, at 3 m 6 percent of the surface illumination was recovered. In the most transparent water on the 10th of August by 180 cm Secchi transparency and 20 percent illumination

TABLE I

Environmental factors

Date	IV. 5.	IV. 18.	V. 2.	V. 16.	V. 31.
Water temperature °C	13	13	15	16	19
Total irradiation during exposal cal/cm²	166	141	227	113	99
Total irradition in the whole day cal/cm²	346	294	519	189	226
Secchi transparency cm	42	45	51	51	55
Illumination at the surface Klux	—	62.4	66.4	40.5	8.1
Illumination in the different depths in percent of the surface illumination					
25 cm	—	50.0	64.7	59.5	63.1
100 cm	—	23.4	26.5	29.8	14.8
200 cm	—	3.9	7.3	8.3	4.2
300 cm	—	0.8	1.0	2.6	1.6
	—	—	—	--	0.9

at 3 m the higher levels were inhibited by light, and the production showed the maximum at 3 m.

In storm the situation was reversed. On the 11th of July, when the illumination at 1 m was only 2 percent, already in this layer and underneath there was no significant production. On the other hand tremendous production was measured at the surface, due to the huge amount of benthic algae, brought up by the storm *(Table II)*.

Similar, but less extreme was the situation during a storm on the 27th of July. On this day 7 percent of the surface light penetrated down 1 meter depth, enabling intensive photosynthesis. At 2 m with 1 percent illumination the production fell to a very low level.

From the means of the 13 days *(Fig. 1)* it appears that the vertical distribution of the algae is even, but the same biomass displays quite different productions in different depths. At 2 m the insufficiency of light is already apparent, while at 3 m only one fourth is produced by the same biomass than at 1 m. The means of the different levels are calculated from values of very different dispersions. At 1 and 2 m there is relatively less variation in the production from one day to the other. At the surface on the other hand the production is usually low owing to inhibition by the excess of light, while in storm it is extremely increased by benthic algae. At 3 m the results show great variability because here serious light insufficiency is caused in two thirds of the cases by turbidity, on the contrary in clear water the production is similar to that of the higher levels. Light saturation at the bottom must be even rarer.

The production as related to surface area *(Fig. 2.)* is relatively even, with values varying between 83 and 168 mg C/m²/4 hours. The differences between the values of the different experimental days are less to be attributed to seasonal changes, than to the different weather conditions of the single days. Smaller differences, resulting from changes in plankton constituents, temperature and water chemism are masked by the effects of the fluctuation in water transparency. Only the last three measurements indicate the autumnal decline of production. The highest values were obtained in the clearest water (13th June, 27th July). In storm the production related to surface area was

VI. 13.	VI. 27.	VII. 11.	VII. 27.	VIII. 10.	VIII. 24.	IX. 7.	IX. 28.
23	19	22	23	24	17	20	14
250	283	95	51	256	164	199	124
485	538	269	221	550	306	399	217
78	55	20	28	110	33	111	41
70.2	66.4	18.0	8.7	54.5	42.5	46.5	38.4
55.6	53.0	40.0	59.0	82.1	61.4	70.8	57.5
30.6	26.5	2.0	6.9	48.2	6.8	43.8	12.5
13.9	6.3	0.0	1.1	32.1	1.4	24.0	2.5
5.5	1.8	0.0	0.1	19.6	0.3	12.5	0.5
2.8	0.9	0.0	0.0	12.1	0.1	8.3	0.3

TABLE II

The biomass of the

	5. IV.	18. IV.	2. V.	16. V.	31. V.
Cyanophyta					
Microcystis flos-aquae	—	—	—	—	—
Aphanizomenon flos-aquae	—	—	—	—	—
Other species	35	3	33	33	20
Total	35	3	33	33	20
Euglenophyta					
Total	45	—	13	3	21
Pyrrophyta					
Cryptomonas erosa	—	—	3	—	—
Ceratium hirundinella	2	16	21	26	29
Peridinium inconspicuum	—	—	—	—	—
Other species	—	—	—	—	—
Total	2	16	24	26	29
Chrysophyta					
Chromulina sp.	5	—	—	1	113
Amphora ovalis	104	37	46	27	20
Cyclotella bodanica	1898	2005	1544	592	1218
Cyclotella ocellata	541	443	200	90	254
Cyclotella quadriiuncta	210	71	111	1	6
Cymatopleura elliptica	32	114	30	19	18
Cymatopleura solea	230	10	12	5	7
Diploneis elliptica	—	—	—	—	—
Melosira granulata	2	18	—	21	86
Navicula gracilis	6	—	—	4	—
Navicula radiosa	114	—	35	—	—
Nitzschia acicularis	684	292	51	17	11
Nitzschia amphibia	—	—	—	27	13
Nitzschia hungarica	45	47	62	92	30
Nitzschia sigmoidea	52	14	43	36	19
Surirella robusta	4	—	32	30	73
Surirella turgida	4	1	30	—	—
Other species	133	71	134	28	176
Total	4064	3123	2330	1242	2044
Chlorophyta					
Closterium aciculare	—	2	—	5	22
Oocystis solitaria	39	11	37	20	49
Other species	54	44	34	21	84
Total	93	57	71	46	155
Sum total of all algae	4239	3199	2471	1350	2269
g/m²	14.8	11.2	8.6	4.7	7.9

low. The benthic algae brought up by the waves can but partly compensate for the darkness of deeper regions.

The mean production during the four hours and the standard error of this mean were 118 ± 8 mg C/m². Owing to the low standard error a 20 per-cent increase in the following years detected by measurements of similar frequency could be regarded as significant difference. The average length of day time in this half-year is 14 hours. To extrapolate the production of the 4 hours of exposure to the whole day time, it was multiplied by 3.5. The mean daily production is 413 mg C/m²/day, the maximal production is 588 mg C/m²/day. According to VINBERG (1961) lakes with 300—700 mg C maximal daily production belong to the mesotrophic category. The maximal daily

phytoplankton 10^6 μ^3/l

13. VI.	27. VI.	11. VII.	27. VII.	10. VIII.	24. VIII.	7. IX.	28. IX.
—	86	—	—	257	—	3	—
—	—	3	6	32	56	199	7
7	139	119	145	103	17	86	64
7	225	122	151	392	73	288	71
36	87	46	20	34	76	26	8
34	48	43	129	47	1	90	11
138	147	341	1022	840	234	870	54
—	134	—	7	4	2	—	—
—	222	38	182	86	19	15	—
172	551	422	1340	977	256	975	65
89	57	46	165	108	56	49	28
9	45	87	33	—	9	—	71
2255	1934	558	335	93	223	—	228
458	384	125	62	10	62	18	29
50	129	—	—	—	—	—	—
34	25	36	58	—	—	—	84
2	6	158	29	—	—	—	24
—	—	—	—	—	—	—	105
242	316	1257	310	1	74	—	4
—	26	284	22	—	—	—	133
—	—	—	—	—	—	—	87
2	15	42	7	—	7	—	39
4	5	110	29	6	25	—	81
—	37	226	40	—	—	—	172
7	2	146	41	—	35	—	32
27	113	1072	786	—	—	—	45
—	189	229	20	—	—	—	17
52	171	431	146	52	135	91	239
3231	3454	4807	2083	270	626	158	1418
134	17	5	60	412	160	162	193
140	26	—	54	13	40	44	30
70	108	178	99	69	92	83	20
344	151	183	213	494	292	289	243
3790	4468	5580	3807	2167	1323	1736	1805
13.3	15.6	19.5	13.3	7.6	4.6	6.1	6.3

production of Lake Balaton falls within this range. According to the yearly gross production, oligotrophic lakes produce 10—30, mesotrophic lakes 30—70 eutrophic lakes 70—200 and hypertrophic lakes 200—400 g C/m^2. During the six months investigated the primary production of Lake Balaton was 0.413 g C/m^2 × 183 = 75.6 g C/m^2/half-year, i.e. the production attained already the yearly level of eutrophic lakes.

The average biomass of the phytoplankton is 10.3 g. The average daily production, if 1 g C corresponds to 10 g biomass was 4.13 g. This means that the mass of phytoplankton is renewed in each 2.5 days. As in other lakes this value is 2—10 days, (GESSNER, 1959), the turnover time of the phytoplankton is relatively short.

TABLE III

The biomass of the phytoplankton at different depths 10^6 μ^3/100 ml

Depth, cm	5. IV.	18. IV.	2. V.	16. V.	31. V.	13. VI.	27. VI.	11. VII.	27. VII.	10. VIII.	24. VIII.	7. IX.	28. IX.
25	344	362	261	169	212	244	460	765	490	129	55	184	214
100	328	296	229	123	235	354	496	620	435	169	103	206	258
200	550	322	134	134	250	449	383	634	391	250	125	151	147
300	434	320	372	131	203	401	455	317	262	273	209	159	120

TABLE IV

The production of the phytoplankton at different depths µg C/100 ml/4 hours

Depth, cm	5. IV.	18. IV.	2. V.	16. V.	31. V.	13. VI.	27. VI.	11. VII.	27. VII.	10. VIII.	24. VIII.	7. IX.	28. IX.
25	3.46	3.35	1.36	3.69	3.99	6.25	1.81	17.53	8.06	1.87	2.53	1.42	3.76
100	6.11	5.99	5.13	4.73	4.66	4.84	3.67	0.60	4.49	2.82	6.02	2.87	3.94
200	4.59	2.58	3.71	3.39	7.50	5.72	5.86	0.38	0.81	4.75	1.05	2.82	1.90
300	0.32	2.43	2.90	0.12	0.83	4.09	1.06	0.42	0.65	7.96	0.28	2.89	0.58

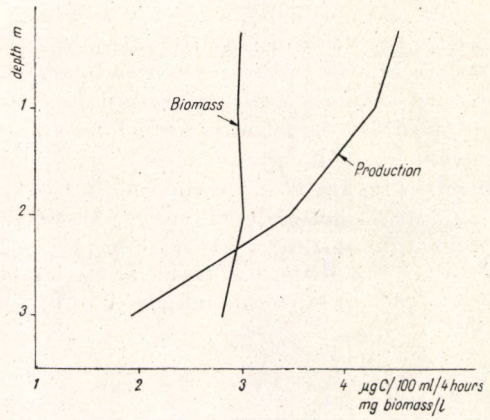

Fig. 1. The average biomass and production of the phytoplankton at different depths

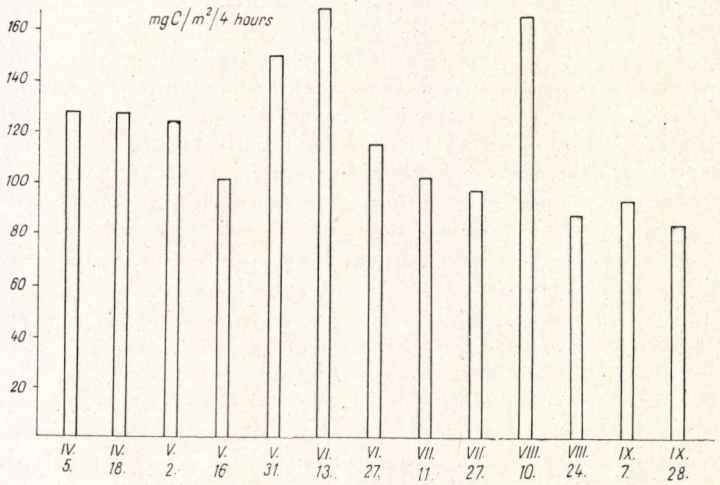

Fig. 2. The primary production of the phytoplankton per unit of lake surface

Due to rapid turnover, the daily production is relatively high as compared to the biomass, and due to the long productive period the yearly production is high as compared to the daily production. This way it is possible, that by relatively low phytomass, the daily production corresponds to the mesotrophic, the yearly production to the eutrophic level. The not too high mass of algae provides more food for other organisms than in lakes with a slower renewal of algae, or with shorter productive period.

The primary production in this half-year, extrapolated to the whole Lake Balaton was some 453 000 metric ton of planctonic algae. This amount is 22 times higher than that estimated by ENTZ (1954) from the low phytomass of the 1940 s (TAMÁS, 1955) for the whole year.

The question may arise, how much did the productivity of the lake change since the first primary production measurements in 1961. It cannot

be answered in an exact manner, since there are essential differences between the two set of experiments. BÖSZÖRMÉNYI et al. (1962) exposed the samples only at 1 m depth for six hours, and measured radioactivity by the GEIGER—MÜLLER tube. In this experiment the production was measured at four different depths, the exposition time was four hours and radioactivity was measured by liquid scintillation.

In order to obtain comparable data the amount of carbon bound by 100 ml sample in one hour at 1 meter depth was calculated. These values were 0.70 and 1.07 μgC/100 ml/h in 1961 and 1973 respectively. Part of this difference may originate in the different techniques. Thus by all probability the productivity of the phytoplankton increased but moderately in the last decade.

Summary

The biomass of phytoplankton, the illumination and primary production were measured fortnightly at four depths.

In the samples 108 alga species, 5 varieties and 1 form were found. Algae were counted by UTERMÖHL microscope. The biomass of each species and the total phytoplankton were calculated from the number of algae and from the volume of individuals. Until the middle of July the plankton was dominated by diatoms, the largest mass was formed by Cyclotella bodanica. Ceratium hirundinella became the dominating species first in the second half of the summer. The average biomass was 10.3 g/m^2.

Transparency is very unstable in the lake, due to the frequent swirling up of the mud. In storm the primary production stops already at 1 m depth because of light insufficiency while after a long calm period in the clear water the deepest layer (3 m) exhibited the highest production.

In general at the surface the production is inhibited be excessive illumination, the maximum is found at 1 or 2 m, and at 3 m the same mass of algae produced four times less than at 1 m, owing to the insufficient light.

The mean production and the standard error of the mean during the four hours expositions were 118 $\pm$ 8 mg C/m^2.

The average daily production was estimated to be 413 mg C/m^2, the primary production during the half-year to 76 g C/m^2.

This primary production corresponds to that of the slightly eutrophic akes.

REFERENCES

BÖSZÖRMÉNYI Z., CSEH E., FELFÖLDY L., SZABÓ E. (1962): A Balatonban C^{14}-módszerrel végzett fotoszintézis mérés módszertani kérdéseiről. — *Annal. Biol. Tihany* **29,** 39—63.

ELSTER, H. J., MOTSCH, B. (1966): Untersuchungen über das Phytoplankton und die organische Urproduktion in einigen Seen des Hochschwarzwalds, im Schleinsee und Bodensee. — *Arch. Hydrobiol. Suppl.* **28,** 291—376.

ENTZ, G., KOTTÁSZ, J., SEBESTYÉN, O. (1937): Quantitative Untersuchungen am Bioseston des Balatons. — *Magyar Biol. Kut. Int. Munkái* **9,** 73—153.

ENTZ B. (1954): A Balaton termelésbiológiai problémái.— *MTA Biológiai és Orvosi Tudományok Osztályának Közl.* **5,** 433—461.

ENTZ B., FILLINGER M. (1961): Adatok a Balaton fényklímájának ismeretéhez. (A víz zavarosságának okairól és kihatásairól.) — *Annal. Biol. Tihany* **28,** 49—89.
ENTZ B., FILLINGER M. (1962): Adatok a Balaton fényklímájának ismeretéhez II. (Fényviszonyok a hóborította befagyott Balaton-vízben.) — *Annal. Biol. Tihany* **29,** 65—74.
FELFÖLDY L., KALKÓ ZS. (1958): A vízalatti fényviszonyok és a fotoszintézis összefüggése a Balatonban 1957 nyarán. — *Annal. Biol. Tihany* **25,** 303—329.
FELFÖLDY L. (1959): A balatonvíz tulajdonságainak vizsgálata algaélettani kísérletekkel. *Annal. Biol. Tihany* **26,** 211—222.
FELFÖLDY L. (1962): Further experiments with algal cultures for determining some properties of water of Lake Balaton. — *Annal. Biol. Tihany* **29,** 85—93.
FELFÖLDY L. (1963): A klorofill-mérés módszertani és elvi kérdései a balatoni eredményekkel kapcsolatban. — *Annal. Biol. Tihany* **30,** 137—165.
GESSNER, F. (1959): Hydrobotanik. — *Deutsch. Ver. d. Wissenschaften, Berlin.*
HÜBEL, H. (1971): Primärproduktion des Phytoplanktons. ^{14}C- oder Radiokohlenstoffmethode. — In: *Ausgewählte Methoden der Wasseruntersuchung VEB Gustav Fischer Verlag, Jena.*
SCHNESEE, W., SCHWARTZ, S. (1971): Plankton. — In: *Ausgewählte Methoden der Wasseruntersuchung VEB Gustav Fischer Verlag, Jena.*
SEBESTYÉN O., TÖRÖK P., VARGA L. (1951): Mennyiségi planktontanulmányok a Balatonon. — *Annal. Biol. Tihany* **20,** 69—125.
SEBESTYÉN O. (1953): Mennyiségi planktontanulmányok a Balatonon. II. Évtizedes változások. — *Annal. Biol. Tihany* **21,** 63—89.
SEBESTYÉN O. (1954): Mennyiségi planktontanulmányok a Balatonon III. Pelagikus Dinoflagellaták biomasszája. (Módszertani tanulmány.) — *Annal. Biol. Tihany* **22,** 185—197.
STEEMANN NIELSEN, E. (1964): Recent advances in measuring and understanding marine primary production. — *J. Ecol.* **52,** 119—130. *(Suppl.)*
TAMÁS G. (1955): Mennyiségi planktontanulmányok a Balatonon. VI. A negyvenes évek fitoplanktonjának biomasszája. — *Annal. Biol. Tihany* **23,** 95—109.
TAMÁS, G. (1967): Horizontale Plankton-Untersuchungen im Balaton. V. Über das Phytoplankton des Sees, auf Grund der im Jahre 1965 geschöpften und Netzfilterproben. — *Annal. Biol. Tihany* **34,** 191—231.
TAMÁS, G. (1969): Horizontal plankton investigations in Lake Balaton. VII. On the phytoplankton of Lake Balaton, based on scooped samples and filtrates taken in 1966. — *Annal. Biol. Tihany* **36,** 257—292.
UTERMÖHL, H. (1958): Zur Vervollkommung der quantitativen Phytoplankton-Methodik. — *Intern. Verein f. theor. u. angewandte Limnologie. Mitteilung* **9,** 1—38.
VINBERG, G. G. (1961): Sovremennoe sostojanie i zadaci izucenija pervicnoj produkcii vodoemov. — In: *Pervicnaja produkcija morej i vnutrennich vod. Minsk,* 1961 11—24.
VOLLENWEIDER, R. A. ed. (1969): A manual on methods for measuring primary production in aquatic environments. *IBP Handbook No.* **12.** *Blackwell Scientific Publ. Oxford and Edinburgh.*
WALLEN, D. G., GEEN, G. H. (1968): Loss of radioactivity during storage of ^{14}C-labelled phytoplankton on membrane filters. — *J. Cons. Perm. Int. Explor. Mer.* **31,** 31—37.

A BALATON FITOPLANKTONJÁNAK ELSŐDLEGES TERMELÉSE 1972. ÁPRILIS—SZEPTEMBERBEN

Herodek Sándor és *Tamás Gizella*

Összefoglalás

Fél éven keresztül kéthetente mértük négy különböző mélységben a fitoplankton tömegét, a megvilágítást és az elsődleges termelést.

A mintákban 108 algafajt, 5 változatot és 1 formát találtunk. Az egyedszámot Utermöhl módszerével határoztuk meg. Az egyedszám és az egyedek átlagos térfogata alapján kiszámítottuk az egyes fajok és az egész fitoplankton biomasszáját. Július köze-

péig a planktonban a kovamoszatok uralkodtak, a legnagyobb tömeget a *Cyclotella bodanica* képezte. A *Ceratium hirundinella* a nyár második felében vált uralkodó fajjá. A biomassza átlaga 10,3 g/m² volt.

A Balaton vizének átlátszósága nagyon változékony az iszap gyakori felkeveredése miatt. Viharban már egy méter mélyen sincs termelés a fényhiány miatt, míg hosszú szélcsend után a víz annyira tiszta volt, hogy a termelés maximuma a legmélyebb (3 m) rétegben alakult ki. Általában a felszínen fénygátlás van, a termelés maximuma egy-két méterre esik, három méter mélyen pedig a fényhiány miatt ugyanakkora algatömeg csak negyedannyit termel, mint egy méteren.

Négyórás expozíció alatt a termelés átlaga és az átlag standard hibája 118 $\pm$ 8 mg C/m² volt. Ebből számítva az átlagos napi termelés 413 mg C/m², a fél év alatti termelés 76 g C/m² volt. A fél évi termelés eléri a természetes eutróf tavak szintjét. Ezt az algatömeg gyors, 2,5 naponkénti megújulása, és a hosszú vegetációs periódus teszi lehetővé.

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BACTERIAL GRADIENTS AT THE SEDIMENT — WATER INTERFACE OF SHALLOW LAKES

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In Lake Balaton the number, biomass and production of the heterotrophic and total bacterioplankton have been investigated intensively from many points of view during the years of 1966—1971 (OLÁH, 1969 a, b; 1970; 1971 a, b, c; OLÁH and VÁSÁRHELYI, 1970 a). However, our knowledge about the bacteriobenthos of this lake is rather scanty. According to our short periodic investigation, in the constantly disturbed water of shallow Lake Balaton the sediment has a significant role in the formation of both the heterotrophic and total bacterioplankton. Therefore, the investigation of bacterial gradients is of special importance at the sediment — water interface, i.e. in the water layers above the bottom, the sediment — water interface taken in the literal sense as well as the deeper sediment layers. In this study we determined the quantitative distribution of the aerobic, anaerobic bacteria and that of the bacteria counted on the membrane filter at the sediment — water interface in three shallow lakes of different trophic levels.

Methods

In Lake Balaton the samples were taken in the Keszthely Bay the most eutrophicated part of the lake and in section "A" (in front of the Institute), which represents the less eutrophicated larger part of the lake. In the highly eutrophic Lake Belső the samples were taken in the deepest part of the lake. In Lake Velence between the state of eutrophy and "senescence", the samples were taken in the reeds-free open water (sampling station "C", OLÁH and VÁSÁRHELYI, 1970 a). Samples were taken in July, 1971 with a MILBRINK's microstatification sampler (1968) into sterile glass or Petri-dish and the determinations were carried out immediately after sampling or in the case of Lake Velence and Keszthely Bay not later than 5 hours. The anaerobic bacterial gradients were determined in January of 1973.

The quantity of bacteria counted on the membrane filter was determined according to KUZNETSOV and ROMANENKO (1963). The distribution of aerobic bacteria at the sediment — water interface was determined with the usual plating method. For plate pouring we have chosen the sodium caseinate agar (OLÁH and VÁSÁRHELYI, 1970 b). Burri-tubes were used to

estimate the number of anaerobic bacteria. To ensure the anaerobic condition, besides using deep agar, the left-over oxygen in the closed tubes was absorbed with alkaline pyrogallol. The aerobic bacteria were cultured at 25° C and the anaerobic bacteria at 38° C. Two culture media were used to estimate the number of anaerobic bacteria. Nutrient II agar with a high organic content:

Beef extract (Difco)	3 g
Peptone (Difco)	5 g
Glucose	10 g
Difco agar	15 g
Distilled water	1000 ml

We used iron sulphite agar (Oxoid) with a lower organic content to count the anaerobic and sulphite-reducing bacteria. The colonies of sulphite-reducing bacteria are black in this medium owing to the iron sulphide precipitation. The agar column was pushed out of the tube, then cut into slices and examined under a microscope.

Results and discussion

Aerobic bacteria

At the end of the 1920s the quantity of bacteria on the sediment surface of the open water was below the value of $1 \cdot 10^3$ cell/g wet sediment (ZIH, 1929) in Lake Balaton. After ten years there was no significant change. On the sediment surface of the open water HARANGHY (1941) found about $1 \cdot 10^3$ cell/g wet sediment both on gelatine and Heyden agar. In this study the number of aerobic bacteria on the sediment surface of the open water reached the value of $1 \cdot 10^5$/g wet sediment *(Fig. 1)*. The comparison of data obtained during the 1920s and 1930s and in this study is reasonable. All these investigations were carried out in July and the same types of media occurred among the culture media which were used for plate pouring. Attention was paid to the selective effect of media (HARANGHY, 1941; OLÁH and VÁSÁRHELYI, 1970 b). The comparison shows that during the first ten years of the period of forty years there was no significant change while in the course of the following thirty years the number of heterotrophic bacteria on the sediment surface increased by two order of magnitude. This very high increase is especially striking, because during the same period the quantity of aerobic bacterioplankton remained on the same order of magnitude (OLÁH, 1969 b).

The gradient of aerobic heterotrophic bacteria at the sediment—water interface of Lake Balaton indicates the low number of cells in the water layer of 11—15 cm above the sediment surface *(Fig. 1)*. The number of aerobic heterotrophic bacteria decreased rapidly in the deeper sediment layers. However, their number even in the sediment layer of 9—11 cm was significant: reached the value of $1 \cdot 10^4$ cell/g. In the sediment of Keszthely Bay the accumulation of organic matter is higher than that in the other part of the lake (PONYI et al., 1972). The input exceeds the output. This is reflected by the quantity of aerobic heterotrophic bacteria living on the sediment surface. Their number reach the value of $3.2 \cdot 10^5$ cell/g wet sediment. We have measured similar values in Lake Velence. In the highly eutrophic Lake Belső the quantity of aerobic heterotrophic bacteria reached the value of $2 \cdot 10^6$

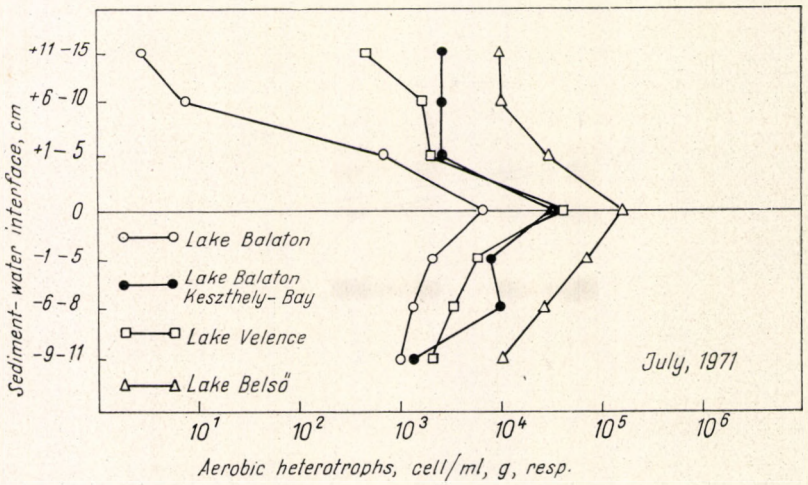

Fig. 1. The quantity of aerobic heterotrophic bacteria at the sediment—water interface of different lakes

cell/g wet sediment on the sediment surface and in the water layer of 11—15 cm above the sediment we have found more bacteria than on the sediment surface of Lake Balaton. In the lakes investigated the quantity of aerobic heterotrophic bacteria decreased rapidly in the deeper sediment layers except the sediment of Keszthely Bay which produced an increase again in the sediment layer of 6—8 cm.

Anaerobic bacteria

We investigated the distribution of anaerobic bacteria at the sediment—water interface only in section "A" of Lake Balaton. On the sediment surface the quantity of anaerobic bacteria counted on iron sulphite agar reached the value of $2 \cdot 10^4$ cell/g wet sediment *(Fig. 2)*. The same value on nutrient II agar was lower by one order of magnitude. During the last thirty years the quantity of anaerobic bacteria also increased on the sediment surface. In the open water sediment HARANGHY (1941) found an average value of $1 \cdot 10^2$ and even in the littoral zone, in the sediment of the reeds his values were below the value of $1 \cdot 10^3$ cell/g. The anaerobic bacterial gradients differ from the gradient of aerobic heterotrophic bacteria. In the water layers we found no anaerobic bacteria except some cells in the water layer immediately above the sediment. The quantity of anaerobic bacteria counted on nutrient II agar was smaller in the oxidized sediment surface than in the reduced sediment layer of 1—5 cm. The distribution of sulphite reducing bacteria was similar. The anaerobic bacteria counted on iron sulphite agar occurred in a larger number on the sediment surface than in the deeper reduced sediment layers. This type of distribution may be explained by the higher proportion of facultative anaerobic bacteria and the anaerobic microenvironment.

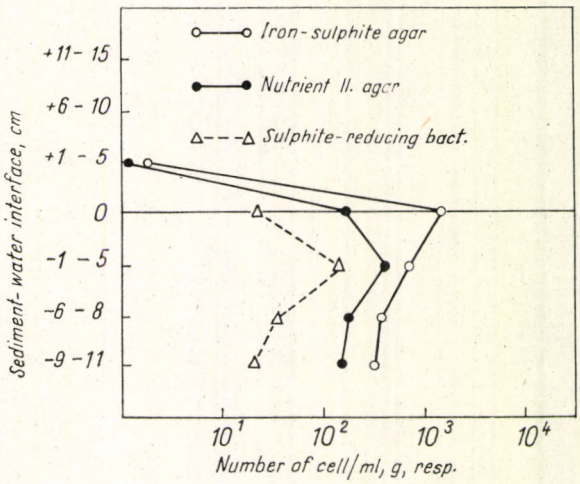

Fig. 2. The quantity of anaerobic heterotrophic bacteria at the sediment—water interface of Lake Balaton

Bacteria counted on membrane filter

A membrane filter variety of the original VINOGRADSKY's method is used more often to estimate the total number of bacteria living in the sediment (KUZNETSOV and ROMANENKO, 1963). According to our present data the quantity of bacteria on the sediment surface of open water in Lake Balaton is lower in July than in the mesotrophic lakes *(Fig. 3)*. The values of $3 \cdot 10^8$ cell/g wet sediment is lower than the values of $0.5—1.5 \cdot 10^9$ cell/g characterizing the mesotrophic lakes (ROMANENKO and ROMANENKO, 1971). How-

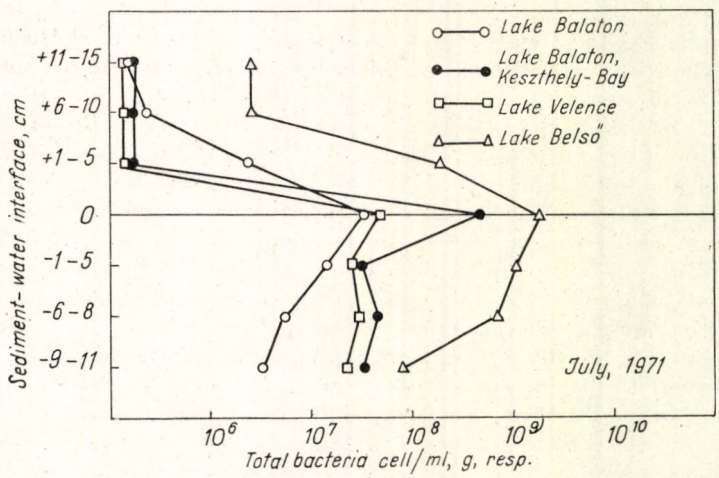

Fig. 3. The quantity of bacteria counted on the membrane filter at the sediment—water interface of different lakes

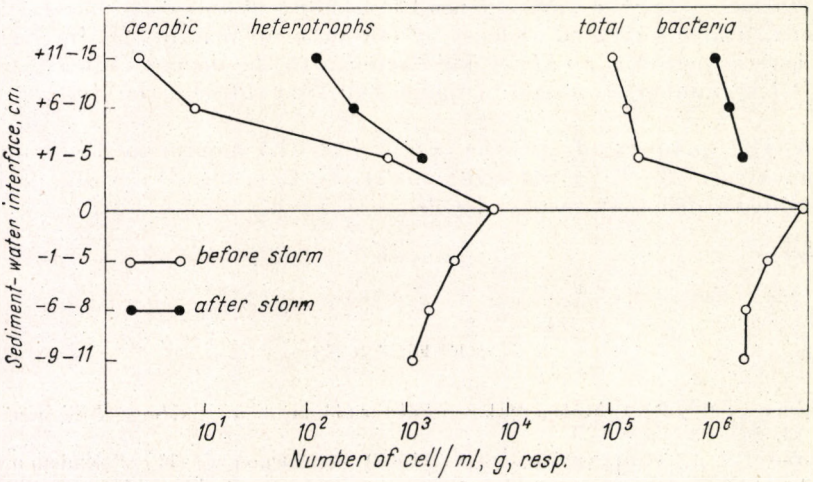

Fig. 4. Storm effect on the quantity of aerobic heterotrophic bacteria and bacteria counted on the membrane filter in the bottom water layers

ever, according to our earlier autumnal investigation the quantity of bacteria on the sediment surface reaches the values of mesotrophic lakes (PONYI et al., 1972). In the Keszthely Bay with a higher trophic level, the quantity of bacteria reached the value of $5 \cdot 10^9$ cell/g characterizing the eutrophic lakes. During our earlier investigation in autumn we found no such a large difference between the Keszthely Bay and the other part of the lake.

