CRYSTALLINE CORPUSCLES IN THE ULTRASTRUCTURE OF MILTIFORM GLIOBLASTOMA

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The electron-microscopical investigation of the glioblastoma multiforme was introduced with the publication by S. H. Nyström (1959). The works of S. Luse (1960), J. C. Navarro and Gullotta (1961), J. Kaizumi (1962) and A. I. Raimondi, S. Mullan and P. J. Evans (1962) and R. Koynov (1965) were published later. All these reports contributed greatly to the enlightening of the ultrastructure of this malignant cerebral tumour. In the present paper, we wish to present the crystalline corpuscles observed in the ultrastructure of the multiform glioblastoma in one patient; such as have not been hitherto described by the authors cited above.

Clinical Observation

Georgi A. N., male aged 44, admitted for treatment at the Nerve Clinic of the Higher Medical Institute in Varna on September 21, 1964 (history of illness № 11732/539). On July 14 the same year he carried a load and on the very same day he sustained urticaria, cough and severe headache accompanied by vomiting. Treatment was carried out at the Therapeutical Department. He responded favourably to the treatment, but refractory headache persisted and for this reason he was refferred to the Neurological Clinic.

Objective examination (21. IX. 1964): somatic state — without deviation whatever from the normal. The neurological state revealed a slight leftside hemiparesis with involvement of the facial musculature; frequent yawning. Psychically, the patient was drowsy, somnolent, but with proper orientation and adequately answering to questions directed to him. The investigation of the eye-bottoms disclosed papillary stasis (+ 3 d) with multiple hemorrhages in the surroundings and within them.

Operated on September 9. 1964: a tumourous mass was detected with pale tinge and soft fragile consistency at a low point of the right temporal region. The tumour seems clearly outlined in temporobasal direction, but frontally — it is not defined and infiltrates the brain substance.

The histological investigation demonstrates multiform glioblastoma.

Electron Microscopy

Material and methods: The material for electron microscopic investigation was obtained during the surgical intervention and was immediately immersed in 1% buffered with veronal acetate osmic tetroxide, and was

furthermore cut in particles measuring 1 mm³. These particles were fixed in the same fixator at 4°C for a duration of two hours. Following dehydration in serial ethyl alcohol, the material was sealed off in butyl-methyl methacrylate mixture (4 : 1) with 1% benzoyl peroxide added for 48 hours at 56°C.

The ultra-thin sections were prepared on ultramicrotome LKB-4800 and were studied with electron microscope JEM 6-C at the Electron Laboratory of the Institute of Human Morphology in Moscow with head of the laboratory Vl. A. Shahlamov and director of the Institute — A. Avtzin to whom I extend my deepest gratitude. Photographic elaboration was carried out at the Centre of Scientific Documentation of the Higher Medical Institute — Varna.

Findings

In one of the tumour cells microbodies were found exhibiting crystalline structure. This cell contains an oval nucleus with a great quantity, uniformly distributed chromatin. The nucleolus is properly represented, located in the middle of the nucleus. The double nuclear membrane was found to be, in some places, with dilatations of the perinuclear reservoir. The cytoplasm is encircled by a membrane, revealing irregularities and protuberances of small pedicles. Mitochondria were found in limited quantity. Their shape is elliptical and they are slightly turguscent. The cristae are reduced and situated mostly along the periphery around the membranes. Their central parts are usually free of content. The endoplasmatic reticulum is scarcely represented under the form of transversely cut canaliculi, with a limited quantity of ribonucleoprotein granules being disclosed along the outer surface of their membrane. Similar granules, single and in groups are seen freely and amidst the network sustance of the hyaloplasm. Numerous roundish and elliptical corpuscles measuring 130—260 mμ, isolated or in groups, are found sparsed almost throughout the entire cytoplasm (Fig. 1). These corpuscles are encosed in a single (ordinary) membrane without ribonucleoprotein granules. In the middle of the corpuscle an electron-proved dense, reticulated substance is detected. Between the latter and the membrane an electron bright space is observed. The field surrounding these corpuscles is often found free of cytoplasmatic formations. The internal content in about 10—15 of these corpuscles displays a crystalline structure. The crystals exhibit oval, square, rectangular and rhomboid forms. Some of them are enclosed in a simple membrane, while others are devoid of such. They are composed of bright and dark bands with periodicity about 75 Å. The length of these bands is most variable in the individual crystals, ranging from 110—200 mμ. The bright bands are about 1.5 times wider than the dark and therefore the width of the bright bands is about 45 Å, and that of the dark—about 30 Å. The dark bands' structure shows irregularities and at places their granular consistency is clearly discerned. In some of the crystals, the bands are oriented in two directions, thus closing an angle between them. The magnitude of the angles, closed between two similar series of bands proves to be different in individual crystals. In some of them it is 45°; in others — 75° and in a third group — 120°. The number of dark bands in some of the crystals is estimated from 14 to 18 (Fig. 2, 3, 4, 5).

