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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

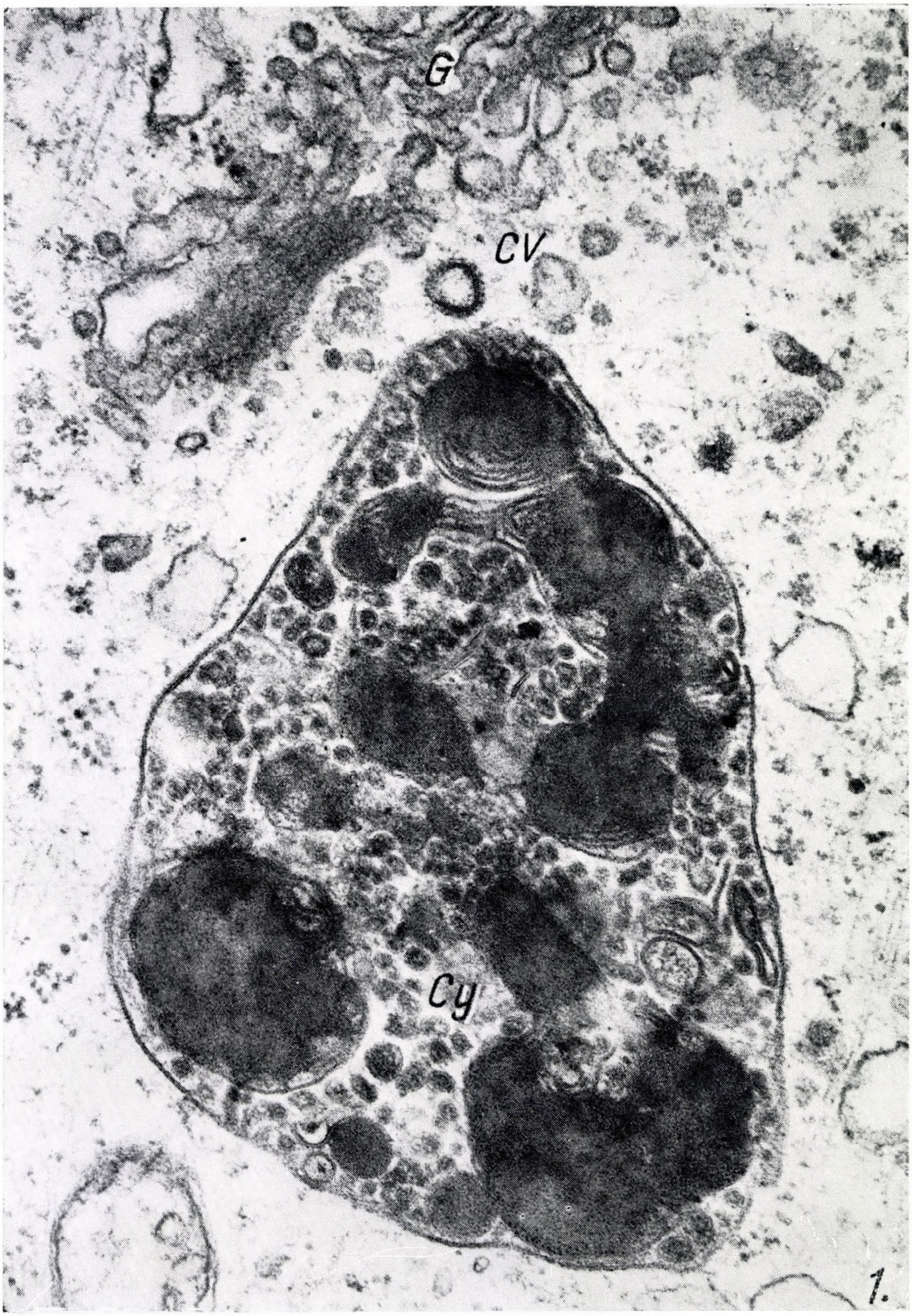

Fig. 1. Detail of an epithelial cell from the central part of the intestine. G — Golgi; Cy — cytosome; CV — coated vesicle; ×58 500

