

Observations on Micronuclei Ultrastructure within Broad Bean (*Vicia faba*) Meristem after γ Ray Radiation

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In spite of the fact that the production of micronuclei by ionizing radiations is well known (Evans, Nearby and Williamson 1959, Heddle and Carrano 1977) there are few data (Koehler 1962, Hugon and Borgers 1966, Hugon, Maisin et Borgers 1966) about cell ultrastructure damages caused by irradiation. The lack of data concerning especially the micronuclei probably comes from several technical difficulties. The micronuclei are smaller than the nucleus and their number is also very small, usually 1-2 (or 3) within a single cell at the broad bean. On the other hand their frequency is low, about 3-10% according to the applied doses of ionizing radiation (Spârchez, Soran and Uray 1979). For this reason their sectioning is quite accidental. At sufficiently high doses (200-300 rad) many cells develop micronuclei, as light microscopy reveal, but on most ultrathin sections no micronuclei can be seen. For this reason it is very difficult to find a correlation between applied doses and the micronuclei ultrastructure at electron microscopical level. We must be content with the chance given by a lot of grids careful examination.

Materials and methods

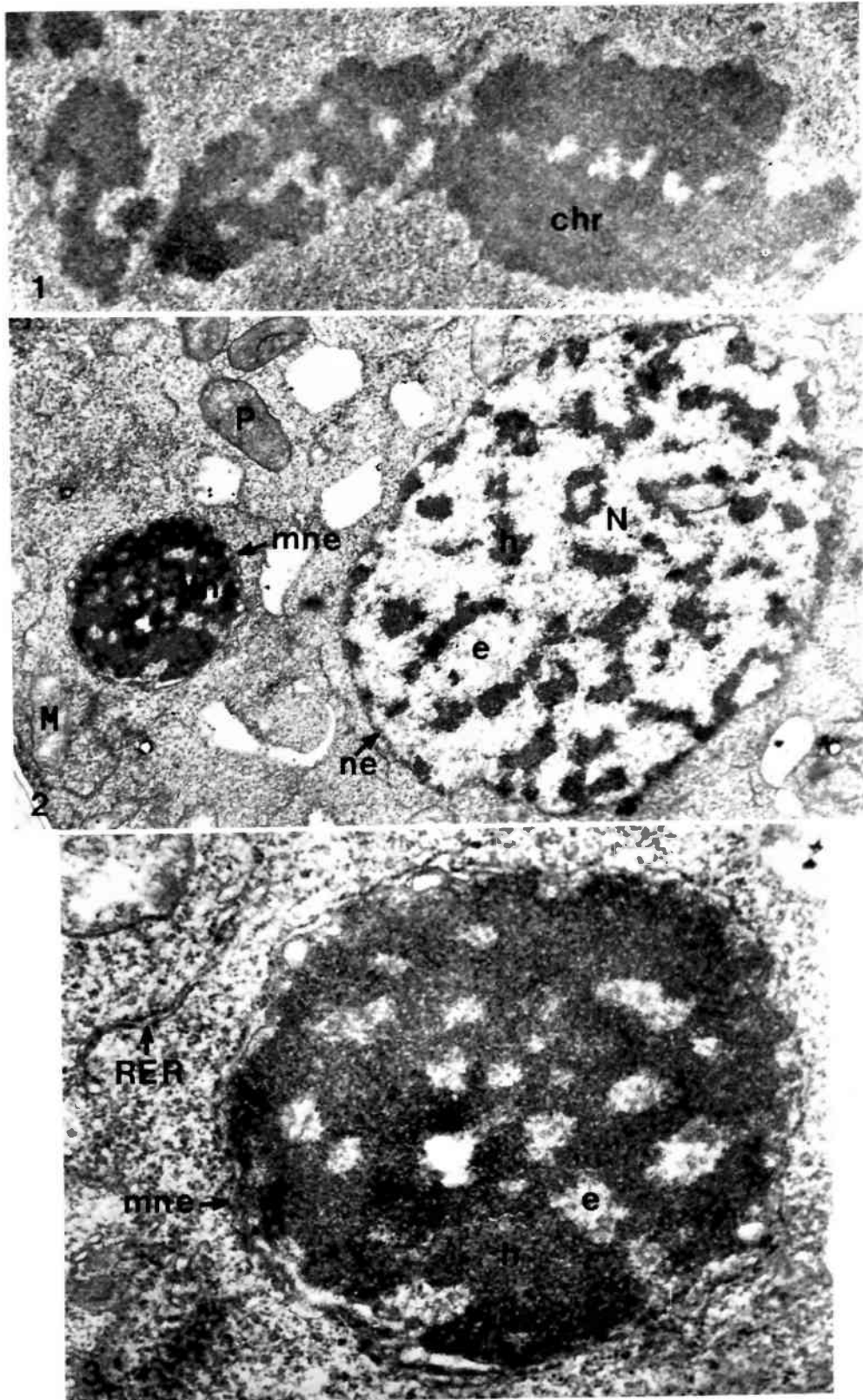
The broad bean (*Vicia faba*) seeds have been soaked with tap water for 24 hours. The germination took place on filter paper which was daily moisted with tap water in Linhardt's dishes at room temperature ($22 \pm 2^\circ\text{C}$). When the seedlings got four days old their primary roots were submitted to various doses (50, 100, 200, 300 and 500 rad) of γ ray. For control we used unirradiated roots.