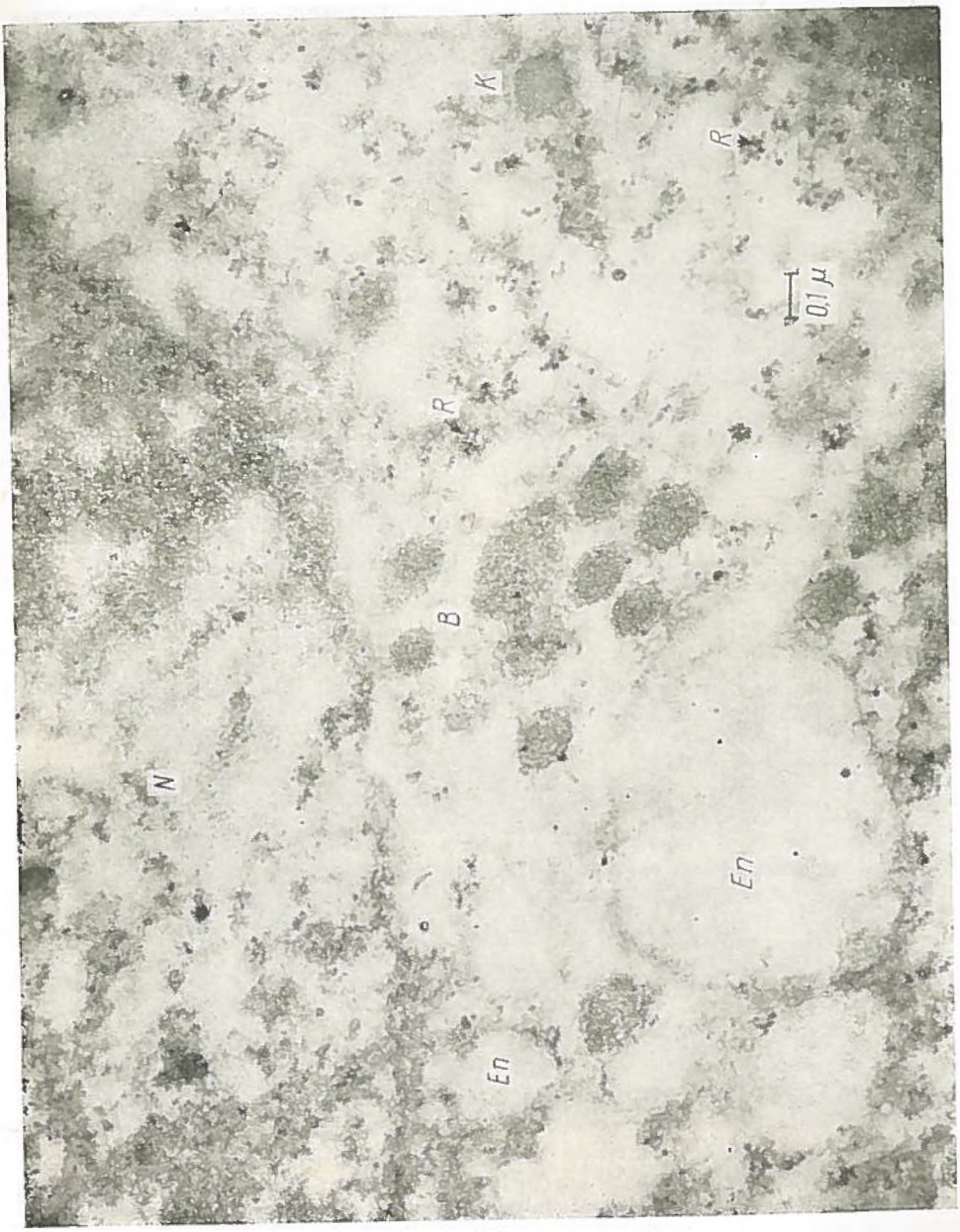

Fig. 1

N — nucleus; *En* — endoplasmatic reticulum; *R* — ribonucleoprotein granules; *K* — crystalline corpuscles. *B* — corpuscles Magnif. 80000×