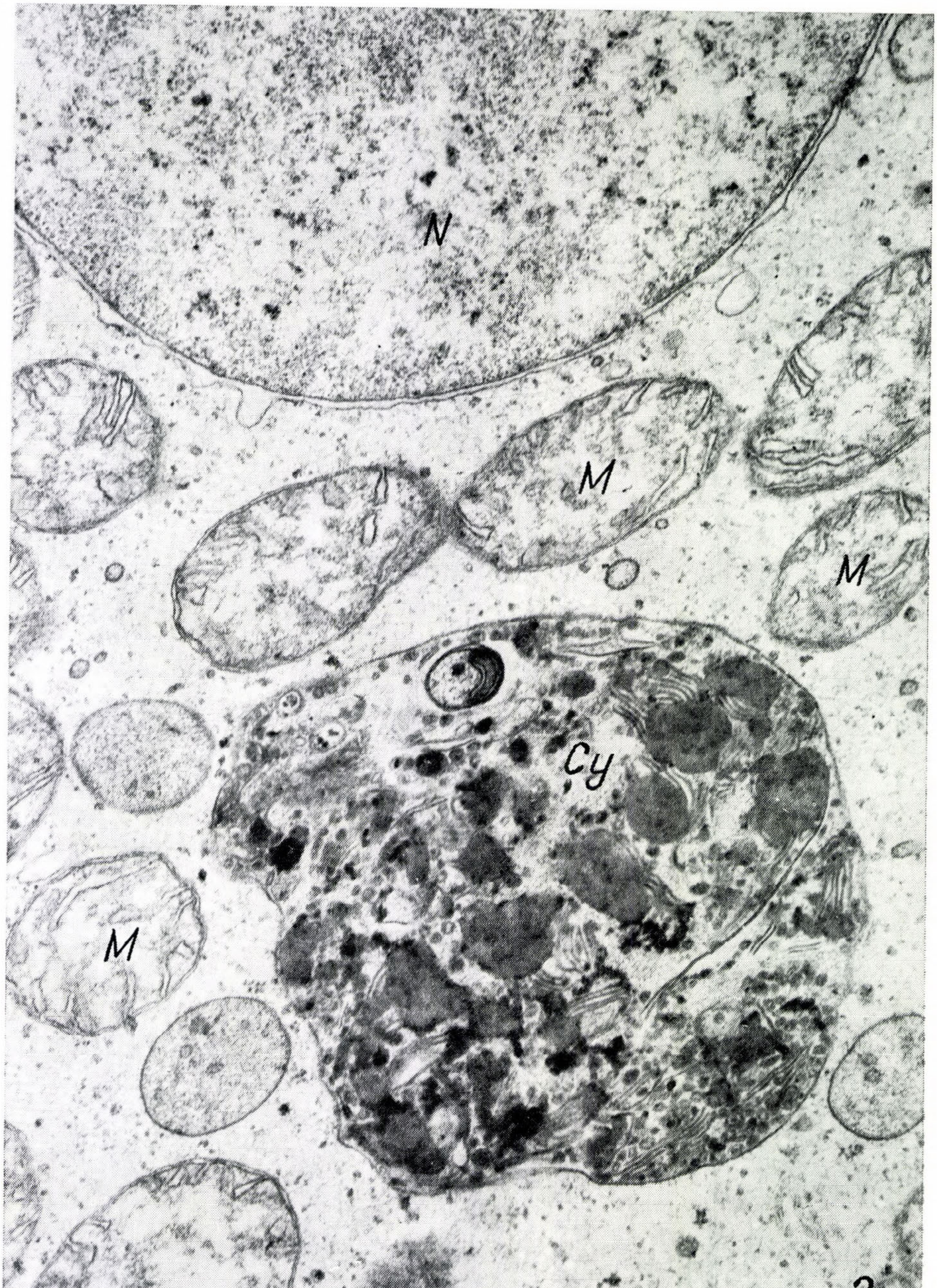

Fig. 2. Detail of an epithelial cell from the kidney. N — nucleus; M — mitochondrium; Cy — cytosome. ×30 000