The number of bacteria living on the sediment surface of the highly eutrophic Lake Belső exceeded the value of $1 \cdot 10^{10}$ cell/g wet sediment.

In the lakes Balaton and Belső the quantity of bacteria counted on membrane filter decreased with depth. In the sediment of Lake Velence and Keszthely Bay the number of bacteria was lower in the deeper sediment layers, however, their number showed no decrease up to the sediment layer of 10 cm. The number of bacteria in the water layers immediately above the bottom decreased rapidly in all three of the investigated lakes. After a storm the number of heterotrophic bacteria increased by two order of magnitude and that of the bacteria counted on membrane filter by one order of magnitude in the water layers immediately above the bottom *(Fig. 4)*. According to our short periodic investigation the storm or strong wind effect may be detectable even in the surface water layers (OLÁH, 1970).

Summary

1. During the last forty years the number of aerobic heterotrophic bacteria on the sediment surface of Lake Balaton increased from a value of $1 \cdot 10^3$ cell/g wet sediment to a value of $1 \cdot 10^5$. During the same period the quantity of the heterotrophic bacterioplankton showed no significant change. Most bacteria occurred in the upper 1 cm layer of the sediment. In the deeper sediment layers and in the water layers above the bottom their number decreased rapidly.

2. During the last thirty years the number of anaerobic bacteria on the sediment surface increased at least by one order of magnitude. In the bottom water layers there was no anaerobic bacteria. In the deeper, reduced sediment layers their number increased or decreased depending on the different media used.

3. The quantity of bacteria counted on the membrane filter increased from a value of $3 \cdot 10^8$/g wet sediment (Lake Balaton) to a value of $1 \cdot 10^{10}$ in lakes with different trophic levels.

REFERENCES

Haranghy L. (1941): Adatok a Balaton bakteriológiájához. — *Magy. Biol. Kut. Munk.* **13,** 57—73.

Kuznetsov, S. I., Romanenko, V. I. (1963): Кузнецов С. И., Романенко В. И.: Микробиологическое изучение внутренних водоемов. — *Изд. АН СССР, Москва—Ленинград.*

Milbrink, G. (1968): A microstratification sampler for mud and water. — *Oikos* **19,** 105—110.

Oláh J. (1969 a): The Quantity, vertical and horizontal distribution of the total bacterioplankton of Lake Balaton in 1966/67. — *Annal. Biol. Tihany* **36,** 186—195.

Oláh, J. (1969 b): A quantitative study of the saprophytic and total bacterioplankton in the open water and the littoral zone of Lake Balaton in 1968. — *Annal. Biol. Tihany* **36,** 197—212.

Oláh, J. (1970): Short periodic changes in the microbial plankton quantity of Lake Balaton. — *Annal. Biol. Tihany* **37,** 199—207.

Oláh, J. (1971 a): Glass effect and the microbial plankton-seston relation in the water of lakes Balaton and Belső. — *Annal. Biol. Tihany* **38,** 153—160.

Oláh, J. (1971 b): The influence of River Zala on the bacteriological condition in Keszthely-Bay (Lake Balaton). — *Annal. Biol. Tihany* **38,** 161—166.

Oláh, J. (1971 c): Weekly changes of the bacterio- and phytoplankton standing stock in Lake Balaton and in the highly eutrophic Lake Belső. — *Annal. Biol. Tihany* **38,** 167—175.

Oláh, J., Vásárhelyi, R. (1970 a): Comparative bacteriological investigation of three shallow Hungarian lakes with different trophic levels. — *Annal. Biol. Tihany* **37,** 223—234.

Oláh, J., Vásárhelyi, R. (1970 b): Comparative nutrient agar studies on the quantitative survey of saprophytic water microorganisms. — *Annal. Biol. Tihany* **37,** 235—246.

Ponyi, J., Oláh, J., Frankó, A. (1972): Distribution of organic matter and bacteria in the upper layer of bottom deposit in the open water of Lake Balaton. — *Annal. Biol. Tihany* **39,** 141—148.

Romanenko, V. I., Romanenko, V. A. (1971): Романенко В. И., Романенко В. А.: К методике определения численности бактерий в иловых отложениях водоемов. — *Микробиология* **40:** 912—915.

Zih S. (1929): Adatok a Balaton vizének baktériumtartalmáról. — *Magy. Biol. Kut. Munk.* **2,** 346—354.

BAKTÉRIUM GRADIENSEK A SEKÉLY TAVAK VÍZ-ÜLEDÉK HATÁRÁN

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Összefoglalás

1. Az elmúlt negyven év során a Balaton üledékfelületén élő aerob heterotróf baktériumok mennyisége $1 \cdot 10^3$ sejt/g-ról $1 \cdot 10^5$ sejt/g-ra emelkedett. Ugyanezen periódus alatt az aerob heterotróf bakterioplankton mennyisége nagyságrendileg nem változott. Legtöbb baktérium az üledék felső 1 cm-es rétegében volt. Az üledékmélységgel, de különösen az üledékfeletti vízrétegben számuk gyorsan csökkent.

2. Az elmúlt harminc év során a Balaton üledékfelületén élő anaerob baktériumok mennyisége legalább egy nagyságrenddel nőtt. Közvetlenül az üledékfeletti vízrétegben nem találtunk anaerob baktériumot. A mélyebb, redukált üledékrétegekben számuk csökkent vagy nőtt az alkalmazott táptalajtól függően.

3. A membránszűrőn számolt baktériumok mennyisége a vizsgált különböző trofitású tavakban $3 \cdot 10^8$ sejt/g nedves üledékről (Balaton) $1 \cdot 10^{10}$ sejt/g nedves üledékre nőtt (Belső tó).

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LIMNOLOGICAL INVESTIGATIONS OF A FISH-POND SUPPLIED WITH SEWAGE-WATER IN THE VICINITY OF LAKE BALATON. I.

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The method of sewage purification in fish-ponds is of old standing. Since the beginning of this century this type of fish-ponds has been maintained in Germany (FALCK, 1935; KISSKALT and ILZHÖFER, 1937; KAUFMANN, 1958; LIEBMANN, 1960). The main point of the procedure is that the organic substances of the sewage-water getting in to the fish-pond after a suitable dilution and distribution, are aerobically destructed by means of bacteria and the biogenic elements formed this way are utilized by the algae. The bacteria and algae propagated during the decomposition of organic substances as well as the organic fragments are incorporated by planktonic animals and other invertebrate organisms, which are in turn consumed by other invertebrates or directly by fish.

In spite of the fact that these fish-ponds supplied with sewage-water function with a good efficiency according to the experience of Germany (IMHOFF, 1956; LIEBMANN, 1960), the results and observations can only be utilize with some difficulties owing to the different conditions in Hungary (CSANÁDY and GREGÁCS, 1965 a). Upon the influence of the advantageous observations obtained in Czechoslovakia (PYTLIK, 1957) several papers have been published in our country which, on the one hand, urged the operating experimental introduction of the postpurification of sewage-water in fish-ponds (WOYNAROVICH, 1959 a, b), and on the other hand, described the principal problems together with home possibilities (DONÁSZY, 1965; CSANÁDY and GREGÁCS, 1965 a). In spite of that, attention was rather focused on the so-called waste stabilization ponds ("Abwasserteich") (UHLMANN, 1962; BRINCK, 1961; CSANÁDY and GREGÁCS, 1965 b), since as against to the fish-pond purification, there is no need of dilution-water in this case, offering a possibility for wider application. According to certain data,the waste stabilization ponds and the fish-ponds supplied with sewage-water cannot be sharply distinguished (PYTLIK, 1957), since in a closed system the culture of carp and tench can be realized even without diluting the water, and what is more, for this purpose even the strongly contaminated waste waters of the food industry can be used.

Neither sewage fish-ponds nor waste stabilization ponds were established in Hungary until 1970 for direct purposes of purification of sewage-waters. Nevertheless, the home literature (CSANÁDY and GREGÁCS, 1965 a, b) reported on the bacteriological, chemical and parasitological relationships of some fish-

ponds loaded with sewage-water and waste stabilization ponds, indicating that these methods could be applied in a useful way even in our country. The authors cited above suggest the establishment of these types of pond especially on the southern shore of Lake Balaton, since "by means of them the sewage-water can practically be kept out of the lake" (CSANÁDY and GREGÁCS, 1965 a, p. 185), and hold as necessary to perform experiments in operational sizes in order to estimate the possibilities. The idea of establishing sewage fish-ponds has arisen even formerly, however, recently some apprehensiveness came to light both abroad and home as regards this method of sewage purification especially in connection with the ponds along the Lake Balaton, containing peat and a huge amount of organic substances (HOLÉNYI, 1962).

The OVH (National Buro for Water Conservancy) agreed to establish experimental ponds in 1970, which is to be built in the region of Fonyód for the utilization of sewage-waters and to start to function in 1973. Until putting this fish-pond into operation, we have investigated the so-called pond of Zardavár No. 1 since 1971, which is a sewage fish-pond not studied before from hydrobiological point of view. The long-term aim of our investigations was to obtain scientific material on the basis of which the research work can be planned with suitable certainty on the experimental ponds starting in 1973.

The investigations were intended to study directly

a) the biomass of bacterio-, phyto- and zooplankton as well as their changes;

b) the biomass of fauna at the bottom of the pond;

c) the growth of fishes;

d) the most important chemical components.

Description of the place of investigations

A fish-pond system of 370 cadastral acre consisting of 3 units serves for the postpurification of the sewage-water of the Fonyód resort centre. The water is conducted here by a main collecting pipe. After lifting over and sedimentation, the water reaches pond No. 1 at one point, which is of 85 cadastral acre. The other two ponds can be filled in with water through the first one, thus, the accidentally remaining contamination can further be destructed.

During the emptying of the ponds, the water gets in a canal called Keleti-Bozót then into Lake Balaton. During this period, the sewage-water is collected in a reserve pond *(Fig. 1)* acting for waste stabilization. The refilling of the ponds takes place from the canal mentioned above containing plentiful clean water.

According to the aims of investigations, the work was concentrated on pond No. 1. The standard places of sampling, except one, fall on the transversal section of the pond. The place of sampling No. 1 was near the inflow of the sewage-water, that of No. 5 near the outflow of the pond, and further 3 places were selected at about identical distances from each other between the two marginal places mentioned above.

Using these places of sampling, the changes can be followed from the inflow up to the outlet of the pond. Samples were taken occassionally from another point of the pond for comparisons *(Fig. 1,* place of sampling No. 6). Thus, 3 samples were at our disposal each from the marginal and open water areas.

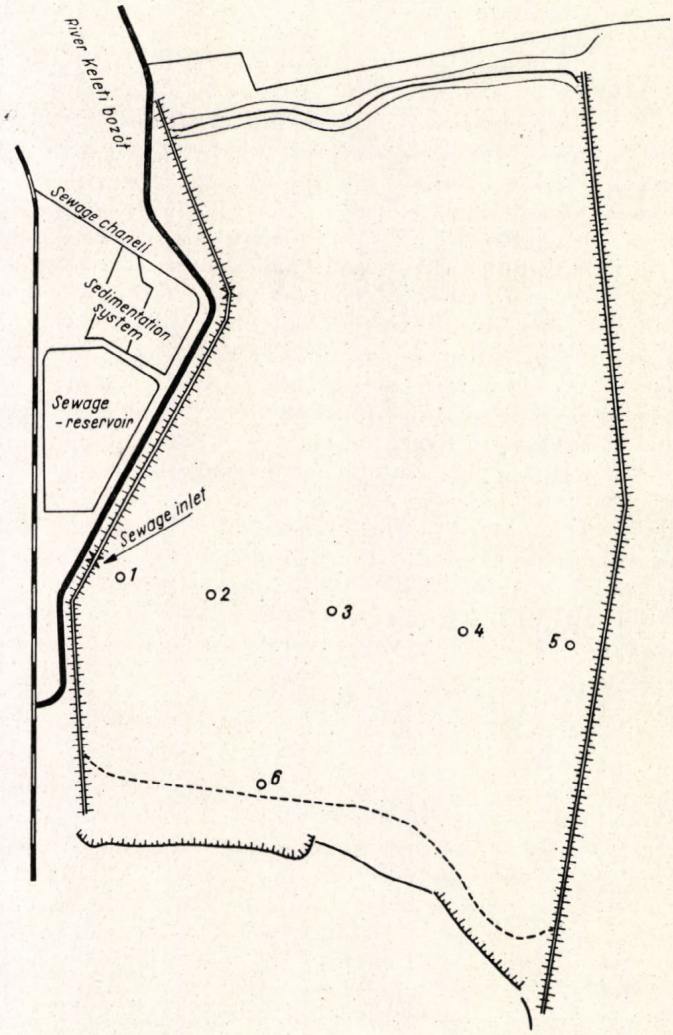

Fig. 1. Places of sampling on pond No. 1 of Zardavár

Methods

1. *The biomass of the planktonic members*

The amount of the total microbial plankton was determined by means of the method of RAZUMOV (1932), whereas the biomass was calculated according to RODINA (1965). For measurements of the phytoplankton biomass, the chlorophyll-method of STRICKLAND and PARSONS (1969) was applied. For the determination of alga species as well as for the evaluation of their percentual distribution, samples filtered through a mesh No. 25 were used. The counting was carried out in a microscope type Uthermöl.

The determination of the zooplankton biomass was performed in light of the works of SEBESTYÉN (1958), NAUWERCK (1963), SCHNESE and SCHWARZ (1970). For qualitative and quantitative analyses of the Rotatoria plankton, the filtrate obtained on a mesh No. 25 from 4.5 l of water was used. At the evaluation of the biomass of the predominant species, the volume values calculated on the basis of models of SEBESTYÉN (1958) concerning the periods of "warm water", as well as the values of length, width and thickness of different species measured under the microscope, were applied. The product of multiplication of those values is considered as a volume given in μ^3 units of a given individual. Since the measurements were carried out on formalin-fixed material, the morphological changes of certain species could not be taken into account (e.g. the retracted foot of Epiphanes, the head-region of Asplanchna, etc.). The chitin-processes and the spines were also neglected (e.g. *Brachionus diversicornis*, or *B. calicyflorus)*. The volume values obtained this way were averaged and multiplied by 1.025 resulting in the wet weight of one rotifer individual in average. The dry weight amounts to 10 percent of that (WINBERG, 1971). The number of individuals per litre was multiplied by this average weight.

For the determination of the biomass of Crustacean plankton, 15 l of drawn water was used concentrated on a bronze-net of 90 μ mesh. The biomass was calculated by multiplying the number of individuals per litre by the value of dry weight measured separately for every species (e.g. the weight of a *Bosmina longirostris* is 2 μg, that of juvenile *Cyclops vicinus* is 5 μg).

2. *The me hods of investigation of the benthos*

The macrobenthos was investigated by using the Eckmann—Birge dredge, the mud was passed through riddles of different mesh (the smallest one of 500 μ). Organisms of this order of magnitude occurred so rarely that the determination of biomass was omitted.

The collecting of meiobenthic samples was also carried out by using the Eckmann—Birge dredge. A mud layer of 1 cm thickness and 40 cm^2 surface area was taken away from the untouched surface of the sample. The material was fixed in 4 percent formalin. The mud sample was put into a measure cylinder of 200 ml and filled up to the sign, then after mixing by shaking the animals were selected and investigated in portions of $25 \times 2 - 4$ ml in counting dishes. The biomass of Nematoda was estimated in dry weight according to the work of WARWICK and BUCHANAN (1971) by calculations. The biomass of the other groups of organisms was not calculated because of their low number of individuals.

3. *Methods of measurements on the fish-production and growth of fishes*

The investigations were mainly carried out on fishes collected during sampling fisheries. The total and standard body length as well as the body weight were measured and conclusions were drawn for the absolute growth from the average values. The allometric growth was calculated from those values (HUXLEY, 1924; cit. BEVERTON and HOLT, 1957). The rate of

growth of body length and weight was characterized by the so-called growth coefficients on the basis of the equations given below (CHAPMAN, 1968; TESCH, 1968):

$$G_w = \frac{\log_e \bar{w}_1 - \log_e \bar{w}_0}{\Delta t}; \quad \text{and} \quad G_l = \frac{\log_e \bar{L}_1 - \log_e \bar{L}_0}{\Delta t}$$

where G_w = growth coefficient of the body weight, G_l = growth coefficient of the body length; w = body weight; L = body length; Δt = time between 0 and 1 (in our case it is 6 months).

The condition of the fishes was determined by using the method of HILE (1936) and LE CREN (1951) on the basis of the following equations:

$$CF = \frac{W}{L^3}; \quad \text{and} \quad CF = \frac{10^6 \times W}{L^3}.$$

At the seasonal changes of the condition the average and the limit values were considered.

The calculation of the actual total mortality was carried out according to RICKER (1958):

$$Z = \frac{-(\log_e N_1 - \log_e N_0)}{\Delta t},$$

where Z = coefficient of the actual total mortality; N = number of individuals at the beginning and at the end; Δt = time (= 0.5 year). From that equation one can determine the coefficient of survival, i.e. the rate of that:

$$S = e^{-Z};$$

as well as the total yearly mortality: A = 1 — S; this applies in this case only to half a year. The "losts" at economical level are given simply as percentages without time-dependence on the basis of the results of fisheries.

The production parameters of Zardavár ponds No. 1, 2 and 3 were evaluated separately between 1964 and 1971, and the net fish-production of each pond was investigated by using the parameters determined above as well as statictical data according to necessity (RICKER, 1958; BACKIEL, 1963; NAGIĘĆ, 1964).

4. The water analyses were carried out according to the Hungarian National Standards. The mud-analyses were performed by the Department of waterphysiology of OMMI.

Results

1. *Qualitative characterization of the water in the sewage fish-pond as well as in the in- and out-flow canals*

Several data summarized in *Table I* and *Table II* on water quality indicate that the fish-pond shows an efficiency of purification of about 40—74 percent. The recipient Keleti-Bozót contains water of good quality 1 km before Lake Balaton. This manifests itself especially in the low O_2-consumption and low ammonium-values.

TABLE I

Some chemical characteristics of the water flowing in and out of the sewage-water fish-pond

Substances investigated (mg/l)	The shaft of the rough screen before the cleaner				The outflow after the sedimentator 1969				The outflow of the pond			
	June	August	Sept.	Oct.	June	August	Sept.	Oct.	June	August	Sept.	Oct.
O_2 consumption (original)	195	245	140	192	90	205	106	152	42	155	71	99
BOI [5]	91	187	99	193	60	136	94	140	25	112	54	108
Dissolved O_2	—	—	—	—	—	1.5	—	—	8.8	7.8	6.3	—
pH	8.0	7.3	7.3	7.5	7.5	7.35	7.35	7.5	8.1	8.1	8.5	7.5
NH_4^+	41	49	54	64.8	51	44	46	54	20	3.0	3.4	48.6
Total dry substance	1438	1260	1545	1097	1234	805	1447	1194	531	577	619	959
Total organic substance	570	747	550	623	382	268	517	519	257	196	338	333
Total mineral substance	868	515	995	728	852	537	830	675	274	381	281	626

TABLE II

Some chemical characteristics of the water of Keleti-Bozót canal reaching Lake Balaton

Substances investigated (mg/l)	1969 June	1969 Sept.	Vehicular bridge of Fonyód		
			1970 April	1970 Nov.	1971 March
O_2 consumption	9.1	5.2	8.0	7.4	2.6
BOI_5	4.1	5.0	5.0	3.0	2.3
Dissolved O_3	6.7	12	7.3	10.4	8.9
pH	8.4	8.0	8.2	8.0	8.1
NH_4^+	0.15	0.2	0.1	0.6	0.1
Total dry substance	456	628	452	606	539

2. *The biomass of bacterioplankton*

The bacterial biomass was of high value during May due to the increasing temperature *(Tables III* and *IV)*. The high spring values were characteristic for the whole pond; no increase in values was noted near the inflow. During nearly one month, by the 9th June, the biomass of the bacterioplankton decreased. The highest value was found at the place farthest from the inflow on the 9th June, together with a low content of bacteria.

TABLE III

Biomass values of the bacterioplankton (g wet weight/m^3) in 1971

	13th May	9th June	23rd June	6th July	10th Aug.	29th Aug.	14th Sept.	12th Oct.	21st Oct.
1.	11	4	21	0.25	4.4	2.8	13.8	20.6	24.1
2.	10.6	3.4	18	0.38	1.4	2.3	12.5	19.7	23.9
3.	9	4.2	14	0.46	1.3	1.8	10.8	21.0	23.3
4.	12	3.8	11	0.51	1.2	1.8	9.2	21.6	25.2
5.	11.8	5.9	12.2	0.55	1.4	3.0	14.9	20.6	21.4
Keleti Bozót	2.6	1.9	2.1	—	—	—	—	—	—

TABLE IV

Biomass values of the bacterioplankton (g dry weight/m^3) in 1971

	13th May	9th June	23rd June	6th July	10th Aug.	29th Aug.	14th Sept.	12th Oct.	21st Oct.
1.	2.2	0.8	4.2	0.05	0.8	0.5	2.7	4.1	4.8
2.	2.1	0.6	3.6	0.07	0.2	0.4	2.5	3.9	4.7
3.	1.8	0.8	2.8	0.09	0.2	0.3	2.1	4.2	4.6
4.	2.4	0.7	2.2	0.10	0.2	0.3	1.8	4.3	5.0
5.	2.3	1.1	2.4	0.10	0.2	0.6	2.9	4.1	4.2
Keleti Bozót	0.5	0.3	0.4	—	—	—	—	—	—

The restarted inflow of sewage-water increased again the biomass of the microbial plankton in the whole pond according to the investigations of the 23rd June. The biomass values surpassed those in high spring. The highest values were obtained near the inflow. Proceeding toward the outlet, the biomass of the bacterioplankton decreased nearly to its half.

Parallel with the blue-green algal bloom, the biomass of the bacterioplankton decreased to an extremely low value (6th July). The bacterioplankton was formed mainly by a single coccoid form of 2—3 μ size (with food-inclusion and often in the state of division).

The biomass of the bacterioplankton remained low even on the 10th August beside the abundant biomass of Anabaena-Microcystis-Oscillatoria-Aphanizomenon species. However, the cocci of 2—3 μ size were changed replaced by filamentous forms. The biomass of bacterioplankton was very low even on the 29th August and the filamentous forms predominated.

The biomass of bacterioplankton was high on the 14th September. The initiated mineralization of Anabaena filaments was indicated by the fact that after the cocci of 2—3 μ size and the filamentous forms, a significant proportion of the bacterioplankton consisted of a form of less than 1 μ of a coccus-streptococcus type. The more or less colonized bacteria were always localized around the destructed Anabaena cells. The terminal cells of the destructed Anabaena filaments were surrounded almost always by the microcolonies of this microorganism.

The biomass of the bacterioplankton remained high on the 12th and 21st October. The decrease of blue-green algae was accompanied by the appearance of diatoms. Parallel to this phenomenon, a thin (less than 1 μ thick) filamentous organism became predominant in the bacterioplankton.

The biomass of bacterioplankton in the Keleti-Bozót was very low during the period of investigations as compared to that of the pond.

In the seasonal change of the biomass of bacterioplankton the very low number of bacteria is the most conspicuous phenomenon observed parallel with the bluegreen algal bloom. The decrease in the number of bacteria accompanying the blue-green algal bloom has also been described during our previous investigations in Lake Balaton and Lake Belső (OLÁH, 1971). It seems to be regular that the destruction and mineralization of the large blue-green algal biomass leads to an extreme increase in the bacterial biomass following the minimum value of it. The complex causal relationships between the blue-green algae and the bacteria described even here will be elucidated in more detail in the future.

On the basis of literary data and considering the bacterial biomass, the waste stabilization pond of Fonyód can be classified as a pond of high productivity and of medium loading.

3. *The changes of biomass of phytoplankton and the percentual composition of the algal groups*

a) Chlorophyll content and phytoplanktonic biomass. Similarly to the biomass of bacterioplankton, the amount of chlorophyll-a decreased after a high spring value *(Table V)*. By the end of June, after the restarting of the sewage-water inflow, the chlorophyll-a content increased again. During July, August and September, the amount of chlorophyll-a extremely increases in

TABLE V

Chlorophyll-a content in µg/litre in 1971

	13th May	9th June	23rd June	6th July	10th Aug.	29th Aug.	14th Sept.	12th Oct.	21st Oct.
1.	95.6	28.2	63	456	464	672	313	150	34
2.	58.4	32.4	114.1	589	603	545	823	150	104
3.	75.2	41.6	92	614	603	522	788	23	58
4.	84	26.9	106	536	487	661	730	11	174
5.	40	21.6	98	519	556	742	812	46	139
Keleti Bozót	16	14	11	—	—	—	—	—	—

consequence of the mass production of blue-green algae. Parallel with the decrease of blue-green algae during October the chlorophyll-a content also significantly decreased.

The chlorophyll-a content is usually lower at the points near the inflow than at the other places of the pond. This decrease in the chlorophyll-a content at the inflow is especially conspicuous in the results of September.

The chlorophyll-a content of the Keleti-Bozót is low as compared to that of the pond.

Apart from the measurements of chlorophyll-a, in some investigations the amounts of chlorophyll-b and c were also measured. At some points the amount of chlorophyll-c reached the value of 200 µg/l indicating an important role of diatoms in the photosynthetic organic production as well as indirectly in the production of oxygen necessary for the aerobic bacteria to decompose organic substances.

The quantitative estimation of phytoplanktonic biomass on the basis of the pigment content is widely known. For this purpose the following formula was used:

$$\text{mg C} = 25 \times \text{mg chlorophyll-a.}$$

The phytoplanktonic biomass and the pigment contents indicate *(Table VI)* an important role of photosynthetic oxygen production in the decrease of organic content of the sewage-water as well as in the processes of decomposition and mineralization. The bacterioplanktonic biomass is somewhat lower in natural, non-contaminated waters or even identical with the phytoplank-

TABLE VI

The biomass values of the phytoplankton in gC/m³ in 1971

	13th May	9th June	23rd June	6th July	10th Aug.	29th Aug.	14th Sept.	12th Oct.	21st Oct.
1.	4.7	1.4	3.1	22.8	23.2	33.6	15.6	7.5	1.7
2.	2.9	1.6	5.7	29.4	30.1	27.2	41.1	7.5	5.2
3.	3.7	2.0	4.6	30.7	30.1	26.1	39.4	1.1	2.9
4.	4.2	1.3	5.3	26.8	24.3	33.0	36.5	0.5	8.7
5.	2.0	1.6	4.9	25.9	27.8	37.1	40.6	2.3	6.9
Keleti Bozót	0.8	0.7	0.5	—	—	—	—	—	—

tonic one. The phytoplanktonic biomass of the waste stabilization pond of Fonyód significantly surpassed that of the bacterioplankton especially during the blue-green algal bloom.

b) Qualitative relationships of algal species *(Table VII)*. The number of algal species determined in the drawn-filtered samples collected at 5 points of the pond No. 1 between the 13th May and 21st October, 1971 as well as in the water of Keleti-Bozót, amounted to 114. They showed the following phylal distribution:

Cyanophyta	14
Euglenophyta	6
Pyrrophyta	3
Chrysophyta	34
Chlorophyta	56
Mycophyta	1
	114

Among the four systematic groups, the species were distributed as follows:

Occurrence of Cynophyta species in the places of sampling of the pond No. 1 during 1971.

	Points of sampling					
	1	2	3	4	5	Keleti-Bozót
13th May		2				
25th May	3	3	3	2	1	1
9th June	5	5	3	5	6	
23rd June	10	4	2	6	5	1
6th July	7	6	9	9	8	1
10th August	6	8	4	8	7	
29th August	5	6	4	9	9	
14th September	6	7	8	7	7	
12th October	10	8	8	5	4	
21st October	6	5	6	6	7	

The number of individuals of the following 4 species gradually increased from July:

Anabaena scheremetievi ELENKIN
Aphanocapsa delicatissima W. et G. S. WEST
Microcystis flos-aquae (WITTROCK) KIRCHNER
Spirulina laxissima G. S. WEST.

All four species prefer the strongly contaminated waters, i.e. they are mesosaprobic organisms. The *Anabaena scheremetievi* reached the total bloom by the end of August and beginning of September. Its exact taxonomical place could be determined on the basis of the persisting cells. Several variants and forms of it also occurred, but, they have not been identified. *Aphanizomenon flos aquae* var. *klebahnii* ELENKIN and *Anabaena spiroides* KLEB. are also characteristic beta-mesosaprobic species. Both occurred rather frequently in the samples.

Euglenophyta

1971	1	2	3	4	5	Keleti-Bozót
25th May		2		1	1	
9th June		1	1			
23rd June	2	2	3		3	
6th July		2	3	1	2	
10th August	1		1	1		
14th September		1	1	1	1	
12th October	2		1	1	1	
21st October	2	1	2	3	2	

Euglena klebsii (LEMM.)MAINX and *Phacus pyrum* (EHR.) STEIN were present in the majority of samples, during the blue-green algal bloom only the *Euglena klebsii* appeared, their number decreased to only a few individuals by August—September.