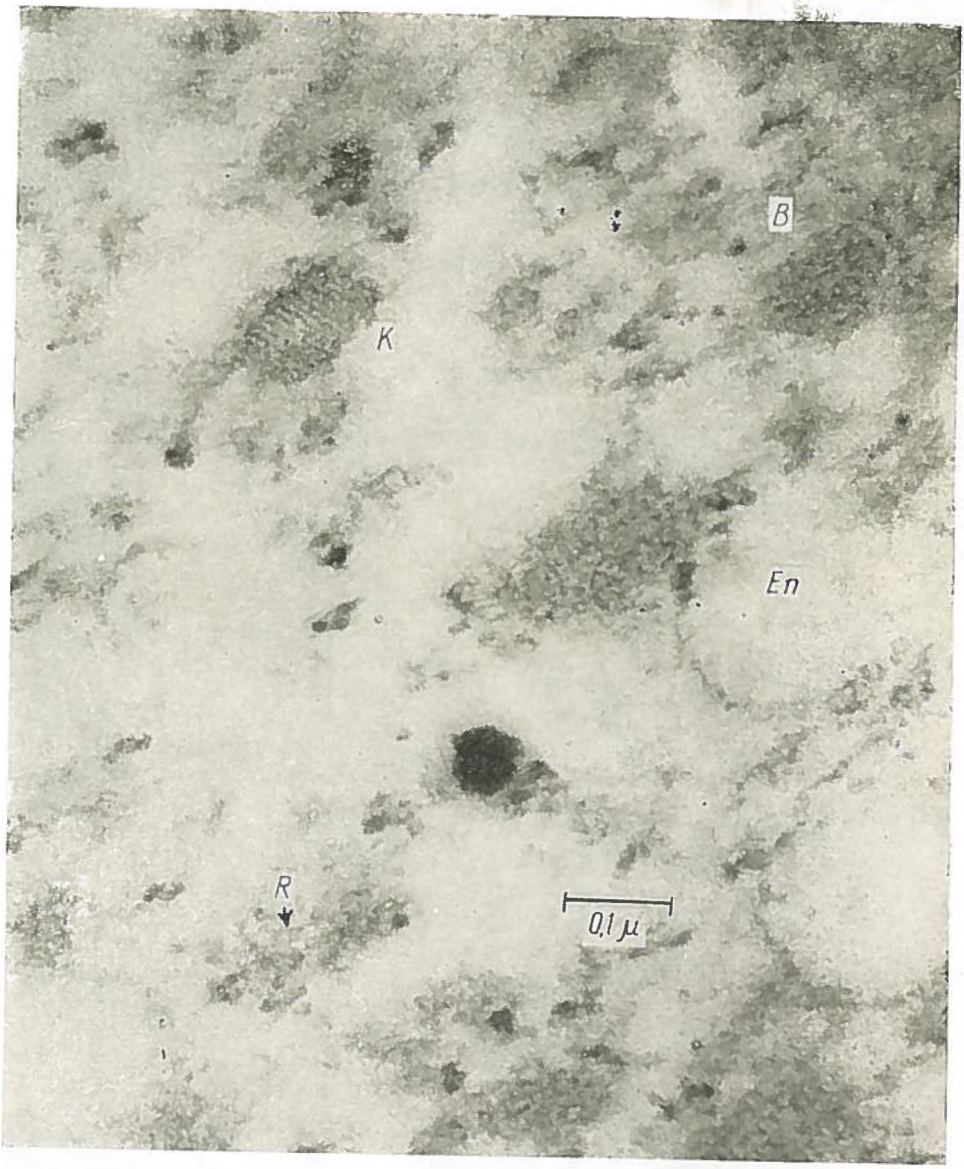

Fig. 2
En — remnants of endoplasmatic reticulum; *R* — ribonucleoprotein granules; *K*—crystalline corpuscles; *B* — corpuscles. Magnif. 200000×

Fig. 3
M — membrane enclosing the crystalline corpuscle; *R* — ribonucleoprotein granules.
Magnif. 180000×

Fig. 4
Crystalline corpuscle with bands intersecting with angle 120°
Magnif. 180000×

Discussion

The crystalline corpuscles are of greatest interest in the electron-microscopical finding herein described, and the discussion in the light of the facts established by the authors is deemed worthwhile.