24 hours after irradiation the top of primary roots (0-2 mm) was fixed for 1 hour in 2% glutaraldehyde and postfixed for 1 hour in 1% OsO_4 . The dehydration of the specimens was made with increasing solutions of acetone and the embedding in W vestopal was performed (Juniper, Cox, Gilchrist and Williams 1970).

The section were made by using LKB III ultramicrotome and then they were double stained with uranyl acetate and lead citrate. The observations were made with Tesla electron microscope (BS-613).

Results and discussions

The elctron microscopy of meristemic cells of broad bean (*Vicia faba*) primary root disclosed after irradiation the existence of two kinds of micronuclei; hetero-



Figs. 1-3. 1, normal chromosome during cell division. chr - chromosome. $\times 20,500$. 2, normal cell nucleus and the heterochromatinic micronucleus. N nucleus, h - heterochromatin, e - euchromatin, ne nuclear envelope, Mn heterochromatinic micronucleus, mne micronuclear envelope, M mitochondrion, P plastid. $\times 9,000$. 3, the ultrastructure of the heterochromatinic micronucleus (the same is in Fig. 2). mne - micronuclear envelope, h - heterochromatin, e - euchromatin, RER - Rough endoplasmic reticulum. $\times 34,000$.

chromatinic and euchromatinic micronuclei and the presence of nuclear fragments which must not be considered micronuclei.

In Fig. 1 the ultrastructure of a chromosome from the broad bean (*Vicia faba*) root meristem can be seen during the process of cell division. We have chosen the picture in order to compare it with that of micronuclei ultrastructure.