Pyrrophyta

1971	1	2	3	4	5	Keleti-Bozót
9th June	1					
6th July			1	1		
10th August				1		
29th August				2	1	
14th September	1	1	1	1	1	
12th October	2	3	1	1	1	
21st October	1		2		2	

The Mallomonas species appeared in the samples from July, whereas none of them were found during the bloom of Anabaena during September. They reappeared in October.

Larger numbers of *Peridinium sp.* are significant at the end of August, September and October. The highest number of individuals was found just during the Anabaena bloom.

Chrysophyta

1971	1	2	3	4	5	Keleti-Bozót
13th May			11			
25th May	12	8	4	2	8	4
9th June	14	10	8	10	10	5
23rd June	12	12	10	15	13	
6th July	11	12	11	13	13	8
10th August	6	5	4	4	7	1
29th August	2	6	3	3	9	
14th September		3	4	1	6	
12th October	8	6	7	4	4	
21st October	7	5	8	5	5	

The majority of species are beta-mesosaprobic. Among the 3 classes of this phylum, 2 species of Xantophyceae *(Botryococcus braunii* and *Tetrakentron*

TABLE VII

The phytoplankton data of fish-pond No. 1 of Fonyódliget between 13th May, 1971 and 21st October, 1971

Symbols: 1—5 places of sampling in the pond; KB = Keleti-Bozót; e = appears; k = low number of individuals; g = frequently occurs; s = numerous; t = mass, algal bloom; β-m = beta-mesosaprobionts; ehl = euryhaline.

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
CYANOPHYTA								
1. *Anabaena scheremetievi* ELENKIN	9th June					e		
	6th July	k	k	k	k	k		
	10th Aug	s	s	s	s	s		
	29th Aug	s	s	s	s	s		β—m
	14th Sept	t	t	t	t	t		
	12th Oct	g	k	k	k	k		
	21st Oct	k	k	k	k	k		
2. *A. spiroides* KLEB.	13th May			e				
	9th June			e		e		
	23rd June	k			k	k		
	6th July				k	k		
	10th Aug		k	k	k	k		β—m
	29th Aug				k			
	12th Oct	k						
3. *Aphanizomenon flos-aquae* var. *klebahnii* ELENKIN	9th June	k	k	k	k			
	23rd June	k	k	k	k			
	6th July	k	k	k	k	k		β—m
	10th Aug		k					ehl
	29th Aug				k	k		
	14th Sept			k		k		
	12th Oct	g	k	k				
	21st Oct	k	k		k	k		
4. *Aphanocapsa delicatissima* W. et G. S. WEST	25th May	k	k	k				
	9th June	k	k		k	k		
	23rd June	s	k	k	k	k		
	6th July	s	k	k	s	k		
	10th Aug	k	k		s	s		
	29th Aug	s	k	k	s	s		
	14th Sept	g	s	s	g	s		
	12th Oct	k	k	k	k	g		
	21st Oct	k	k	k	k	k		
5. *A. elachista* W. et G. S. WEST	25th May				k			
	23rd June	k					k	
	29th Aug				k	s		
6. *Chroococcus limneticus* LEMM.	25th May	k						
	9th June	k	k	k	k	k		
	23rd June	g	k		k	k		
	6th July	k	k	k	k	k		
	10th Aug	k	k	k	k	k		
	29th Aug		k	k	k	k		
	14th Sept	k	k	k		k		
	12th Oct	k	k	k	k			
	21st Oct		k			k		

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
7. *Gomphosphaeria lacustris* CHOD.	9th June	k				k		
	23rd June	k	k		k			
	6th July	k	k	k	k	k		β—m
	10th Aug	k						
8. *Lyngbya limnetica* LEMM.	25th May	k	k	k	k	g	k	
	9th June	k	k		k	k		
	23rd June	k			k	k		
	6th July	k	k	k	k	k		
	10th Aug	k	k		g	k		
	29th Aug	k	k	k	k	k		
	14th Sept	g	k	k	k	k		
	12th Oct	k	k	k				
	21st Oct	k		k		g		
9. *L. martensiana* MENEGH.	13th May			k				
	25th May		k					
	23rd June	k						
	6th July			k				
	10th Aug					k		β—m
	14th Sept	k						
10. *Merismopedia glauca* (EHR.) NAEG.	6th July				k		k	
	10th Aug		k		k			
	29th Aug		k		k	k		ehl
	14th Sept		k	k	k			
	12th Oct	k				k		
11. *Microcystis flos-aquae* (WITTROCK) KIRCHNER	25th May			k				
	9th June		k		k			
	23rd June	k				k		
	6th July	k		k	k	s		β—m
	10th Aug	k	k	s	k	s		ehl
	29th Aug	k	k			s		
	14th Sept	k	g	g	s	g		
	12th Oct	k	k	k	k	k		
12. *Oscillatoria princeps* VAUCHER	23rd June	k						
	12th Oct	k	k	k				
	21st Oct			k	k	k		α—m
13. *Romeria elegans* (WOLOSZ.) KOCZW.	6th July			k		k		
	10th Aug				k			
	29th Aug					k		
	14th Sept				k			
14. *Spirulina laxissima* G. S. WEST	29th Aug	k	g		k			
	14th Sept		k	k	k	k		
	12th Oct	k	k	k	k	k		
Euglenophyta	21st Oct	k		k	k	k		
15. *Euglena ehrenbergii* KLEBS	23rd June			e				

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
16. *E. klebsii* (LEMM.) MAINX	23rd June			k		k		
	6th July		k	k	k	k		
	10th Aug			k	k			
	14th Sept		k	k	k	k		
	12th Oct	k		k	k	k		
	21st Oct	k	k	k	k	k		
17. *E. oxyuris* SCHMARDA	23rd June		k					
	6th July		k	k				
18. *Phacus acuminatus* STOKES	25th May		k		k			
	6th July			k				
	21st Oct				k			
19. *P. pseudonordstedtii* POCHM.	25th May		e					
	23rd June	e				e		
	6th July					e		
20. *P. pyrum* (EHR). STEIN.	25th May					k		
	9th June		k	k				
	23rd June	k	k	k		k		
	10th Aug	k						
	12th Oct	k				k		
	21st Oct	k		k	k	k		
PYRROPHYTA Cryptophyceae								
21. *Mallomonas acaroides* PERTY	6th July			k	k			
	10th Aug				k			
	12th Oct		k					
	21st Oct				k	k		
22. *M. tonsurata* TEIL.	29th Aug				k	k		
	12th Oct	k	k					
	21st Oct			k		k		
Dinophyceae								
23. *Peridinium* sp.	9th June	k						
	29th Aug				k			
	14th Sept	k	k	g	k	k		
	12th Oct	k	k	k	k	k		
	21st Oct	k		k		k		
CHRYSOPHYTA Xantophyceae								
24. *Botryococcus braunii* KÜTZ.	9th June	k						
	23rd June				k			
	6th July		k	k	k	k		
	10th Aug			k		k		
25. *Tetrakentron tribulus* GEITLER	9th June					k		
	23rd June	k	k		k			
	6th July		k	k	k	k		
	10th Aug					k		
Chrysophyceae								

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
26. *Amphichrysis compressa* KORCSH.	29th Aug		k			k		
	12th Oct	k	k	k				
	21st Oct	k	k	k		k		
27. *Chromulina* sp.	9th June	e						
	12th Oct			e				
28. *Rhizochrysis limnetica* G. M. SMITH	9th June	k	k	k		k		
	23rd June			k		k		
	6th July	k		k	k	k		
	10th Aug			k	k			
Bacillariophyceae								
29. *Amphora ovalis* KÜTZ.	25th May		e				e	
	9th June	k	k	k				
	23rd June	k	k	k	k	k		
	6th July	k			k	k	k	
	10th Aug	e						
	14th Sept		e					
30. *Anomoeoneis sphaerophora* (KÜTZ) PFITZNER	9th June				e			
	23rd June					e		
	10th Aug					e		
31. *Cocconeis diminuta* PANT.	9th June				e			
32. *C. placentula* EHR.	13th May				k			
	25th May	k					k	
	9th June	k	k	k		k	k	
	23rd June			k	k			
	6th July		k				s	
	21st Oct	k						
33. *Cyclotella meneghiniana* KÜTZ.	25th May	k	k	k		k		
	9th June	k	k	k	k			
	23rd June	k	k	k		k		
	6th July	k	k	k	k	k	k	β—m
	10th Aug	k	k		k			
	29th Aug	k	k	k		k		
	14th Sept					k		
	12th Oct	k	k	k	k			
	21st Oct	k	k	k	k			
34. *C. ocellata* PANT.	9th June						e	
	23rd June				e			
	6th July					e		
35. *Cymatopleura solea* (BRÉB.) W. SMITH	25th May			e				
	23rd June	k	k	k		k		
	6th July	k		k				
	29th Aug	k				k		
	14th Sept		k	k				
	21st Oct	e						

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
36. *C. prostata* (BERK.) CLEVE	9th June					k		
	23rd June	k	k	k	k			
	6th July			k			s	
	10th Aug	k	k					
37. *Cymbella cymbiformis* (KÜTZ.) V. HEURCK	9th June				e	e		
	23rd June				e			
	10th Aug		e					
38. *Epithemia sorex* KÜTZ.	25th May					k		
	23rd June					k		
	6th July				k	k		
	10th Aug	k						
	14th Sept		k		k			
39. *Fragilaria construens* (EHR.) GRUN.	13th May				k			
	25th May	g	g					
	9th June	k	k		k	k	k	
	23rd June	g	k	k	k	k		
	10th Aug	k	k	k	k			
	29th Aug		k		k	k		
	21st Oct			k				
40. *Gomphonema lanceolatum* EHR.	9th June				e			
41. *Gyrosigma kützingii* (GRUN.) CLEVE	25th May	k	k	k		k		
	9th June	k	k	k	k			
	23rd June		k		k	k		
	6th July	k	k		k	k		
	10th Aug					k		
	29th Aug				k			
42. *Melosira granulata* (EHR.) RALFS	25th May	k						
	9th June	k			k	k		
	23rd June	k	k		k			
	6th July		k	k	k			
	10th Aug	k			k		k	
	29th Aug		k	k		k		
	14th Sept					k		
	21st Oct	k		k		k		
43. *M. granulata* var. *angustissima* MÜLL.	13th May				k			
	25th May	k						
	9th June							
	14th Sept					k		
	12th Oct	k		k		k		
	21st Oct		k	k	k			
44. *M. granulata* var. *angustissima* f. *spiralis* MÜLL.	13th May				g			
	9th June	k	k	k		k		
	23rd June	k	k			k		
	6th July	k	k	k	k	k		
	10th Aug		k	k				
	29th Aug		k		k	k		
	14th Sept			k				
	12th Oct	k	k	k		k		
	21st Oct	k		k	k			

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
45. *M. varians* C. A. Ag.	13th May				k			
	25th May	k	k			k		
	9th June		k			k		
	23rd June				k			β—m
	6th July				k		k	
	14th Sept		k	k				
46. *Navicula cryptocephala* Kütz.	13th May				e			
	25th May						e	
	6th July						e	β—m
	29th Aug					e		
	14th Sept			e				
47. *N. hungarica* var. *capitata* (Ehr.) Cleve	13th May				k			
	25th May		k					
	9th June						k	
	23rd June	k				k		
	14th Sept					k		
48. *Nitzschia acicularis* W. Smith	13th May				k			
	25th May	k	k	k	k	g		
	9th June	k			k	k		
	23rd June	k	k	k	k	k		β—m
	6th July	k	k	k	k	k	k	
	12th Oct	s	s	s	s	s		
	21st Oct	s	s	s	s	s		
49. *N. sigmoidea* W. Smith	13th May				k			
	25th May	k						
	9th June	k		k				
	23rd June	k			k	k		
	6th July		k			k		
	29th Aug			k				
	14th Sept					k		
50. *Pinnularia maior* Kütz.	25th May	k						
	23rd June				k			
	6th July		k					
	12th Oct	k						
51. *Rhizosolenia longiseta* Zach.	25th May				k	k		
	9th June	k	k		k	k		
	23rd June			k				
	6th July	k		k	k	k		
	10th Aug					k		
52. *Strauoneis alabamae* Heiden	13th May				e			
	25th May	e				e		
	6th July	e						
53. *Stephanodiscus hantzschii* Grun.	29th Aug					k		
	12th Oct	k	k	k				β—m
	21st Oct					k		

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
54. *Surirella robusta* var. *splendida* (Ehr.) V. Heurck	25th May						k	
	9th June	k	k	k				
	23rd June	k	k		k	k		
	6th July		k			k		
	10th Aug					k		
	29th Aug					k		
55. *S. tenera* Greg.	25th May		k					
	23rd June		k					
	6th July	k						
	10th Aug					k		
56. *Syndera acus* Kütz.	6th July		k					
57. *S. acus* var. *angustissima* Grun.	13th May					k		
	25th May				k			
	23rd June			k				
	14th Sept					k		
	12th Oct	s	s	s	s	s		
	21th Oct	s	s	s	s	s		
Chlorophyta								
Volvocales								
58. *Chlamydomonas* sp.	9th June					k		
59. *Pandorina morum* Bory	9th.June						k	β—m
Chlorococcales								
60. *Actinastrum hantzschii* Lagerh.	13th May				k			
	25th May	k	k	k	k			
	9th June					k		
	29th Aug		k					β—m
	12th Oct	k	k	k		k		
	21st Oct	k		k	k	k		
61 *Ankistrodesmus falcatus* (Corda) Ralfs	13th May				k			
	25th May	k	k		k	k	k	
	9th June		k					
	23rd June		k					
	6th July	k	k				k	β—m
	10th Aug			k	k			
	29th Aug		k			k		
	14th Sept					k		
	12th Oct	g	k		k	k		
	21st Oct		k	g	g	k		
62. *A. falcatus* var. *acicularis* (A. Braun) G. S. West	25th May					e		
	13th May				e			
	12th Oct			e				
63. *A. falcatus* var. *mirabile* W. et G. S. West .	9th July					e		

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
64. *A. lacustris* (CHOD.) OSTENF.	25th May		k	k	k	k		
	9th June	k	k	k	k	k		
	23rd June	k		k	k	k		
	6th July	k	k	k	k	k	k	
	12th Oct	k	k					
65. *A. longissimus* (LEMM.) WILLE	9th June					k		
	23rd June				k			
	12th Oct	k	k					
66. *Chodatella balatonica* SCHERFFEL	9th June					k		
67. *C. ciliata* (LAGERH.) LEMM.	9th June	k	k	k	k	k		
	23rd June	k		k	k	k		β—m
	6th July			k	k			
	10th Aug		k					
	29th Aug					k		
68. *C. quadriseta* LEMM.	25th May		k		k			
	21st Oct	k						
69. *Coelastrum microporum* NAEG.	25th May		k	k				
	9th June		k	k	k	k		
	23rd June				k			
	6th July			k	k		k	β—m
	10th Aug	k	k		k	k		
	29th Aug	k	k	k	k	k		
	14th Sept	k	k		k	k		
	12th Oct	k		k		k		
	21st Oct	k	k	k	k			
70. *C. sphaericum* NAEG.	9th June	e						
	10th Aug				e			
	29th Aug					e		
71. *Crucigenia tetrapedia* (KIRCHN.) W. et G. S. WEST	9th June	k	k		k			
	23rd June		k		k	k		
	6th July			k		k		β—m
	12th Oct	k				k		
	21st Oct	k	k	k				
72. *Dictyosphaerium pulchellum* WOOD	13th May				k			
	9th June	k	k					
	23th June		k		k	k		
	6th July				k	k		β—m
	10th Aug			k				
	12th Oct			k				
	21st Oct			k				
73. *Golenkinia radiata* CHOD.	9th June	e						
	23rd June		k					
	6th July			k				
	10th Aug	k	k			k		
	29th Aug	k	k	k	k	k		
	14th Sept				k	k		
	12th Oct		k	k	k	k		
	21st Oct	k	k	k	k	k		

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
74. *Micractinium pusillum* FRESENIUS	10th Aug	k				k		
	10th Aug	k				k		
	14th Sept		k	k	k	k		
	12th Oct		k	k	k	k		
	21st Oct			k	k	k		
75. *Oocystis solitaria* WITTR.	25th May			k				
	9th June		k	k	k	k	k	
	23rd June	k		k	k	k		
	6th July	k	k	k	k	k		
	10th Aug	k						
	29th Aug			k				
	14th Sept						k	
76. *Pediastrum boryanum* (TURPH.) MENEGH.	25th May	k	k					
	9th June	k	k					
	23rd June			k			k	
	6th July	k						β—m
	10th Aug	k	k	k				
	29th Aug		k		k			
	12th Oct	k		k		k		
	21st Oct			k	k	k		
77. *P. boryanum* var. *granulatum* (KÜTZ.) A. BRAUN	13th May				k			
	25th May	k	k	k	k	k	k	
	9th June	k		k	g	g	k	
	23th June	k	k		k	k		
	6th July	k	k	k	k	k		
	10th Aug		k	k	k	k		
	29th Aug			k		k		
	14th Sept	k	k	k	k	k		
	12th Oct		k					
	21st Oct	k	k	k				
78. *Pediastrum simplex*	25th May	k		k	k	k		
	9th June	k	k	k	k	s		
	23rd June	k	k	k	k	k	k	
	6th July	k	k	k	k	k		
	10th Aug	k	k	k	k	k	k	
	29th Aug	k	k	k	k	k		
	14th Sept	k	k	k	k	k		
	12th Oct	k	k	k	k	k		
	21st Oct	k	k	k	k	k		
79. *P. tetras* (EHR.) RALFS	13th May				k			
	9th June	k	k	k	g	k		
	23rd June	k		k	k	k		
	6th July	k	k	k	k	k	k	
	10th Aug	k			k			
	29th Aug	k	k	k	k			
	14th Sept	k				k		
	12th Oct	k	k	k	k	k		
	21st Oct		k	k		k		

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
80. *Scenedesmus acuminatus* (LAGERH.) CHOD.	13th May				g			
	25th May	g	k	k	k	k	k	
	9th June	k	k	k	k	k		
	23rd June	k	k	k	k	k	k	
	6th July			k	k	k	k	
	10th Aug	k				k		
	12th Oct	k	k	k	k	k		
	21st Oct	k	k	k	k	k		
81. *S. carinatus* var. *polycostatus* HORTOB. et NÉMETH	9th June	k	k	g	g			
	23rd June	k	k	g	g	k		
	6th July	k	k	k	k	s		
	10th Aug	k	k	k	k	k		
	29th Aug	k	k	k	k	k		
	14th Sept	k	k	k	k	k		
	12th Oct	s	g	k	k	k		
	21st Oct	k		k	k	k		
82. *S. denticulatus* LAG.	6th July		k					
83. *S. dispar* BRÉB.	9th June			k				
	12th Oct					k		
84. *S. ecornis* (RALFS) CHOD.	23rd June		k		k			
	6th July		k		k	k		
85. *S. ecornis* var. *disciformis* CHOD.	23rd June		k					
	21st Oct		k					
86. *S. ellipsoideus* CHOD.	23rd June			k		k		
87. *S. intermedius* CHOD.	9th June	k	k	k	k			
	23rd June	k		k	k	k		
	6th July			k	k	k	k	
	10th Aug		k		k	k		β—m
	29th Aug					k		
	14th Sept		k	k				
	12th Oct	k	k	k		k		
	21st Oct	k		k				
88. *S. quadricauda* (TURP.) BRÉB.	13th May				s			
	25th May	s	k	k		s	k	
	9th June	s	k	s	s	s	k	
	23rd June	s	s	s	s	s	k	
	6th July	s	s	s	s	s	k	β—m
	10th Aug	s	k	k	s	s		
	29th Aug	s	s	s	k	s		
	14th Sept	s	s	s	k	s		
	12th Oct	s	s	s	s	s		
	21th Oct	s	s	s	s	s		
89. *S. quadricauda* var. *biornata* KISS	25th May	k						
	9th June		s	k		s		
	23rd June		k					
	6th July		k		k	k		
	10th Aug	k	k	k	k			
	29th Aug	k	k		k	k		
	14th Sept					k		
	12th Oct	k			k	g		
	21st Oct	g			g			

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
90. *Scenedesmus quadricauda* var. *biornata* f. *gigantica* UHERKOV.	9th June	g	s	g	g	s		
	23rd June	g	g	g	g	g		
	6th July	k	k	k	k	s	k	
	10th Aug	k	k	k		k		
	29th Aug	k	k	k	k	k		
	14th Sept	k	k	k	k	k		
	12th Oct	k	k	k	k	g		
	21st Oct	g	k	k				
91. *S. quadricauda* var. *longispina* (CHOD.) G. M. SMITH	13th May				s			
	25th May	s	k	k	s	g		
	9th June	s	k	s	s	s	k	
	23th June	s	s	s	s	s	k	
	6th July	s	s	s	s	s	k	
	10th Aug	k	k	k	s	s		
	29th Aug	ss	s	k		s		
	14th Sept	s	k	g	k	k		
	12th Oct	s	s	g	k	k		
	21st Oct	k	k	k		k		
92. *S. pannonicus* HORTOB.	9th June	k	k					
	23rd June	k	k			k		
	26th July			k	k	k		
	29th Aug		k			k		
93. *S. spinosus* CHOD.	13th May				s			
	25th May	k	g		k	s		
	9th June	k	s	k	k	g	k	
	23rd June	g		g	s	k		
	6th July	k	k	g		k		β—m
	10th Aug	k				k		
	29th Aug				k			
	14th Sept	k						
	12th Oct			k	k	k		
	21st Oct	k		k	k	k		
94. *Selenastrum gracile* REINSCH	9th June					k		
	6th July				k		k	
	29th Aug	k						
	14th Sept	k	k	k				
95. *Tetraedron caudatum* var. *incisum*. LAGERH.	13th May				k			
	25th May	k	k	k	k	g		
	9th June	k	g	k	g	k		
	23rd June	g	k	k	k	g		
	6th July	k	k	k	g	k		
	10th Aug		k	k	k	k		
	29th Aug	k	k		k	k		
	14th Sept	k		k				
	12th Oct	k		k	k	k		
	21st Oct	k	k	k		k		
96. *T. cruciatum* (WALLICH) W. et G. S. WEST	9th May	k	k					
	10th Aug			k				

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
97. *T. hastatum* var. *palatinum* LEMM.	9th June		k	k	k			
	23rd June	k	k					
	6th July	k	k	k				
	10th Aug	k	k	k				
	29th Aug	k	k					
	14th Sept			k				
	21st Oct		k					
98. *T. minimum* (A. BRAUN) HANSG.	13th May				g			
	25th May	k	k	k		k		
	9th June	k	g	k	s	k	k	
	23rd June	g	k	k	k	g		
	6th July	k	k	k	g	g	k	
	10th Aug	k	k	k	k	k		
	29th Aug	k	k	k	k	k		
	14th Sept	k	k	k	k	k		
	12th Oct	k	k	k	k	k		
	21st Oct	k	k	k	k			
99. *T. muticum* (A. BRAUN) HANSG.	9th June	k	k			k		
	23rd June	k	k	k	k	k		
	6th July	k		k	k	k		
	10th Aug		k		k			
	12th Oct					k		
	21st Oct	k		k	k			
100. *Tetraedron proteiforme* (TURNER) BRUNNTH.	9th June					e		
101. *T. regulare* KÜTZ.	13th May				k			
	9th June	k		k	k	k		
	23rd June	k	k	k		k		
	10th Aug	k	k					
	29th Aug		k		k	k		
	14th Sept				k	k		
	12th Oct	k	k	k		k		
	21st Oct		k		k	k		
102. *Tetrastrum heteracanthum* (NORDST.) CHOD.	25th May		k			k		
103. *T. staurogeniaeforme* (SCHROED.) LEMM.	13th May				k			
	9th June	k	k	g	k	k	k	
	23rd June	k		k		k		
	6th July	k	k	k	k	k		β-m
	29th Aug					k		
	12th Oct	k	k	k	k			
	21st Oct	k	k	k	k	k		
104. *Treubaria triappendiculata* BERN.	9th June	k	k	k	k	k		
	23rd June	k		k	k	k		
	6th July	k		k	k	k		
	10th Aug	k				k		
	29th Aug			k				
	12th Oct		k	k				
Zygnematales	13th May				k			

TABLE VII *(continued)*

Species	Date of sampling	1.	2.	3.	4.	5.	KB	Note
105. *Closterium acerosum* var. *elongatum* BRÉB.	12th Oct	k		k				
106. *Cosmarium* sp. (I.)	25th May					k		
	9th June	k			k	k		
	23rd June	k	k		k	k		
	6th July	k	k		k	k		
107. *Cosmarium* sp. (II.)	9th June	k		k	k	k		
	23rd June				k			
	6th July				k	k		
108. *Cosmarium* sp. (III.)	6th July					k		
	10th Aug	k						
	29th Aug	k	k					
109. *Staurastrum furcigerum* BRÉB.	25th May					k		
	9th June					k		
	23rd June			k		k		
	6th July	k	k	k	k	k		
	10th July		k					
110. *S. gracile* RALFS	9th June					k		
	29th Aug				k			
	14th Sept	k				k		
	12th Oct					k		
	21st Oct		k					
111. *S. paradoxum* MEYEN	25th May		k		k			
	9th June	k	k	k		k		
	23rd June	k	k	k	k	k		
	6th July	k	k	k	k			
	10th Aug.					k		
	29th Aug					k		
112. *Spirogyra* sp. (I.)	13th May				g			
	9th June		k		k	k		
113. *Spirogyra* sp. (II.)	13th May				k			
	9th June		k			k	k	
	25th June						k	
MYCOPHYTA								
114. *Planktomyces békefii* GIMESI	13th May				k			
	10th Aug	k						
	12th Oct				k			
	21st Oct		k			k		

tribulus) occurred and reached a higher number of individuals during July—August. None of them were found before and after the end of August.

Among the 3 representatives of Chrysophyceae, *Rhizochrysis limnetica* was very frequent during June beginning of August. During the blue-green algal bloom it disappeared from the samples. *Amphychrysis compressa* occurred

in the samples of August. It be came rare during the intense Anabaena bloom in September and was again frequent in the samples of places No. 1, 2 and 3 in October.

Pelagic, benthic and epiphytic species appeared among the species of Bacillariophyceae. The pelagic *Melosira granulata* and its variant as well as its spirale form were present from May till the end of October, their number significantly decreased during the Anabaena bloom, whereas later on many empty shells were found. In the October samples the variant and the spiral form reached a higher number of individuals. The *Cyclotella meneghiniana* was very frequent in all samples except the period of algal bloom in September. The individuals of *Fragilaria construens* floating in forms of ribbon and the fragile *Rhizosolenia longiseta* reached their highest frequency from May till the beginning of August, during the algal bloom they occurred only sporadically. After the mass appearance of blue-green algae none of them were found again.

The *Nitzschia acicularis* and *Synedra acus* var. *angustissima* were of rather high frequency from May till June. None of them were found during July and August, whereas their number abruptly increased by October at all 5 places of sampling.

Stephanodiscus hantzschii preferring strongly eutrophic waters appeared in masses after the Anabaena bloom in October.

Chlorophyta

1971	1	2	3	4	5	Keleti-Bozót
13th May			17			
25th May	12	15	11	12	14	4
9th June	28	29	24	23	34	10
23rd June	23	22	22	25	25	6
6th July	21	22	25	27	27	11
10th August	19	18	13	15	14	
29th August	15	19	12	14	18	
14th September	13	11	12	11	15	
12th October	21	19	22	15	23	
21st Oct.	19	18	22	16	15	

The 56 species of green algae found in the samples can be divided into 3 groups according to their frequency, similarly as the diatoms:

1. Those occurring in all samples and showing often a high number of individuals, e.g. *Coelastrum microporum*, *Pediastrum* species, *Scenedesmus quadricauda* and its variants and forms, *Tetraedron* species. These were not influenced by the Anabaena bloom.

2. Those influenced strongly by the blue algal bloom, their number of individuals decreased to be sporadic during August—September. Their number is higher before and after the algal bloom, e.g. *Scenedesmus spinosus*, *Tetrastrum staurogeniaeforme*, *Treubaria triappendiculata*.

3. Two subgroups can be distinguished: *a)* Significant number of individuals during May—June—July, i.e. before the Anabaena bloom, e.g. *Ankistrodesmus lacustris*, *Chodatella ciliata*, *Oocystis solitaria*, *Staurastrum*

furcigerum and *S. paradoxum. b)* Higher number of individuals appear just during and after the Anabaena bloom, e.g. *Golenkinia radiata, Micractinium pusillum.*

Mycophyta

1971	1	2	3	4	5	Keleti-Bozót
10th August	1					
29th August		1				
14th September				1		
21st October	1				1	

The *Planctomyces békefii* was of higher frequency before and after the Anabaena bloom in the plankton of the pond.

The total number of algal species in the pond and the canal shows the following distribution:

1971	1	2	3	4	5	Keleti-Bozót
13th May			30			
25th May	27	28	18	17	24	9
9th June	48	45	36	38	50	15
23rd June	47	40	37	46	46	7
6th July	39	42	49	51	50	20
10th August	33	31	22	29	28	1
29th August	22	32	19	28	37	
14th Sept.	20	23	26	22	30	
12th Oct.	43	36	39	26	33	
21st Oct.	35	30	40	30	32	

The percentual distribution of the algal phyla according to their number of individuals in the pond No. 1 on the basis of the samples of May—October, was as follows:

13th May	
Cyanophyta	5%
Chrysophyta	15%
Chlorophyta	80%
	100%

25th May	
Cyanophyta	10%
Euglenophyta	4%
Chrysophyta	26%
Chlorophyta	60%
	100%

9th June	
Cyanophyta	15%
Euglenophyta	5%
Pyrrophyta	1%
Chrysophyta	19%
Chlorophyta	60%
	100%

23rd June	
Cyanophyta	12%
Euglenophyta	8%
Chrysophyta	20%
Chlorophyta	60%
	100%

9th July	
Cyanophyta	12%
Euglenophyta	5%
Pyrrophyta	3%
Chrysophyta	20%
Chlorophyta	60%
	100%

10th August	
Cyanophyta	30%
Euglenophyta	2%
Pyrrophyta	2%
Chrysophyta	25%
Chlorophyta	40%
Mycophyta	1%
	100%

29th August	
Cyanophyta	50%
Pyrrophyta	5%
Chrysophyta	14%
Chlorophyta	30%
Mycophyta	1%
	100%

14th September	
Cyanophyta	60%
Euglenophyta	3%
Pyrrophyta	3%
Chrysophyta	10%
Chlorophyta	23%
Mycophyta	1%
	100%

12th October	
Cyanophyta	20%
Euglenophyta	5%
Pyrrophyta	5%
Chrysophyta	35%
Chlorophyta	35%
	100%

21st October	
Cyanophyta	20%
Euglenophyta	5%
Pyrrophyta	4%
Chrysophyta	30%
Chlorophyta	40%
Mycophyta	1%
	100%

4. *The change of biomass and percentual composition of the zooplankton during the periods of investigations*

a) Rotatoria plankton

The average volume of 9 predominating Rotatoria species was calculated by multiplication of measures of length, width and thickness.

