

ELECTRON MICROSCOPIC INVESTIGATION OF GLIOBLASTOMA MILTIFORME

R. Koynov

The history of electron microscopical investigations initiates with the report made by Nyström, S. H. (3). Next followed the publications by S. Luse (1), Navaro, J. C. and Gullotta (2), Raimondi, A. J., Mullan, S. and Evans, J. P. (4).

All studies referred to have thrown some light upon the ultrastructure of this particular type of tumour, but, it must be added at once that as yet, a comprehensive description and elucidation of its fine structure has not been achieved and therefore going into details in this respect would be useful.

The present report represents a clinical, histological and electron microscopical investigation of two multiform glioblastomas.

Clinical Observations

Case report — Jeliu, M. M., aged 63, accepted for treatment on March 3, 1964; history of illness № 501/142 — 64 — Neurosurgical Clinic — ISUL (Postgraduate Medical Training Institute).

Past history: since two months he experiences a strong thirst and urinates very often. Complains of persistent dryness in the mouth. Fifteen days ago sustained a headache attack with pains strongly pronounced in the sinciput. The fits were of various duration and were favourably influenced by analgetics. Lately the thirst disappeared.

Somatic state: RR — up to 170/120.

Neurological state: leftside spastic hemiparesis with involvement of the facial and sublingual nerves.

Psychical state: apathy-abulia syndrome, lack of initiative, very reluctant in fulfilling orders received, delays answers, emotionally pale.

Investigations: fundus oculi — rightside papillary stasis — 2 d
leftside papillary stasis — 1 d

Operated upon March 3, 1964 — tumour of the right fronto-parietal region.

Case report — Rumen T. H., aged 3 years. Accepted on April 4, 1964 at the Neurosurgical Clinic — ISUL; history of illness № 3401/274.

Past history: On December 24, 1963 the child was accepted with evidence for space occupying process (headache, epileptic fits, rightside hemiparesis, stasis in eyebottoms — 4—5 d) on account of which he underwent operation on December 31, 1964. A large cystic tumour was found which was partially extirpated. On February 7, 1964 the papillary stasis were reduced to + 1 d. Since March 25, 1964 the headache complaints appearing every 1—2 days

were renewed. The headache was intensified furthermore in the past 5—6 days. The child was completely relaxed, without appetite and somnolent.

Somatic state: Head with enlarged sizes, not uniformly haired. Operative scar is disclosed in the left temporo-parietal region. Pulse — 100/min. From the neurological state: Muscle tone weakened bilaterally, slight rigidity of cervical musculature. Fundi oculorum — slightly edematous. Histological diagnosis of biopsy material obtained during the second operation: glioblastoma multiforme.

Electron Microscopy

Material and method: The material was obtained during surgical intervention and was immersed in 1% buffered with veronal acetate osmic tetroxide and cut into small particles, each measuring 1 mm³. The latter were fixed in the same fixator at 4°C for a duration of two hours. Following dehydration in serial ethyl alcohol, the material was embedded in butyl-methyl methacrylate mixture in a proportion 4 : 1 with 1% benzoyl peroxide added for 48 hours at 56°. The ultra-thin sections in one of the tumours were cut on ultramicrotome LKB and studied on electron microscope JEM 5 Y at the Electron Laboratory-Institute of Poliomyelitis and Viral Encephalitis in Moscow under the direction of N. M. Shestopalova with the valuable assistance of V. N. Reingold; the second tumour was treated with the microtome of Sitte (Reichert) and microscope Tesla B. S. 242 A at the Institute of Morphology — Bulgarian Academy of Sciences — Sofia, directed by academician A. Hadjiolov, with the valuable assistance of A. Mihaylova-Boyadjieva. I wish to express my deepest thanks to all members of staffs listed above for their assistance.