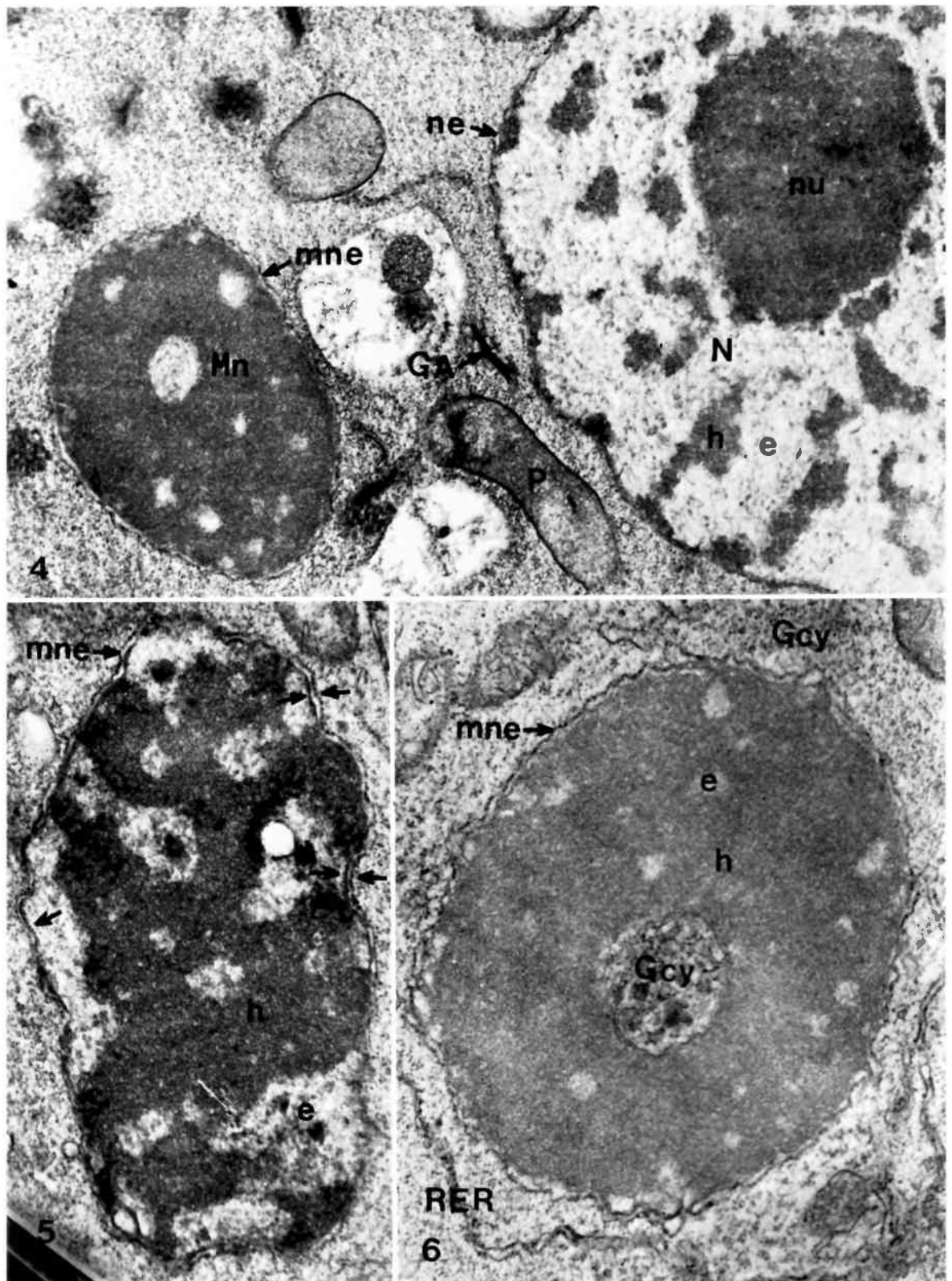
In Fig. 2 and Fig. 4 the cell nucleus and a micronucleus were sectioned together during the interphase period. It can be clearly seen that the nucleus has a normal ultrastructure (Frey-Wyssling and Mühlethaler 1965, Chentsov and Polyakov 1974) with nucleolus, heterochromatin, euchromatin and bilayer envelope.

Unlike the nucleus the micronucleus shows the highest density of heterochromatin very similar with that of the chromosome. We considered this kind of micronucleus as a heterochromatinic micronucleus. Inside of heterochromatinic micronucleus very small amount of euchromatin may be found.

