ANNAL. BIOL. TIHANY | 39 | 3—11 | HUNGARIA 1972

EFFECT OF ACTINOMYCIN-D ON THE MONOAMINE CONTENT OF THE CENTRAL NERVOUS SYSTEM IN THE FRESH-WATER MUSSEL *ANODONTA CYGNEA* L. AS REVEALED BY FLUORESCENCE HISTOCHEMISTRY

KÁROLY ELEKES and IMRE ZS.-NAGY

Biological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary

Received: 4th February, 1972

The assumption that non-cholinergic mediation plays the primary role in the nervous system of Pelecypoda has been supported by numerous experimental data (SALÁNKI, 1963; SALÁNKI et al., 1966; ZS.-NAGY, 1964; ZS.-NAGY and SALÁNKI, 1965). Serotonin (5HT) and catecholamines (CA) have been demonstrated by the Falck-fluorescence method in the ganglia of various *Anodonta* species (DAHL et al., 1962; 1966; ZS.-NAGY, 1967; 1968) and a significant quantity of dopamine (DA) has been found by spectrofluorimetry (SWEENEY, 1963; HIRIPI, 1972). Investigations have revealed that apart from 5HT, DA may have an important role as a transmitter, in the central nervous system of the fresh-water mussel (ZS.-NAGY, 1967; 1968).

Former investigations indicated that actinomycin D (AD) influenced the periodic activity of fresh-water mussel and simultaneously decreased the 5HT-content of ganglia (SALÁNKI et al., 1968). Our electronmicroscopic investigations (ELEKES and ZS.-NAGY, 1971) proved that the protein-synthetizing system of the nerve cells as well as the formation of dense-core vesicles (DCV) are affected by AD.

Therefore, it seemed to be necessary to investigate the effect of AD on the central nervous system of fresh-water mussel even by means of fluorescence microscopic histochemistry, in order to compare the ultrastructural alterations with those of the localization of biogenic monoamines.

Material and methods

The investigations were carried out on the ganglia of 18—20 cm long specimens of *Anodonta cygnea* L. The experimental animals were injected with 2 ml AD (100 μg/ml) into the foot 3 times per day. The controls (9 animals) were given the same quantity of Balaton-water. The animals were put into 3 groups each consisting of four specimens and killed in 8, 24 and 48 hours following the treatment. The ganglia were frozen in isopentane cooled by liquid nitrogen and subsequently freeze-dried in an apparatus type HVG 1 (Ilmenau, GDR) cooled by CO_2-aceton mixture at an end vacuum of 10^{-5} Torr. The freeze-drying of 12 pairs of ganglia required approximately 7 hours.

The freeze-dried ganglia were treated with dry formaldehyde vapours (FALCK, 1962; FALCK and OWMAN, 1965). The paraformaldehyde was stored at a relative humidity of 60 per cent (HAMBERGER et al., 1965) and the formaldehyde treatment lasted 1 hour at 80°C. The material was subsequently placed in paraffin for 10 minutes at 56°C, then cooled at room temperature. Serial sections of 6—8μ were cut and examined either uncovered or covered with Entellan (Merck) + 10% xylene.

Zeiss Nfpk microscope was used as a fluorescence microscope with BG 3 and OG 1 light filters. HBO 50 mercury-vapour lamp served as a light source.

Results

It is characteristic of the fluorescence picture of the ganglia of normal, uninjected animals that the cells displaying a yellow fluorescence (containing 5HT) predominate in the cortex, while the cells fluorescing green (containing CA) occur only rarely. On the other hand, the neuropile shows only green fluorescence. During our experiments we failed to find any significant difference between the fluorescence pictures of the ganglia of untreated animals and those injected with Balaton-water *(Figs 1* and *2)*.