Findings

The cells of the multiform glioblastoma exhibit most variable forms and sizes. Zones are encountered of irregularly oval cells and other — with multiangular, elongated in a peculiar fashion cells etc. Occasionally, the cell seems made up of a nucleus and a narrow band of cytoplasm, in other places the cytoplasm is localized at one end of the cell under the form of shapeless bulging, and thirdly, long cytoplasmic bodies are detected running throughout the entire electronogram. On the whole, the idea of „multiforme“ fully justifies its meaning as a denomination of the cellular pattern of this type of tumours. It must be pointed out however, that between the individual cells there are invariably two cell membranes with intercellular space measuring 160—180°. In between separate cells often spaces are established in which structure is non-existent and yet, the amorphous rete-form matter is disclosed with sparsely cellular fragments — most likely micronecrotic areas.

The nuclei exhibit most varying forms and sizes. Oval, irregularly rounded, spindle-shaped, inflated in peculiar manner, twisted and elongated and occasionally with the appearance of sand-glass tumour. Folds in the nuclear surface are a common finding. Often from the nucleus, outgrowths

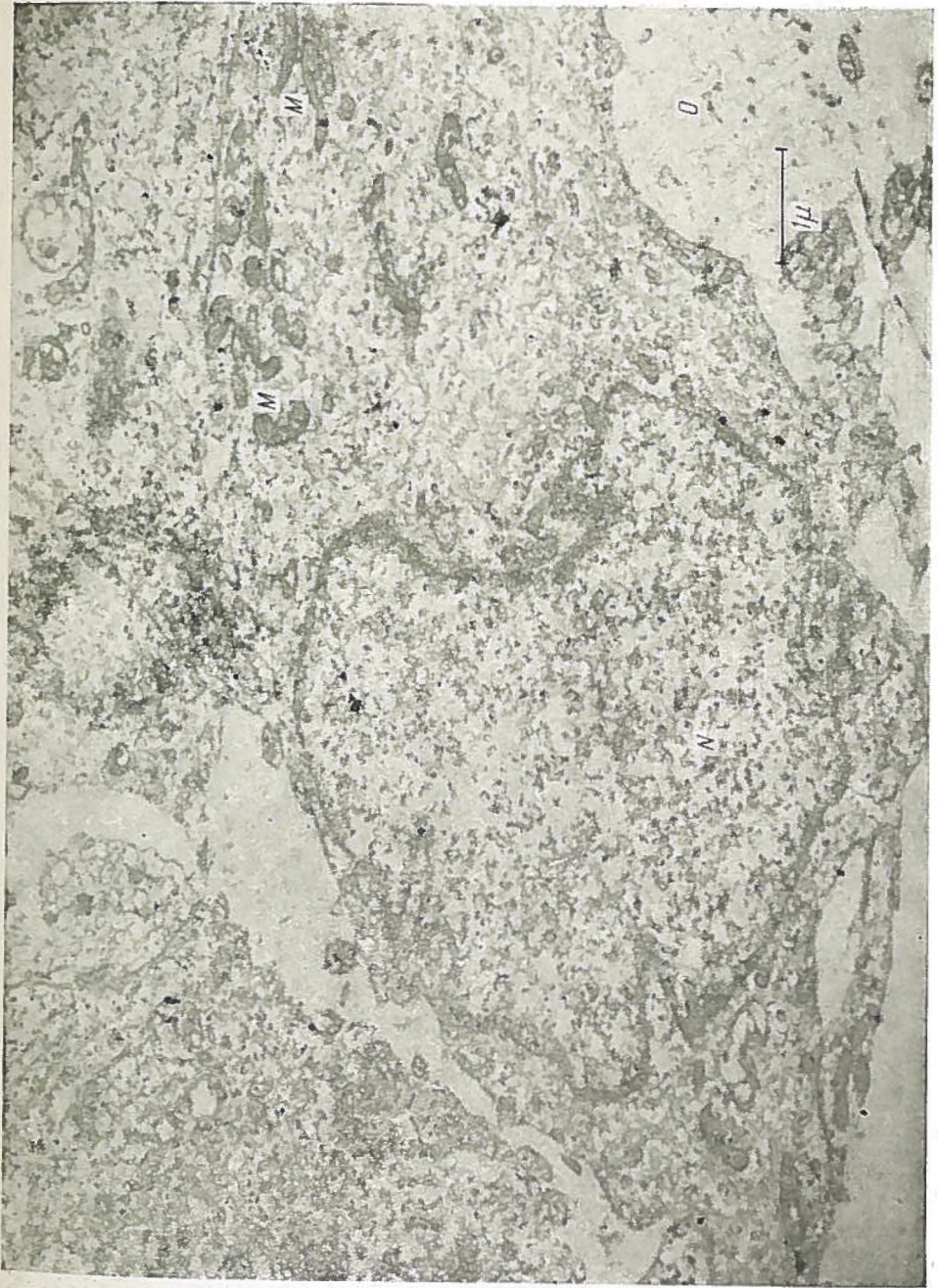


Fig. 1

N — nucleus; *n* — small nucleus; *M* — mitochondria; *O* — necrotic foci. Magnif. 19380 x