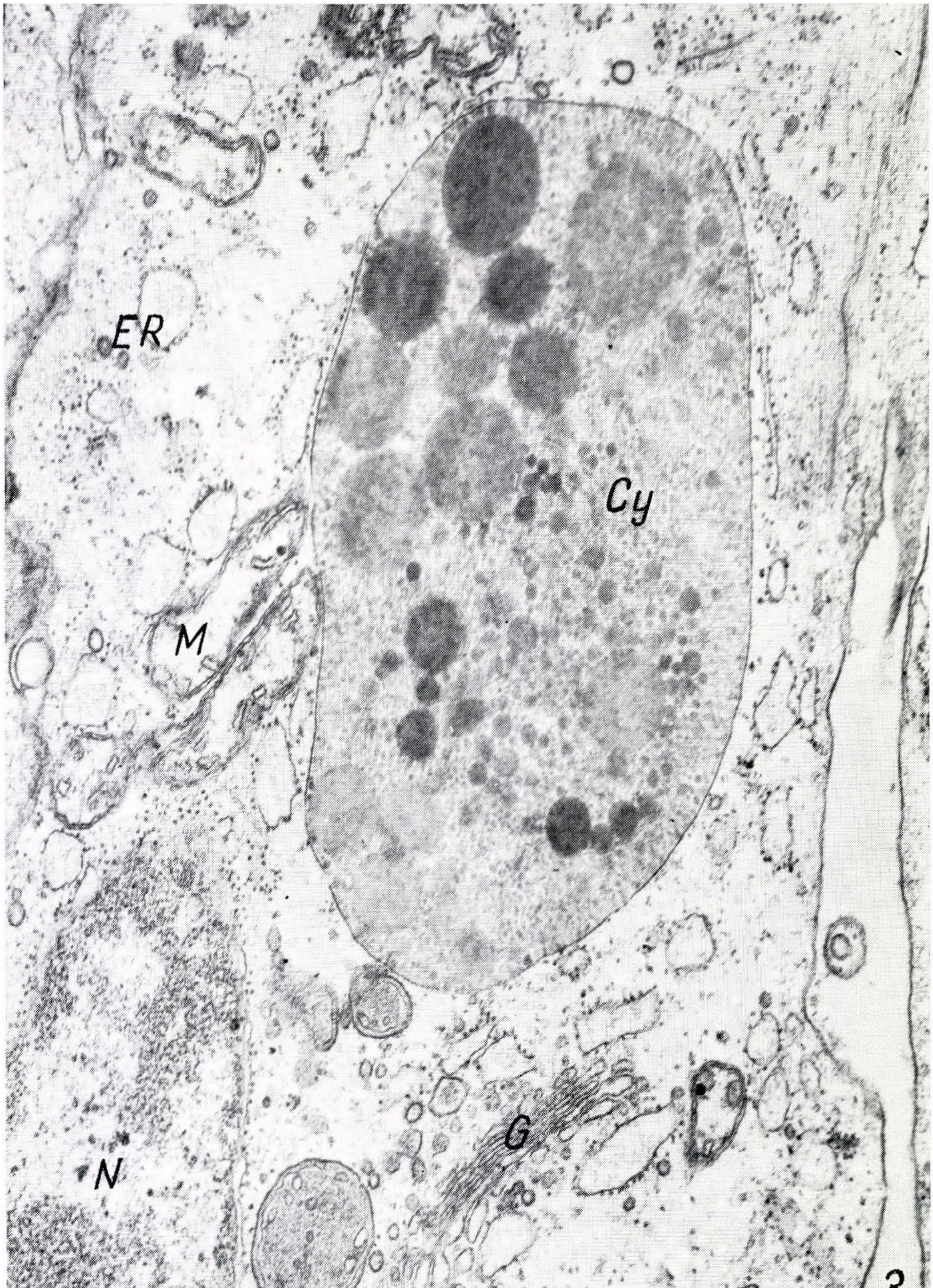

Fig. 3. Detail of a cell from the hepatopancreas. N — nucleus; M — mitochondrium; Cy — cytosome; G — Golgi; ER — endoplasmic reticulum. $\times 30\,000$