Brachionus calyciflorus	10 688 986 μ^3
Brachionus diversicornis	3 696 491 μ^3
Anuraeopsis fissa	138 727 μ^3
Epiphanes macrourus	2 153 005 μ^3
Brachionus angularis	1 213 946 μ^3
B. calyciflorus amphiceros	2 997 164 μ^3
Proalides tentaculatus	131 604 μ^3
Pedalia mira	943 622 μ^3
Asplanchna sieboldi	100 737 294 μ^3

Considering the volume values determined by SEBESTYÉN, the average value of the volume of one Rotatoria individual (except Asplanchna) amounts to 1 622 444 μ^3. Including the Asplanchna, this value would be above 7 millions which is wholly unreal. Therefore, we used the former value for the calculations of the biomass.

The Rotatoria biomass shows various distribution within the transversal section both in space and time *(Tables VIII* and *IX)*. The lowest value were always obtained at the inflow except in a few cases (6th July, 29th August and 12th October).

13th May: The predominant species are identical both qualitatively and in quantitative rates at the places of sampling No. 2—5. At point No. 1, *Conochilus unicornis* was substituted by *Brachionus quadridentatus*. Predominant species: *Keratella cochlearis, Pompholyx sulcata, Keratella tecta, Conochilus unicornis, Brachionus angularis, Brachionus quadridentatus.*

TABLE VIII

The distribution of the Rotatoria biomass (g/m³) in pond No. 1 in 1971

Date of sampling	Points of sampling				
	1	2	3	4	5
13th May	0.400	1.019	1.173	1.055	0.832
25th May	1.537	3.682	3.169	3.432	3.179
9th June	0.270	0.295	0.410	0.280	0.338
23th June	0.165	0.166	0.253	0.209	0.231
6th July	0.534	0.469	0.491	0.342	0.317
10th Aug	0.372	0.507	0.460	0.303	0.776
29th Aug	1.598	0.928	1.112	0.837	0.741
14th Sept	0.830	1.025	0.886	1.046	1.120
12th Oct	0.393	0.407	0.417	0.350	0.196
21st Oct	0.165	0.233	0.183	0.170	0.194

TABLE IX

Changes of the quantitative section of pond No. 1 during in 1971 (individuals per litre)

Points of sampling	Date of sampling									
	13. V.	25. V.	9. VI.	23. VI.	6. VII.	10. VIII.	29. VIII.	14. IX.	12. X.	21. X.
1.	2408	9 241	1624	991	3209	2237	9610	4991	2361	990
2.	6127	22 143	1776	998	2818	3050	5580	6167	2450	1403
3.	7055	19 115	2464	1524	2953	2767	6685	5331	2508	1099
4.	6344	20 638	1684	1260	2056	1823	5031	6288	2106	1022
5.	5004	19 119	2034	1392	1908	4664	4457	6737	1181	1167

25th May: The predominant species are identical on all the 5 places of sampling. Their quantitative distribution shows identical rates on points No. 2—5 and differs only on No. 1. Predominant species: *Pompholyx sulcata, Brachionus diversicornis, Keratella tecta, Brachionus calyciflorus dorcas, Keratella quadrata.*

The horizontal distribution of the predominant species was roughly uniform on the whole area of the pond in those two times of sampling.

19th June: Only two predominant species are common in all places of sampling *(Keratella tecta, Pompholyx sulcata).* Further predominant species: *Filina longiseta, Brachionus angularis, Brachionus quadridentatus, Brachionus diversicornis, Keratella quadrata.*

23rd June: Four of the 5 predominant species appeared at all 5 points of sampling. The quantitative rates of the predominant species, however, strongly varied. Predominant species: *Keratella tecta, Pompholyx sulcata, Brachionus angularis, Polyarthra vulgaris, Filina longiseta, Keratella cochlearis, Brachionus calyciflorus dorcas, Trichocerca pusilla.*

6th July: Polyarthra vulgaris and *Pompholyx sulcata* occurred in the highest rates at points No. 1, 2 and 3, whereas at No. 4 and 5, *Keratella tecta*

and *Brachionus angularis* represented the largest mass. The predominant species were qualitatively identical on places No. 3, 4 and 5, however, their rates were different to each other. Predominant species: *Pompholyx sulcata, Polyarthra vulgaris, Keratella tecta, Brachionus angularis, Filinia longiseta, Keratella cochlearis.* The last one was represented by its subspecies, *K. c. macracantha* on places No. 1 and 2. Apart from the predominant species listed above, the *Brachionus budapestinensis* also appeared. At point No. 2, *Keratella quadrata* predominated instead of *K. cochlearis.*

10th August: Among the predominating species, the first place was occupied by *Filina longiseta* at all sampling points, its number of individuals varied between 1073—1846 per litre. Apart from it, 5 species were found occurring at least at 4 points in predominant quantities: *Brachionus calyciflorus amphiceros, Keratella tecta, Anuraeopsis fissa, Proalides tentaculatus, Polyarthra vulgaris.* From the last two the former and latter were absent at points No. 2 and 1, respectively. Other predominant species: *Trichocerca pusilla* in places No. 1, 2, 4 and 5; *Brachionus calyciflorus anuraeiformis* at points No. 2, 4 and 5; *Brachionus angularis* at points No. 4 and 5; *Brachionus budapestinensis* at point No. 2; *Pedalia mira* at No. 3; a *Bdelloidea sp.* and *Asplanchna sieboldi* at No. 5.

29th August: The highest number of individuals was yielded by *Epiphanes macrourus* (2034—2599 per litre) at points No. 2—5. The number of predominant species amounts to 8 *(Epiphanes macrourus, Polyarthra vulgaris, Anuraeopsis fissa, Keratella tecta, Filinia longiseta, Keratella cochlearis, Pedalia mira, Proalides tentaculatus).* Five of them occurred at all 5 points of sampling, two of them *(Keratella cochlearis* and *Proalides tentaculatus)* were absent from points No. 4, and 1, and so was *Pedalia mira* from No. 1 and 2. On this basis the pond can be regarded as uniform in the period of investigation. Wide quantitative differences occurred, however, just in this period (the total number of individuals per litre was almost twice as high at point No. 1 than at the others).

14th September: Among the predominant species, the quantitative rates of *Anuraeopsis fissa* and *Keratella tecta* were nearly identical at the sampling point of the transversal section. The number of predominant species was 10 *(Anuraeopsis fissa, Keratella tecta, Polyarthra vulgaris, Filinia longiseta, Epiphanes macrourus, Keratella cochlearis, Brachionus calyciflorus amphiceros, Brachionus angularis, Chromogaster ovalis, Proalides tentaculatus);* 6 were common at all points of sampling, *Brachionus calyciflorus amphiceros* was absent at point No. 2, and so was *Brachionus angularis* at No. 1 and 3. Chromogaster and Proalides were present in a dominant quantity only at point No. 5.

12th October: The number of predominating species decreased to 5, and to 3 at point No. 1. The transversal section can be regarded as uniform from the view point of the composition of the qualitative Rotatoria plankton. Predominant species: *Polyarthra vulgaris, Brachionus angularis, Brachionus calyciflorus amphiceros, Keratella tecta, Keratella cochlearis.*

21st October: The number of predominating species was 3 and 2 at point No. 1. As regards the qualitative composition of Rotatoria plankton, the 5 sampling point were identical. The quantitative relationships were balanced. Predominant species: *Brachionus angularis, Brachionus calyciflorus amphiceros, Polyarthra vulgaris.*

The number of species encountered during the whole investigation was 62.

1. *Asplanchna amphora* Western
2. *Asplanchna brightwelli* Gosse
3. *Asplanchna girodi* de Guerne
4. *Asplanchna sieboldi* (Leydig)
5. *Anuraeopsis fissa* (Gosse)
6. *Bdelloidea* sp.
7. *Brachionus angularis* Gosse
8. *Brachionus budapestinensis* Daday
9. *Brachionus calyciflorus calyciflorus* Pallas
10. *Brachionus calyciflorus anuraeiformis* Brehm
11. *Brachionus calyciflorus amphiceros* (Ehrbg)
12. *Brachionus calyciflorus dorcas* Brehm
13. *Brachionus calyciflorus dorcas spinosa* (Wierzejski)
14. *Brachionus diversicornis diversicornis* (Daday)
15. *Brachionus leydigi* Cohn
16. *Brachionus plicatilis* Müller
17. *Brachionus rubens* Ehrenberg
18. *Brachionus quadridentatus* Hermann
19. *Brachionus quadridentatus ancylognathus* Schmarda
20. *Brachionus quadridentatus brevispinus* Ehrenberg
21. *Brachionus quadridentatus cluniorbicularis* Skorikov
22. *Brachionus quadridentatus rectangularis* (Lucks)
23. *Brachionus quadridentatus rhenanus* (Lauterborn)
24. *Cephalodella gibba* (Ehrbg)
25. *Chromogaster ovalis* (Bergendal)
26. *Colurella adriatica* (Ehrbg)
27. *Colurella colurus* (Ehrbg)
28. *Colurella obtusa* (Gosse)
29. *Colurella obtusa aperta* Haner
30. *Collotheca* sp.
31. *Conochilus unicornis* Rousselet
32. *Euchlanis deflexa* (Gosse)
33. *Euchlanis dilatata lucksiana* Hauer
34. *Epiphanes macrourus* Barrois & Daday
35. *Filinia longiseta* (Ehrenberg)
36. *Keratella cochlearis* Gosse
37. *Keratella cochlearis macracantha* Lauterborn
38. *Keratella cochlearis macracantha micracantha* Lauterborn
39. *Keratella tecta* Gosse
40. *Keratella quadrata* Müller
41. *Lecane bulla* Gosse
42. *Lecane closterocerca* Schmerda
43. *Lecane hamata* Stokes
44. *Lecane lunaris* (Ehrbg)
45. *Lecane stenroosi* Meissner
46. *Lepadella ovalis* Müller
47. *Lepadella patella* Müller
48. *Lophocaris salpina* (Ehrbg)
49. *Monommata longiseta* Müller
50. *Mytilina mucronata* Müller
51. *Pedalia mira* Hudson
52. *Platyias quadricornis* (Ehrenberg)
53. *Philodina* sp.
54. *Polyarthra vulgaris* Carlin
55. *Pompholyx sulcata* Hudson
56. *Proalides tentaculatus* de Beauchamp
57. *Proales* sp.
58. *Rotatoria* sp.
59. *Trichocerca dixon-nuttalli* Jennings
60. *Trichocerca pusilla* Jennings
61. *Trichocerca stylata* Gosse
62. *Trichotria pocillum* Müller

Considering the whole period of investigations, we may state the following.

1. The characteristic species of the open water plankton such as *Keratella cochlearis, Pompholyx sulcata, Keratella tecta, Keratella quadrata* predominating at the beginning of May, completely disappear by August, and are not found in significant quantities even later.

2. The appearance of *Pompholyx sulcata* in the water indicates a temperature of about 20° C and contamination. Its disappearance by August cannot be explained by the change in temperature but can be connected only with the increase of pollution. This is also proved by the occurrence of this species in Lake Balaton (it is absent in the Keszthely Bay, Zánkai and Ponyi, 1970; 1972).

3. *Filinia longiseta* is of a cold stenothermic character in Lake Balaton, whereas in the pond investigated it grows better at higher temperatures.

b) Crustacean plankton

The changes of the Crustacean biomass show a uniform pattern in the pond. The development of Cladocera mass results in a maximum at the early

TABLE X

Quantitative changes of biomass of the Crustacea-plankton in the transversal section of pond No. 1 (the values are in g/m³) in 1971

Date of sampling	Groups of crabs	Points of sampling					
		1.	2.	3.	4.	5.	KB
	Cladocera	0.092	0.060	0.140	0.088	0.098	—
13th May	Copepoda	0.129	0.220	0.494	0.215	0.179	—
	Total Crustacea	0.221	0.280	0.634	0.303	0.277	—
	Cladocera	0.252	1.266	0.870	0.630	0.860	—
25th May	Copepoda	0.320	0.340	0.423	0.562	0.354	—
	Total Crustacea	0.572	1.606	1.293	1.192	1.214	—
	Cladocera	4.466	4.450	5.066	3.140	1.910	0.674
9th June	Copepoda	0.579	0.453	0.497	0.845	0.293	0.229
	Total Crustacea	5.045	4.903	5.563	3.985	2.203	0.903
	Cladocera	2.130	1.684	3.526	4.244	3.956	0.164
23rd June	Copepoda	0.212	0.439	0.442	0.577	0.599	0.041
	Total Crustacea	2.342	2.123	3.968	4.821	4.545	0.205
	Cladocera	0.400	4.110	1.730	2.097	1.140	0.166
6th July	Copepoda	0.221	0.472	0.437	0.105	0.343	0.021
	Total Crustacea	0.621	4.582	2.167	2.202	1.483	0.187
	Cladocera	0.060	0.064	0.036	0.054	0.010	—
10th Aug	Copepoda	0.189	0.283	0.323	0.291	0.314	—
	Total Crustacea	0.249	0.347	0.359	0.345	0.324	—
	Cladocera	0.024	0.030	0.026	0.001	0.010	—
29th Aug	Copepoda	0.280	0.184	0.164	0.098	0.084	—
	Total Crustacea	0.304	0.214	0.190	0.099	0.094	—
	Cladocera	0.006	0.006	0.016	0.010	0.004	—
14th Sept	Copepoda	0.121	0.116	0.112	0.117	0.040	—
	Total Crustacea	0.127	0.122	0.128	0.127	0.044	—
	Cladocera	0.006	0.002	—	—	—	—
12th Oct	Copepoda	0.026	0.045	0.065	0.036	0.045	—
	Total Crustacea	0.032	0.047	0.065	0.036	0.045	—
	Cladocera	0.014	0.004	0.006	0.002	0.008	—
21st Oct	Copepoda	0.094	0.043	0.079	0.090	0.058	—
	Total Crustacea	0.108	0.047	0.085	0.092	0.066	—

summer (about 4 g per m³) and abruptly decreases by August. Later on its amount is minimal *(Fig. 2, Table X)*. The Copepoda biomass is much lower (0.2—0.5 g per m³) during spring and early summer seasons than that of Cladocera, however, from August when the biomass of Cladocera is insignificant, that of Copepoda is considerable.

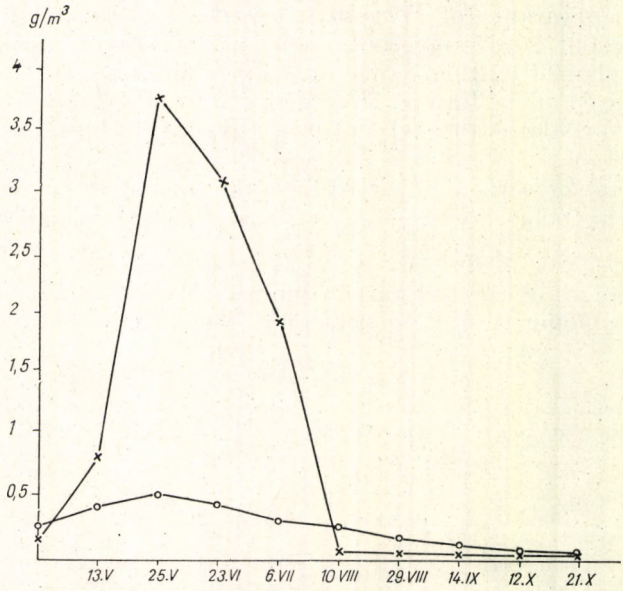

Fig. 2. The change of biomass of Cladocera and Copepoda in the whole section of investigation. x—x—x Cladocera; o—o—o Copepoda

The Cladocera biomass consists practically of *Bosmina longirostris* and *Chydorus sphaericus (Table IX)*. The rare occurrence of *Daphnia hyalina* (and generally the species of *Daphnia)* is connected with the fact that these animals possessing a fine filtering apparatus hardly at all, or can not live in waters rich in corpuscular organic substances (moorish waters). Their filtering apparatus becomes clogged and consequently they die.

The biomass of Copepoda is composed essentially of *Acanthocyclops vernalis* and *Cyclops vicinus (Table XI)*. Both species possess a high ecological tolerance. The former is expressis verbis carnivorous (feeds on small larvae of water insects and worms), whereas the latter lives on the smaller members of the plankton (algae, Rotatoria) as well as on other plant fragments.

During the investigations 18 Cladocera, 6 Ostracoda and 7 Copepoda species, varieties and forms were identified *(Table XII)*.

TABLE XI

The seasonal occurrence of the planktonic Crustacea species in pond No. 1

Species	13. V.	25. V.	9. VI.	23. VI.	6. VII.	10. VIII.	29. VIII.	14. IX.	12. X.	21. X.
Cladocera										
Daphnia hyalina var. *lacustris* SARS	+	+	+++	++						
Simocephalus vetulus (O. F. MÜLLER)										
Ceriodaphnia pulchella SARS			+	+		++				
Moina rectirostris (LEYDIG)							+			
Scapholeberis kingi SARS	+	+								
Macrothrix laticornis (JURINE)										
Ilyocryptus sordidus LIÉVIN										
Ilyocryptus agilis KURZ			+							
Leydigia leydigii (LEYDIG)										+
Leydigia acanthocercoides (FISCHER)									+	
Chydorus sphaericus (O. F. MÜLLER)	+++	++++	++++	++	+			+	+	
Pleuroxus trigonellus (O. F. MÜLLER)										
Alona affinis LEYDIG										
Alona quadrangularis (O. F. MÜLLER)										
Alona guttata SARS		+								
Alona rectangula rectangula SARS		+	+							
Bosmina longirostris f. *cornuta* JURINE	+++	++++	++++	++++	++++	++	+	+	+	+
Bosmina longirostris f. *curvirostris* FISCHER										+
Copepoda										
Macrocyclops albidus (JURINE)								+		
Paracyclops fimbriatus (FISCHER)										
Cyclops vicinus ULJANIN	++++	++	++		+		+	+	+	++
Acanthocyclops vernalis (FISCHER)	+	++	++	++	+	++	+	+	+	
Thermocyclops crassus (FISCHER)	+	++		+			+			
Cyclops sp. copepodid stadium	++	+++	+++	+++	+++	++	++	++	+	
Cyclops nauplius	++++	++	++++	+++	++	++	+	+	++	+++

Explanation: Mass ++++; Numerous +++; Little ++; Sporadic +

TABLE XII

The occurrence of Crustacea species in pond No. 1 and in the canal Keleti-Bozót in 1971

Species	Pond No. 1		Keleti-Bozót
	plankton	mud	
Cladocera			
Bosmina longirostris f. *cornuta*	+	+	+
Bosmina longirostris f. *curvirostris*	+		
Chydorus sphaericus	+	+	+
Pleuroxus trigonellus			+
Macrotrix laticornis		+	
Ilyocryptus sordidus		+	
Ilyocryptus agilis	+	+	
Scapholeberis kingi	+		
Cladocera			
Ceriodaphnia pulchella	+		
Daphnia hyalina var. *lacustris*	+		
Simocephalus vetulus	+		+
Moina rectirostris	+		
Alona guttata	+		
Alona rectangula	+	+	
Alona affinis	+	+	+
Alona quadrangularis	+	+	+
Leydigia leydigii	+	+	+
Leydigia acanthocercoides	+	+	
Ostracoda			
Lymnocythere inopinata		+	
Cyclocypris laevis	+	+	+
Cypridopsis vidua		+	
Cyclocypris ovum		+	
Cypria ophtalmica	+	+	
Darwinula stevensoni		+	
Copepoda			
Macrocyclops albidus	+		
Cyclops vicinus	+		
Acanthocyclops vernalis	+		
Thermocyclops crassus	+		
Paracyclops fimbriatus		+	+
Eucyclops serrulatus			+
Nitocra hibernica			+

5. *Changes of the dissolved O_2 in the transversal section of pond No. 1*

The measurements of oxygen concentrations carried out 10 times *(Fig. 3)* revealed that the values were more or less different at the points of sampling at the same time. As a rule, higher values were obtained in the central regions while near the shore they were lower. The oxygen concentration of point No. 1 (at the inflow) showed significant differences 3 times (23rd June, 29th August and 15th September) as compared to the other place near the shore, i.e. No. 5.

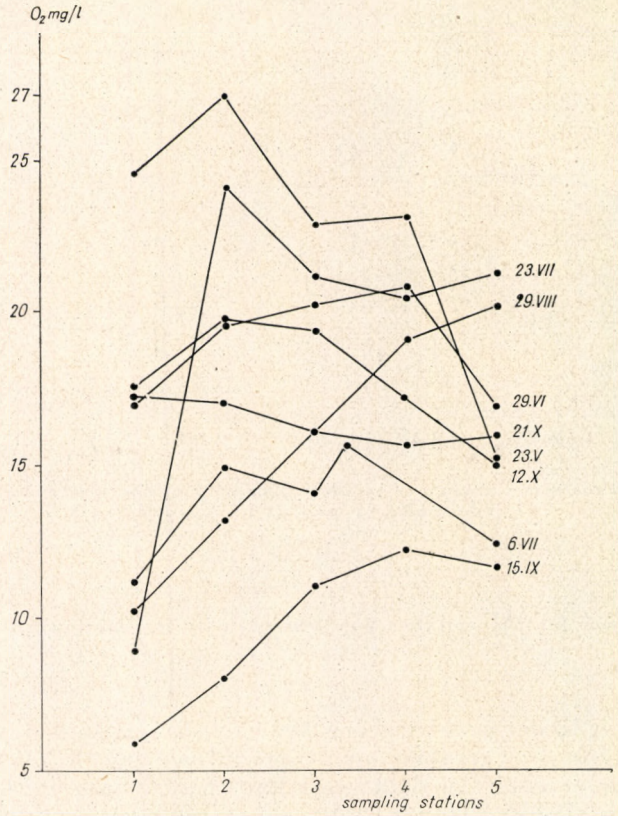

Fig. 3. The quantities of the oxygen dissolved in the water at the places of sampling of pond No. 1

The values were 9, 10 and 6 at point No. 1, respectively, whereas at the other place 21, 20 and 12 mg/litre, i.e. just twice as high. The difference is presumably due to the effect of the inflowing sewage-water.

The data of *Fig. 3* indicate that the oxygen concentration of the water is of decreasing tendency from May till September and it increases again only from October. At the beginning of May, the average O_2 concentration was 36 mg/litre, whereas at the end of May and in June it amounted to 19—22 mg/l. During July, August and September, the average value varied between 10—15 mg/l. It increased again to reach the early summer value during October.

On the 6th July and 29th August the oxygen content analyzed throughout a day (24 hr). It was found that in the middle of the pond (point No. 3) the oxygen supply was sufficient for the fishes even in the "critical" period of early morning, however, at the point of inflow it is so low in August (1 mg/l) to be insufficient for them *(Fig. 4)*. Furthermore, the oxygen supply is better generally in the middle of the pond than near the shore. Similar observations were made in other fish-ponds by HANNAN and ANDERSON (1971).

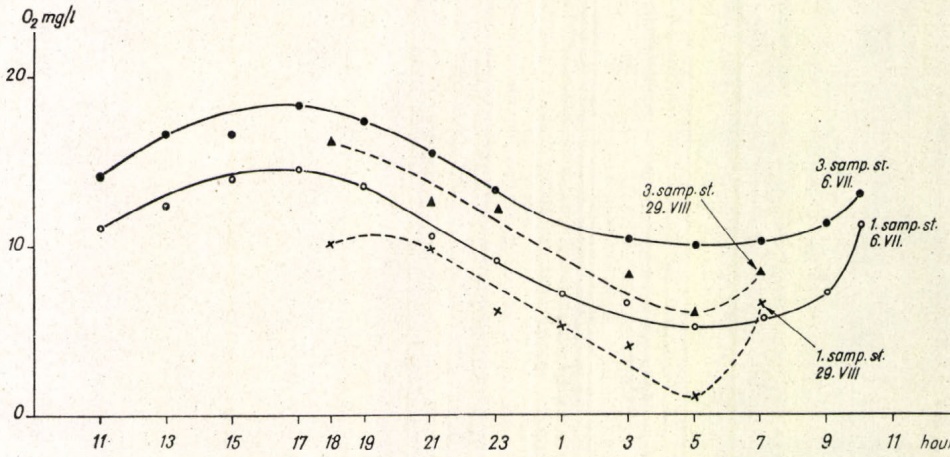

Fig. 4. The quantitative change of oxygen dissolved in the water during 24 hr at two points (No. 1 and 3) of pond No. 1

6. *Some reasons for the changes in biomasses of the more important groups of plankton*

According to our investigations, the bacteria, algae, Rotatoria, Crustacea represent the groups of greatest importance influencing the planktonic biomass to the highest extent. The data regarding the biomass of those groups are summarized in *Table XIII.*

In the beginning of May, the biomass is composed in a nearly identical proportion of the algae (3.5 g per m^3) and bacteria (2.2 g per m^3), followed by Rotatoria (0.89) and last comes the Crustacea plankton (0.32). Upon the influence of the relatively high algal and bacterial biomasses, mainly the Rotatoria showing a quick propagation increase among the members of the zooplankton, then it is followed by a large increase of Crustacea (Bosmina) plankton. Presumably, the algal and bacterial biomasses further decrease on the effect of zooplanktonic biomasses.

The organic substances arriving with the sewage-water in an increasing quantity from the beginning of July, induced an extensive Anabaena bloom. On its effect the bacterioplankton as well as the Rotatoria and Crustacea biomasses were reduced *(Fig. 5, Table XIII)*. The Anabaena biomass reached its maximum in the middle of September. Between July and September, only the Rotatoria biomass is considerable, apart from the algal mass, the former displayed a maximum at the end of August (1 g per m^3). The bacterial mass becomes significant only after the decrease of algae. The Crustacea biomass completely lost its significance from August owing to the algal bloom. Significant differences appeared between the points of sampling as regards the biomass of the total plankton (bacteria, algae, Rotatoria and Crustacea) from July when the loading with sewage-water increased *(Table XIII, Fig. 6)*. The relatively lowest biomass value was found during the whole period of

TABLE XIII

Quantitative changes of the total planktonic biomass in pond No. 1 during 1971 (g dry weight/m³)

Date	Groups of organism	Points of sampling					Average
		1.	2.	3.	4.	5.	
13. V.	Bacteria	2.20	2.10	1.80	2.40	2.30	2.16
	Algae	4.70	2.90	3.70	4.20	2.00	3.50
	Rotatoria	0.40	1.02	1.17	1.05	0.83	0.89
	Crustacea	0.22	0.28	0.63	0.30	0.28	0.34
25. V.	Bacteria	—	—	—	—	—	—
	Algae	—	—	—	—	—	—
	Rotatoria	1.54	3.68	3.17	3.43	3.18	3.00
	Crustacea	0.57	1.61	1.29	1.19	1.21	1.17
9. VI.	Bacteria	0.80	0.60	0.80	0.70	1.10	0.80
	Algae	1.40	1.60	2.00	1.30	1.60	1.58
	Rotatoria	0.27	0.29	0.41	0.28	0.34	0.32
	Crustacea	5.04	4.90	5.56	3.98	2.20	4.33
23. VI.	Bacteria	4.20	3.60	2.80	2.20	2.40	3.04
	Algae	3.10	5.70	4.60	5.30	4.90	4.92
	Rotatoria	0.16	0.17	0.25	0.21	0.23	0.20
	Crustacea	2.34	2.12	3.97	4.82	4.54	3.55
6. VII.	Bacteria	0.05	0.07	0.09	0.10	0.10	0.08
	Algae	22.80	29.40	30.70	26.80	25.90	27.12
	Rotatoria	0.53	0.47	0.49	0.34	0.32	0.43
	Crustacea	0.62	4.58	2.17	2.20	1.48	2.21
10. VIII.	Bacteria	0.80	0.20	0.20	0.20	0.20	0.32
	Algae	23.20	30.10	30.10	24.30	27.80	27.10
	Rotatoria	0.37	0.51	0.46	0.30	0.78	0.48
	Crustacea	0.25	0.35	0.36	0.34	0.32	0.32
29. VIII.	Bacteria	0.50	0.40	0.30	0.30	0.60	0.42
	Algae	33.60	27.20	26.10	33.00	37.10	31.40
	Rotatoria	1.60	0.93	1.11	0.84	0.74	1.04
	Crustacea	0.30	0.21	0.19	0.10	0.09	0.17
14. IX.	Bacteria	2.70	2.50	2.10	1.80	2.90	2.40
	Algae	15.60	41.10	39.40	36.50	40.60	34.64
	Rotatoria	0.83	1.02	0.89	1.05	1.12	0.98
	Crustacea	0.13	0.12	0.13	0.13	0.04	0.11
12. X.	Bacteria	4.10	3.90	4.20	4.30	4.10	4.12
	Algae	7.50	7.50	1.10	0.50	2.30	3.78
	Rotatoria	0.39	0.41	0.42	0.35	0.20	0.35
	Crustacea	0.03	0.05	0.06	0.04	0.04	0.04
21. X.	Bacteria	4.80	4.70	4.60	5.00	4.20	4.66
	Algae	1.70	5.20	2.90	8.70	6.90	5.08
	Rotatoria	0.16	0.23	0.18	0.17	0.19	0.19
	Crustacea	0.11	0.05	0.08	0.09	0.07	0.08

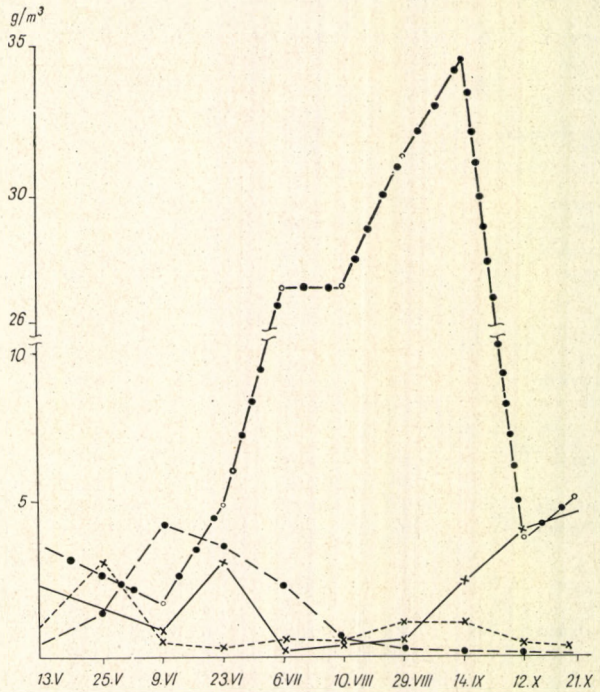

Fig. 5. The changes in the biomass of the most important planktonic groups at all the points of the pond No. 1. o— Bacteria; o—.—.—o Algae; o— — —o Rotatoria; o — —o Crustacea

investigations at point No. 1 (at the inflow) and the highest at point No. 2. The averages are as follows:

Point No. 1	14.96 g/m³
Point No. 2	19.18 g/m³
Point No. 3	18.05 g/m³
Point No. 4	17.82 g/m³
Point No. 5	18.52 g/m³

The differences in planktonic composition as well as of biomass values must be looked for in the quantity and quality of the sewage-water. The insignificant role of Crustacea plankton during summer and the lower biomass value near the inflow may indicate the inlet of a considerable amount of detergents into the system. The practically complete absence of Daphnia species as well as the secondary role of Cladocera during the summer season involves a limited transportation of the increased algal biomass along the food chain. These questions will be discussed later in connection with fish production.