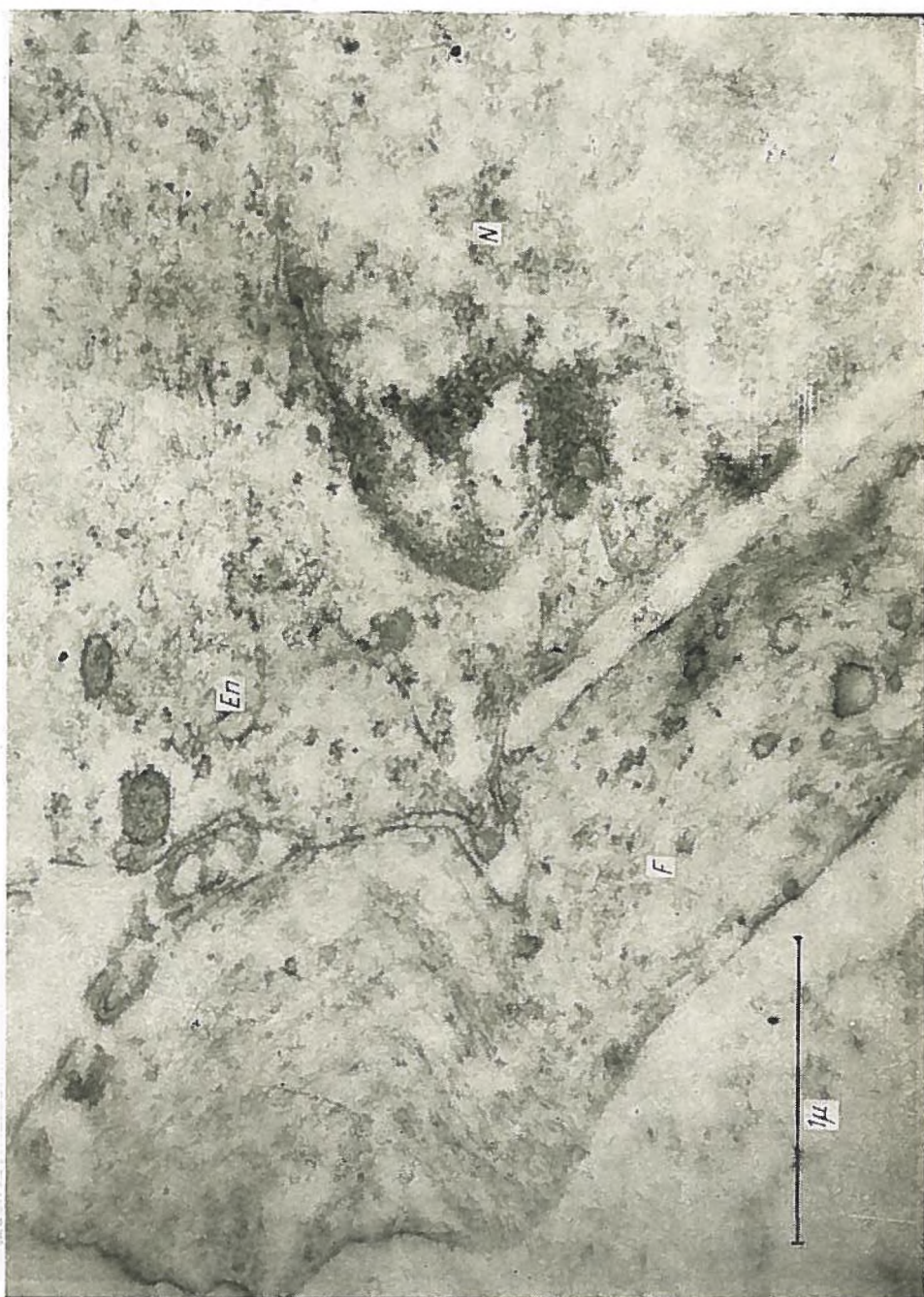


Fig. 2

N — nucleus with peculiar folds; *M* — dense small mitochondria; *En* — fragments of endoplasmic reticulum; *F* — outgrowth with glyofibrils. Magnif. 67510x