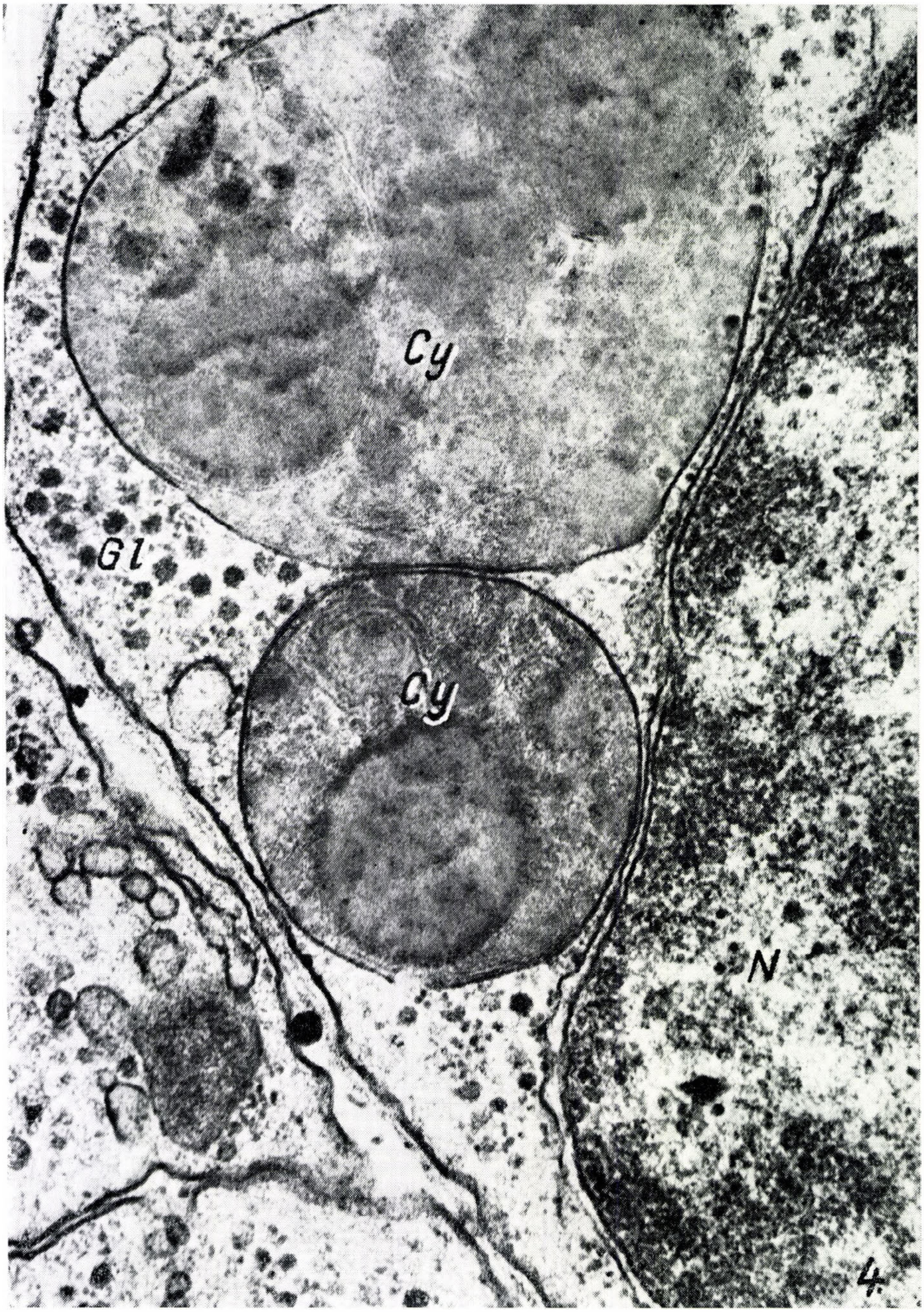

Fig. 4. Detail of an interstitial connective tissue cell of the heart musculature. N — nucleus; Gl — glycogen; Cy — cytosome. ×58 500