Eight hours after treatment essential changes occurred first in the cortex in comparison to the control ganglia. The cells of green fluorescence being otherwise rare even in the control ganglia, wholly disappeared. Also the

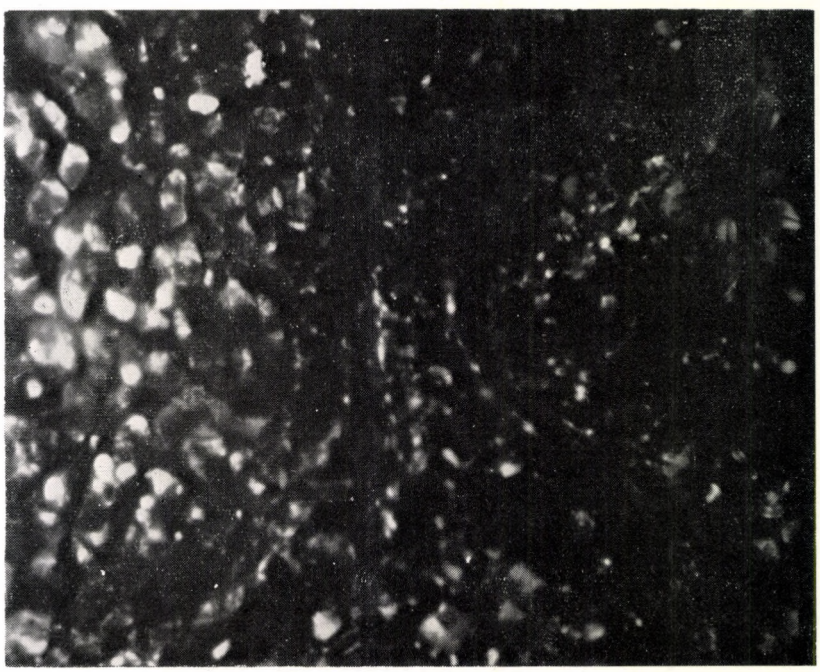

Fig. 1. Nerve cells containing 5HT from the pedal ganglion of control animals (injected with Balaton-water). ×300

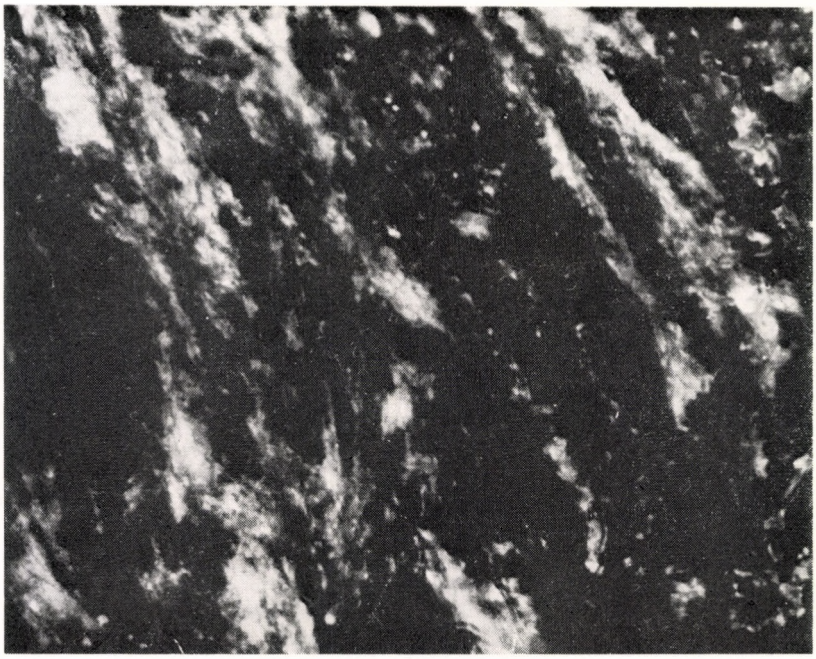

Fig. 2. The picture of control (injected with Balaton-water) neuropile. Pedal ganglion. ×300