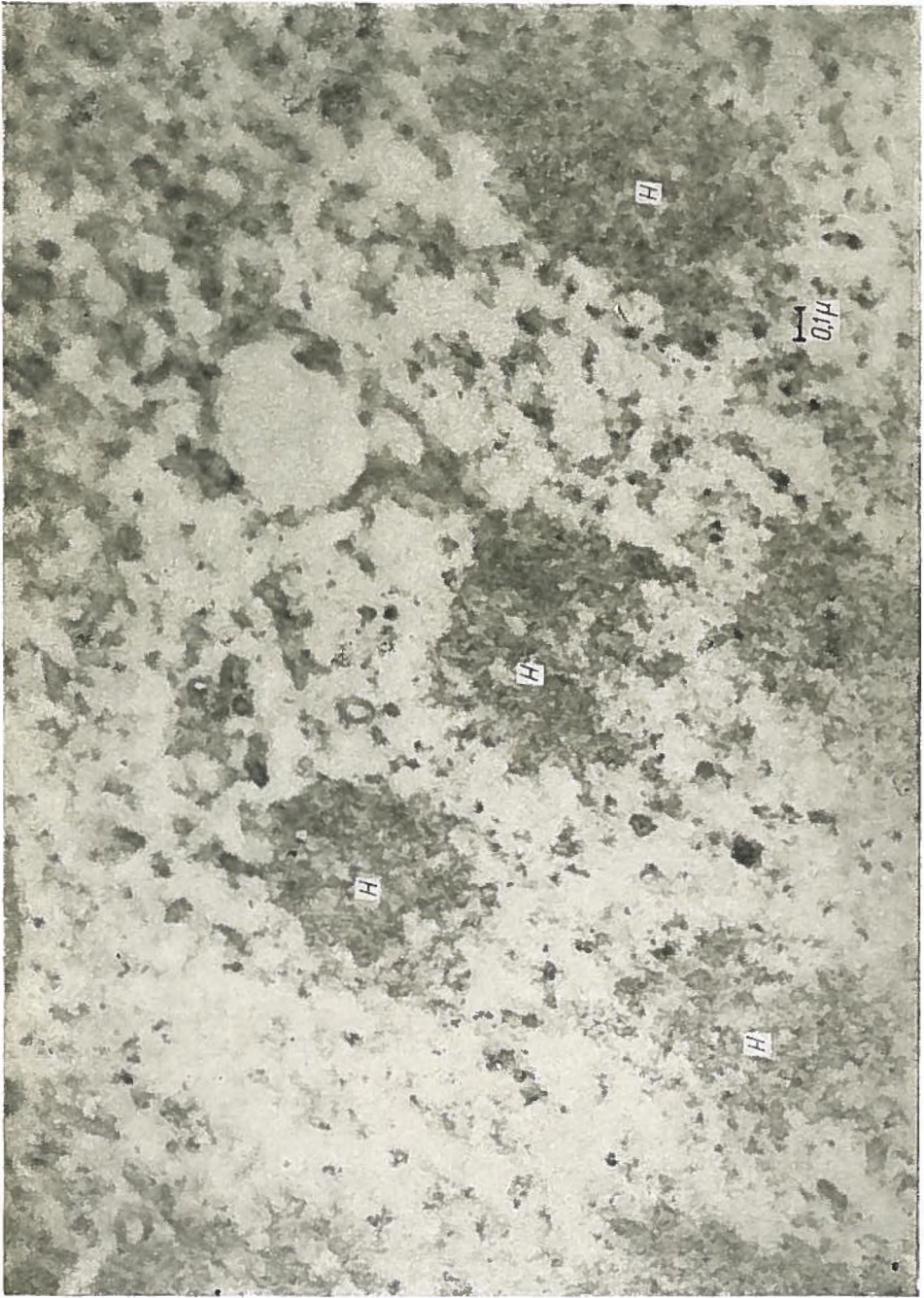


Fig. 3
Chromatin stains in the cytoplasm-chromosomes. Magnif. 67510x

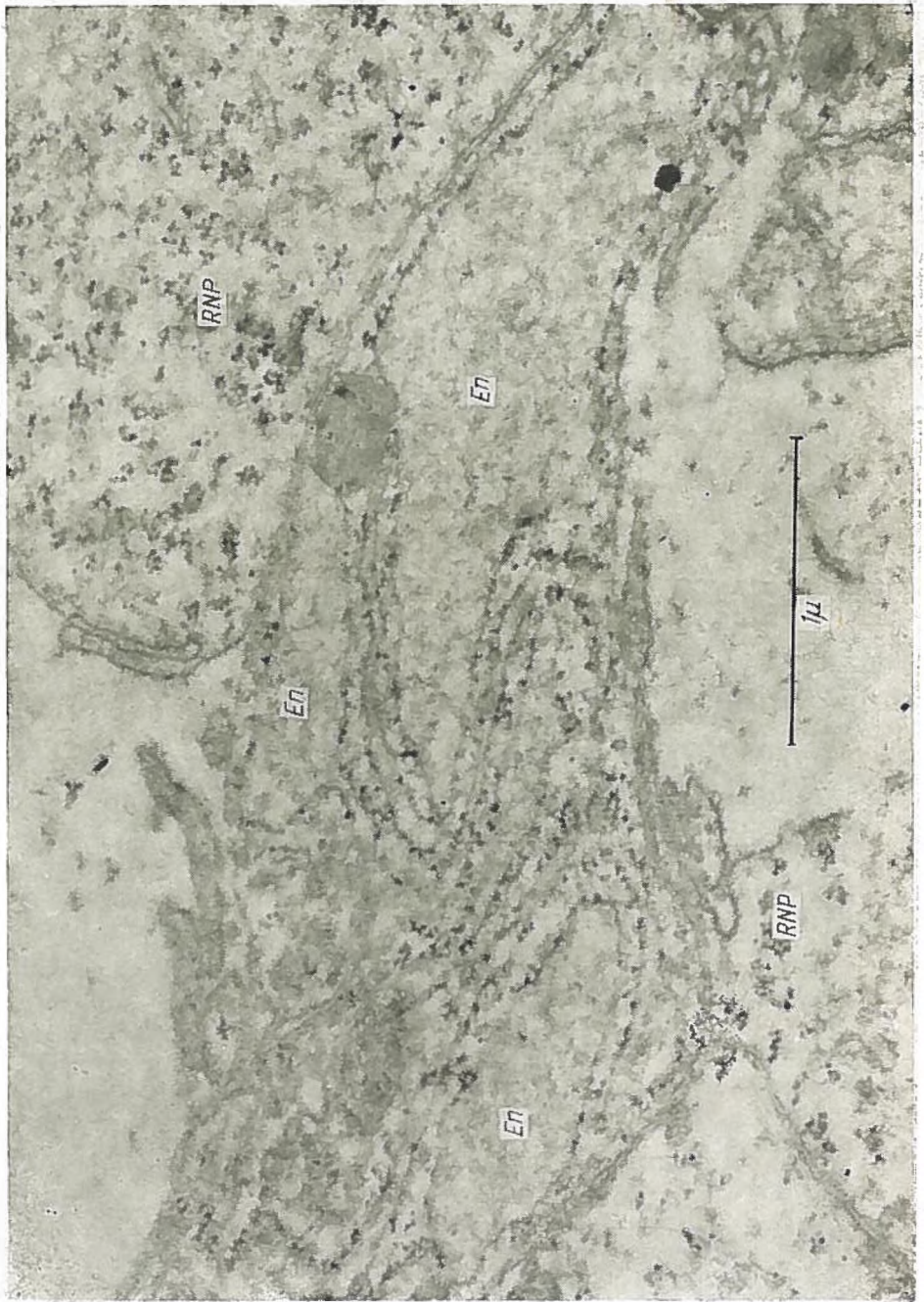


Fig. 4

En — endoplasmic reticulum with dilatations filled up with rete-like substance; *RNP* — ribonucleoprotein granules, freely lying. Magnif. 67510x

stick out of most variable shape or depressions are observed filled up of cytoplasmatic content. The size of several nuclei measured displays 3—4—6—8—10 μ of the long diameter.

Usually, the nuclear membrane is double with clearly outlined space of the perinuclear reservoir between them. The perinuclear reservoir width is about 140Å. In the latter often extensions are encountered reaching 420—440Å. Areas are furthermore noted where merely a single-layer nuclear membrane is present, with the cytoplasmatic membrane missing most frequently. Areas are likewise disclosed of cells where the nucleus is represented by chromatin accumulated in 6—8 separate spots of irregular angulated, elliptical shape, without vaginae and freely lying in the cytoplasm, measuring 1.2 to 0.75 μ .