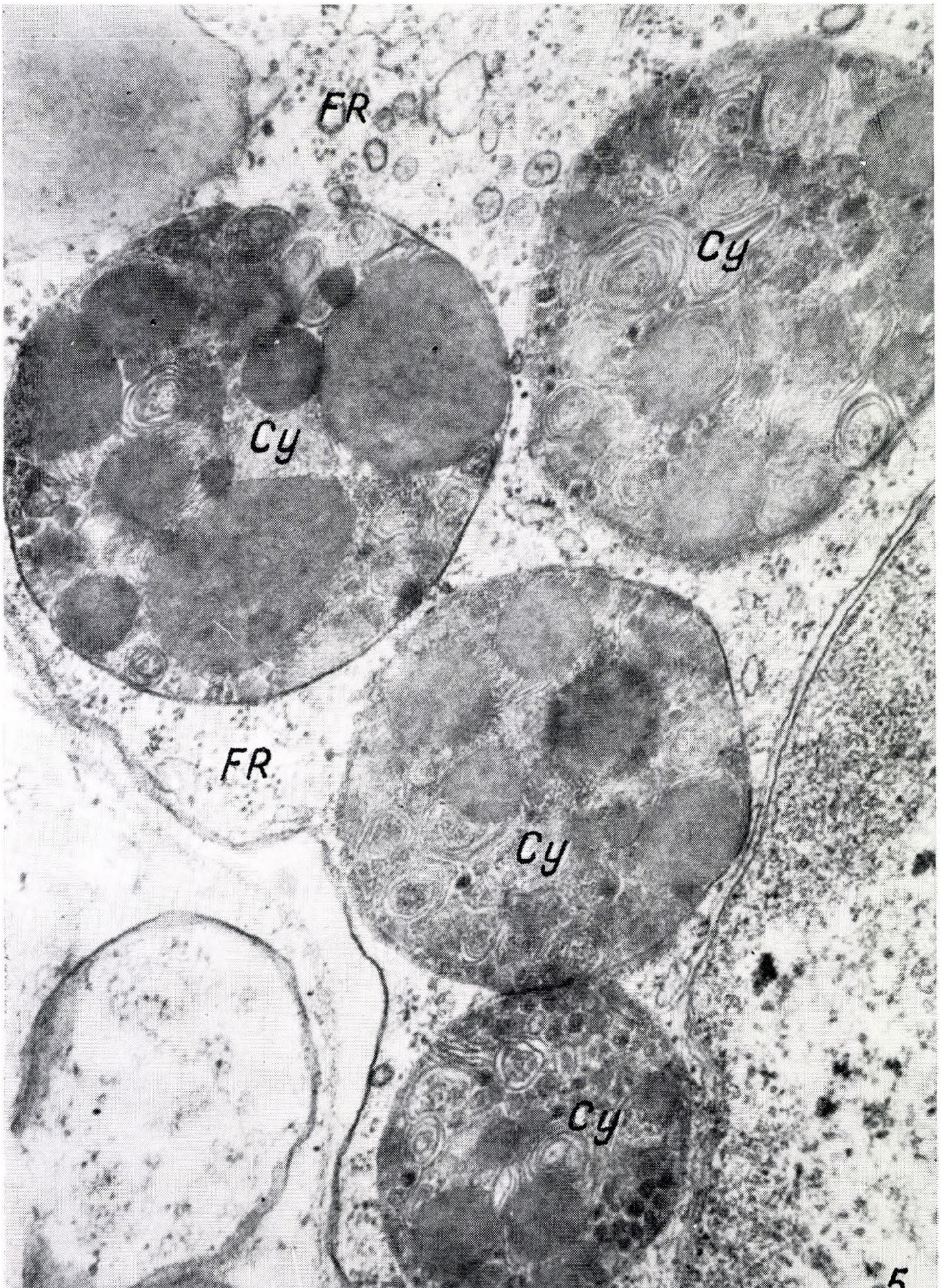

Fig. 5. Detail of a fibrocyte from the subepithelial connective tissue of the foot. N — nucleus; Cy — cytosome; FR — free ribosomes. ×48 500