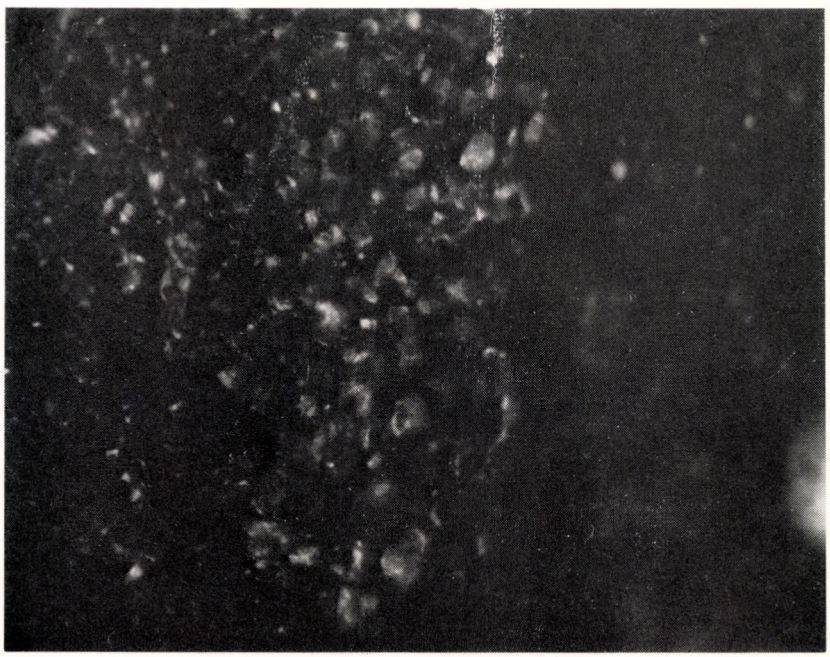

Fig. 3. Detail of the cellular layer 8 hours following AD injection. The disappearence of cells containing 5HT can be seen. Note the abundance of cells showing autofluorescence. Pedal ganglion. ×300

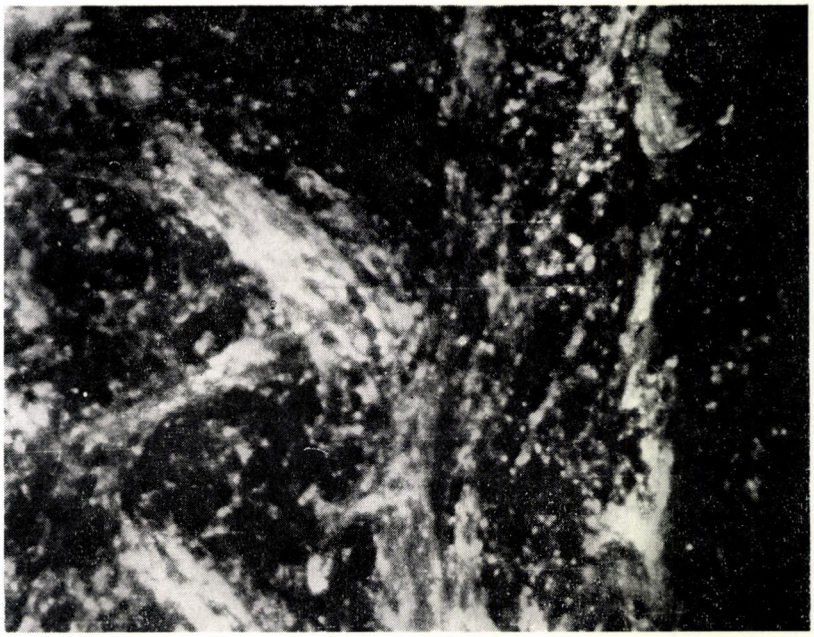

Fig. 4. Eight hours after treatment the green fluorescence indicating DA becomes stronger in the neuropile of the visceral ganglion. × 220