The chromatin is sparsed throughout the entire nucleus, but often nuclei are observed with chromatin deposited mainly along the periphery. It displays finely granular and fibrous character. Along with fine grains, 2—5-fold bigger grains are also observed.

In a great number of cells a well shaped, usually single, small nucleus is noted. It has irregular oval or longitudinal form, occasionally squeezed in the middle. In some of the cells the small nucleus is made up of varying in form and size few patches connected in between or located closely to each other.

The cytoplasm contains a fibrous matrix with sparsed therein single (isolated) or groups of ribonucleoprotein granules — polyribosomes.

Mitochondria are established in varying quantities, scattered in the cell cytoplasm and its outgrowths. Their shape is ellipsoid or oval depending on the section. In some of the latter a three-layer vagina is disclosed and the septae with transverse to their longitudinal axis disposition. In some of them vacuoles are detected with dense filling up between the septae. The longitudinal dimension of some of the measured mitochondria is 0.3, 0.6 and 1.0, and the transverse — 0.3—0.5 μ . Darkly tinted mitochondria are also encountered without being possible to distinguish the septa. The fact is worth mentioning that around the sites where mitochondria are localized, dense mitochondria in particular, the nucleus exhibits pronounced depressions and outgrowths.

The endoplasmatic reticulum in some areas is adequately represented by its membrane, outlining the channels and tank-shaped dilatations filled up of netlike fibrous substance.

The Golgi apparatus in some of the cells is hardly distinguishable, under the form of chanrel-shaped vesicles, and in most of the cells it is absent.

Moreover, in the cytoplasm electron proved consistent corpuscles are noted with dimersions 370/220 μ to 450/300 μ . It is difficult to determine the nature of these corpuscles. It is probable that they represent lysosomes.

Corpuscles, the size of mitochondria are also present in the cytoplasm in which spherical, electron proved empty formations or formations with a dense mass in the middle are observed. The nature of these formations is likewise difficult for ascertaining. It might be assumed that mitochondria are concerned with peculiar arrangement of the cristae.

Peculiar fibrous formations are further found in the cytoplasm of the cells greatly resembling a horse's tail. In other instances, glial fibers fill up the entire outgrowth.