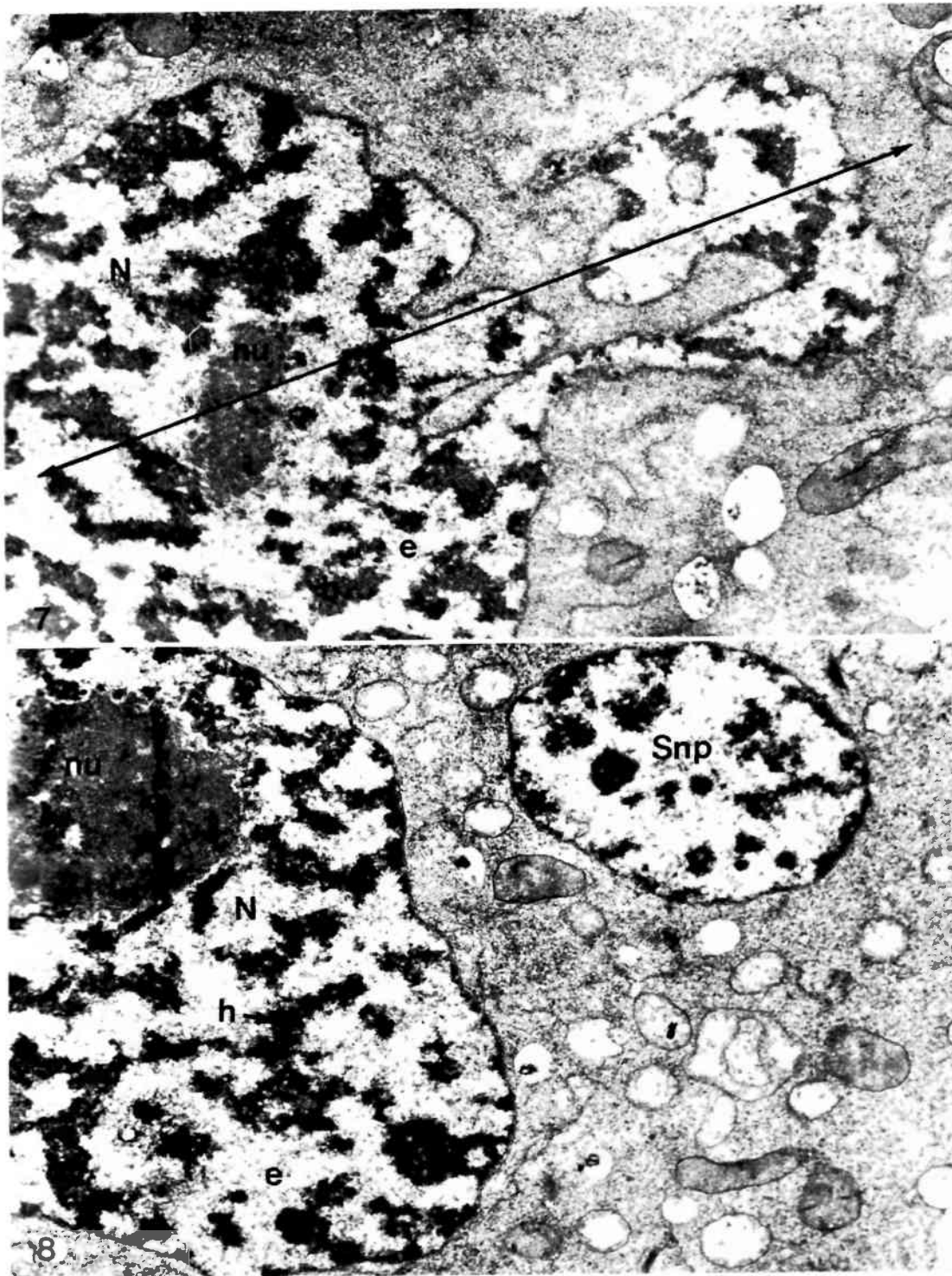
The heterochromatinic micronucleus as well as the nucleus is sure rounded by a bilayer envelope which has many ribosomes attached on its outer membrane. In Fig. 6 the ultrastructure of a heterochromatinic micronucleus shows a small area filled with ground cytoplasm. This proves that the surface of the micronucleus is very irregular having several protuberances and invaginations. The whole ultrastructure of this micronucleus demonstrates Heddle and Carrano's (1977) hypothesis according to which usually or some of the micronuclei are chromosomal acentric fragments (see Fig. 3). So the heterochromatinic micronuclei (Figs. 2-4, 6) correspond to acentric fragments of chromosomes with very packed heterochromatin.