In 1961/63 N. M. Shestopalova and V. N. Reingold reported on the presence of needle-shaped (spicular, spiked) crystals in the epithelial cells of the intestinal mucosa fo white albino rats, previously contaminated with poliomyelitis virus per os, and in 1963, the same authors in collaboration with V. M. Borisov, described identical crystals in the epithelial cells of the intestinal mucosa of newborn healthy albino rats. However, the crystals found out by the cited authors prove to be larger and rather long — needle-shaped. Their transverse section is reported to be 0.5, 0.2, 0.3 μ and length ranging from 2—5—6μ. Along with that, the alternating periodicity of white and black bands proves to be 55—65Å. Secondly, the needle-shaped crystals are enclosed by a membrane, densely sparsed with ribosome granules (ribonucleoprotein granules). Thirdly, is should be pointed out that the disposition of the crystals in the canals and reservoirs of the endoplasmatic reticulum warrants the assumption that these crystals are a product of the activity of ergastoplasm, membrane and ribosomes, which are known to be responsible for the nucleoprotein synthesis. Thus the authors reach the conclusion that the crystals observed are of nucleoprotein nature.

Fig. 5
Crystalline corpusclex.
Magnif. 290400×

The crystalline formations found by the American writers H. F. Shipney, P. H. Libermann, F. W. Foot, F. W. Stewart (1964) are likewise worth of special attention, besides the findings just described.

The cited authors give a description of the ultrastructure of crystals found in the cytoplasm of the cells of an alveolar sarcoma. First and foremost, it is necessary to point out that big crystals are concerned in the latter case, visible under light microscope also. The shape of these crystals is rhomboid, rod-shaped or pincer-like. In many instances they are seen sur-

rounded, as in our experience, by a simple membrane. Alternation of white and bright bands here too, is recorded with 100Å periodicity, arrangement of the bands in two directions with intersection under approximately 75° angles and granularity of the dark bands' structure. Finally, using biochemical methods of staining, the authors establish that these particular crystals are composed of the complex protein-carbohydrate.

Against the background of the above statements, it is inferred that the crystals herein described represent protein crystalline corpuscles of a new type. They differ from the needle-shaped crystals, found in the intestinal epithelial cell of albino rats, observed by N. M. Shestopalova, V. N. Reingold and V. M. Borisov, as well as from the crystals in the alveolar sarcoma reported by H. S. Shipney and assoc. From the former they are differentiated insofar form, size, internal structure of periodicity and presence of a double direction in the disposition of bright and dark bands are concerned. Besides that, the needle-shaped crystals are recovered from the ergastoplasmatic reticulum, whereas the crystalline corpuscles in the multiform glioblastoma may be found outside the endoplasmatic reticulum. They also differ in size from the alveolar sarcoma crystals. While the crystals of the alveolar sarcoma are so great as to be rendered readily visible under light microscope, the crystals of the multiform glioblastoma display the size of virus corpuscles. In addition, differences are also established in their internal structure. The periodicity of the crystalline corpuscles observed by us is lower, whereas the band intersection angles are several. They bear resemblance insofar in both instances (crystallines in alveolar sarcoma and in multiform glioblastoma) the membranes enclosing the crystalline structures show the nature of a simple membrane. The membranes referred to by Shipney and assoc. resemble, insofar structure is concerned, that of the Golgi apparatus. It should be pointed out however, that an adequately formed Golgi apparatus in these cells was not established. Except for features non characteristic of the Golgi apparatus, both crystalline corpuscles and corpuscles of analogous characteristics without visible crystalline arrangement of the internal content as well are observed, usually located perinucleary, sparsed in various areas of the cytoplasm and in a larger amount as compared to the Golgi apparatus.

What do these crystalline corpuscles represent? If analogy is made with the reported in literature two types of crystals, it might be assumed that they likewise possess a protein structure and even protein-carbohydrate. If logical reasoning is the path chosen, it could be assumed that as protein formations, they ought be produced by the ribonucleoprotein formations. The presence of ribosomes along the membranes of the needle-shaped crystals in the intestinal mucosa of the rats explains the origin of the same. The absence of ribonucleoprotein granules along the membrane of the crystals protographed by Shipney and assoc. and in those of the multiform glioblastoma as well, commits us to search their origin in a different site. If assumed that their membrane bears the characteristic features of the Golgi apparatus, it could easily be recongized that the Golgi apparatus here too accounts for condensing the protein content, similarly to the production of hormones, occurring in glandular cells. Under similar conditions of protein production, however, it should be anticipated that the ribonucleoprotein granules, situated along the membranes and those freely found in the hyaloplasm will be in abundant quantities. The latter picture was not observed