7. *Investigations on the benthos in the transversal section of pond No. 1*

a) Detailed chemical analyses of an average sample. The mud sample taken between points No. 1 and 2, i.e. near the shore is be classified to be an

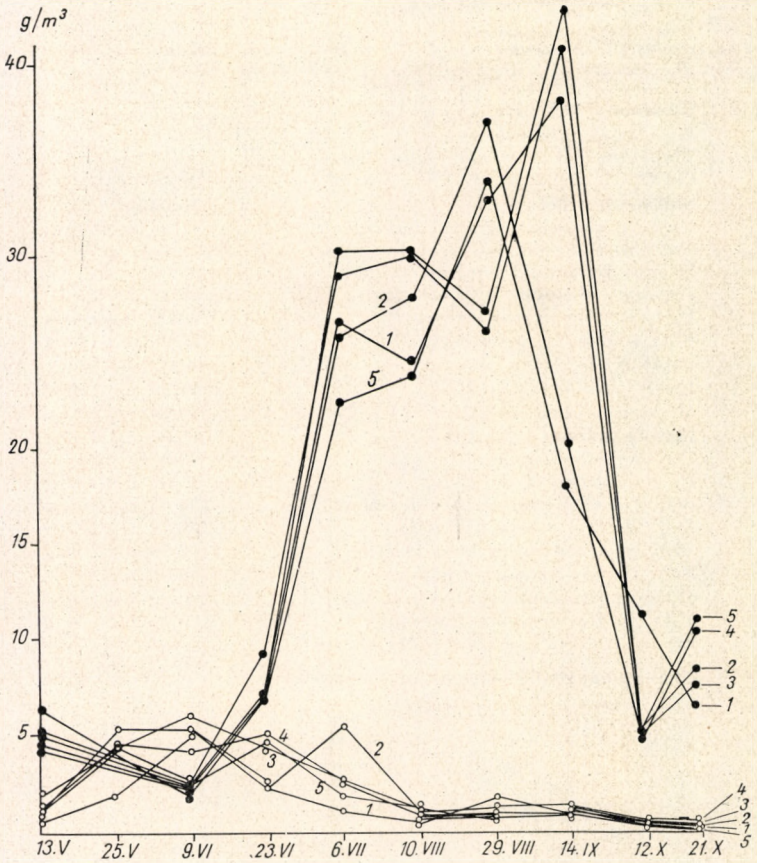

Fig. 6. The upper curves 1—5 show the biomass changes of algae and bacteria, the lower ones those of the total zooplankton at the five points during the whole period of investigations

organic mud *(Tables XIV* and *XV)*. It proved to be of rather inhomogeneous composition during the analyses.

Its reaction was neutral, although contained 22,4 percent $CaCO_3$. This originates mainly in fragmented shells of mussels and snails.

In the mud saturated up to the level of flow (K_A number: 170), 0.11 percent water-soluble salts were measured, indicating a tendency to salt-accumulation.

The compact fractions contained more Fe^{3+}, SO_4^{2+} and SiO_2 than the disintegrated one.

The fraction of the mud sedimentable by boiling (percentage of the particles of less than 0.02 mm) was low, 16.03 percent, indicating a relatively high amount of non-humificated organic matter in the mud, contradicting the results obtained by humid decomposition and burning.

Among the interchangeable cations of the mud, Ca^{2+} predominated, however, the amount of Mg^{2+} was almost the same, while that of Na^+ and K^+ was relatively low.

TABLE XIV

The results of investigations of the mud-samples taken near the shore (between the points of sampling Nos. 1—2) of the pond of Fonyód (1971)

Reaction: 7.10 pH	
K_A (H_2O)	170
Total amount of water-soluble salts	0.11%
$CaCO_3$	22.40%
Sluiceable fraction	16.03%
Fe^{3+} in the disintegrated fraction	41.94 mg/100 g
Fe^{3+} in the compact fraction	1358.90 mg/100 g
Humus by humid decomposition	23.20%
Organic substance by burning	56.42%
P_2O_5 soluble in aqua regia	192.00 mg/100 g
K_2O soluble in aqua regia	235.00 mg/100 g
Total N	900.00 mg/100 g
Easily soluble P_2O_5-(Al-P)	52.00 mg/100 g
Moderately soluble P_2O_5-(Al-P)	39.10 mg/100 g
Hardly soluble P_2O_5-(Al-P)	13.00 mg/100 g
Binding of phosphorous	90.00%
Easily soluble K_2O-(Al-K)	21.00 mg/100 g
SO_4^{2-} in the disintegrated fraction	134.43 mg/100 g
SO_4^{2-} in the compact fraction	3696.79 mg/100 g
SiO_2 in the disintegrated fraction	12.50 mg/100 g
SiO_2 in the compact fraction	104.06 mg/100 g
P_2O_5 in the disintegrated fraction	180.00 mg/100 g
P_2O_5 in the compact fraction	150.00 mg/100 g

The exchangeable cations of the mud:

	mg/100 g
Ca^{2+}	1010.00
Mg^{2+}	541.04
Na^{+}	34.75
K^{+}	26.50

The water-extract analyses of the mud are only informative results, since they give preliminary prognosis to a certain extent to the secondary quality of the water, and allows us to calculate the order of magnitude of dissolution of the mud components during the refilling of the fish-pond.

The large O_2-consumption of the water-extract is considerable in this respect. The amounts of inorganic nitrogen, PO_4^{3-} and Fe^{3+} are remarkable in the aqueous extracts. In a mud-water of 1 : 5 extract 455.6 mg/l salts were dissolved.

The chemical type of the aqueous extract is characterized by Ca-hydrocarbonate.

The mud is rich in N and phosphorous and contains small amounts of K_2O soluble in aqua regia, as the morassy soils do in general. As compared to the soluble P_2O_5, even the soluble K_2O is inconsiderable. The amount of P_2O_5 soluble only in aqua regia is nearly identical with the P_2O_5 content of the compact and humificated fractions (even the disintegrated fraction contains a considerable amount of P_2O_5). Twenty-seven percent of the P_2O_5 soluble in aqua regia is easy to dissolve. Using a continuous dissolution, the second results in 39.10, the third in 13.00 mg P_2O_5 per 100 g. Therefore, more than a half of the P_2O_5 soluble in aqua regia can be dissolved to different degrees (easily, moderately and hardly).

TABLE XV

The data of analysis of the water extract of the mud prepared using a water : mud rate of 5 : 1 (1971)

Reaction: 7.30 pH

	mg/l/200 g mud
O_2-consumption (0.01 n $KMnO_4$)	56.98
Total degree of hardness	12.01
Soluble sulphide	4.87
Calculated H_2S	0.98
Alkalinity L°	2.73
Na^+	40.09
K^+	6.3
Ca^{2+}	37.5
Mg^{2+}	29.5
Fe^{3+}	1.7
NH_4^+	3.0
HCO_3^-	166.6
Cl^-	11.3
SO_4^{2-}	128.3
PO_4^{3-}	0.8
SiO^{2-}	17.8
NO_2^-	6.6
NO_3^-	5.3
Sum of cations	118.9
Sum of anions	336.7
Cations and anions together	455.6

During the laboratory experiments of dunging the applied amount of P_2O_5 (10 mg/100 g) was bound to 90 percent, the binding, however, was weak, it was easy to liberate, observed during the continuous dissolution of the dunged mud. The bound P_2O_5 was given back during the second or third dissolution. Therefore, the mud samples of Fonyód contain considerable amounts of N, P_2O_5, Fe^{3+} and SO_4^{2-}. Large O_2 consumption and P_2O_5 as well as N amounts can be precalculated during refilling.

b) Quantity and quality of the benthic organisms. According to their sites they can be divided into 3 groups: macro-, micro- and meiobenthos. The term macrobenthos involves the Chironomida-Tubifex, the meiobenthos the Nematoda-Cladocera-Copepoda, while the microbenthos includes the algae and unicellular animals. We call the macrobenthos above 2 mm, the meiobenthos between 2 and 0.1 mm.

Macrobenthos

The points of transversal section investigated proved to be very poor in macrobenthic organisms. Only near the inflow (No. 1) have we found a few Tubifex samples. The almost complete absence of the macrobenthos can be explained by the activity of carps searching for these organisms.

Meiobenthos

Only the Nematoda showed a higher frequency in this group, whereas the other organisms occurred only sporadically *(Table XVI)*.

It deserves special interest that the Nematoda were almost without exception juvenile forms. This obviously indicates that the fish have optimal

TABLE XVI

The occurrence of the members of meiobenthos in the transversal section of the pond

	Place of sampling No. 1.					Place of sampling No. 2.				
	13. V.	9. VI.	6. VII.	29. VIII.	12. X.	13. V.	9. VI.	6. VII.	29. VIII.	12. X.
Nematoda	367	17	114	17	47	17	—	19	28	64
Oligochaeta	—	—	—	—	—	33	—	3	—	—
Cladocera	—	—	—	—	—	—	—	8	3	3
Ostracoda	17	17	3	—	—	17	—	—	3	—
Copepoda	50	33	14	17	8	—	—	5	—	—
Diptera	—	—	—	—	—	—	—	—	—	—
Total	434	67	131	34	55	67	—	35	34	67

accers to the benthic organisms. On this basis one can clearly explain the sporadic occurrence of the organisms larger in size than the Nematoda (Cladocera, Copepoda, Oligochaeta, Diptera). This peculiar phenomenon can only be explained by the fact that the toxic substances (e.g. detergents) of the inflow are fixed by the mud surface of point No. 1. Therefore, Cladocera being relatively sensitive to environmental effects, immediately die.

One can point out the interesting occurrence of the mud-living Cladocera. Whereas these animals are absent in the mud near the inflow, their number increases towards the other side of the pond.

Hydracarina and Tardigrada organisms occur sporadically in the benthos.

Biomass values were only calculated for the more frequent Nematoda *(Table XVII)*. The data indicate that the largest biomass value was yielded by point No. 1, i.e. the inflow region during the whole period of investigation.

TABLE XVII

Changes of Nematoda biomass in pond No. 1 (mg/m²) during 1971

Points of sampling	Dates of sampling					Average
	13. V.	9. VI.	6. VII.	29. VIII.	12. X.	
1.	6.0	0.3	2.0	0.3	0.8	1.8
2.	0.3	—	0.3	0.5	1.1	0.5
3.	0.5	0.3	0.1	0.5	0.1	0.3
4.	0.3	0.8	0.1	0.3	0.1	0.3
5.	2.2	0.5	0.3	0.1	0.5	0.7

The reason is that this group is less sensitive to environmental changes than the Cladocera, and there are many species among them preferring the medium of high organic content. Considering the fact that apart from the spring and early summer seasons, the zooplanktonic biomass plays only a secondary role beside the phytoplankton, the members of the meiobenthos may be of higher importance in the nutrition of fish than it had been assumed before the investigations.

investigated in 1971. (Values are given in individuals/dm², rounded of figures)

Place of sampling No. 3.					Place of sampling No. 4.					Place of sampling No. 5.				
13. V.	9. VI.	6. VII.	29. VIII.	12. X.	13. V.	9. VI.	6. VII.	29. VIII.	12. X.	13. V.	9. VI.	6. VII.	29. VIII.	12. X.
33	17	5	30	3	17	50	8	17	5	133	33	19	5	36
—	—	3	—	3	33	—	11	—	—	33	—	3	5	—
—	17	5	—	—	—	33	8	—	—	—	117	150	3	—
17	33	—	—	—	—	—	—	—	—	—	—	5	—	—
50	83	5	3	—	17	—	19	8	11	17	50	5	—	—
17	—	—	—	—	—	—	—	—	—	—	17	3	—	3
117	150	18	33	6	67	83	46	25	16	183	217	185	13	39

It should be noted that the mud of the pond is inclined to form hydrogen sulphide affecting the quantitative relations of certain benthic organisms. This question will be treated in more detail later in connection with the destruction of fish.

8. *Investigations on the growth of the fish population*

a) Evaluation of the productivity of ponds on the basis of parameters of production.

Only the data of highest significance will be treated from the voluminous *tables (XVIII* and *XIX, a + b)* in order to characterize the ponds.

Pond No. 1 *(Table XX, Fig. 7):*

The production of the pond showed significant differences depending on the amount of fish recovered as well as on the mortality during 1964—1971.

Considering the total amount of fish recovered (106—286 q), the production forms a variable part of the average biomass, namely 8.59—67.42 percent, related to the natural loss. During 1971, this rate much surpassed the results of the earlier years, amounting to 204.6 percent. The fishes introduced were mainly carp and tench, whereas in May 1971 sheat-fish as well as fry of white and spotty grass-carps were also introduced. The natural loss of carp was significant at the first-summer individuals, 37—61 percent, while in the case of older ones, it amounted to only 15—20.4 percent. The natural production of the pond varied between 36—150.2 kg/cadastral acre (in 1971 107.7 kg) achieved by consuming 1.4—2.6 kg food per 1 kg fish flesh. Considering the number of introduced fishes, this seems to be a better efficiency of the food than in the other ponds (see *Tables XVIII* and *XIX b)*. The pre-sedimented sewage-water diluted with that of the Keleti-Bozót gets in directly into pond No. 1. The quality and daily amount is not yet known, however, it may significantly affect the natural production and the parameters seem to indicate even higher productions. The carp and other fishes cultured in that pond tolerate the loading with sewage-water, as far as we are informed, mass destruction of fishes has not been observed because of the sewage-water inflow. Considering that the pond has a bottom of boggy character and is rich in phytoplankton, the introduction of grass-eating species during 1971 was a fair bid for the future.

TABLE XIX/b

Recovery statistics of fish during 1964—1971 from

	A	B	Planted material			
			Carp	Other fishes	Total	Total
			kg			
1964	1	88	2 537	1122	3 659	10 632
	2	132	6 330	274	6 604	20 430
	3	142	10 559	479	11 038	15 456
total:		366	19 426	1875	21 301	46 518
1965	1	88	2 860	—	2 860	22 214
	2	132	9 364	839	10 203	6 027
	3	142	6 660	533	7 193	5 234
total:		366	18 884	1372	20 256	33 475
1966	1	88	10 600	—	10 600	28 556
	2	132	19 915	613	20 528	39 323
	3	142	16 480	—	16 480	22 149
total:		366	46 995	613	47 608	90 028
1967	1	88	9 200	1123	10 323	24 397
	2	132	14 000	997	14 997	18 703
	3	142	13 680	2100	15 780	29 720
total:		366	36 880	4220	41 100	72 820
1968	1	88	9 693	1036	10 729	16 971
	2	132	13 467	3057	16 524	19 343
	3	142	16 581	1506	18 087	18 852
total:		366	39 741	5599	45 340	55 166
1969	1	75	10 400	—	10 400	16 853
	2	84	8 594	348	8 942	22 912
	3	90	2 958	—	2 958	1 241
total:		249	21 952	348	22 300	41 006
1970	1	75	7 794	—	7 794	11 561
	2	84	13 060	305	13 365	29 647
	3	90	9 013	675	9 688	12 280
total:		249	29 867	980	30 847	53 488
1971	1	75	10 006			10 827

w = White grass-carp; sp = Spotty grass-carp; sh = Sheat-fish; * = 1-summer-old

Pond No. 2 *(Table XXI)*

This pond had had an area of 132 cadastral acres between 1964 and 1969, then it was restricted to 84 cad. acres. The number of introduced fishes as well as the amounts recovered were higher excepting the years of 1965 and 1967, than in the previous pond. The percentual value of the loss amounted to 4.1—44.2 at the first-summer fishes, whereas at the older ones it was much higher, 6.5—64.9 percent. The pond offered an outstanding production in 1965, when the introduced amount of fishes was 60 q and in spite of the high mortality, it increased to 120 q by the end of the year involving a production of 169.3 percent achieved by a food consumption of 3.51 kg/1 kg fish flesh. The natural

the fish-ponds of Zardavár

Increase of the pond per cad. acre		Number of missing carps from the initial stock in percentage		Consumed food in maize value kg	Amount of food per 1 kg fish flesh, kg	Natural production of the pond per cad. acre in kg
From carp	Total	At 1-summer-old ones	At older ones			
kg		kg				
—	120	57	—	26 011	2.44	36
—	155	—	45	50 355	2.46	46
—	106	—	77	16 107	1.04	74
—	127	57	63	92 473	1.90	55
228	222	37	—	31 475	1.41	150.2
41	44	—	64.9	21 292	3.51	—
29	37	97.3	—	9 453	1.60	17.8
66	91	86.8	64.9	62 220	1.86	42.9
318	324	60	8.9	60 046	2.10	129.5
286	290	4.1	—	74 790	1.90	132
153	159	71.8	60.5	49 304	2.22	56.7
242	246	59.9	37.8	184 140	2.04	102.2
282	277	56.2	—	55 425	2.27	82.8
132	137	—	30	41 939	2.24	42
203	209	45.9	—	61 294	2.06	76
195	199	49	30	158 658	2.18	67.7
191	192	60.9	20.4	39 789	2.34	63
135	142	—	40.5	50 461	2.61	36.2
141	133	—	—	66 096	3.65	—
151	151	60.9	24.5	156 346	2.83	28.7
—	224	50.9	—	31 176	1.8	—
—	272	—	6.5	42 044	1.8	—
13	13	—	7.9	12 228	9.9	—
—	—	—	—	85 448		—
147	154	—	15	30 034	2.6	39
332	353	44.2	—	57 862	1.9	156
125	136	—	29.2	40 878	3.3	—
202	215	44.2	23.5	128 774	2.4	66.7
	295	44.7* 5,3 w 26,4 sp. 40,3 sh.		49 253	2.22	107.7

production of the pond was 36.2—156 percent, its average biomass production varied between 32.33—169.3 percent.

Pond No. 3 *(Table XXII)*

It is of lower planting than that of No. 2. (except 1967), having an area of 142 cadastral acres between 1964—1969 and 90 cad. acres from 1969. The values of mortality were high, nevertheless the amounts of produced fish widely varied: 42—455 q. The production, accordingly, amounted to 53.1—238.4 percent. The amount of food consumed fluctuated between 1.04—9.9 kg

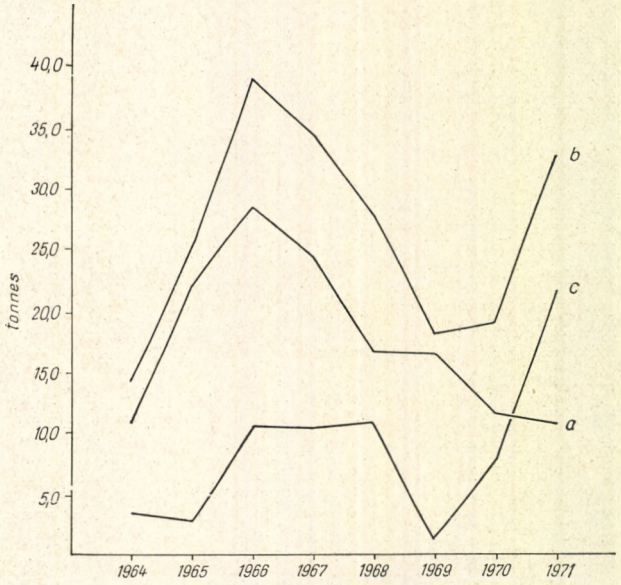

Fig. 7. The data of fish production in pond No. 1 of Zardavár during 1964—71. a = material planted; b = recovered material; c = production

TABLE XX

The productivity of pond No. 1 of Zardavár (Fonyód) during 1964—71 (without wild fish)

Year	Area of the pond cad. acre	The amount of planted matter kg	Total increase per 1 cad. acre kg	Rate of missing fish from the initial stock in percentage		Total recovery kg	Production of the biomass	
				A	B		kg	%
1964	88	10 632	120	57	—	14 291	3 659	34.4
1965	88	22 214	222	37	—	25 074	2 860	12.87
1966	88	28 556	324	60	8.9	39 156	10 600	37.12
1967	88	24 397	277	56.2	—	34 720	10 323	42.31
1968	88	16 971	192	60.9	20.4	27 700	10 729	63.22
1969	75	16 853	224	50.9	—	18 300	1 447	8.59
1970	75	11 561	154	—	15	19 355	7 794	67.42
1971	75	10 827	295	44.7	—	32 978	22 151	204.59

Note: Rate of missing fish from the initial stock in percentage, A = one-summer-old, B = at older ones

per 1 kg fish flesh representing a high value. During the years of 1965—66 and 1969 this pond was of the lowest level of planting among the three, however, it offered the highest relative production in 1965 and 1969, corresponding to an increase of weight of 30—72 q. Nevertheless, the production depends on the intense feeding. During seven years between 1964—70, the survived portion of the introduced fish-mass produced an increase in weight of about 30—181 q.

TABLE XXI

The productivity of pond No. 2 of Zardavár (Fonyód) during 1964—71 (without wild fish)

Year	Area of the pond cad. acre	The amount of planted matter kg	Total increase per 1 cad. acre kg	Rate of missing fish from the initial stock in percentage		Total recovery kg	Production of the biomass	
				A	B		kg	%
1964	136	20 430	155	—	45	27 034	6 604	**32.33**
1965	136	6 027	44	—	64.9	16 230	10 203	169.29
1966	136	39 323	290	4.1	—	59 851	20 528	52.20
1967	136	18 703	137	—	30	33 700	14 997	80.18
1968	136	19 343	142	—	40.5	35 867	16 524	85.43
1969	84	22 912	272	—	6.5	31 854	8 942	39.05
1970	84	29 647	353	44.2	—	43 012	13 365	45.08

TABLE XXII

The productivity of pond No. 3 of Zardavár (Fonyód) during 1964—71 (without wild fish)

Year	Area of the pond cad. acre	The amount of planted matter kg	Total increase per 1 cad. acre kg	Rate of missing fish from the initial stock in percentage		Total recovery kg	Production of the biomass	
				A	B		kg	%
1964	142	15 456	106	—	77	26 494	11 038	71.42
1965	142	5 234	37	97.3	—	12 427	7 193	137.43
1966	142	22 149	159	71.8	60.5	38 629	16 480	74.41
1967	142	29 720	209	45.9	—	45 500	15 780	53.10
1968	142	18 852	133	—	—	36 939	18 087	95.94
1969	90	1 241	13	—	7.9	4 199	2 958	238.36
1970	90	12 280	136	—	29.2	21 968	9 688	**78.79**

TABLE XXIII

The productivity of ponds Nos. 1—3 of Zardavár (Fonyód) during 1964—71 (without wild fish)

Year	Area of the pond cad. acre	The amount of planted matter kg	Total increase per 1 cad. acre kg	Rate of missing fish from the initial stock in percentage		Total recovery kg	Production of the biomass	
				A	B		kg	%
1964	366	46 518	127	57	63	67 819	21 301	45.79
1965	366	33 475	91	86.8	64.9	53 731	20 256	60.51
1966	366	90 028	246	59.9	37.8	137 636	47 608	52.88
1967	366	72 820	199	49	30	113 920	41 100	56.44
1968	366	55 166	151	60.9	24.5	100 506	45 340	82.19
1969	249	41 006	—	—	—	63 306	22 300	54.38
1970	249	53 488	215	44.2	23.5	84 335	30 847	57.67

General evaluation of ponds Nos. 1—3 *(Table XXIII)*

The summarized area of the ponds was 366 cad. acres until 1968, then 249 from 1969. The amounts planted varied between 335—900 q. The increase of weight was 91—246 kg per cad. acre. Except 1964, the mortality was higher at the first-summer carp (44.2—86.8 percent) than at the older ones (23.5—64.9 percent). The total amount of fish caught was between 633—1376 q including a total biomass production of 202—467 q, i.e. 52.9—60.5 percent showing extreme values of 45.8—82.2 percent. It can be regarded in general that the ponds taken either collectively or even individually are of medium fish-production as compared to other ponds. However, it seems to be likely that perhaps by an increased sewage-water consumption and by mass plantation of lower number of species mainly of grass-carps, the production can be increased up to 80—100 percent in each pond or even above that. For this reason one has to know more about the connections between sewage-water loadings and fish-production, since only a few data are known at present concerning this problem. The joint effect of duck-cultivation and sewage-water consumption should also be cleared up in pond No. 1, first of all, in order to increase the production of the grass-eating species.

b) Investigations on the growth of carp in pond No. 1.

During 1971 altogether 679 two-summer-old and 263 fry-carps were investigated in order to estabilsh the rate of growth *(Table XXIV)*. Although the performance of samplings could not always be carried out, sufficient amount of data have been obtained during June, September and November in pond No. 1 regarding the growth of carps *(Table XXIV)*. The results prove that in

TABLE XXIV

The fish-material studied in pond No. 1 of Zardavár with a view to the rate of growth (1971)

	June	Sept.	Nov.	Total (pc)
Carp 2-summer-old	103	280	296	679
Carp 1-summer-old	42	107	114	263
White grass-carp	—	26	37	63
Tench	—	35	35	70
Crucian carp	—	48	31	79
Total:	145	496	513	1154

spite of the large amount of food consumed in this pond, carps grow relatively slowly and rather unevenly. This concerns both the body length and weight and the slow growth is reflected by the low or medium results of production mentioned above. The growth of length of the second-summer carps remains far below the attainable values and even the rate of growth of the fry is unsatisfactory *(Fig. 8)*. A similar conclusion is gained when investigating the coefficients of equations calculated for the allometric connections of the body weight and length. The value of regression coefficient b changes seasonaly (2.4—2.6), it has never reached the value of 3.0, i.e. the body mass (the so-called specific weight) of the fish is significantly lower than the average achieved in other fish-ponds *(Fig. 9)*.

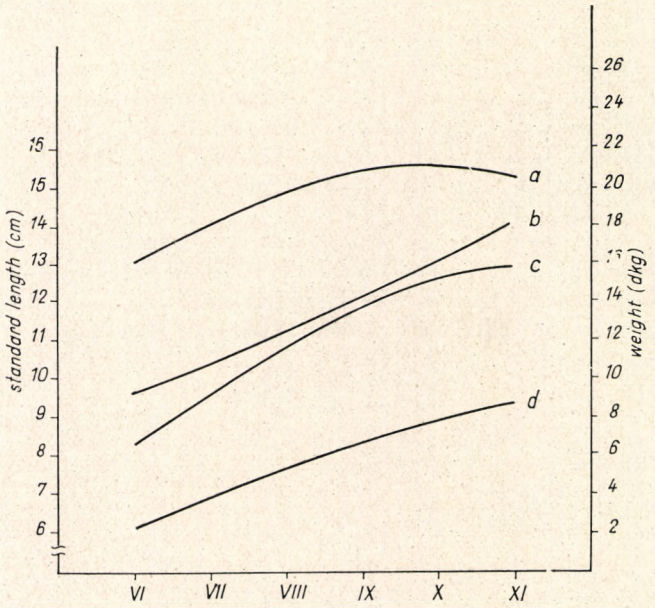

Fig. 8. The increase of body length and weight of carp in pond No. 1 of Zardavár. a = increase of standard length of two-summer-old carp; b = increase of body weight of two-summer-old carp; c = increase of standard length of carp fry; d = increase of body weight of carp fry

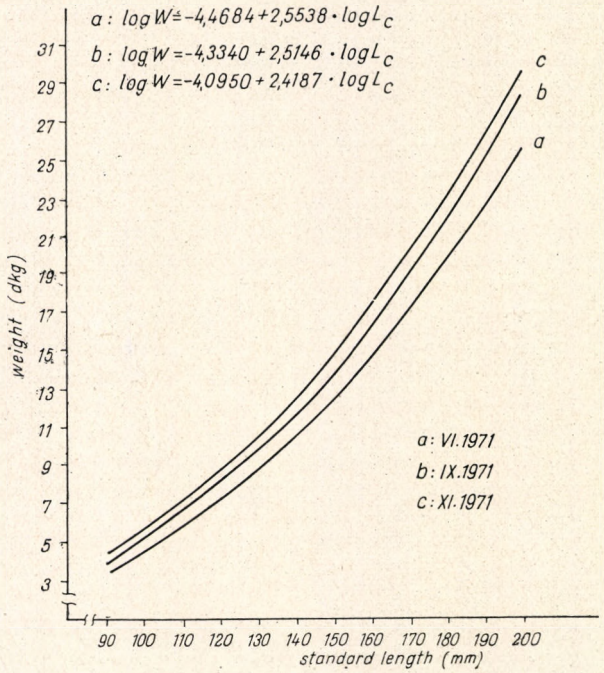

Fig. 9. The ratio of body weight and length of carp during the period of investigation (pond No. 1). W = body weight in dkg; L_c = standard length in mm

The coefficient of body-weight increase varied between $G_w = 1.39-2.46$ at the second-summer carps during the period of investigations, whereas the same regarding body length was $G_l = 0.26$ showing a much slower increase *(Table XXV)*. The rate of growth of the fry significantly surpasses the above values ($G_w = 2.58$; $G_l = 0.91$).

The condition of the carps changes according to season, it seems to decline by the end of the year. The values of CF were 3.2367—4.6654 in June (average 4.2010), 3.0440—4.9202 in September (average 3.814) and 2.666—5.370 in November (average 3.956). The extreme values display great differences among the fishes of the same age. Beside those numerical values, the population cannot be regarded as uniform attributable to nutritional and ecological connections.

TABLE XXV

The rates of mortality and survival of fish-species as well as the growth coefficients of body weight and length in pond No. 1 (1971)

	Z	G_w	G_l	S	A
Carp 2-summer-old	—1.1912	2.4642	0.2652	30.42	69.58
fry	—	2.5756	0.9062	—	—
Tench	—3.7002	1.3054	—	2.47	97.53
White grass-carp	—0.1069	5.9994	—	89.58	10.42
Spotty grass-carp	—0.9082	3.2325	—	40.25	59.75
Sheat-fish	—1.0780	4.6614	—	33.96	66.04

Z = instantaneous total coefficient of mortality (RICKER, 1958), G_w and G_l = coefficients of growth of weight and length, respectively, (CHAPMAN, 1968; TESCH, 1968), S = survival rate in percentage, A = total annual mortality in percentage (in our case, involves 0.5 year).