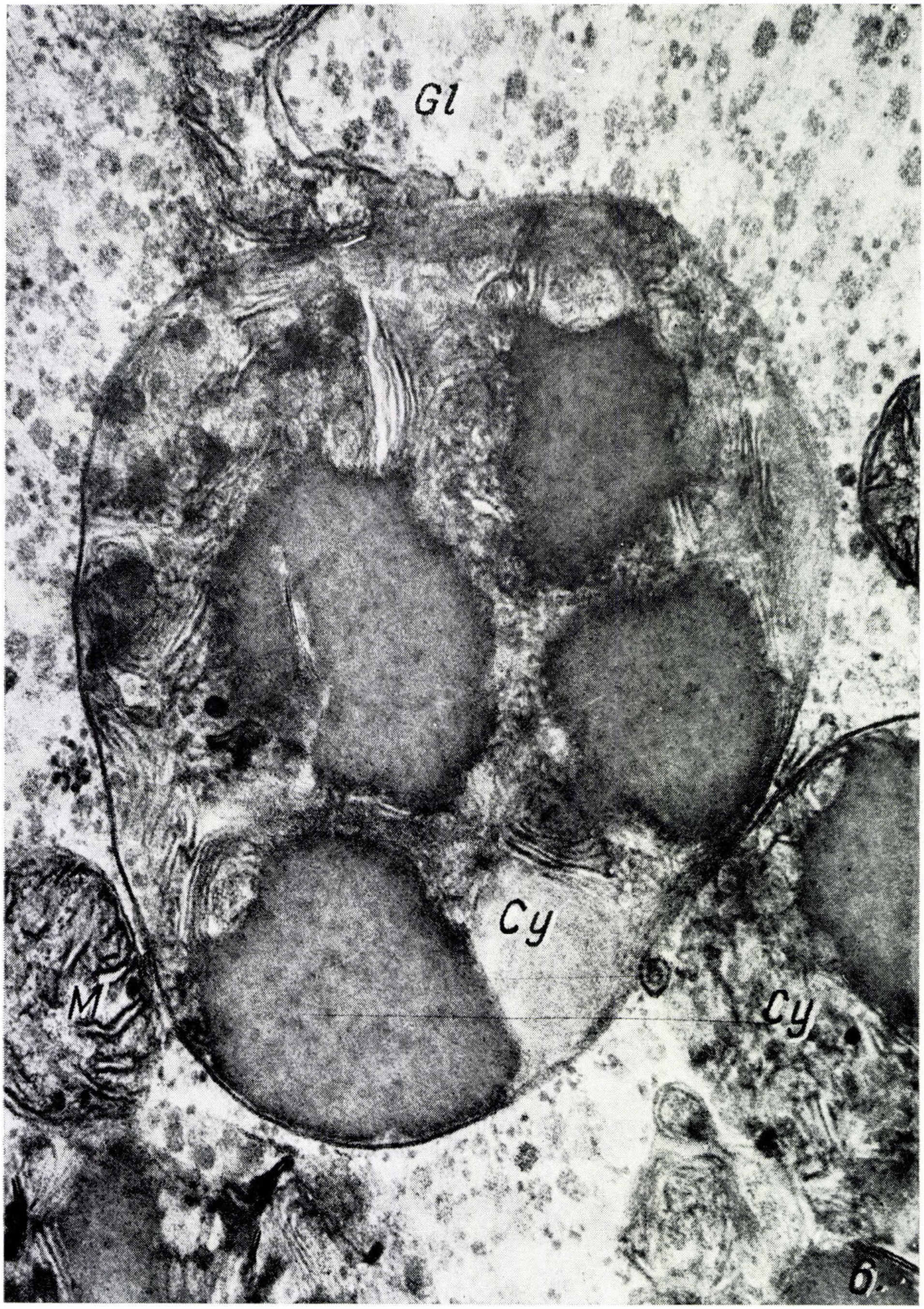

Fig. 6. Detail of a heart muscle cell from the ventricle. Gl — glycogen; M — Mitochondrium; Cy — cytosome. ×58 500