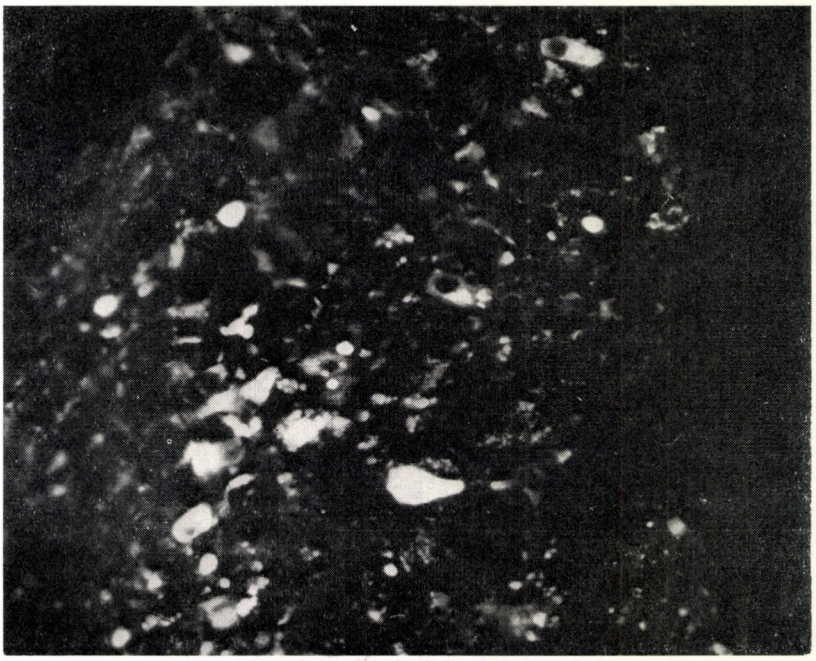

Fig. 5. Group of "green" cells fluorescing intensely in the cerebral ganglion 24 hours after treatment. ×300

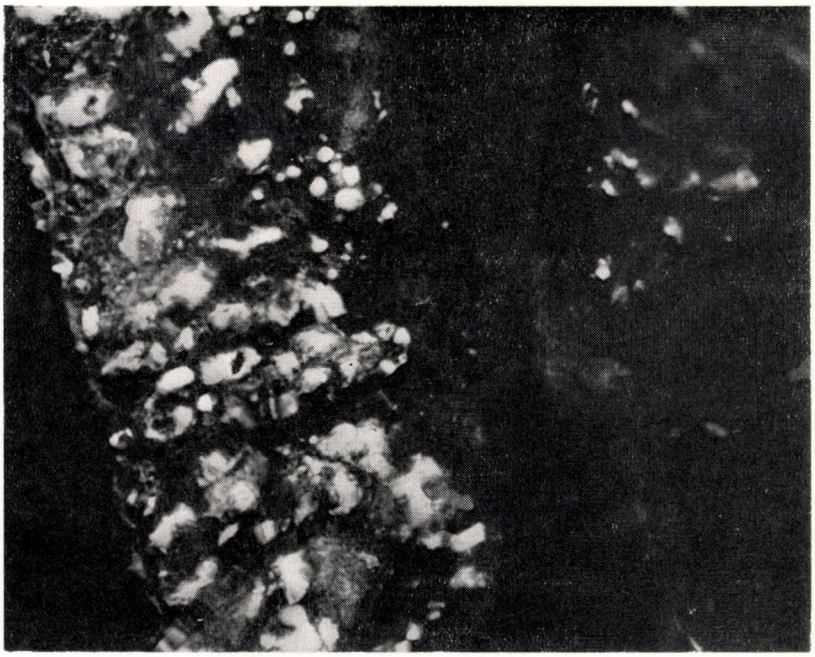

Fig. 6. Rarely occurring group of nerve cells containing 5HT and fluorescing very strongly in the visceral ganglion 24 hours after treatment. ×300