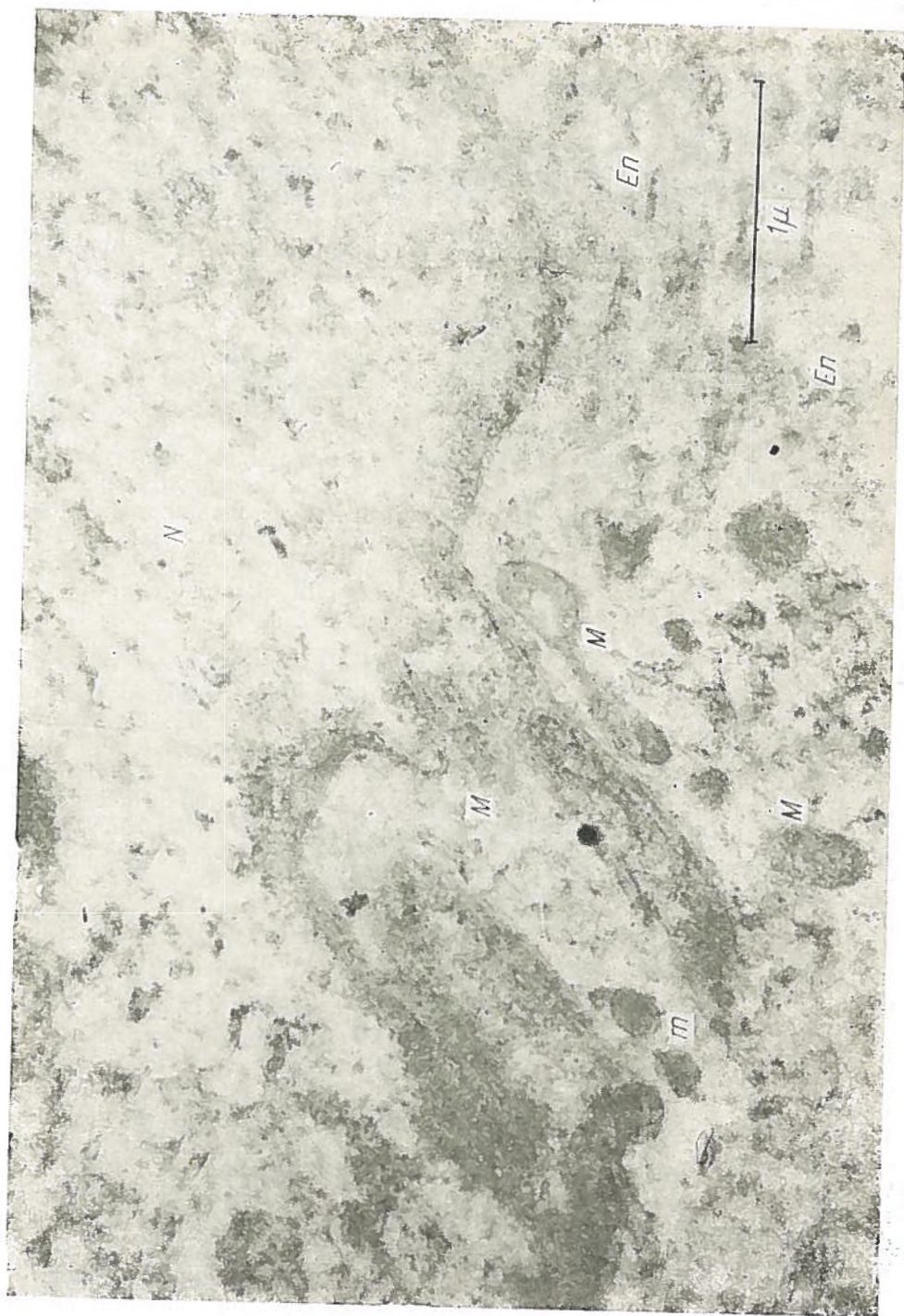


Fig. 5

N — nucleus with odd outgrowths surrounding dense mitochondria. *M* — mitochondria.
Magnif. 54950

Adjacent to some of the cells different patterns of transversely cut myelin fibers are observed of varying form and diameter.