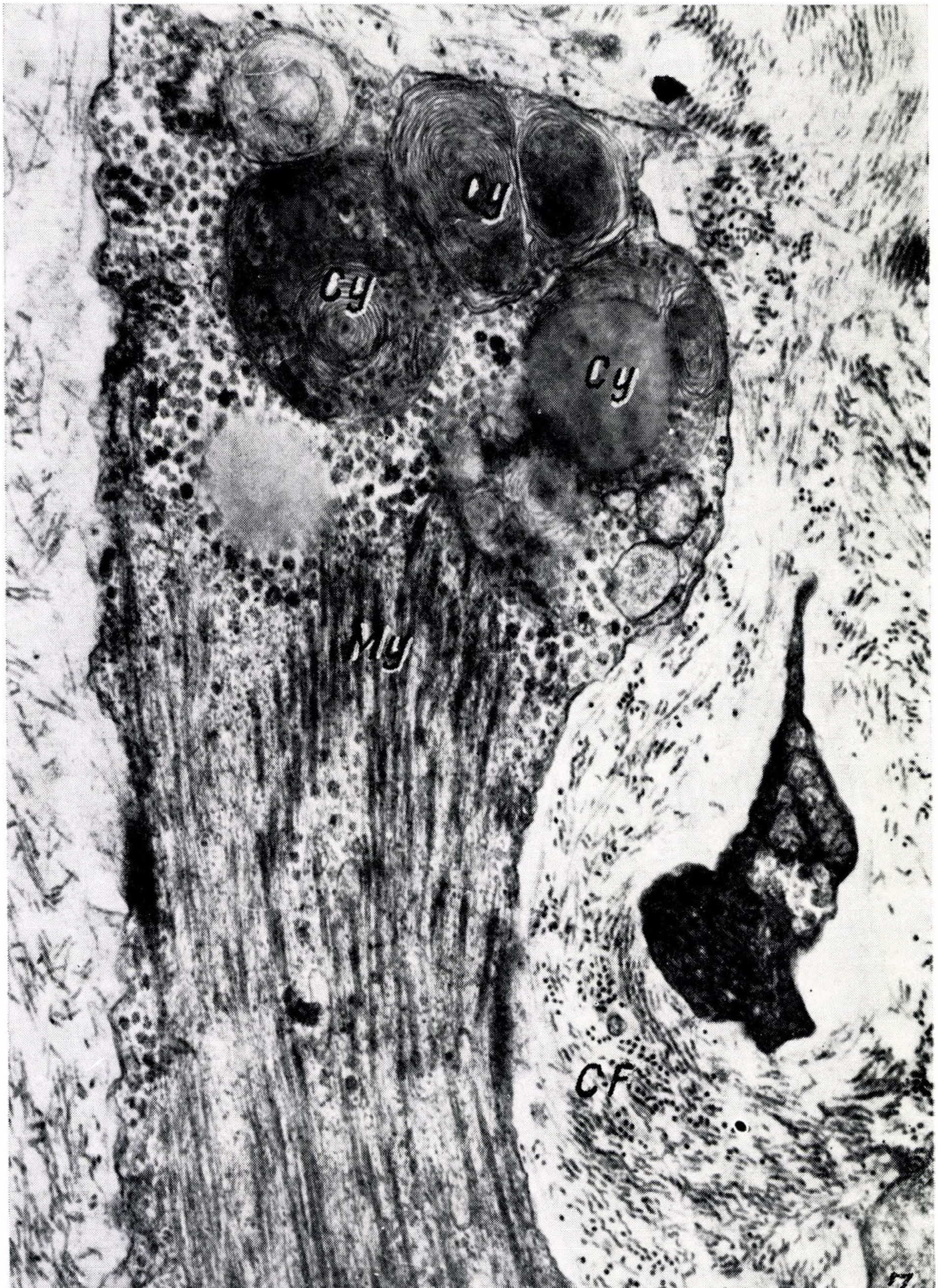

Fig. 7. Detail of the subepithelial layer of the oral palp containing collagen and scattered muscle cells. My — myofilaments in the muscle cell; Cy — cytosomes; Gl — glycogen; CF — collagen fibres. ×30 000