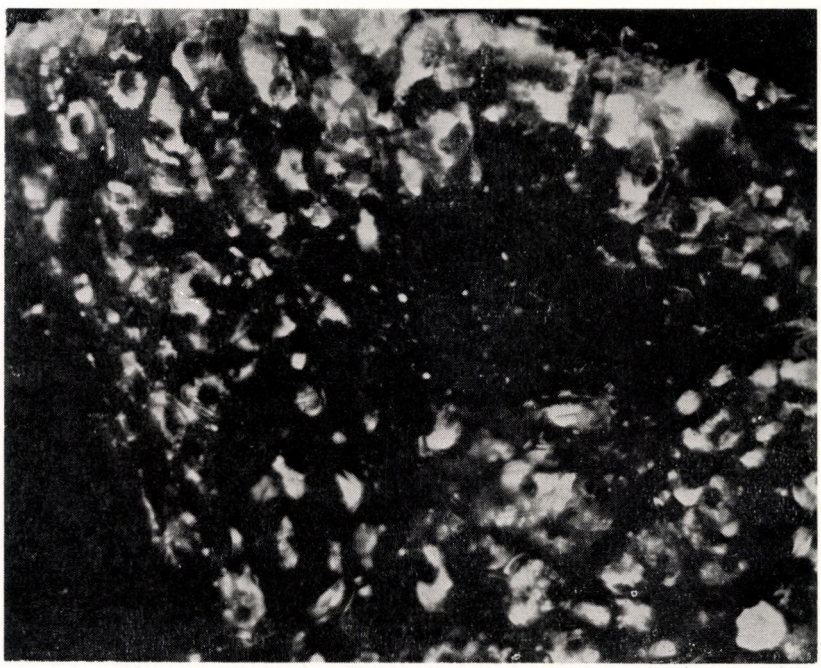

Fig. 7. Fourty-eight hours following AD treatment, 5HT containing cells showing yellow fluorescence appear again in the perikaryon layer in great masses. Visceral ganglion. ×300

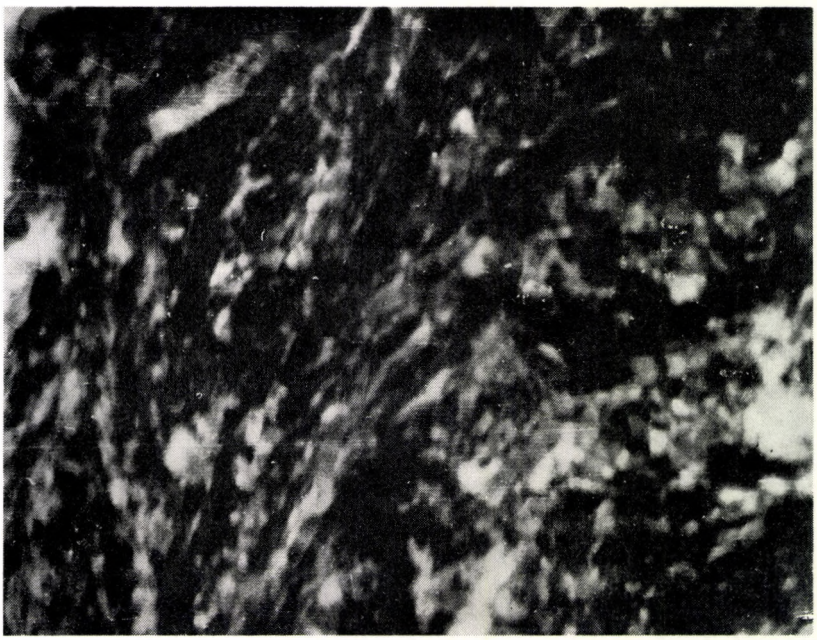

Fig. 8. Two days after treatment intensely fluorescing fibers and varicosities can be observed in the neuropile. Visceral ganglion. ×560

intensity of the yellow fluorescence decreased and the majority of the cells showed only autofluorescence, though the intensity of the latter seemed to be weaker compared to the control *(Fig. 3)*. In the neuropile the green fluorescence indicating DA decreased to some extent. However, it should be noted, that this observation cannot be considered as an unequivocal result, since in some places we observed an almost totally empty neuropile, whereas in regions of other depth the picture of the neuropile did not differ from that of the control animals. Nevertheless, 8 hours following the treatment, the green fluorescence was even stronger in the neuropile of the visceral ganglion than in that of the control *(Fig. 4)*.