The value of the actual total mortality is high at the two-summer-old carps ($Z = -1.1912$), the loss was 69.6 percent during a period of 6 months accordingly the survival rate was only 30.4 percent. The calculated values of mortality and survival differ more or less from the real values, since they describe mathematically a change between an initial (t_0) and a final (t_1) point of time, i.e. they express the time dependence of a logarithmic decrease in the number of individuals. The seasonal variations of the values cannot be followed, since the exact determination of the number of individuals is possible only on the basis of data of planting and recovery because of the large area of the ponds and the insufficient technical facilities.

Data regarding the growth of carps allow us to draw the conclusion that this species may play only a secondary role in the waste stabilization ponds of Fonyód, if the increase of fish production is intended at a higher consumption of sewage-water.

c) Observations on the growth of other fishes

Apart from the carps, white and spotty grass-carps as well as sheat fish were introduced into pond No. 1 in 1971. At the same time, mainly tench, a smaller number of pike-perch and pike were fished representing the natural production of the pond. The crucian carp was the most significant among the

wild fishes, nevertheless, it plays no role in the statistic. Apart from the carps, observations were carried out to study the growth of tench, the crucian carp, the white and spotty grass-carps and partly of sheat fish. The results are summarized briefly as follows:

Tench: Both the body and length increased more intensely than in the case of carps *(Fig. 10)* according to the relationships calculated for the allometric growth at first- and second-summer as well as fry individuals. The allometric exponent significantly differs from 3.0. As against the carp, this species is of continuous growth during the whole period of investigations, thus at the end of autumn, from September till November, the body length increased about 2—2.5 cm and the body weight about with one third. The actual coefficient of the increase of body weight is of relatively low value ($G_w = 1.3054$). The mortality is high (97 percent), accordingly, the survival rate is only 2.47 percent.

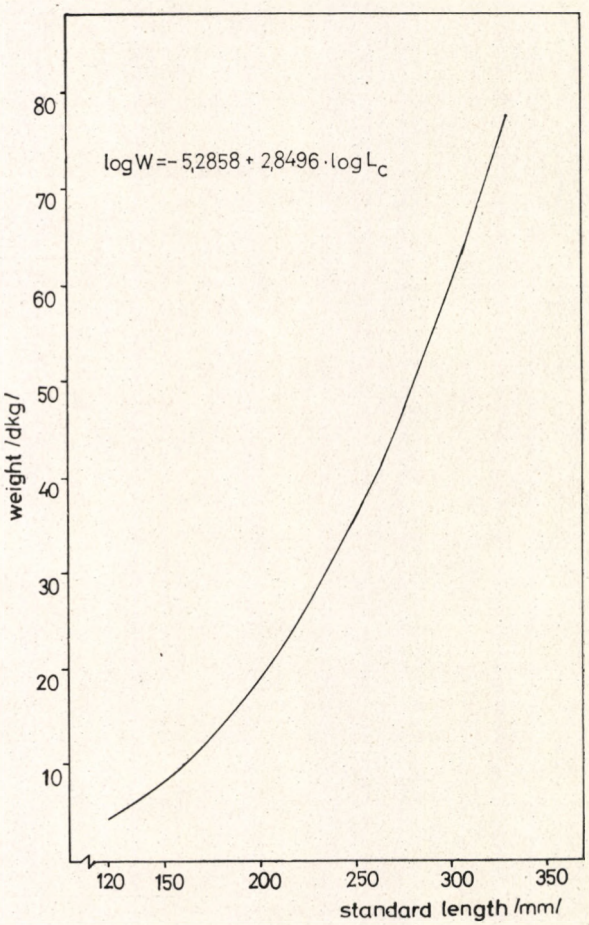

Fig. 10. The allometric relationship of body weight and length of tench (see for other explanations *Fig. 9)*

Crucian carp: It is considered to be a junk-fish in fish-ponds, since it is a food-competitor of carps and other useful fishes. It gets into the pond with the filling-water and because of its extraordinary prolification and intense food-consumption it may have a considerable effect on the growth of carps. Two groups of size occurred in pond No. 1 (first-summer-old and fry) with a size-difference of 6—7 cm *(Table XXVI)*. The equation calculated for the connection of body-weight and length indicates that the increase in body size compared to the other species, best approaches the isometric value (b = = 2.954) *(Fig. 11)*.

TABLE XXVI

The average values of body sizes of fish investigated during the period of investigation in 1971

	June			September			November		
	L_c	L_t	W	L_c	L_t	W	L_c	L_t	W
Carp 2-summer-old	130	161	9.3	154	189	14.2	153	190	14.0
fry	82	103	2.4	118	144	6.2	145	180	12.5
							129	161	8.7
White grass-carp	—	—	—	253	305	31.9	—	—	—
Tench	—	—	—	175	210	13.0	149	180	9.3
							199	235	20.3
							293	348	53.3
Crucian carp	—	—	—	115	142	5.0	163	203	15.4
							109	136	5.2

L_c = standard length in mm; L_t = total body length in mm; W = body weight in dkg.

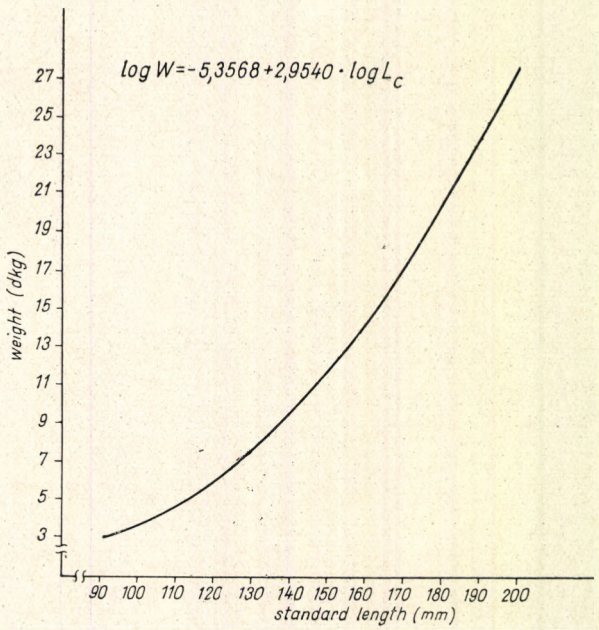

Fig. 11. The allometric relationship of body weight and length of crucian carp (see for other explanations *Fig. 9)*

TABLE XVIII

Planting statistics of fish into the ponds of Fonyód-Zardavár (1964—1971)

	A	B	C	D	Carp						Tench		Sheat-fish				Pike-perch				White amur		White grass-carp		Spotty grass-carp		Planted together kg
					Mature female		2-summer-old		1-summer-old		Fry		Breeding		Fry		Breeding		Fry		Breeding		Fry		Fry		
					pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	
1964	1	88	250	8.0	71	354	176 000	1 408	74 000	2 183	—	1122	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5 067
			840	2.9																							
	2	136	496	11.0	—	—	66 100	7 111	—	—	—	180	—	—	1760	274	—	—	—	—	—	—	—	—	—	—	7 565
	3	142	500	7.4	234	762	94 200	7 608	—	—	—	—	—	—	1900	299	—	—	—	—	—	—	—	—	—	—	8 669
total:		366			305	1116	336 300	16 127	74 000	2 183	—	1302	—	—	3660	573	—	—	—	—	—	—	—	—	—	—	21 301
1965	1	88	795	4.0	—	—	—	—	70 000	2 860	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2 860
	2	136	316	20.0	350	960	41 750	8 404	—	—	—	—	1272	747	—	—	430	92	—	—	—	—	—	—	—	—	10 203
	3	142	2250	2	63	533	—	—	330 000	6 600	—	—	—	—	2500	60	—	—	—	—	—	—	—	—	—	—	7 193
total:		366			413	1493	41 750	8 404	400 000	9 460	—	—	1272	747	2500	60	430	92	—	—	—	—	—	—	—	—	20 256
1966	1	88	2270	2.8																							
			114	50.0	—	—	10 000	5 000	200 000	5 600	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10 600
	2	136	154	11.4																							
			255	54.0	—	—	34 750	17 537	20 920	2 378	—	96	531	249	480	40	280	200	570	28	—	—	—	—	—	—	20 528
	3	142	535	2.8																							
			500	20.6	—	—	70 300	14 480	76 000	2 000	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16 480
total:		366					115 050	37 017	296 920	9 978	—	96	531	249	480	40	280	200	570	28	—	—	—	—	—	—	47 608
1967	1	88	2270	2.8	1500	3600	—	—	200 000	5 600	—	1123	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10 323
	2	136	316	32.0	—	—	43 000	14 000	—	—	—	590	265	177	—	—	425	230	—	—	—	—	—	—	—	—	14 997
	3	142	3500	2.0	1500	4100	—	—	498 940	9 580	—	2100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15 780
total:		366			3000	7700	43 000	14 000	698 940	15 180	—	3813	265	177	—	—	425	230	—	—	—	—	—	—	—	—	41 100
1968	1	88	3200	2.8																							
			100	13.0	105	443	8 800	1 150	281 600	8 100	—	1036	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10 729
	2	136	880	10.0	500	2400	119 900	11 067	—	—	—	1481	—	—	1100	230	—	—	—	—	802	1346	—	—	—	—	16 524
	3	142	535	15.0	1305	5423	75 950	11 158	—	—	—	1278	—	—	1100	228	—	—	—	—	—	—	—	—	—	—	18 087
total:		366			1910	8266	204 650	23 375	281 600	8 100	—	3795	—	—	2200	458	—	—	—	—	802	1346	—	—	—	—	45 340
1969	1	75	2893	4.6	—	—	—	—	217 030	10 070	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	10 070
	2	84	431	24.0	—	—	36 200	8 594	—	—	—	124	277	166	—	—	66	34	—	—	—	—	—	—	—	—	8 942
	3	90	9		829	3288	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3 288
total:		249			829	3288	36 200	8 594	217 030	10 070	—	124	277	166	—	—	66	34	—	—	—	—	—	—	—	—	22 300
1970	1	75			—	—	32 795	7 794	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	7 794
	2	84			—	—	441	215	353 600	12 845	—	242	—	—	1045	63	—	—	—	—	—	—	—	—	—	—	13 365
	3	90			—	—	43 254	9 013	—	—	—	675	—	—	—	—	—	—	—	—	—	—	—	—	—	—	9 688
total:		249			—	—	76 490	17 022	353 600	12 845	—	917	—	—	1045	63	—	—	—	—	—	—	—	—	—	—	30 847
1971	1	75	4500	3.5	—	—	—	—	288 500	10 006	—	—	1800	120	—	—	—	—	—	—	—	—	30 000	380	20 000	321	10 827
	2	84	550	21.0	—	—	46 110	9 779	—	—	—	—	1650	990	—	—	—	—	—	—	—	—	—	—	—	—	10 769
	3	90	4500	1.5	—	—	9 000	1 041	342 300	4 335	50 000	2080	—	—	—	—	—	—	—	—	3000	28	—	—	—	—	8 084
total:		249			—	—	55 100	10 820	630 800	14 941	50 000	2080	3450	1110	—	—	—	—	—	—	3000	28	30 000	380	20 000	321	29 680

A = number of ponds; B = area of the pond in cad. acres; C = planted per cad. acre; D = average weight in dkg.

TABLE XIX/a

Recovery statistics of fish during 1964—1971 from the fish-ponds of Zardavár

	A	B	Carp								Recovered material						Tench			Sheat-fish				Pike-perch		White amur		Pike		Crucian carp	Others	Total (without wild fish)
			Class I/b 1.5—2 kg		Class II 1—1.5 kg		Class III 0.6—1 kg		Class IV below 0.6 kg		Mature female		Breeding of 2-summer-old		Fry		Total	above 10 dkg	below 10 dkg	Mat. fem. and for selling		Breeding mature f. and fry		mat. fem. and fry				Above and below 1 kg				
			pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	kg	kg	kg	pc	kg	pc	kg	pc	kg	pc	kg	pc	kg	kg	kg	kg
1964	1	88	—	—	284	349	—	—	—	—	150	602	30 556	10 735	—	—	11 731	643	803	—	—	—	—	90	21	—	—	4050	1093	—	—	14 291
	2	132	—	—	8 223	8 799	14 289	11 044	9 943	5129	—	—	—	—	—	—	24 972	—	108	—	—	1232	1196	—	—	—	—	1410	758	—	—	27 034
	3	142	1 538	2 925	8 225	9 965	7 101	6 126	137	67	72	333	9 460	3 471	6 650	1194	24 081	—	338	—	—	909	672	—	—	—	—	4370	1403	—	—	26 494
total:		366	1 538	2 925	16 732	19 158	21 390	17 170	10 080	5196	222	935	40 016	14 206	6 650	1194	60 784	643	1249	—	—	2141	1868	90	21	—	—	9830	3254	—	—	67 819
1965	1	88	—	—	730	930	1 450	1 070	510	310	—	—	44 090	22 160	6 550	750	24 910	—	96	—	—	500	48	210	20	—	—	—	—	100	350	25 074
	2	132	249	302	7 452	8 759	6 960	5 074	—	—	197	643	660	377	—	—	14 778	210	—	500	824	315	141	280	200	—	—	—	200	—	—	16 230
	3	142	—	—	5 354	6 232	2 170	1 640	—	—	129	747	—	—	14 370	1618	10 994	430	—	121	139	—	—	360	8	—	—	—	775	—	—	12 427
total:		366	249	302	13 536	15 921	10 580	7 784	510	310	326	1390	44 750	22 537	20 920	2378	50 622	640	96	621	981	815	189	850	228	—	—	—	975	100	350	53 731
1966	1	88	5 482	10 387	2 315	3 233	1 310	1 036	—	—	—	—	80 000	24 000	—	—	38 656	300	200	—	—	—	—	—	—	—	—	—	—	—	—	39 156
	2	132	6 060	11 925	21 300	31 059	9 450	8 520	—	—	—	—	18 000	7 398	—	—	58 902	120	—	300	424	400	252	250	137	—	—	—	16	100	50	59 851
	3	142	2 193	3 439	13 188	15 592	10 782	8 593	1 550	734	—	—	21 392	9 828	—	—	38 186	—	—	—	—	—	—	—	—	—	—	—	443	13	—	38 629
total:		366	13 735	25 751	36 803	59 884	21 542	18 149	1 550	734	—	—	119 392	41 226	—	—	135 784	420	200	300	424	400	252	250	137	—	—	—	459	113	50	137 636
1967	1	88	490	784	1 771	2 245	755	755	88	46	1300	3350	84 440	26 830	—	—	34 010	—	710	—	—	—	—	—	—	—	—	—	—	—	889	34 720
	2	132	1 000	1 800	10 000	14 000	15 000	14 400	—	—	—	—	4 000	1 800	—	—	32 000	992	200	200	300	—	—	220	200	—	—	10	8	—	—	33 700
	3	142	—	—	—	—	—	—	—	—	1200	3500	27 000	39 000	—	—	42 500	1500	1500	—	—	—	—	—	—	—	—	—	—	—	—	45 500
total:		366	1 490	2 584	11 771	16 245	15 755	15 155	88	46	2500	6850	358 440	67 630	—	—	108 510	2492	2410	200	300	—	—	220	200	—	—	10	8	—	889	113 920
1968**	1	88	—	—	7 000	5 000	—	—	—	—	80	500	11 000	21 000	—	—	26 500	400	800	—	—	—	—	—	—	—	—	—	—	—	—	27 700
	2	132	235	268	8 620	5 831	—	—	—	—	366	1565	62 460	23 144	100 000	1006	31 814	—	1030	11	27	288	135	20	25	733	2821	38	15	—	—	35 867
	3	142	—	—	—	—	—	—	—	—	668	1583	103 140	35 135	—	—	36 718	—	—	—	—	340	212	10	9	—	—	—	—	—	516	36 939
total:		366	235	268	15 620	10 831	—	—	—	—	1114	3648	275 600	79 279	100 000	1006	95 032	400	1830	11	27	628	347	30	34	733	2821	38	15	—	516	100 506
1969			—	—	—	—	—	—	—	—	—	—	73 200	18 300	—	—	18 300	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18 300
	1	75	359	382	320	251	—	—	—	—	89	298	32 800	7 883	—	—	8 814	—	—	120	87	—	—	—	—	—	—	100	52	—	760	8 953
	2	84	14 204	18 677	10 090	8 317	—	—	—	—	—	—	9 536	3 783	—	—	30 777	—	—	250	401	—	—	150	63	—	—	348	613	229	—	31 854
	3	90	127	576	—	—					554	2074	441	215	22 700	706	3 571	—	152	—	—	—	—	—	—	—	—	1070	476	—	12	4 199
total:		249	14 690	19 635	10 410	8 568	—	—	—	—	643	2342	115 977	30 181	22 700	706	61 462	—	152	370	488	—	—	150	63	—	—	1518	1141	229	772	63 306
1970	1	75	3 016	3531	15 697	10 786	9 170	3 962	—	—	—	—	—	—	23 100	549	18 828	—	180	1	6	—	—	629	290	—	—	60	51	—	—	19 355
	2	84	—	—	—	—	—	—	—	—	—	—	197 240	41 014	—	—	41 014	—	802	—	—	806	635	100[m]	124[m]	—	—	540	437	1012	—	43 012
	3	90	490	578	11 524	8 663	18 610	7 745	—	—	—	—	—	—	167 200	3328	20 314	—	1407	—	—	17	32	1379	207	—	—	8	8	—	—	21 968
total:		249	3 506	4 109	27 221	19 449	27 780	11 707	—	—	—	—	197 240	41 014	190 300	3877	80 156	—	2389	1	6	823	667	2108	621	—	—	608	496	1012	—	84 335
1971	1	75											159 400	19 053			19 053	7860	628	24	9	1050	720	730	70	28 420[w] 12 720[Sp]	7254 5148	350	60			32 978

Notes: ** = estimated values; w = white grass-carp; Sp. = spotty grass-carp, breeding; m = mature female for selling (see pike-perch in 1970)

White and spotty grass-carp: their growth could not be followed by means of samplings because of their motility, only the data of planting and recovery could be used. Grass-eating fishes were first introduced in pond No. 1 in 1971 and this action proved to be of surprisingly successful result. Considering the very high value of the coefficient of the body-weight increase ($G_w = 5.9994$) of the grass-carp, surpassing by far all other fish species, it can be regarded as the most suitable for the given circumstances. Apart from its quick growth, even the low rate of mortality (10.4 percent) is advantageous, the survival is 89.6 percent. It reached a body weight of about 8—10-times higher than the average weight at planting by the end of the first year, and the body length of 4—5 cm increased to 27—32 cm. There was no observation at our disposal regarding the behaviour of this species in sewage-water ponds, thus, further investigation is needed to determine the number of individuals of maximal planting.

The spotty grass-carp proved to be also of successfully plantable and quickly increasing species, although it does not the former in any respect. The coefficient of increase in body weight was $G_w = 3.2325$, the rate of mortality was about 60 percent, accordingly the survival was only 40 percent.

Sheat fish: The coefficient indicating the growth of body weight ($G_w = 4.6614$) was between those of the two grass-eating species, however, its mortality was higher (66 percent). From the flesh-production point of view in waste-stabilization ponds this species is insignificant.

d) The reasons for the high mortality of fishes in the pond of Zardavár

Among the data of pond No. 1 investigated in more details, the high, yearly changing rates of mortality of planted carps, first of all of fry are especially conspicuous. Because of its importance, this problem should be more thoroughly analyzed.

On the basis of home literary data (VÁMOS et al., 1963) it is known that the mass destruction of fish was observed in the ponds having an acidic, boggy bottom extremely rich in organic substances. Hydrogensulphide can easily be formed in those waters during the summer season. This compound is not formed from the protein decomposition but as a result of activity of sulphate-reducing bacteria in the mud. The initial point of the process is the fermentation of the plant residues (cellulose) in the mud, offering the hydrogen and the organic substances for the reduction processes transforming the sulphate ion into hydrogensulphide.

The H_2S in the water forms ferrous-sulphide (FeS) as long as the water contains dissolved iron, and in the form of a black deposit it is sedimented at the bottom. If the water layer above the mud contains O_2, the FeS will be oxidized (rost-brown colour). What is more, under oxidative circumstances there exist the possibility that the H_2S could be transformed into untoxic elementary sulphur.

If anaerobic conditions prevail at the bottom, the redox-level increases from the mud into the water. In such cases the amount of molecular hydrogen represents one of the main factors of the intense activity of the sulphate-reducing bacteria.

If the prolonged warm weather is followed abruptly by a cool period, the oxygen content of the cool water increases in pressing down the redox-

level. As a consequence, the reductive layer having been so far anaerobic, abruptly becomes aerobic and quick processes of oxidation will start resulting in the formation of sulphuric acid which liberates H_2S.

The formation of H_2S is usually accompanied by algal bloom, mainly by a blue-green alga due to increased respiration induced by H_2S, as a consequence of which their specific weight decreases and they will rize to the surface.

The chemical analyses of mud and water of pond No. 1 prove (the extremely high organic substance-, iron- and sulphate-content of the mud, the periodically occurring oxygen-shortage at the mud-surface) that the mud and water of that pond are inclined to form H_2S, since all the factors of sulphate-reduction are present together. These facts can explain even the high mortality of fishes.

General discussion

When evaluating the results, it should be borne in mind that they were obtained only during one year. The relations and phenomena observed cannot be applied without any further restrictions to other seemingly identical aqueous biotopes.

Pond No. 1 analyzed in details represents a biotope rich in organic substances, nitrogen and phosphorous, having a boggy bottom. The investigation of the planktonic biomass indicates that during the summer season when the loading with sewage-water is of the highest rate, the biomass of blue-green algae predominates (Anabaena bloom). In spite of the good N and P supplies, the water of the pond is poor in natural fish-food (Chironomida, Tubifex, Crustacea plankton, etc.) because of the reductive processes taking place at the water-mud interface and the boggy mud of disadvantageous structure.

On the basis of parameters of production of several years, the growth and production of carps in pond No. 1 was lower than even the values obtained in the fish-ponds of medium production. The rate of mortality of carps especially of the fry was very high, although it varied annually. That rate was much lower in case of second-summer carps, however, nevertheless the value of 15—20 percent represents a considerably loss. The high mortality can probably be explained by the locally formed hydrogensulphide. Namely, H_2S induced a mass destruction of fish in that pond during the early sixties. Certain amount of detergents may also get in into the pond and cause destruction. The reasons of the slow development of carps can be searched mainly in the poor bottom-fauna and zooplankton.

The grass-carp first planted in 1971 showed the highest productivity on the basis of both the rate of growth and the low mortality. The quick growth can be explained not only by the large amount of phytoplankton and its consumption but also by the utilization of the boggy bottom and its fauna (meiobenthos) being useless or inaccessible for the other fish species (carp, tench). The observations of soviet researchers prove that the grass-carp develops extremely well in ponds of boggy bottom where there is no firm flora. The intestinal content of fish included peat occasionally up to 80 percent. Using a mixed plantation, the rate of feeding of the carp indicates no

considerable consumption of carp-feed by the grass-carps. On the basis of that observation, it seems to be likely that the grass-carps planted into pond No. 1 were feeding to a considerable extent on peat and its fauna apart from the phytoplankton. Home experiences proved that in the ponds where a nearly optimal plantation of grass-eating fishes was applied, the increase of production could be reached without any essential increase of dunging and feeding, i.e. first of all the natural production increased. The natural production of carps based mainly on the protein of zooplankton does not limit the plantation with grass-eating fishes even when considering economical points of views. Apart from the poorness of the pond investigated in proteins of animal origin, the condition is of special significance that the mass-production of phytoplankton representing the main food for the grass-eating fishes is greatly enchanced by the duck-cultivation and post-purification of sewage-waters carried out in the pond. Considering that under such conditions the carp produces flesh only according to the rate of feeding, it is in a disadvantageous position as against to the grass-eating fish, therefore, its maintenance in that pond is uneconomic. One of the aims of our investigations was even the selection of the suitable species of fish. On the basis of our observations and the data of production we are convinced that for the utilization of sewage-waters in fish-ponds, the white grass-carp are the most suitable. Beside the disadvantageous morphometric and soil-characters of the pond, the role of the grass-eating fishes is underlined by the fact that they need no animal proteins except in the first several months of their life and even during that period hardy or not at all, therefore, they do not load the natural ability of the pond for carp-production. In the case of the white grass-carp, the ability of the pond to produce algal plankton can be intensified almost without limitation even beside duck-cultivation and supply with sewage-water of suitable mixture, as long as it does lead not to the worsening of the water quality and to the lowering of the oxygen-supply. The relationships of the optimal density of population and the load with sewage-water, representing the keyproblems of the post-purification of sewage-waters by fish-ponds, will be studied in experimental waste-stabilization ponds during the following years (and a final evaluation will be given only on the basis of those future experiments).

Summary

1. The distribution of the total planktonic biomass is uneven in the transversal section of pond No. 1, namely it is about 4—7 percent lower in the vicinity of the inflow of sewage-water than at other places.

2. The zooplankton plays a secondary role in the planktonic community during the warm-water period as compared to the algal biomass.

3. The almost complete absence of Daphnia species as well as the se condary role of the other Cladocera during the summer season indicate a limited transfer of the algal biomass increased by the sewage-water along the food-chain.

4. On the basis of the bacterial biomass, pond No. 1 can be classified to be of high productivity and medium loading.

5. The boggy soil of the pond is rich in organic substances (N, P_2O_5) iron and sulphate ions. There exist a danger of the formation of H_2S because of the O_2-shortage at the mud-surface during summer.

6. The data of production of the ponds testify that carp-production is generally high and apart from the varying mortality, it depends on the quantity and quality of the fishes planted as well as on the feeding. Pond No. 1 but the other two also are qualified to be of medium or low productivity.

7. The rate of growth of different fishes varied in pond No. 1; the increase of body weight and length of carp lagged that of other fishes. Its mortality was high, survival rate was low.

8. The slow growth of carp can be explained by the poorness of the natural food of animal origin.

9. The white and spotty grass-carps consuming algae and small benthic animals displayed intense growth, showed a lower mortality and the best adaptation to the given circumstances.

REFERENCES

BACKIEL, T. (1963): Wzrost i próba oceny smiertelnosci boleni lovionyvh w Wisle. — *Rocz. Nauk. Roln.* **84**-B-2: 215—239.

BEVERTON, R. J. H., HOLT, S. J. (1957): On the dynamics of exploited fish populations. — *Fishery Invest. London* **19,** 533.

BRINCK, C. W. (1961): Operation and maintenance of sewage lagoons. — *Water and Sewage Works* 466—468.

CHAPMAN, D. W. (1968): Production, pp. 182—196. In: Ricker, W. E. (Ed.) Methods for assessment of fish production in fresh waters. — *IPB Handbook* No. **3,** *Blackwell Sci. Publ. Oxford.*

CSANÁDY, M., GREGÁCS, M. (1965 a): Über die hygienischen Fragen der Abwasserbeseitigung in Fischteichen. — *Hidrológiai Közl.* **4,** 179—186. (In Hungarian with Russian and German summary.)

CSANÁDY, M., GREGÁCS, M. (1965 b): Oxidation ponds. — *Hidrológiai Közl.* **10,** 469—476. (In Hungarian with Russian and German summary.)

DONÁSZY, E. (1965): Theoretische Fragen der Abwasserbeseitigung in Fischteichen.— *Hidrológiai Közl.* **4,** 173—178. (In Hungarian with Russian and German summary.)

FALCK, T. (1935): Die Entwicklung der Abwasserreinigung in Fischteichen. — *Ges. Ing.* **58,** 6—12.

HANNAN, H. H., ANDERSON, B. T. (1971): Predicting the diel oxygen minimum in ponds containing macrophytes. — *The Progressive Fish-Culturist* **33,** 45—47.

HILE, R. (1936): Age and growth of the cisco *Leucichthys artedi* (LE SUEUR), in lakes of the northeastern highlands. — *Bull. Bur. Fish. U.S.* **48,** 211—317.

HOLÉNYI, L. (1962): Sewage disposal problems around Lake Balaton. — *Hidrológiai Közl.* **6,** 493—500. (In Hungarian with Russian and English summary.)

IMHOFF, K. (1956): *Taschenbuch der Stadtenwässerung. — Oldenbourg, München,* **16,** *Aufl.' (cit. ap.* LIEBMANN, 1960).

KAUFMANN, J. (1958): Chemische und biologische Untersuchungen an den Abwasserfischteichen von München. — *Z. f. angew. Zollogie* **45,** 433—481.

KISSKALT, K., ILZHÖFER, H. (1937): Die Reinigung von Abwasser in Fischteichen. — *Archiv. f. Hygiene* **118,** 1—65.

LE CREN, E. D. (1951): The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). — *J. anim. Ecol.* **20,** 201—219.

LIEBMANN, H. (1960): Biologie der Abwasserfischteiche. Biolgie der Abwasserteiche. — In: LIEBMANN: *Handbuch der Frischwasser- und Abwasser-Biologie* **2,** 531—550. VEB Gustav Fischer Verlag, Jena.

NAGIĘĆ, M. (1964): Wzrost i próba oceny smiertelnosci sandacza *(Lucioperca lucioperca* (L.)*)* z Wisly. — *Rocz. Nauk. Roln.* **84**-B-2: 329—345.

NAUWERCK, A. (1963): Die Beziehungen zwischen Zooplankton und Phytoplankton im See Erken. — *Symb. Bot. Upsalienses* **17,** 1—163.

Oláh, J. (1971): Weekly changes of the bacterio- and phytoplankton standing stock in Lake Balaton and in the highly eutrophyc Lake Belső. — *Annal. Biol. Tihany* **38,** 167—175.

Pytlik, R. (1957): Oczyszczanie sciekow domowych oraz organichnych sciekow przemyslowych metoda stawow akumulacyjnych i asymilacyjnych. — *Biul. P. A. N. Zaklad Biologiie Stawow (Kraków)*, 119—132.

Razumov, A. S. (1932): Разумов А. С.: Прямой метод учета бактерий в воде. — *Микробиология* **I:** 131—146.

Ricker, W. E. (1958): Handbook of computation for biological statistics of fish populations. — *Bull. Fish. Res. Bd. Canada* **119,** 300.

Rodina, A. G. (1965): Родина А. Г.: Методы водной микробиологии. — Изд. «*Наука*», *Москва—Ленинград* p. 363.

Schnese, K., Schwarz, S. (1970): B. Methoden zur Analyse aquatischer Lebensgruppen. Plankton. — 1—30 (in: *Ausgewählte Methoden der Wasseruntersuchung, Band II, VEB Gustav Fischer Verlag, Jena).*

Sebestyén, O. (1958): Quantitative plankton studies on Lake Balaton VIII. Biomass calculation on open water Rotatoria. — *Annal. Biol. Tihany* **25,** 267—279. (In Hungarian with Russian and English summary.)

Strickland, J. D. H., Parsons, T. R. (1968): A practical handbook of seawater analysis. — *Fish. Res. Bd. Canada, Bull.* **167.**

Tesch, F. W. (1968): Age and growth, pp. 93—120. In: Ricker, W. E. (Ed.) *Methods for assessment of fish production in fresh waters. — IBP Handbook* No. **3,** *Blackwell Sci. Publ. Oxford.*

Uhlmann, D. (1962): Oxydationsgräben und Oxydationsteiche. — *Wissensch. Ztsch. d. Karl-Marx-Univ. Leipzig,* **11,** 187—199.

