

This shows a small necrotic vein within an area of cold induced cortical softening. The endothelial cells (E) are about to undergo structural disintegration but the vascular basement membrane (VB) is still preserved. The perivascular space is stuffed with extravasated erythrocytes. The distended glial basement membrane (GB) appears totally denuded from astrocytic foot-plates. Nevertheless, it is obviously able to prevent further spreading of the perivascular hemorrhage.  $\times 5170$ .

the perivascular space. Under the pressure of the extravasated erythrocytes, the denuded glial basement membrane may become distended; however, even under these conditions, it usually yields no evidence of actual rupture (Figure). On the contrary, it appears to be, like the vascular basement membrane, a very resistant structure, able to prevent, at least for some time, spreading of perivascular hemorrhages into the surrounding necrotic neuropile, even after all cellular barriers have broken down.

Light microscopy has long since shown us that the blood vessels are the stablest tissue components in an area of cerebral necrosis. The electron microscope has confirmed this classical observation, and has further pointed out that the most resistant structural elements of the vessel walls themselves, are the basement membranes including the perivascular glial ones. Our present findings, which partially corroborate the results of previous electron microscopic studies on traumatic, radiation, and anoxic-ischemic brain lesions<sup>4,5</sup>, reveal that both vascular and glial basement membranes can 'survive' for quite some time without being associated with any of the living cells from which they were originally elaborated.

**Zusammenfassung.** An der Grosshirnrinde von Goldhamstern wurde durch lokale Kälteeinwirkung eine umschriebene Erweichung hervorgerufen. Im Zentrum derselben wiesen nicht nur die neuronalen und gliösen Gewebekomponenten, sondern häufig auch die Blutgefässe schwerste nekrotische Strukturveränderungen auf. Bemerkenswerterweise blieben selbst nach dem völligen Untergang der Gefässwandzellen und der perivaskulären Astrozytenfußstücke die vasalen und gliösen Basalmembranen eine gewisse Zeit erhalten. Bei grösseren kortikalen Blutgefässen konnte sogar wiederholt beobachtet werden, dass die von astrozytären Zytoplasmafortsätzen bereits völlig entblösten gliösen Basalmembranen noch in stande waren, die Ausbreitung massiver perivaskulärer Erythrozytenansammlungen in das umgebende nekrotische Neuropil zu verhindern.

K. BLINZINGER, A. MATSUSHIMA  
and A. P. ANZIL

*Max-Planck-Institut für Psychiatrie,  
8 München 23 (Germany), 24 April 1969.*

<sup>4</sup> H. HAGER, *Die feinere Cytologie und Cytopathologie des Nervensystems dargestellt auf Grund elektronenmikroskopischer Befunde* (G. Fischer Verlag, Stuttgart 1964).

<sup>5</sup> C. P. HILLS, *Am. J. Path.* 44, 531 (1964).

## Special Forms of Amitotic Nuclear Division in Striated Muscle and Other Insect Tissues

Numerous examples of amitotic nuclear division have been reported by different authors<sup>1-3</sup> in several animal tissues in normal and pathological or experimental circumstances. Up to the present we have not found any literature concerning the amitotic nuclear division in insect tissues, where it is a frequent process. The main purpose of this paper is to describe briefly some amitotic pictures found in insect tissues and to point out – as a preliminary report – the observation of a new type of complex amitotic nuclear division in insect striated muscle cells.

Adult specimens and embryos of Diptera, Hymenoptera, Lepidoptera and Coleoptera were studied in the present work. Microscopical preparations were performed by 2 different methods: (a) Fragments of the insect tissue were placed in a centrifuge tube and homogenized lightly by means of a glass rod and then the tissue pulp was suspended in 5 ml distilled water. The suspension containing

the isolated cells and tissue fragments was collected and centrifuged at 1000 rpm for 3 min. The supernatant was aspirated off. Methanol-glacial acetic acid (3:1) fixative was added without disturbance of the pellet at the bottom of the centrifuge tube. After 2 h fixation the cells were suspended, centrifuged and resuspended in fresh fixative. Slides were prepared by the air-drying technique of ROTHFELS and SIMINOVITCH<sup>4</sup>. (b) Fragments of insect

<sup>1</sup> E. GRUNDMANN, *Allgemeine Zytologie. Eine Einführung in die funktionelle Morphologie der Zelle* (Georg Thieme Verlag, Stuttgart 1963).

<sup>2</sup> N. WEISSENFELS, *Z. Zellforsch. mikrosk. Anat.* 62, 667 (1964).

<sup>3</sup> N. WEISSENFELS and E. A. LÖBBECKE, *Naturwissenschaften* 54, 178 (1967).

<sup>4</sup> K. U. ROTHFELS and L. SIMINOVITCH, *Stain Technol.* 33, 73 (1958).

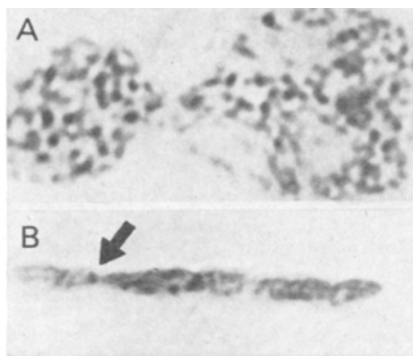


Fig. 1. (A) Mesenchymal cell nuclei dividing amitotically. A clear chromatinic strand connecting the 2 originated nuclei is present. (B) Continuous chain of several connected nuclei in a myoblast. The arrow signals a chromatinic strand connecting a separating nucleus to the rest of the structure. Adult mosquito specimen (*Culex bonariensis* Brèthes). Feulgen staining.  $\times 1000$ .