in the cell studied by us, the most striking feature being that the spaces, adjacent to these corpuscles contained still lesser amount or virtually none ribonucleoprotein granules. We were further impressed by the fact that the structure of the cytoplasmatic content was generally found to be altered not merely as regards insufficient representation of endoplasmatic reticulum, but also insofar degenerative turgid mitochondria and absence of structure whatsoever around them were concerned. Along with that, the structure, of crystalline corpuscles appeared to be comparatively more preserved. Thus, it was inferred that the formations described represent foreign bodies, developing at their own expence and not conformed to the needs of the cells. The fact that they were discovered in a single cell merely, whereas in the neighbouring cells they were absent is a further support of the latter suggestion. It was inferred therefore, that these corpuscles bear a viral character. Their size is likewise in accordance with the size of already known and accordingly photographed vira. If a comparative study is undertaken of the particles observed with the known vira, it should be stressed that they are comparable merely with so-called myxovira. The plague virus among birds, grippe, parotitis, Newcastle and Senday disease vira pertain to the myxovira. The vira listed consist of centrally situated ribonucleoprotein, surrounded by lipoprotein covering. Their size ranges from 70 to 500 mμ. The molecular organization of the nucleoprotein in the same shows a spiral characteristics (B. K. Weinstein and A. Kiselev). On the ground of the facts described, it could be assumed that the dark bands in the structure of the multiform glioblastoma crystalline corpuscles represent the spiral of the molecular organization of nucleoproteins in the virus corpuscle, whereas the covering of the crystals reveals lipoprotein character. A further indication for a similar structure is the stratification and lowering of some of the terminal bands in one of the crystalline figures, demonstrating their layer structure.

Naturally, all suggestions hitherto made do not go beyond the limits of hypothesis. Further histo- and cytochemical, X-ray and electron-structure investigations as well as virusological studies should be carried out in order to be able to corroborate or reject the suppossitions referred to.

REFERENCES

1. Luse, S. Electron microscopic studies of brain. — *Neurology*, 1960, 10, 881—905.
2. Navarro, J., F. Gullotta. Contribution of electron microscopy to the knowledge of glioblastoma. — *Arch. Ital. Anat. Istol. Path.*, 1961, 35, 365—378.
3. Nyström, S. H. Electron microscopical structure of the wall of small blood vessels in human multiform glioblastoma. — *Nature*, 1959, 184/4679, 65.
4. Raimondi, A. J., S. Mullan and J. P. Evans. — Human brain tumors and electron microscopic study. — *J. Neurosurg.*, 1962, 19, 731—753.
5. Shipkey, H. F., P. H. Liberman, F. W. Foote and F. W. Stewart. Ultrastructure of alveolar soft part sarcoma. — *Cancer*, 1964, v. 7, No. 7, p. 821—830.
6. Вайнштейн, Б. К. и Н. А. Киселев. Строение вирусов. Сб. Вирусология и имунология. Издат. „Наука“, Москва, 1964, с. 8.
7. Койнов, Р. Електронномикроскопско проучване на мултиформения глиобластом (под печат), 1965.
8. Шестопалова, Н. М., В. Н. Рейнгольд и В. М. Борисов. Субмикроскопическая структура игольчатых кристаллов эпителиальных клеток слизистой кишечника и их положение в системе эндоплазметического ретикулума. Доклады Академии наук СССР, 1963, т. 153, 2, 454.

КРИСТАЛЛИЧЕСКИЕ ТЕЛЬЦА В УЛЬТРАСТРУКТУРЕ МУЛЬТИФОРМЕННОЙ ГЛИОБЛАСТОМЫ

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РЕЗЮМЕ

Автор сообщает об одном собственном наблюдении над ультраструктурой мультиформенной глиобластомы, где устанавливает наличие кристаллических тел. Величина последних от 130 до 260 mμ. Они состоят из темных и светлых полосок с периодичностью приблизительно в 75Å, обвитые простыми мембранами. Исходя из структуры кристаллов и литературных данных, автор принимает, что они белкового происхождения. Рассуждая над их сущностью, он допускает, что они являются продуктом клетки или имеют вирусное происхождение.