Twenty-four hours after treatment generally groupped nerve cells showing an intense green fluorescence appeared in the perikaryon layer *(Fig. 5)*. A certain part of the originally "yellow" cells showed henceforward only a weak autofluorescence, while very rarely groups of cells showing strong fluorescence were also visible *(Fig. 6)*. On the other hand, very intense green fluorescence and varicosities could be observed in the neuropile. Fourty-eight hours subsequent treatment the yellow fluorescence characteristic of 5HT appeared again in the nerve cells almost everywhere *(Fig. 7)*. The picture observed in the neuropile was similar to that of the 24 hours treatment *(Fig. 8)*.

Discussion

Former investigations (DAHL, 1962, 1966) have proved that the yellow and green fluorescence revealed by fluorescence histochemical methods in the nervous system of Molluscs correspond to the 5HT and DA respectively (nor-

adrenaline represents only a very small part of the whole quantity of the CA) (SWEENEY, 1963; HIRIPI, 1972). Our electron microscopic results (ELEKES and ZS.-NAGY, 1971) showed that the AD influenced the ultrastructure of *Anodonta* ganglia in a complex way. Among others the changes manifested themselves in the alterations of the submicroscopic structure of nucleolus, endoplasmic reticulum and GOLGI apparatus. This indicates first of all that the function of the protein-synthetizing system of the nerve cells was damaged by AD. The results of our fluorescence histochemical investigations generally agree with those reached by electron microscopy.

Eight hours after AD treatment the number of the cells of yellow and green fluorescence strongly decreased in the cellular layer and the latter even completely disappeared. The cells observed this time, showing a very weak autofluorescence obviously correspond to those possessing ultrastructurally only a weakly active cytoplasm.

On the effect of treatment also the 5HT content of nerve cells decreases, however, it cannot be established unequivocally the morphological change of what subcellular elements is connected directly with this phenomenon. Therefore, on the basis of our investigations the subcellular localization of 5HT can even further be regarded as questionable. Nevertheless, the experimental results do not contradict the assumption that it is related to the endoplasmic reticulum (ZS.-NAGY et al., 1965).

The diminution of the DA content was indicated by the disappearence of green fluorescence in the somas as well as by its decrease in some places of the neuropile. The ultrastructural changes of DCV observed at the same time in both places (ELEKES and ZS.-NAGY, 1971) indicate, that DA may be stored in DCV. At the same time, however, the fact, that much more cells contained DCV 24—48 hours following treatment, than cells of green fluorescence were visible, postulates two possibilities: 1. In *Anodonta* ganglia DCV are not uniform from the point of view of monoamine content. Since at this time the yellow fluorescence characterizing 5HT was already more or less developed, it might be assumed, that it is also related to DCV, at least to a certain group of them. 2. It can also be supported, that DCV appearing this time are not yet quite complete and represent only the synthetizing apparatus which will be able to synthetize DA only later.

To reach final decision of the problem further detailed investigations are needed.

The investigation of the ganglia of 24 and 48 hours showed that AD caused indeed a reversible alteration in the monoamine content and synthesis in the nerve cells, but there is a significant difference between the restoration of 5HT and DA dontent of the cells. Large number of green cells appeared again already twenty-four hours after treatment, whereas the intense yellow fluorescence of the cells could be observed only later indicating that the complete restoration of 5HT synthesis needs a significantly longer period of time than that of DA. In spite of this, the observation that 24 hours following treatment, cell groups of very strong yellow fluorescence were rarely visible, suggests the possibility that the effect of AD on 5HT containing cells depends on the momentary state of the cells and 5HT synthesis.