Vámos, R., Zsolt, J., Ribiánszky, M. (1963): Waterbloom and Fish-Decay. — *Hidrológiai Közl.* **6,** 528—533. (In Hungarian with German and English summary.)

Warwick, R. M., Buchanan, J. B. (1971): The meiofauna off the coast of Northumberland II. Seasonal stability of the nematode population. — *J. mar. biol. Ass. U.K.* **51,** 355—362.

Winberg, G. G. (1971): Symbols, units and conversion factors in studies of fresh water productivity. — *IBP Central Office*

Woynárovich E. (1959 a): Szennyvizek hasznosítása a halgazdaságban. — *Halászat* **6,** 48.

Woynárovich E. (1959 b): Létesíthető-e szennyvíz-tógazdaság Magyarországon? — *Halászat* **6,** 64.

LIMNOLÓGIAI VIZSGÁLATOK EGY BALATON MELLETTI SZENNYVIZES HALASTÓBAN I.

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Összefoglalás

1. A teljes plankton biomassza megoszlása az 1. sz. tó keresztmetszetében egyenetlen, a szennyvízbeömlését körülvevő vízterületen 4—7%-kal kisebb, mint egyéb helyeken.

2. A plankton társulásban meleg víz idején a zooplankton alárendelt szerepet játszik az alga-biomasszához képest.

3. *Daphnia* fajok szinte teljes hiánya, valamint a többi Cladocera alárendelt szerepe a nyári időszakban, azt jelenti, hogy a szennyvíz által megemelt alga-biomasszának a továbbjutása a táplálékláncmentén korlátozott.

4. A bakterioplankton biomasszája alapján az 1. sz. tó nagy produktívitású, közepesen terhelt tavak közé sorolható.

5. A tó tőzeg talaja szerves anyagban (N, P_2O_5), vas- és szulfátionban gazdag. Nyáron az iszap felszínén fellépő O_2 hiány miatt a kénhidrogén képződésének veszélye fennáll.

6. A halastavak termelési adatai arról tanúskodnak, hogy a ponty-produkció általában magas és változó mortalitás mellett mindenkor a telepített halanyag mennyiségétől, minőségétől, valamint a takarmányozástól függ. Az 1. sz. — de a két másik tó is — közepes vagy alacsony produktívitású tónak minősül.

7. Az 1. sz. szennyvízoxidációs tóban az egyes halfajok növekedési sebessége eltérő, a ponty testhossz és testsúlygyarapodása egyaránt elmarad egyéb halastavi adatoktól. Mortalitása magas, életben maradási százaléka alacsony.

8. A ponty lassú növekedésének oka a természetes, állati eredetű táplálék szegénysége.

9. A fehér és pettyes busa mint az algák és apró iszaplakó állatok fogyasztói intenzív növésű, alacsonyabb mortalitású, az adott körülményekhez legjobban alkalmazkodó fajok.

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THE BIOMASS OF ROTATORIA IN LAKE BALATON

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The investigations on Lake Balaton having been recommenced since 1965, have estimated the horizontal distribution of Rotatoria plankton over the whole lake (P.-ZÁNKAI and KERTÉSZ, 1967; P.-ZÁNKAI and PONYI, 1970; 1971; 1972), and on the other hand, they were connected to the former works (SEBESTYÉN, et al., 1951; SEBESTYÉN, 1953) concerning the quantitative and qualitative relationships of the Rotatoria in the open water in front of Tihany.

The present paper was intended at describing the changes of Rotatoria biomass on the basis of comparisons with the former investigations based on the data of population density obtained during 1965—67.

Methods

The volume-values determined by SEBESTYÉN (1958) were used for our calculations, namely those of the "forms of warm water" according to the possibilities, since the samples were collected from May to November in each year. The specific weight of the animals was taken for unity and the biomass was expressed in mg wet weight/m^3.

During 1966—67, three parallel samplings were made, therefore, the values of the number of individuals per liter obtained during the evaluation of the samples, were averaged when calculating the biomass. For comparisons, the data of SEBESTYÉN (1958) concerning the months from May till November were also averaged and expressed in the same unit of measure.

According to our previous investigations (P.-ZÁNKAI and KERTÉSZ, 1967; P.-ZÁNKAI and PONYI, 1970; 1971; 1972), the lake can be divided into two areas considering the qualitative and quantitative relations of Rotatoria, namely the south-eastern part, i.e. the Keszthely Bay and its surrounding (segments "M" and "K"), as well as the north-eastern basin to the line of Ságpuszta—Balatonszemes (segments "G", "A" and "E"). The data of population density of segments "M" and "K" were averaged and the biomass values calculated from them were compared with the averages of segments "G", "A" and "E". This way, the changes of Rotatoria biomass are treated in view of the two main areas of Lake Balaton.

Results

The biomass of *Keratella cochlearis* showed a maximum in August of all three years in the area "M + K" *(Table I.)*. In the other part of the lake ("G + A + E"), the density of population increased twice during both 1966 and 1967, the values of May were four or nearly five times higher than those of August, respectively. During the same time, the south-western part of the lake displayed only a single mass development. In 1965, very high density of individuals as well as values of biomass were found in the Keszthely Bay and its surrounding, which in absence of parallel samplings is assumed to be a result of collecting from shoals. The distribution of this species can be regarded as uniform over the entire lake during the years of investigations.

TABLE I

Quantitative distribution of Keratella cochlearis along five transversal sections of Lake Balaton
($w = 1.22 \cdot 10^{-4}$ mg fresh)

Date	i/m³ Collecting place		Biomass (fresh) mg/m³	
	M + K	*G + A + E*	*M + K*	*G + A + E*
1965, VI.	1 000	20 700	0.1	2.5
VII.	2 100	8 500	0.2	1.0
VIII.	168 000	27 500	20.5	3.3
IX.	20 000	40 300	2.4	4.9
X.	2 500	35 000	0.3	4.3
average:	38 720	26 400	4.7	3.2
1966, V.	13 000	149 000	1.6	18.2
VI.	25 300	309 000	3.1	3.8
VII.	58 200	39 800	7.1	4.8
VIII.	61 000	36 100	7.4	4.4
IX.	16 000	19 400	1.9	2.4
X.	23 200	25 700	2.8	3.1
XI.	3 100	16 000	0.4	1.9
average:	28 542	44 271	3.4	5.5
1967, V.	21 000	130 000	2.6	15.9
VI.	21 000	28 000	2.6	3.4
VII.	29 200	16 200	3.5	1.9
VIII.	60 500	33 600	7.4	4.1
IX.	4 800	6 900	0.6	0.8
X.	1 800	18 100	0.2	2.2
average:	23 050	38 800	2.8	4.7
average of three years	23 189	36 823	3.6	4.4

The species *Keratella cochlearis tecta* was formerly considered to be an autumnal form in Lake Balaton on the basis of its occurrence (SEBESTYÉN, 1958). However, it displayed a characteristic summer development during the three years, the values of biomass were the highest in July and mainly in August *(Table II)*. The density of population increased only once during the three years overall the lake, the rates of which and the biomasses were different

TABLE II

Quantitative distribution of Keratella cochlearis tecta along five transversal sections of Lake Balaton ($w = 1.31 \cdot 10^{-4}$ mg fresh)

Date	i/m³ Collecting place		Biomass (fresh) mg/m³	
	$M + K$	$G + A + E$	$M + K$	$G + A + E$
1965, VI.	0	1 700	0	0.2
VII.	1 100	14 000	0.1	1.8
VIII.	83 000	330 000	10.9	4.3
IX.	3 100	12 500	0.4	1.6
average:	20 040	13 860	2.6	1.7
1966, V.	0	5 400	0	0.8
VI.	7 800	15 000	1.0	2.0
VII.	101 000	48 700	13.3	6.4
VIII.	199 000	31 900	26.0	4.2
IX.	6 100	9 300	0.8	1.2
X.	14 000	9 300	1.8	1.2
XI.	2 100	5 500	0.3	0.7
average:	47 142	17 871	6.1	2.3
1967, V.	0	3 100	0	0.4
VI.	11 000	18 000	1.4	2.4
VII.	23 000	12 300	3.0	1.6
VIII.	43 300	21 700	5.7	2.8
IX.	7 800	8 900	1.0	1.2
X.	3 100	7 200	0.4	0.9
average:	14 700	18 866	1.9	1.5
average of three years	27 294	14 532	3.5	1.8

on the two areas of the lake. The very low biomass values of the south-western part characterized by two segments, observed in July, increased to their high multiple by July and August, then decreased again nearly to the former values by September. The increase of biomass was of much lower rate in the other region of the lake, during the last year of investigation no maximum appeared, the mass was uniformly distributed during the period of June—September. Comparing the yearly averages, large differences between the two areas of water were found only in 1966. This results in twice as high biomass in the Keszthely Bay and its surroundings when comparing the averages of three years.

The population density of *Keratella quadrata* was higher in the north-eastern areas of water ("G + A + E") during the spring and autumn of all the three years (May, June in 1965; October, November) than in the Keszthely Bay and its surrounding *(Table III)*. Considerable masses of this species appeared just during the spring and autumn months on this part of the lake. This result supports the finding of SEBESTYÉN (1958) who regards this species an early spring form on the basis of collections across segment "A", as well as the occurrence of population maxima. However, during the summer periods (e.g. July—September of 1966 and 1967), the biomass of the species was 2—24 times higher in segments "M + K", than in the other regions. The

TABLE III

Quantitative distribution of Keratella quadrata along five transversal sections of Lake Balaton ($w = 6.61 \cdot 10^{-4}$ mg fresh)

Date	i/m³ Collecting place		Biomass (fresh) mg/m³	
	M + K	*G + A + E*	*M + K*	*G + A + E*
1965, VI.	510	1 300	0.3	0.9
VII.	2 100	1 100	2.2	0.7
VIII.	18 000	0	11.9	0
IX.	7 500	1 300	5.0	0.8
X.	510	1 100	0.3	0.7
average:	5 724	960	3.9	0.7
1966, V.	1 600	4 500	1.1	3.0
VI.	50 700	2 100	33.5	1.3
VII.	16 700	2 100	11.0	1.3
VIII.	25 700	4 000	17.0	2.7
IX.	24 000	2 300	15.9	1.5
X.	16 700	7 400	11.0	4.9
XI.	810	4 300	0.5	2.8
average:	19 458	3 814	12.8	2.5
1967, V.	21 000	57 000	13.9	37.7
VI.	9 100	3 100	5.9	2.0
VII.	9 500	4 300	6.3	2.8
VIII.	18 000	2 400	11.9	1.6
IX.	6 300	410	4.2	0.3
X.	510	2 300	0.3	1.5
average:	10 735	12 101	7.1	7.6
average of three years	11 969	2 812	7.9	3.4

average values of three years showed a more than double difference between the two areas of water in favour of the Keszthely Bay and its surrounding.

The biomass of *Polyarhtra vulgaris* strongly varied during the three years following each other on both areas of water *(Table IV)*. A certain regularity was only observed over the entire lake during all three years, in so far as the population increased during the autumn. Apart from that, the values of biomass were high in June in the Keszthely Bay and its surrounding, as well as in May and August in the north-eastern part. The former investigations in segment "A" (SEBESTYÉN, 1953) indicated the months July—August as well as May, December and September when this species reached the highest numbers per liter. Comparing the yearly averages of biomass values found in the two areas of the lake reveals that this species occurs in larger mass in segments "G—E". The difference is sometimes small (1966) but it can even reach a double level.

The biomass of *Pompholyx sulcata* showed the largest difference between the two areas, since it was negligible in the Keszthely Bay and its surrounding, whereas even 20 mg/m³ occurred in other regions *(Table V)*. It is a characteristic species of summer development, its maximal masses occur during July

TABLE IV

Quantitative distribution of Polyarthra vulgaris along five transversal sections of Lake Balaton ($w = 3.83 \cdot 10^{-4}$ mg fresh)

Date	ind/m³ Collecting place		Biomass (fresh) mg/m³	
	M + K	*G + A + E*	*M + K*	*G + A + E*
1965, VI.	1 000	3 300	0.4	1.3
VII.	0	0	0	0
VIII.	0	0	0	0
IX.	49 000	35 700	18.8	13.7
X.	49 000	172 000	18.8	65.9
average:	19 800	42 200	7.6	16.2
1966, V.	21 000	91 900	7.7	35.2
VI.	23 800	5 700	9.1	2.2
VII.	3 200	29 000	1.2	11.1
VIII.	9 500	52 100	3.6	21.0
IX.	36 000	25 900	13.8	9.9
X.	75 700	11 300	29.0	43.2
XI.	6 400	12 900	2.4	7.3
average:	25 085	48 114	9.5	18.5
1967, V.	23 000	29 000	8.8	11.1
VI.	31 000	18 000	11.9	6.9
VII.	2 800	22 100	1.1	8.5
VIII.	9 100	64 000	3.4	24.5
IX.	5 800	31 200	2.2	11.9
X.	51 500	43 400	19.7	16.6
average:	20 533	34 616	7.8	13.2
average of three years	21 806	41 643	8.3	16.0

tnd August. Its distribution was uniform in segments "G—E" during all the ahree years on the basis of comparisons of the yearly average biomass values.

Both the density of individuals and the biomass of *Kellicottia longispina* are uniformly low in both parts of the lake. Its highest mass appears in May in accordance with former literary data (SEBESTYÉN, 1953; 1958) .The yearly averages show no significant differences between the two areas, whereas the comparisons of the years revealed small differences only.

Discussion

During the three years of investigations, the *Polyarthra vulgaris* occupied the first place among the Rotatoria of the open water of the lake as regards biomass values. It was followed by *Keratella quadrata* and *Pompholyx sulcata (Tables III, IV* and *V)*, i.e. the mass is formed by a species of medium volume but high density as well as by an other one of large volume and relatively of lower number of individuals. According to former investigations (SEBESTYÉN, 1958), *Polyarthra* and *Pompholyx* showed the highest biomass values even during other years in the north-eastern basin of the lake.

TABLE V

Quantitative distribution of Pompholyx sulcata along five transversal sections of Lake Balaton
($w = 3.11 \cdot 10^{-4}$ mg fresh)

Date	i/m³ Collecting place		Biomass (fresh) mg/m³	
	$M + K$	$G + A + E$	$M + K$	$G + A + E$
1965, VI.	0	7 300	0	2.3
VII.	0	67 000	0	20.8
VIII.	0	35 500	0	11.0
IX.	0	38 300	0	11.9
X.	0	12 500	0	3.9
average:	0	32 120	0	9.9
1966, V.	210	47 000	0.1	14.6
VI.	17 000	56 000	5.3	17.4
VII.	700	54 100	0.2	16.8
VIII.	0	64 500	0	20.1
IX.	0	17 000	0	5.3
X.	830	7 300	0	2.3
XI.	0	810	0	0.2
average:	2 677	35 244	0.8	10.9
1967, V.	3 000	25 100	0.9	7.8
VI.	2 000	36 000	0.6	11.2
VII.	1 500	69 000	0.5	21.4
VIII.	0	30 300	0	9.4
IX.	0	17 700	0	5.5
X.	0	14.300	0	4.4
average:	1 083	32 066	0.3	9.9
average of three years	626	33 143	0.4	16.2

However, *Keratella quadrata* has never been of such a high density. Since at each three points of each three segments nearly uniform number of individuals per liter were obtained, one can exclude the possibility of collecting from shoals, and one has to accept the wide propagation of this species over the entire area of water.

Systematic quantitative investigations of Rotatoria plankton involved only the open water area in front of Tihany (segment "A") before 1965. According to our results having been obtained so far, this segment represents well the area of the whole north-eastern basin, and on the other hand, its Rotatoria fauna is similar to that of the line Ságpuszta—Balatonszemes (segment "G") both qualitatively and quantitatively. Therefore, one can conclude on the basis of changes appearing in segment "A" that similar phenomena also occur in the larger part of the open water of the lake ("G + A + E").

In order to be able to compare the recent biomass data with the former ones, the values of segment "A" were separated from the other two segments belonging to the north-eastern basin. Analyzing the changes of biomass of certain species present in the plankton with higher number of individuals *(Table VI)*, one can establish that the mass of *Keratella cochlearis* increased as compared to that of years 1936—49, it remained practically unchanged from

TABLE VI

Changes of Rotifera biomass in the water-area in front of the Biological Institute (transversal section "A")

Year	*Keratella cochlearis*	*Keratella c. tecta*	*Keratella quadrata*	*Polyarthra vulgaris*	*Pompholyx sulcata*	*Kellicottia longispina*	*Trichocerca pusilla*	Total biomass mg/m³
1936	1.1	0.3	0.7	1.5	2.5	0.4	0	6.5
1937	1.0	0.4	1.3	2.3	3.1	0.6	0	8.7
1938	2.2	0.4	2.0	1.5	4.3	1.7	0	12.1
1947	0.8	3.5	1.3	17.6	4.0	0.4	1.4	29.0
1949*	0.8	1.0	0.7	2.7	1.5	1.3	0.4	8.4
1951	6.3	6.1	1.3	15.3	9.6	0.2	2.1	40.9
1965**	2.4	1.1	0.3	5.4	13.2	0	0	22.4
1966	6.0	2.2	1.5	19.5	11.7	0.6	0.5	42.0
1967	4.3	1.3	8.0	10.2	9.8	1.4	0.5	35.5

* an unusual subsidence of the open water during that time
** relatively few samplings

1951. A similar pattern was shown even by *Pompholyx sulcata*. The biomass of *Keratella c. tecta* increased until 1951 and started to decrease only during recent years, whereas that of *Keratella quadrata* was practically unchanged from the first year of investigation, apart from the higher value of 1967 which however, could not be evaluated because of the absence of further investigations. The *Polyarthra* from the 1940s, the *Kellicottia* during the whole period of investigations occur in the Rotatoria plankton with a nearly constant mass. *Trichocerca pusilla* could be collected in almost identical masses since its propagation to the open water (SEBESTYÉN, 1953; 1958).

The total biomass of Rotatoria continuously increased until 1951 in segment "A" representing the larger part of the lake. Since that time a stagnation has appeared instead of a further increase, the reason is unknown. However, different hypotheses can be outlined (cf. PONYI and P.-ZÁNKAI, 1972, pp. 136—137).

Summary

Among the most frequent rotifers of the lake three species *(Polyarthra vulgaris, Keratella quadrata* and *Pompholyx sulcata)* represent the highest biomass values.

On the basis of average biomasses of years 1965, 1966 and 1967, *Polyarthra vulgaris* is of the highest importance showing a value of 8.3 and 14.1 mg/m³ in the Keszthely Bay and its surrounding as well as in the other parts of the lake. *Keratella quadrata* and *Pompholyx sulcata* display different distribution of biomass in the two areas: the former occurred in 7.9 mg/m³ in the samples taken from the Keszthely Bay and its surrounding (segments "M+K"), the latter in 0.4 mg/m³. In the segments representing about two thirds of the lake (segments "G + A + E"), the former showed 3.5 while the latter 10.3 mg/m³ biomass value.

In the larger part of the open water (segments "G + A + E"), the total biomass of the Rotatoria gradually increased till the 1950s from 6.5 up to 40.9 mg/m³, then it remained at nearly identical level until 1967 (38.7 mg/m³).

REFERENCES

PONYI, J. E., P.-ZÁNKAI, N. (1972): Investigations on planktonic Crustacea in Lake Balaton V. Horizontally occurring quantitative changes in the different areas of the lake in 1965—1966. — *Annal. Biol. Tihany* **39,** 131—139.

SEBESTYÉN O., TÖRÖK P., VARGA L. (1951): Mennyiségi plankton tanulmányok a Balatonon. *Annal. Biol. Tihany* **20,** 69—126.

SEBESTYÉN O. (1953): Mennyiségi planktontanulmányok a Balatonon. II. Évtizedes változások. — *Annal. Biol. Tihany* **21,** 63—69.

SEBESTYÉN O. (1958): Mennyiségi planktontanulmányok a Balatonon VIII. Biomassza számítások nyíltvízi Rotatóriákon. — *Annal. Biol. Tihany* **25,** 267—279.

P.-ZÁNKAI, N., KERTÉSZ, GY. (1967): Horizontal plankton investigations in Lake Balaton VI. A study of the open water Rotatoria of Lake Balaton, based on collecting in 1965. — *Annal. Biol. Tihany* **34,** 255—275.

P.-ZÁNKAI, N., PONYI, J. E. (1970): The quantitative proportions Rotifera plankton in Lake Balaton in 1967. — *Annal. Biol. Tihany* **37,** 291—308.

P.-ZÁNKAI, N., PONYI, J. E. (1971): The horizontal distribution of Rotifera plankton in Lake Balaton. — *Annal. Biol. Tihany* **38,** 285—304.

P.-ZÁNKAI, N., PONYI, J. E. (1972): Quantitative relationships of the Rotatoria plankton in Lake Balaton during 1965—1966. — *Annal. Biol. Tihany* **39,** 189—204.

KEREKESFÉRGEK (ROTATORIA) BIOMASSZÁJA A BALATONBAN

P.-Zánkai Nóra és *Ponyi Jenő*

Összefoglalás

A tó leggyakoribb kerekesférgei közül biomassza értéket tekintve 3 faj *(Polyarthra vulgaris, Keratella quadrata, Pompholyx sulcata)* a legjelentősebb.

1965, 1966 és 1967 évek átlagos biomasszája alapján a legfontosabb a *Polyarthra vulgaris*, melynek értéke a Keszthelyi-öböl és környékén 8,3, a többi részen 14,1 mg/m^3 volt. A *Keratella quadrata* és a *Pompholyx sulcata* biomasszájának megoszlása a tó két vízterületén eltérő; az előbbi faj biomasszája a Keszthelyi-öböl és környékén vett mintákban (M + K terület) 7,9 mg/m^3, az utóbbié 0,4 mg/m^3 volt. A tó kb. kétharmad részét reprezentáló G + A + E szelvényeken a *Pompholyx* biomassza értéke 10,3 mg/m^3, a *Keratella quadrata*-é 3,5 mg/m^3 volt.

A tó nyíltvizének nagyobbik részén (G + A + E szelvények) az 1930-as évektől kezdődően a kerekesférgek összes biomasszája az 1950-es évekig fokozatosan emelkedett 6,5 mg/m^3-ről 40,9 mg/m^3-ig, majd ettől kezdve 1967-ig közel azonos szinten maradt (38,7 mg/m^3).

CHRONICLE

The new four-year's research plan started in 1972 in our Institute which is the continuation of the earlier experimental work. The investigations follow the main topics of research of the Hungarian Academy of Sciences corresponding to the themes "Bioregulation" and "Biosphere". Accordingly research on the "*Regulation of the physiological processes*" carried out in the *Department of Experimental Zoology* focussed attention on the neurohumoral regulations in the invertebrate animals, while in the *Department of Hydrobiology*, hydrobiological problems of Lake Balaton and its catchment area were studied.

Results of the work performed by the members of the two Departments were published partly in *Annal. Biol. Tihany 40*, and partly in various Hungarian and foreign journals (See *Annal. Biol. Tihany*, 1973, 40, p. 301). The list of scientific lectures held in 1972 by the scientific staff of the Institute is published in *Annal. Biol. Tihany*, 40, pp. 302—303.

In the sessions of the General Meeting of the Hungarian Academy of Sciences in 1972 Dr. JÁNOS SALÁNKI, Director of the Institute, Dr. KATALIN S.-RÓZSA, Dr. IMRE ZS.-NAGY and Dr. ELEMÉR LÁBOS senior scientific research workers were awarded the Academy Prize for establishing and developing in Hungary investigations on invertebrate neurobiology in the Department of Experimental Zoology. Neurobiological investigations were started in 1962, when the present Director of the Institute was appointed them to be Director of the Institute and at the same time the head of the Department of Experimental Zoology. Dr. JÁNOS SALÁNKI won his degree of candidate of Biological Sciences (Ph. D.) in Moscow in the laboratory headed by academician H. S. KOSHTOYANTS prominent representative of the school of Soviet comparative physiology, then in 1970 he won the degree of Doctor of Biological Sciences in Hungary. Dr. KATALIN S.-RÓZSA was also the follower of Professor H. S. KOSHTOYANTS and she won the degree of Candidate of Biological Sciences in Moscow in 1961. Dr. IMRE ZS.-NAGY and Dr. ELEMÉR LÁBOS finished University Medical School in Debrecen in 1961, the former from 1963, the latter between 1962—1970 were investigating invertebrate neurobiology at Tihany. Dr. I. ZS.-NAGY won his Candidate degree of Biological Sciences in 1967 and Dr. E. LÁBOS the same in 1969 on the basis of the investigations carried out in our Institute. The scientific publications of the Prize winners can be found in the volumes of *Annal. Biol. Tihany* published between 1963—1972, and in the other scientific journals listed in the above volumes.

Dr. JENŐ PONYI, head of the Department of Hydrobiology on the competition for the "Prize of Research work" conducted by the General Secretary of the Hungarian Academy of Sciences won the Prize on the basis of his work entitled "Investigations on crustacean and molluscan remains in the upper sedimentary layer of Lake Balaton". The Prize winner paper was published in **38**th volume of *Annal. Biol. Tihany*.

During 1972 the following members of the scientific staff received the title of University Doctor or won the candidate degree of Biological Sciences:

1. On the 20th of April, ISTVÁN KISS on the basis of his work "Different forms of the generation of rhythm and types of the chemical sensitivity of the giant neurones identified in CNS of *Lymnaea stagnalis*" obtained a title of University Doctor as the Department of Comparative Physiology of the Eötvös Loránd University, Budapest.

2. On the 16th of October, TIBOR KISS obtained his University Doctorship on the basis of his work "Microelectrophysiological and morphological investigations on the snail heart muscle cells" at the Department of Comparative Physiology of the Eötvös Loránd University, Budapest.

3. On the 27th of November, Dr. JÁNOS OLÁH won the degree of Candidate of Biological Sciences with his dissertation: "The mass and production of the plankton of microorganisms and the trofic condition in Lake of Balaton".

The Institute's permanent staff is 54 persons, comprising 18 scientific research workers, 14 technical assistants, 6 administrative and 16 other workers.

The following changes took place in the scientific staff of the Institute: on the 1st of May, ISTVÁN KISS, on the 1st of October, KÁROLY ELEKES and TIBOR KISS were appointed scientific research workers to the Department of Experimental Zoology. On the 1st of September, JÁNOS NEMCSÓK biologist was appointed scholar assistant scientific worker to the Department of Experimental Zoology and at the same time JUDIT N.-HORVÁTH biologist was appointed assistant scientific research worker to the Department of Hydrobiology.

Inland scientific connections

The Institute had inland connections with several scientific Institutes of the Academy and University Departments, realized by cooperations and consultations.

The following Hungarian scientists worked in our Institute as visiting research workers in 1972: Prof. E. BIRÓ, Biochemical Institute of the Eötvös Loránd University, Budapest; Prof. GY. BOT, Department of Medical Chemistry of University Medical School, Debrecen; Prof. B. CSILLIK, Department of Anatomy, Histology and Embriology of University Medical School, Szeged; Dr. G. CZEGLÉDY-JANKÓ, OKI, Budapest; Prof. O. FEHÉR, Physiological Department of József Attila University, Szeged; Dr. F. JÓLESZ, Kandó Kálmán Higher Technical School of Electrical Industry, Budapest; Dr. E. KNYIHÁR, Department of Anatomy, Histology and Embriology of University Medical School, Szeged; Prof. M. NEMESSURI, Institute of Sports, Budapest; Dr. A. PUPPI, Central Laboratory of Zootechnic of the Biophysic Institute of POTE, Pécs.

Similarly to the previous years Dr. J. SALÁNKI, Director of the Institute held lectures at the Department of Comparative Physiology of Eötvös Loránd University, Budapest for students-biologists, which was entitled "Excitation at membrane level".

Similarly to the previous years, in 1972, several university students performed here the experimental part of their theses submitted for certification at the University, In the summer period eight university students joined the experimental work of the Institute. Dr. ZS.-NAGY IMRÉNÉ, TÓTH VALÉRIA assistant, who was corresponding student at the teacher's training course in biology and chemistry at the Kossuth Lajos University, Debrecen between 1966—1972 performed her thesis submitted for certification at the Zoological Department of the Institute.

Among the scientific workers of the Institute Dr. I. ZS.-NAGY senior scientific research worker spent one week as an inland study tour in the Department of Pathological Anatomy of POTE, Pécs.

Scientific connections with foreign Institutes and research workers

In 1972 within the frame-work of an official agreement mutual work was done with the Czechoslovakian Academy of Sciences on: "Light and electronmicroscopic structures of neurohaemal organ of insects" and on "Quantitative analysis of the nerve processes of invertebrates". The first theme was investigated jointly with the Institute of Entomology (Prague), the second with the Institute of Physiology (Prague). To study the second theme Dr. T. J. SKVARIL engineer visited our Institute in the frame of a study tour.

Travels abroad

1. Dr. P. BIRÓ, scientific research worker finished his four-month study tour on the 30th of April, spent in the Institute of Freshwater Biology in Borok (Soviet Union).

2. Dr. B. ENTZ, senior scientific research worker, has continued his work as a UNESCO expert in UAR.

3. T. KISS assistant scientific research worker in June spent two weeks in the Physiological Institute of the Ukrainian Academy of Sciences at Kiev (Soviet Union).

4. ILONA B.-MUSKÓ assistant scientific research worker was working in the Department of Insect Physiology of the Institute of Entomology of the Czechoslovakian Academy of Sciences (Prague) between the 9th and 28th of October.

5. Dr. J. OLÁH scientific worker attended a Symposion on "Detritus and its ecological role in aquatic ecosystems" organized by IBP-UNESCO between the 23rd and 27th of May in Pallanza (Italy).

6. Dr. KATALIN S.-RÓZSA senior scientific research worker and Dr. J. SALÁNKI Director of the Institute were invited to deliver lectures at the Zoological Department of the Basel University, Basel (Switzerland); then Dr. K. S.-RÓZSA was on a study tour in the Zoological Department of the Jena University, Jena (GDR) between the 1st and 19th of June, subsequently, she attended the methodical course held by ICRO-EMBO in the topic of Biophysic of the membranes in Bern (Switzerland) between the 12th and 29th of September.

7. Dr. J. SALÁNKI, Director of the Institute, Dr. K. S.-RÓZSA senior scientific research worker, I. VARANKA scientific worker, T. KISS assistant scientific research worker, I. VADÁSZ scholar scientific worker and M. VÉRÓ electronic engineer attended the International Congress of Biophysics held in Moscow (Soviet Union) between the 4th and 14th of August. Dr. J. SALÁNKI took part in the work of the Meeting of COMECON experts in the frame of cooperation on the Biophysics of Membranes held in Reinhardsbrunn (GDR) between the 28th and 31st of May.

8. M. VÉRÓ electronic engineer visited the Laboratory of Neurocybernetics of the Physiological Institute of the Czechoslovakian Academy of Sciences (Prague) and Physiological Institute of the Slovakian Academy of Sciences (Bratislava) between the 2nd and 21st of October.