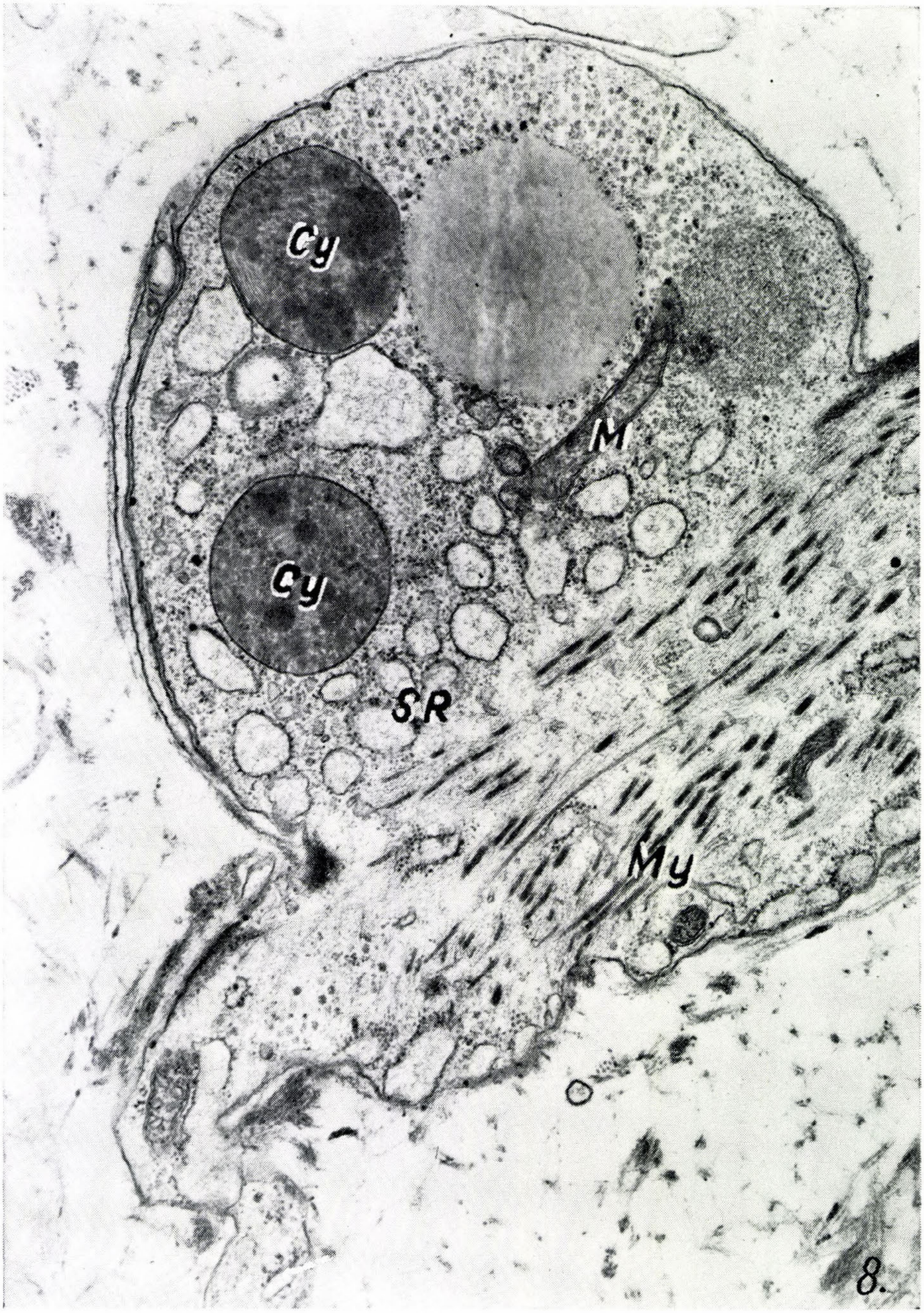

Fig. 8. Detail of a muscle cell from the intestinal wall. My — myofilaments; SR — sarcoplasmic reticulum; Cy — cytosome; M — mitochondrium. $\times 25\,000$

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 cytosomes 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.

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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 több-kevesebb 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.