In Fig. 5 a micronucleus with a particular structure is shown. It has an intermediate ultrastructure between the normal structure of the nucleus and the ultrastructure of heterochromatinic micronucleus. We called this kind of micronucleus as euchromatinic micronucleus because inside of it more euchromatin is to be found. A special feature of this micronucleus is the existence of several ribosomes on the both sides of the envelope (see the arrows in Fig. 5). The existence of these ribosomes on the inner side of the envelope is unknown for nuclear envelope (Palade 1955, 1956, Palade and Siekevitz 1956, Sabatini, Tashiro and Palade 1966, Hereward 1974, Lishnevskaya 1977) and it arises a puzzling problem for micronuclear envelope origin in the case of euchromatinic micronucleus. This particular structure makes very difficult to understand the actual origin of euchromatinic micronucleus and of its envelope. Anyway the euchromatinic micronucleus is not a simple piece or fragment of acentric chromosome. We suppose that it may be built probably of 1 to 2 chromosomes during the late telophase. The presence of ribosomes on the both sides of the envelope suggest something about its origin and according to Barer, Joseph and Merck (1959) probably it may be formed by endoplasmic reticulum.

In Figs. 7 and 8 the ultrastructure of a fragment of nucleus is shown. The line in Fig. 7 suggests the probable direction for sectioning which may result in a picture similar to that in Fig. 8. From these two pictures it comes obvious that in this case a nucleus fragment and not a micronucleus had been sectioned. The nuclear fragments have a similar structure as normal nucleus and they must not be misinterpreted as micronuclei.



Figs. 4-6. 4, normal cell nucleus and the heterochromatinic micronucleus with more heterochromatin. N = nucleus, h - heterochromatin e - euchromatin, ne = nuclear envelope, nu = nucleolus, Mn - micronucleus, mne - micronuclear envelope, P = plastid, GA = Golgi Apparatus. $\times 18,000$. 5, the ultrastructure of the euchromatinic micronucleus with the ribosomes on the both sides of the micronuclear envelope (see the arrows). $\times 27,000$. 6, the heterochromatinic micronucleus with ground cytoplasm (Gcy) inside of it. $\times 27,000$.



Figs. 7-8. 7, an amoeboid nucleus after 100 rad γ irradiation and post-treatment with cysteamine (50 mg/100 ml distilled water). The line shows the direction of a probable section which may give the picture on Fig. 8. $\times 10,000$. 8, a nucleus with sectioned nuclear fragment (Snp) nearby which must not be misinterpreted as a micronucleus. $\times 10,000$.

Résumé

La méristème radical de *Vicia faba* a été irradié avec des rayons γ appliqués par doses de 50, 100, 200, 300 et 500 rads pendant la quatrième jour à la suite de la germination. Après 24 heures l'extrémité de la racine a été fixée avec glutaraldehyde et acid osmique et puis incluse en W vestopal. Dans des plusieurs sections les micro-

nuclées (micronoyaux) sont présents et leurs ultrastructures peuvent être étudiés. Au point de vue ultrastructurale les micronoyaux actuels sont des fragments chromosomales acentriques. Une séparation nette parmi les micronoyaux et les autres fragments du noyau normal sera bienvenue.

Summary

The root meristem of broad bean (*Vicia faba*) fourth day old with γ ray in doses of 50, 100, 200, 300 and 500 rad was irradiated. After 24 hours the root tip was fixed with glutaraldehyde and osmic acid and after that embedded in W vestopal. On several sections micronuclei were present and their ultrastructure could be studied. From ultrastructural point of view the heterochromatinic micronuclei are acentric chromosomal fragments and the euchromatinic ones probably not. A clear separation between micronuclei and other fragments of the normal nucleus was proved.

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