9. Dr. NÓRA P.-ZÁNKAI and Dr. S. HERODEK scientific research workers were invited to visit the Ecological Institute of the Polish Academy in Sciences in Warsaw between the 11th and 18th of December.

The following scientific research workers visited the Institute or spent longer periods of time here during 1972:

Dr. U. BASILE, Department of Construction of the Biological Apparatus at the University of Milano, Milano, Italy; Dr. S. BERNOT, Department of Neurology at the University of Würzburg (GFR); Dr. L. BOLIS, Physiological Department of the University of Rome, Italy; Dr. BRYLINSKI, Institute of Hydrobiology, Olsztyn-Polska, Poland; Dr. T. P. CIRULIS, Institute of Evolutionary Physiology and Biochemistry, Leningrad, Soviet Union; Dr. A. CENTAMORE, Institute of Genetics, Rome, Italy; Dr. C. L. DEELDER, Fisheries Laboratory, Ijmuiden, Holland; Dr. T. KITAGAWA, University Medical School, Nisimachi, Gonado City, Japan; Dr. D. LABIC, Institute of Molecular Pathology, Paris, France; Dr. R. S. LEEUWIN, Pharmacological Department of the Amsterdam University, Amsterdam, Holland; Dr. M. MACKAY, London University, London, UK; Dr. P. MARKKANEN, Biochemical Laboratory of the Research Centre of Finland, Helsinki, Finland; Dr. L. MARTON, Institute and Museum of History and Technology, Washington, USA; Dr. R. NORDMANN, Biochemical Department of University Medical School, Paris, France; Dr. A. B. NOVIKOFF, Albert Einstein College of Medicine, New York, USA; Dr. B. M. OKUJAWA, Institute of Clinical and Experimental Neurology, Tbilisi, Soviet Union; Dr. C. C. ROSS, University of South Carolina, Spartamsurg, USA; Dr. J. J. SAWTELL, Bethany College, Bethany, USA; Dr. L. SEVEUS, LKB-AB, Bromma, Sweden; Dr. G. TAUTERMANN, Department of Zoology of the University of Innsbruck, Austria; Dr. H. UDE, Zoological Department of the Jena University, Jena, GDR; Dr. H. VOLKMER, Physiological Department of the Jena University, Jena, GDR; Dr. H. J. SEEWALD, Physiological Department of the Jena University, Jena, GDR; Dr. K. WIECKOWSKI, Institute of Geography of the Polish Academy of Sciences, Warsaw, Poland.

Meetings

In 1972 the following meetings were held at the Institute:

1. Winter School in the field of nuclear physics organized by the Central Research Institute of Physics of the Hungarian Academy of Sciences from the 24th to 28th of January (25 participants).

2. The meeting of the Section of Biomechanics of the Physiological Training Scientific Council dealing with the "Automatism of the human movements" between the 4th and 6th of May (40 participants).

3. Meeting of the Committee of water protection in Lake of Balaton on the 30th of May with 50 participants.

4. Symposium on the mechanisms of neurovegetative transmission between 19th and 24th of June (52 participants).

5. Summer course for the pupils of secondary schools with biological specialization was organized by the Biological Department of the Hungarian Academy of Sciences between the 27th and 30th of June (27 participants).

6. The IVth International Continuation Course of Hydrobiology between the 10th and 17th of July (20 participants).

7. First conference on the properties of membranes between the 4th and 6th of September (25 participants).

8. The course on the topic of the "Regulation of cell division" held by the Morphological and Cytological Committee between the 13th and 16th of September (50 participants).

9. The "Hydrobiological days" were organized by the Hungarian Hydrobiological Society and the Hydrobiological Department of the Institute between the 5th and 7th of October (50 participants).

10. Course on the topic "Development of the IVth generations computer system" was organized by the 1st Department of the Natural Sciences of the Hungarian Academy of Sciences between the 30th of November and 15th of December (53 participants).

Improvement of research facilities

The equipment park was completed in 1972 among others by NE-230. x-y Recorder; 4-channel pen-recorder, KUTESZ-146; tape punch Perfomon-30; tape reader Readman-1000; 4-channel FM/DR data recorder TEAC, R-200; Preparative centrifuge K-24, GDR; Submabine Photometer, Model-310, GM, Instr. Corp. USA; industrial permanent pH-meter, MMG-MOSION. The modernization of the aquarium-room was finished and as a result the thermoregulation of the aquarium was completed (5—30° C).

A two-channel data recorder was made in our workshop.

Library

At the end of the year the Institute's Library comprised 45483 volumes: book = 4853 units, journals = 30 326 units and reprints = 10 304 units. The Institute's Year Book — *Annal. Biol. Tihany* — Vol. **39** (1972) was sent to 645 Institutes and private persons all over the world. In exchange the Library received about 346 different journals and publications.

Miscellaneous

Our Institute participated in the International Fair held in Budapest in May, 1972. In the Pavillion of the Hungarian Academy of Sciences our research program was made public and the equipments constructed at the Institute were exhibited.

KRÓNIKA

Az Intézetben 1972-ben indult az új négyéves kutatási terv, mely közvetlen folytatását képezi a korábban folytatott vizsgálatoknak. A kutatások csatlakoznak az MTA kutatási főirányához „Bioreguláció", ill. „Bioszféra" témakörökben. Ennek megfelelően a *Kísérletes Állattani Osztály* munkája az *„Életfolyamatok szabályozása"* c. fő feladaton belül a neurohumorális szabályozás törvényszerűségeinek tanulmányozására irányult gerinctelen állatokon, míg a *Hidrobiológiai Osztály* a Balaton és vízgyűjtő területeinek hidrobiológiai vizsgálatát folytatta.

A két osztály tudományos tevékenységét tükröző tanulmányok részben az *Annal. Biol. Tihany* **40**. kötetében, részben más hazai és külföldi folyóiratokban kerültek publikálásra (l. *Annal. Biol. Tihany*, 1973, **40**, 301). Az 1972-ben tartott tudományos előadások jegyzéke az *Annal. Biol. Tihany* 40. kötetének 302—303 oldalán kerül felsorolásra.

Az MTA 1972. évi közgyűlésén Dr. Salánki János intézeti igazgató, valamint Dr. S.-Rózsa Katalin, Dr. Zs.-Nagy Imre és Dr. Lábos Elemér tudományos főmunkatársak Akadémiai Díj-ban részesültek a Kísérletes Állattani Osztályon folyó Gerinctelen Neurobiológiai kutatási irányzat hazánkban történt kifejlesztéséért, illetve az abban való részvételért. Az Intézetben 1962 óta folynak neurobiológiai kutatások, amikor az Intézet jelenlegi igazgatója az Intézet élére került és egyben a Kísérletes Állattani Osztály vezetője lett. Dr. Salánki János a szovjet összehasonlító élettan ismert képviselőjének, H. Sz. Kosthoyants akadémikusnak Intézetében, Moszkvában kandidált 1959-ben, majd 1970-ben a biológiai tudományok doktora fokozatot Magyarországon szerezte meg. Dr. S.-Rózsa Katalin ugyancsak Kosthoyants professzor tanítványa volt és 1961-ben védte meg kandidátusi disszertációját, Moszkvában. Dr. Zs.-Nagy Imre és Dr. Lábos Elemér a Debreceni Orvostudományi Egyetemen végeztek 1961-ben, az előbbi 1963 óta, az utóbbi 1962—1970 években vett részt a Tihanyban kialakított gerinctelen neurobiológiai kutatásban. Zs.-Nagy Imre 1967-ben, Lábos Elemér 1969-ben védte meg kandidátusi disszertációját az Intézetben folytatott vizsgálatok eredményeként. A díjazottak tudományos munkái megtalálhatók az *Annal. Biol. Tihany* 1963—1972 években megjelent köteteiben, illetve az ezekben felsorolt, más folyóiratokban.

Dr. Ponyi Jenő a Hidrobiológiai Osztály vezetője a MTA főtitkára által Kutatási Díjra kiírt pályázaton „A Balaton felső üledékrétegéből származó rák (Crustacea) és puhatestű (Mollusca) maradványok vizsgálata" c. pályamunkája díjazásban részesült. A díjazott munka az *Annal. Biol. Tihany* **38**. kötetében (1971) került publikálásra.

Az év folyamán az alábbi kutatók szereztek egyetemi doktori címet, illetve kandidátusi fokozatot:

1. Kiss István 1972. április 20-án „A ritmusgenerálás különböző formái és a kémiai érzékenység típusai *Lymnaea stagnalis* központi idegrendszerében identifikálható óriás neuronokon" c. témakörből Budapesten az ELTE Összehasonlító Élettani Intézetében egyetemi doktori címet szerzett.
2. Kiss Tibor 1972. október 16-án „Mikroelektrofiziológiai vizsgálatok éticsiga szívizomrostjain" c. értekezésével Budapesten az ELTE Összehasonlító Élettani Intézetében egyetemi doktori címet nyert.
3. Oláh János 1972. november 27-én megvédte „A mikrobiális plankton mennyisége, produkciója és a trofikus állapot a Balatonban" c. kandidátusi értekezését.

Az Intézet személyi állománya 54 fő, mely a következőképpen oszlott meg: kutató 18, kutatási segéderő 14, adminisztratív 6, egyéb 16.

Az Intézet kutatói állományában az alábbi változások történtek: Kiss István tudományos segédmunkatárs 1972. május 1-től, Elekes Károly és Kiss Tibor tudományos segédmunkatársak 1972. október 1-től tudományos munkatársi kinevezést kaptak. 1972. szeptember 1-én Nemcsók János biológus gyakornokként a Kísérletes Állattani Osztályra, N.-Horváth Judit biológus pedig segédmunkatársként a Hidrobiológiai Osztályra kapott kinevezést.

Belföldi tudományos kapcsolatok

Az Intézet számos egyetemi és akadémiai intézettel tartott fenn kapcsolatot, mely konkrét együttműködés vagy konzultáció formájában realizálódott.

Hazai kutatók közül az alábbiak dolgoztak Intézetünkben 1972-ben:

Prof. Biró Endre, ELTE Biokémiai Intézete, Budapest; Prof. Bot György, DOTE Orvosvegytani Intézet, Debrecen; Prof. Csillik Bertalan, SZOTE Anatómiai Intézet, Szeged; Dr. Czeglédi-Jankó Géza, OKI Budapest; Prof. Fehér Ottó, JATE Állattani Intézet, Szeged; Dr. Jólesz Ferenc, Kandó Kálmán Villamosipari Műszaki Főiskola; Budapest; Dr. Knyihár Erzsébet, SZOTE Anatómiai Intézet, Szeged; Prof. Nemessuri Mihály, Testnevelési Főiskola, Budapest; Dr. Puppi András, PÓTE Biofizikai Intézet Központi Zootechnikai Laboratórium, Pécs.

Dr. Salánki János intézeti igazgató ez évben is speciálkollégiumot tartott az ELTE biológia szakos hallgatóinak „Elemi ingerület" címmel.

Korábbi évekhez hasonlóan 1972-ben több egyetemi hallgató készítette pályamunkájának kísérletes részét Intézetünkben. A nyári hónapokban 8 egyetemi hallgató kapcsolódott be az Intézet munkájába. Az Intézetben készítette egyetemi szakdolgozatát Dr. Zs.-Nagy Imréné, Tóth Valéria asszisztens, aki a Debreceni Kossuth Lajos Tudományegyetemen biológia-kémia tanári szak levelező hallgatója volt 1966—1972 között.

Az Intézet kutatói közül Dr. Zs.-Nagy Imre tudományos főmunkatárs, a PÓTE Kórbonctani Intézetében töltött egy hetet belföldi tanulmányúton.

Külföldi tudományos kapcsolatok

Az egyezményes témák keretein belül 1972-ben a Csehszlovák Tudományos Akadémiával folyt közös munka „Rovarok neurohaemális szerveinek fény és elektronmikroszkópos szerkezete", valamint „Gerinctelenek idegi folyamatainak kvantitatív analízise" c. témakörből. Az előbbi téma az Entomológiai Intézettel (Prága), az utóbbi aFiziológiai Intézettel (Prága) közösen folyt. A második téma kidolgozására Dr. T. J. Skvaril mérnök tanulmányútra Intézetünkbe látogatott.

Az Intézet kutatói közül az alábbiak utaztak külföldre:

1. Dr. Biró Péter tudományos munkatárs április 30-án fejezte be négyhónapos tanulmányútját a Belvizek Biológiai Problémái-t kutató Intézetben, Borokban (Szovjetunió).

2. Dr. Entz Béla tudományos főmunkatárs folytatta munkáját mint UNESCO szakértő az Egyesült Arab Köztársaságban.

3. Kiss Tibor tudományos segédmunkatárs júliusban két hetet töltött az Ukrán Tudományos Akadémia Élettani Intézetében, Kievben (Szovjetunió).

4. B.-Muskó Ilona tudományos segédmunkatárs október 9—28 között a Csehszlovák Tudományos Akadémia Entomológiai Intézetének Rovarfiziológiai Osztályán (Prága) dolgozott.

5. Dr. Oláh János tudományos munkatárs részt vett az IBP-UNESCO által szervezett szimpoziumon „Detritus and its ecological role in aquatic ecosystems" témából május 23—27 között Pallanzában (Olaszország).

6. Dr. S.-Rózsa Katalin tudományos főmunkatárs és Dr. Salánki János intézeti igazgató meghívásra előadást tartottak a Baseli Egyetem Zoológiai Intézetében (Svájc) február 27—március 5 között, Dr. S.-Rózsa Katalin június 1—14 között tanulmányúton volt a Jénai Egyetem Zoológiai Intézetében (NDK), és részt vett az ICRO-EMBO szervezésében rendezett metodikai kurzuson Membrán Biofizika tárgykörből szeptember 12—29 között, Bernben (Svájc).

7. Dr. SALÁNKI JÁNOS intézeti igazgató, S.-RÓZSA KATALIN tudományos főmunkatárs, KISS TIBOR tudományos segédmunkatárs, VADÁSZ ISTVÁN központi gyakornok és VÉRÓ MIHÁLY vezető mérnök részt vettek a Nemzetközi Biofizikai Kongresszuson, Moszkvában augusztus 4—14 között (Szovjetunió). SALÁNKI JÁNOS május 28—31 között KGST szakértői tanácskozáson vett részt a Membrán Biofizikai Együttműködés keretein belül Reinhardsbrunnban (NDK).

8. VÉRÓ MIHÁLY vezető mérnök a Csehszlovák Tudományos Akadémia Prágai Fiziológiai Intézetének Neurokibernetikai Laboratóriumát (Prága) és a Szlovák Tudományos Akadémia Fiziológiai Intézetét (Bratislava) látogatta meg október 2—21 között.

9. Dr. P.-ZÁNKAI NÓRA és Dr. HERODEK SÁNDOR tudományos kutatók december 11 és december 18 között meghívásra a Lengyel Tudományos Akadémia Ekológiai Intézetét (Varsó) látogatták meg.

Az alábbi külföldi kutatók tettek látogatást vagy töltöttek hosszabb időt az Intézetben:

Dr. U. BASILE Milánói Egyetem Biológiai-Készülékeket Előállító részlege, Olaszország; Dr. S. BERNOT a Würzburgi Egyetem Neurológiai Részlete, NSZK; Dr. L. BOLIS Római Egyetem Élettani Intézete, Róma, Olaszország; D. N. BRYLINSKI Hidrológiai Intézet Olsztyn-Polska, Lengyelország; Dr. T. P. CIRULIS, Evolúciós Élettani és Biokémiai Intézet, Leningrád, SZU; Dr. A. CENTAMORE, Genetikai Intézet, Róma, Olaszország; Dr. C. L. DEELDER, Halászati Laboratórium, Ijmuiden, Hollandia; Dr. T. KITAGAWA, Orvostudományi Egyetem, Nisimachi, Gonado City, Japán; Dr. D. LABIC, Molekuláris Pathológiai Intézet, Párizs, Franciaország; Dr. R. S. LEEUWIN, Amsterdami Egyetem Farmakológiai Intézete, Amsterdam, Hollandia; Dr. M. MACKAY, Londoni Egyetem, London, Anglia; Dr. P. MARKKANEN, Finnországi Kutatóközpont Biokémiai Laboratóriuma, Helsinki, Finnország; Dr. L. MARTON, Történelmi és Technológiai Múzeum, Washington, USA; Dr. R. NORDMANN, Orvosegyetem Biokémiai Intézete, Párizs, Franciaország; Dr. A. B. NOVIKOFF, Albert Einsteinről elnevezett Orvos Kollégium, Bronx-New York, USA; Dr. B. M. OKUJAWA, Klinikai és Kísérletes Neurológiai Intézet, Tbiliszi, SZU; Dr. C. C. ROSS, Dél-Karolinai Egyetem, Spartamsurg, USA; Dr. J. J. SAWTELL, Bethani Kollégium, Bethany, USA; Dr. L. SEVEUS, LKB-AB Bromma, Svédország; Dr. G. TAUTERMANN, Innsbrucki Egyetem Zoológiai Intézete, Innsbruck, Ausztria; Dr. H. UDE, Jénai Egyetem Zoológiai Intézete, Jéna, NDK; Dr. H. VOLKMER, Jénai Egyetem Élettani Intézete, Jéna, NDK; Dr. H. J. SEEWALD, Jénai Egyetem Élettani Intézet, Jéna, NDK; Dr. K. WIECKOWSKI, Lengyel Tudományos Akadémia Földrajzi Intézete, Varsó, Lengyelország.

Rendezvények

1972-ben az alábbi rendezvényekre került sor az Intézetben:

1. Téli iskola a magfizika tárgyköréből az MTA Központi Fizikai Kutatóintézetének rendezésében január 24—28 között, 25 résztvevővel.

2. A Testnevelési Tudományos Tanács Mozgásbiológiai Szekciójának ülése „Az emberi mozgás automatikája" témából május 4—6 között, 40 résztvevővel.

3. Balatoni Vízvédelmi Bizottság ülése május 30-án 30 résztvevővel.

4. Neurovegetatív transzmissziós Mechanizmusok Szimpoziuma 52 fő részvételével, június 19—24 között.

5. Középiskolás diákok nyári tanfolyama június 27—30 között, 27 fő részvételével, az MTA II. Főosztályának rendezésében.

6. IV. Nemzetközi Hidrobiológiai Továbbképző Tanfolyam július 10—17 között, 20 résztvevővel.

7. Első kerekasztal konferencia membranológiai tárgykörből szeptember 4—6 között, 25 fő részvételével.

8. „A sejtosztódás szabályozása" c. tanfolyam a Morfológiai és Citológiai Bizottság rendezésében szeptember 13—16 között, 50 résztvevővel.

9. Hidrobiológus Napok október 5—7 között 50 fő részvételével, a Magyar Hidrobiológiai Társaság és az Intézet Hidrobiológiai Osztályának rendezésében.

10. „IV. generációs számítógéprendszerek fejlesztése" c. tanfolyam az MTA Természettudományi I. Főosztály rendezésében 53 fő részvételével, november 30—december 1 között.

Kutatási feltételek fejlődése

1972-ben vásárolt jelentősebb műszerek, kutatási eszközök:
NE-230. X-Y Recorder; KUTESZ-146 4-csatornás kompenzográf; Perfomon-30. Perforátor; Readmom-1000 lyukszalagolvasó; TEAC, R-200, 4-sávos FM/DR mágneses adatrögzítő; K-23 (NDK) Preparatív centrifuga; MODEL-310, Submabine Photometer (GM. Instr. Corp. USA); MMG-MOSION ipari folyamatos pH-mérő. Befejeződött az akváriumszoba korszerűsítése, melynek eredményeként az akváriumok hőszabályozása (5—30 C°) megoldást nyert.

Az Intézetben készült kutatási eszköz: FM/DR 2-sávos FM/DR mágneses adatrögzítő.

Könyvtár

Az évvégi összesítés alapján az Intézet Könyvtárának állománya 45 483 egység. Ebből könyv: 4853 db, folyóirat: 30 326 kötet és különlenyomat: 10 304 db.

Az Intézeti Évkönyv — *Annal. Biol. Tihany* — **39.** kötetét 645 címre küldtük meg, melyért cserébe 346 kiadvány érkezett.

Egyebek

Intézetünk részt vett az 1972 májusában megrendezett Budapesti Nemzetközi Vásáron. Az MTA kiállítási pavilonjában ismertette az Intézet kutatási területét és a kutatásokhoz az Intézetben kifejlesztett műszereket.

LIST OF PAPERS PUBLISHED ELSEWHERE AS IN VOL. 39 OF OUR ANNALES

Biró, P.: *Neogobius fluviatilis* in Lake Balaton — a *Pontic Caspian* goby new to the fauna on central Europe. — *J. Fish. Biol.* (1972) **4,** 249—255.

Borovyagin, V. L., J. Salánki, I. Zs.-Nagy: Ultrastructural alterations in the cerebral ganglion of *Anodonta cygnea* L. induced by transection of cerebrovisceral connective. — *Acta biol. Acad. Sci. hung.* (1972) **23,** 31—45.

Elekes, K., P. Péczely: Light and electron microscopic investigations on the median eminence of the pigeon after TSH and PTU treatment. — *Z. Zellforsch.* (1972) **134,** 337—349.

Herodek, S.: Formation of diglycerides of long turnover time from labelled acetate and glucose in rat tissues. — *Lipids* (1972) **7,** 572—575.

Herodek, S., G. Csákváry: Effect of dietary fatty acids on the desaturation of stearic acid in rat liver. — *Acta Biochim. Biophys. Acad. Sci. hung.* (1972) **7,** 207—213.

Kiss, T., K. Elekes: Myo-neural junctions in the ventricle of the snail *Helix pomatia.* — *Acta Biol. Acad. Sci. hung.* (1972) **23,** 207—210.

Oláh J.: Aljzatcsere és táplálkozás közötti kapcsolat a *Potamophylax retundipennis* Brauer lárvájánál (Trichoptera). — *Állattani Közl.* (1972) **59,** 106—110.

S.-Rózsa, K., I. V.-Szőke: Ion mechanisms of the resting and action potentials in the heart of some insect species. — *Comp. Biochem. Physiol.* (1972) **41A,** 495—506.

S.-Rózsa, K., I. V.-Szőke: The effect of bioactive substances on the heart muscle cell membranes on *Locusta migratoria migratorioides.* — *Acta Physiol. Acad. Sci. hung.* (1972) **41,** 27—36.

Salánki, J.: Serotonin in the neuronal regulation of the bivalve mollusc *Anodonta cygnea* L. In: *Recent developments in neurobiology in Hungary III.* (Ed. by K. Lissák) (1972) pp. 67—89.

Salánki, J., I. Varanka: Central determination of the rhythmic adductor activity in the fresh-water mussel *Anodonta cygnea* L. (Pelecypoda). — *Comp. Biochem. Physiol.* (1972) **41A,** 465—474.

Verzár, F., I. Zs.-Nagy, N. Masera: Agl-dependent differences in thermal denaturation of nucleoproteins in situ in the nerve cells of the bivalve *Mytilus galloprovincialis* (Mollusca) as revealed by electron microscopy. — *Mech. Agl. Development* (1972) **1,** 199—211.

Zs.-Nagy, I., V. L. Borovyagin: Organization of the cytosomal membranes of molluscan neurons under normal and anaerobic conditions as revealed by electron microscopy. — *Tissue and Cell* (1972) **4,** 73—84.

Zs.-Nagy, I., M. Ermini: Oxidation of $NADH_2$ by the lipochrome pigment of the tissues of the bivalve *Mytilus galloprovincialis* (Mollusca, Pelecypoda). — *Comp. Biochem. Physiol.* (1972) **43B,** 39—46.

Zs.-Nagy, I., M. Ermini: ATP-production in the tissues of the bivalve *Mytilus galloprovincialis* (Pelecypoda) under normal and anoxic conditions. — *Comp. Biochem. Physiol.* (1972) **43B,** 583—600.

LIST OF SCIENTIFIC LECTURES IN 1972 YEAR

Biró P.: A fogassüllő-populáció táplálékfogyasztása és energiatranszformációja a Balatonban. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

Biró P.: *Neobius fluviatilis* a Balatonban. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

Czeglédi-Jankó G., Ponyi J., Csonti F: A Balaton peszticid szennyezettségi dinamikájának néhány kérdése. — *XV. Jubileumi Balatoni Közegészségügyi Napok, Siófok*, 1972. május 5.

Elekes K., Kiss T., S.-Rózsa K.: Ca-hiány okozta ultrastrukturális változások *Helix pomatia* szivében. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

Frankó A., Ponyi J.: A szén és nitrogén arányának változása a Balaton felső iszaprétegében. — *MHT Limnológiai Szakosztálya, Budapest*, 1972. április 28.

Hajdu L., Oláh J.: Adatok a Planctomycesek ismeretéhez. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

Herodek S., Tamás G.: Az elsődleges termelés vizsgálata a Balatonban 1972-ben. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

Herodek S., Tamás G.: A balatoni fitoplankton elsődleges termelésének vizsgálata ^{14}C módszerrel. — *Magyar Biológiai Társaság Botanikai Szakosztályának* 963. *szakülése, Budapest*, 1972. október 24.

Hiripi L., Salánki J.: Seasonal and activity-dependent changes of serotonin level in the central nervous system of molluscs. — *Symposium on Neurovegetative Mechanisms, Tihany*, 1972. June 19—24.

Hiripi L., Salánki J., Zs.-Nagy I., B.-Muskó I.: Biogén monoaminok szubcelluláris lokalizációja *Anodonta cygnea* L. központi idegrendszerében. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

Hiripi L., Salánki J.: Szezonális és aktívitásfüggő szerotoninszint változások Molluszkák központi idegrendszerében. — *X. Biológiai Vándorgyűlés, Szeged*, 1972. augusztus 28—30.

Kiss I., Salánki J.: Az ion-miliő szerepe a spontán aktívitás generálásában *Lymnaea stagnalis* identifikált óriás neuronjain. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

Kiss T., S.-Rózsa K.: Egy- és kétvegyértékű ionok szerepe *Helix pomatia* szívizomsejtjeinek potenciálgenerálásában. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

Kiss, T., S.-Rózsa, K.: Ion dependence of the resting and action potentials in the heart muscle cells of the snails, *Helix pomatia* L. — *IV. International Biophysics Congress, Moscow, Soviet Union*, 7—14, August, 1972.

Nagy-Vezekényi, K., Zs.-Nagy, I.: Further investigations on the ultrastructure of psoriasis. — *XIV. International Congress of Dermatology, Venice*, 1972. május 22—27.

Oláh, J.: Leaching, colonization and stabilization during detritus formation. — *IBP-UNESCO Symposium "Detritus and its ecological role in aquatic ecosystems". Pallanza* 1972. 23—27. May.

Oláh J.: Kilúgozódás, kolonizáció és stabilizáció a detrituszképződés folyamatában (a detritusz mennyisége és tápértéke a Balatonban). — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

PONYI J.: A zooplankton változása a Balatonban az utóbbi évtizedekben. — *Állattani Szakosztály, Budapest*, 1972. január 7.

PONYI J.: A Balaton eutrofizálódásának néhány kérdése. — *X. Biológiai Vándorgyűlés, Szeged*, 1972. augusztus 28—30.

PONYI, J.: Über die Balaton-Forschung in den Jahren 1969—72. — *XXIII. Dunakutató Kongresszus tihanyi látogatásakor*, 1972. szeptember 23.

PONYI J., BIRÓ P., MURAI K.: A balatoni vágódurbincs *(Acerina cernua* L.) tápláléka, növekedése és bélparazitái. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

PONYI J., BIRÓ P., OLÁH J., P.-ZÁNKAI N., KISS GY., CSEKEI T.: A fonyódi szennyvíz halastavi utótisztítás néhány biológiai tanulsága. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

P.-ZÁNKAI N.: Az *Eudiaptomus gracilis* táplálkozási viszonyairól és optimális táplálékkoncentrációról. — *XIV. Hidrobiológus Napok, Tihany*, 1972. október 5—7.

P.-ZÁNKAI N.: A modern hidrobiológia aktuális problémái. — *Középiskolás diákok továbbképzése, Tihany*, 1972. június 29.

P.-ZÁNKAI, N.: Ekszperimentaljnoe isszledovanie pitanija *Diaptomus gracilis* c pomosju C-14. — *PAN Ecológiai Intézete Varsó*, 1972. december 16.

S.-RÓZSA, K.: Humoral aspects of heart regulation of invertebrates. — *Colloq. of Zool. Institute, Basel Univ. Basel, Switzerland*, 1972. 1st of March.

S.-RÓZSA K.: Visszajelzések természete és jellemzése *Helix pomatia* diffúz miogén ritmusú szivében. — *MYT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

S.-RÓZSA K., SALÁNKI J.: Afferens és efferenes kapcsolatok a központi idegrendszer neuronjai és a szív között *Helix pomatian*. — *X. Biológiai Vándorgyűlés, Szeged*, 1972. augusztus 28—30.

SALÁNKI, J.: Mechanisms of endogeneous rhythms in invertebrates. — *Colloq. of Zool. Institute, Basel Univ. Basel, Switzerland*, 1972. 1st of March.

SALÁNKI J.: Nyugalmi és akciós potenciál ionelmélete. — *I. Membrán konferencia, Tihany*, 1972. szeptember 4—6.

SVIDERSKII, V. L., VARANKA, I.: Functional and structural characteristics of the descending pathways of the flight system in Insecta. — *IV. International Biophysics Congress Moscow, Soviet Union*, 7—14 August, 1972.

TAMÁS G.: A Balaton fitoplanktonja és mikrofitobentosza biomassza értékeinek változásai az 1960-as években. — *Magyar Biológiai Társaság Botanikai Szakosztályának 963. szakülése, Budapest*, 1972. október 24.

VADÁSZ I., ELEKES K., SALÁNKI J.: Br-neuron fiziológiai és morfológiai jellemzői *Helix pomatia* L. központi idegrendszerében. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

VADÁSZ, I., SALÁNKI, J., VÉRÓ, M.: Some characteristics of the activity generation in the Br-cell of the snail *Helix pomatia* L. — *IV. International Biophysics Congress, Moscow, Soviet Union*, 7—14 August, 1972.

VARANKA I., SVIDERSKII V. L.: Légáramlási receptorok interneuronjainak választípusai vándorsáskán *(Locusta migratoria migratorioides)*. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

ZS.-NAGY I.: Mitochondriális folyamatok mitochondriumokon kívül gerinctelenek idegszövetében. — *MATE és Rtg-optikai Szakosztály Elektronmikroszkópos Szakcsoportja, Budapest*, 1972. február 11.

ZS.-NAGY I., ERMINI M.: Energiatermelés anoxiális endogén oxidáció útján *Mytilus galloprovincialis* (Mollusca, Pelecypoda) szöveteiben. — *MÉT* 38. *Vándorgyűlése, Budapest*, 1972. május 31—június 3.

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Műszaki szerkesztő: Sós Attila
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