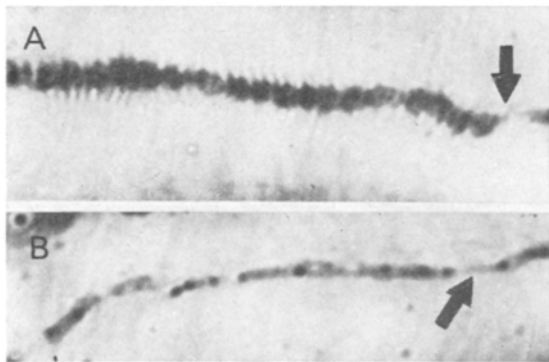


Fig. 2. (A and B) Clear distinct figures of multiple amitotic nuclear division in cord-like nuclear structures (Polykaryonema) of young muscle fibers. In (A) multiple periodical constrictions can be seen. The arrows indicate commissural chromatinic filaments. Adult fly specimen (*Dermatobia cyaniventris* Macquart). Feulgen staining. About  $\times 3000$ .

flight muscle were carefully excised, placed in albumenized slides, squashed and fixed for 15 min in Carnoy's fluid. The smears obtained by these 2 methods were stained with Feulgen, Giemsa and Toluidine blue stainings.

The study of the cytological preparations disclosed distinct nuclear pictures corresponding to different stages of amitotic division. Giant nuclei were observed in many cases 10 times longer than normal and showing periodic constrictions. Frequently the 2 or more originated nuclei – in many cases different in size – were connected by a strand or by a tenuous chromatinic filament (Figure 1A).

In striated muscle fibers the increase in size preceding the amitotic nuclear division occurs along the long axis of the nucleus, giving rise to very typical nuclear structures which we have found in myoblasts and young muscle fibers of the insects studied (Figures 1B and 2). These consist of truly chromatinic cords, varying in diameter, which by successive constrictions and fragmentations originate the rows of independent nuclei found in the central axis of the adult striated muscle fibers.

We suggest the term *Polykaryonema* to designate this cord-like or filamentous nuclear structure which by successive divisions will give origin to several nuclei. We propose the term *clasmatotenesis* or *clasmatotenic division* for this new type of complex amitotic nuclear division.

**Résumé.** On décrit quelques aspects morphologiques de l'appareil nucléaire des fibres musculaires striées des insectes. Le nom Polykaryonéma est proposé pour désigner les noyaux filamenteux géants qui par fragmentations successives produisent des chaînes de noyaux indépendants, placées dans le centre des fibres musculaires adultes. Ce phénomène offre un bon exemple de division nucléaire amitotique.

T. P. PESSACQ

*Instituto de Investigación de Ciencias Biológicas,  
Montevideo (Uruguay), 14 February 1969.*

## Feulgen-Cytophotometric Determination of DNA Content in the Germ Cell Nuclei of the Female Chicken Embryo During Premeiosis

In a previous work<sup>1,2</sup>, one of us investigated DNA synthesis during premeiosis in the ovarian germ cells of the chicken embryo both in vitro and in vivo, using the autoradiographic technique, following the incorporation of <sup>3</sup>H-thymidine. The successive developmental germ cell stages found during this period in the cortex of the ovary of the chicken embryo are represented in Figure 1. Only during the preleptotene stage of the germ cells, chiefly occurring in the central part of the ovarian cortex of 15- to 17-day-old embryos, does nuclear incorporation of <sup>3</sup>H-thymidine take place<sup>3</sup>. Germ cells with a reticulated nucleus, characterized by a more regular and much finer chromatin distribution than the oogonia at interphase, do not incorporate the DNA-precursor. Large numbers of these cells are found in the central part of the ovarian cortex of 14- and 15-day-old embryos. Since there is morphological evidence of a transition between cells with a reticulated nucleus and cells in the early preleptotene stage<sup>4</sup>, we consider the former to be the pre-

cursors of the latter. The meiotic divisions of the female germ cells in the chicken are concluded months or years after the observed <sup>3</sup>H-thymidine incorporation wave. Hence, this DNA synthesis may represent:

(1) A premeiotic reduplication of the germ cell nuclear DNA; (2) a metabolic DNA synthesis<sup>5</sup> ('specific gene amplification')<sup>6</sup> or DNA turnover; (3) a combination of these two hypotheses. The present study was undertaken to check these explanations by comparing the DNA content of reticulated nuclei and leptotenes.

<sup>1</sup> M. CALLEBAUT et R. DUBOIS, C. r. heb. Séanc. Acad. Sci., Paris 261, 12 (1965).

<sup>2</sup> M. CALLEBAUT, *Experientia* 23, 419 (1967).

<sup>3</sup> M. CALLEBAUT, *J. Embryol. exp. Morph.* 18, 299 (1967).

<sup>4</sup> G. C. HUGHES, *J. Embryol. exp. Morph.* 17, 513 (1963).

<sup>5</sup> H. ROELS, *Int. Rev. Cytol.* 19, 1 (1966).

<sup>6</sup> D. BROWN and I. B. DAWID, *Science* 160, 272 (1968).