Summary

The effect of AD on the monoamine content of the central nervous system of *Anodonta cygnea* L. was investigated by fluorescence histochemical method. Eight hours after treatment the 5HT and DA content of the nerve cells strongly decreased. At the same time, the intensity of the fluorescence of the neuropile decreased only in some places. The cells showing green fluorescence appeared again already 24 hours following treatment, while the restoration of 5HT synthesis needed more time (48 hours). The reconciliation and differences of the results are discussed by the authors in relation to their former electron microscopic data.

REFERENCES

Dahl E., B. Falck, M. Lundquist, C. Mecklenburg (1962): Monoamines in mollusc neurons. — *Kungl. Fysiografiska Sällskapets i Lund Förhand.* **32,** 89—91.

Dahl E., B. Falck, C. Mecklenburg, H. Myhrberg, E. Rosengreen (1966): Neuronal localization of dopamine and 5-hydroxytriptamine in some Mollusca. — *Z. Zellforsch.* **71,** 489—498.

Elekes K., I. Zs.-Nagy (1971): Effect of actinomycin-D on the ultrastructure of the central nervous system of fresh-water mussel *Anodonta cygnea* L. *Annal. Biol. Tihany* **38,** 11—30.

Falck, B. (1962): Observations on the possibilities of the cellular localization of monoamines by fluorescence method. — *Acta physiol. scand.* **56,** Suppl. 197, 1—26.

Falck B., C. Owman (1965): A detailed methodological description of the fluorescence method for the cellular demonstration of biogenic monoamines. — *Acta univ. Lund Sectio II.* **7,** 1—23.

Hamberger B., T. Malmfors, C. Sachs (1965): Standardization of paraformaldehyde and of certain procedures for the histochemical demonstration of catecholamines. — *J. Histochem. Cytochem.* **13,** 147.

Hiripi L. (1972): Catecholamines in different tissues of fresh-water mussel *Anodonta cygnea* L. — *Annal. Biol. Tihany* **39,** 13—20.

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., L. Hiripi, E. Lábos (1966): Specific cholinesterase activity in the central nervous system of *Anodonta cygnea* L. — *Annal. Biol. Tihany* **33,** 143—150.

Salánki J., I. Zs.-Nagy, L. Hiripi (1968): Nissl-substance and serotonin level in the ganglia of fresh-water mussel after treatment with actinomycin-D, in relation to activity regulation. — *Comp. Biochem. Physiol.* **27,** 817—825.

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

Zs.-Nagy I. (1964): Electron-microscopic observation 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. Hung.* **18,** 1—8.

Zs.-Nagy I. (1968): 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* 1968. pp. 69—84.

Zs.-Nagy I., J. Salánki (1965): Histochemical investigations of cholinesterase in different Molluscs with reference to functional conditions. — *Nature* **206,** 842—843.

Zs.-Nagy I., K. S.-Rózsa, J. Salánki, I. Földes, L. Perényi, M.Demeter: (1965): Subcellular localization of 5-hydroxytriptamine in the central nervous system of Lamellibranchiates. — *J. Neurochem.* **12,** 245—251.

ACTINOMYCIN-D HATÁSA A TAVI KAGYLÓ *ANODONTA CYGNEA* L. KÖZPONTI IDEGRENDSZERÉNEK MONOAMIN TARTALMÁRA. FLUORESCENS HISZTOKÉMIAI VIZSGÁLATOK

Elekes Károly és *Zs.-Nagy Imre*

Összefoglalás

Actinomycin-D kezelés *Anodonta cygnea* L. központi idegrendszerének monoamin tartalmára való hatását vizsgáltuk fluorescens hisztokémiai módszerrel. A kezelés után 8 órával az idegsejtek szerotonin, illetve katekolamin (dopamin) tartalma erősen lecsökken. Ugyanakkor a neuropil fluorescenciájának intenzitása csak helyenként mérséklődik. A zöld fluorescenciát mutató sejtek a kezelés után már 24 órával újra megjelennek, míg a szerotonin szintézis helyreállása hosszabb időt (48 óra) vesz igénybe. Szerzők diszkuttálják az eredmények egyeztethetőségét és eltéréseit a korábbi elektronmikroszkópos adataikhoz képest.