Discussion

The electron microscopic finding in glioblastoma multiforme herein described demonstrates the presence of two opposite to each other processes: one is a process of rapid enlargement of cytoplasmatic and in particular nuclear content and simultaneous multiplication, and a second process of desintegration of individual cytoplasmatic organelles, cytoplasm and nuclei.

The process of quick nuclear growth is proved by the finding of varying in size nuclei. Some nuclei are several times bigger than others, assuming the appearance of giant nuclei. On their behalf, many of the nuclei exhibit substantial inequalities, protuberances, depressions, outgrowth of rather unique shape, revealing the presence of high dynamism in the processes of metabolism between the nucleus and the cytoplasm. At some places the quick growth of the nucleus runs against mitochondria and not being able of removing them, it encloses them with bizarre curvings and protuberances. Some of the nuclei seem empty or with insufficient quantity chromatin, a fact which might readily be ascribed to their quick growth. The nucleolus is similarly substantially enlarged. In addition, 7—8 individual chromatin spots — chromosomes might be disclosed in the cytoplasm, situated one next to the other, indicating the presence of dividing forms.

The presence of a great quantity of ribonucleoprotein granules in the cytoplasm is worth mentioning, along the membrane as well as those found freely lying in the matrix of the cytoplasm. Naturally, the latter finding should be related to the intense production of proteins in these particular cells.

Parallel to the process of growth of the cellular content, phenomena are also observed of degeneration. The nuclear membranes are moved away from each other, dilatations are formed between them and one vagina (or both) disappear. The disappearance of the nuclear membranes, besides as a process of degeneration; might be also considered as a process of cellular division.

A reduced number and vacuolization of mitochondria is established in the cytoplasm, and on the other hand, presence of small and dark young forms — promitochondria. Fragments of the membranes are encountered with ribonucleoprotein granules, vacuoles and bare fields, accordingly determined as micronecrotic areas.

We would like to lay emphasis on a characteristic feature of the tumours discussed which has not been considered by the writers in previous descriptions of their fine structure. The problem is concerned of the unusual structure of the endoplasmatic reticulum. At certain places of the cytoplasm, vast fields are seen surrounded by a membrane on the outer aspect of which ribonucleoprotein granules are arranged. The composition of these fields represents rather consistent, net-like substance, composed of fine filaments with most variable orientation and small grains, 3—4 — fold smaller than the grains located along the membrane. Similar expansions and content of endoplasmatic reticulum was neither observed in other electron microscopic

pictures (mainly cerebral tumours), nor reports were found in the literature reviewed. Together with the description of this finding, the question is also posed for its significance. Bearing in mind that the endoplasmatic reticulum presents a system of minute channels, by way of which substances are received and eliminated, the fact of being established in an unusually great amount and consistency could be assumed as a manifestation of enhanced metabolism.

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ЭЛЕКТРОННО-МИКРОСКОПИЧЕСКОЕ ИЗУЧЕНИЕ МУЛЬТИФОРМЕННОЙ ГЛИОБЛАСТОМЫ

Р. Коунов

РЕЗЮМЕ

Автор описывает клиническую картину, обычную и электронно-микроскопическую находку в двух случаях мультиформенной глиобластомы. Электронно-микроскопически отмечается находка, указывающая на наличие двух процессов: усиленного роста и размножения и процесса распада. Усиленный рост проявляется в увеличении ядра и ядрышка, множестве перегибов, выемок и выпячиваний ядра и большом количестве рибонуклеиновых гранул. Процесс распада выявляется в дегенеративном изменении и небольшом количестве митохондрий, наличии фрагментов мембраны с рибонуклеопротеидными гранулами и полей запустения.

Наряду с этим автор обращает внимание на наличие обширных полей с плотной сеткообразной субстанцией, огороженных мембранами эндоплазматического ретикулума. В отношении ее допускаются две возможности: или она представляет собой необычное поступление продуктов обмена, или, что это вироплазма.