Formation and Division of Binucleated Cells in Kidney Cell Cultures of Microtus Agrestis

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Summary. Epithelial kidney cell cultures of Microtus agrestis contain 10 to 25% binucleated cells. Observations of living cells under the phase contrast microscope showed that binucleated cells can arise by nuclear mitosis without cytoplasmic division. When binucleated cells divide the two nuclei are highly synchronized as they enter mitosis. In mitosis the chromosomes of both nuclei combine to a common metaphase plate leading to polyploid cells. In one case a tripolar spindle was seen after formation of a metaphase by the chromosomes of the two nuclei of a binucleated cell. This tripolar mitosis resulted in one binucleated and one mononucleated cell. The DNA-content (Feulgen photometry) and the distribution of heterochromatic bodies of the nuclei were corresponding to a tetraploid, a triploid and a haploid chromosome set. This suggests the possibility of somatic segregation of complete haploid sets.

In tissue cultures derived from epithelial kidney cells of Microtus agrestis a high incidence of bi- and multinucleated cells is found. The frequency of binucleated cells ranges from 10 to 25% in primary cultures and does not change after subcultivation. The mode of their division and formation were studied by direct observation of living cells and on fixed preparations.

Methods

Cell suspensions were made from pieces of kidney by trypsinisation and were seeded in petri dishes containing coverglasses. The medium consisted of 90 parts Eagles MEM, 10 parts fetal calf serum and antibiotics. Observations of living cells were made in a Sykes-Moore tissue chamber (Bellco) fitted into a heated microscope stage. For permanent preparations the cells were fixed in methanol/acetic acid 3:1, and stained with pararosanilin-methylgreen or according to Feulgen. Photometric DNA measurements were made on Feulgen preparations with a microdensitometer (BARR and STROUD). The diploid G 1-value ("2c") was estimated from measurements of 60 mononucleated cells in each preparation.

Observations

Structure of Binucleated Cells

In preparations of fixed cells the two nuclei of the binucleated cells are usually seen in close vicinity within the cell but are clearly separated by their nuclear membranes. Mononucleated diploid cells of Microtus agrestis may contain two conspicuous chromatin bodies derived from the two large heterochromatic sex chromosomes. The chromatin bodies from different tissues vary in shape and

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density but variation may also be seen in nuclei from one tissue (Schmid et al., 1965; Per a u. Wolf, 1967). The heterochromatic bodies of the two nuclei within a binucleated cell usually show a remarkable similarity in shape, density and position.

**Formation of Binucleated Cells**

To date our observations of living cells revealed two examples of the formation of a binucleated cell.

In the first case a mononucleated cell entered mitosis and completed it until early telophase. But at the end of mitosis cytokinesis and cleavage of the cytoplasm did not take place. This resulted in a binucleated cell. After fixation and Feulgen staining each of the two nuclei showed two heterochromatic bodies indicating a diploid chromosome complement (Figs. 1–6).

In the second case a binucleated cell gave rise to one binucleated and one mononucleated cell (see below).

**Division of Binucleated Cells**

In fixed preparations occasionally two mitotic figures can be observed within one cell. In most of these cells both nuclei are in prophase but only on rare occasions two separate metaphase or anaphase figures are found. This indicates that in most binucleated cells the chromosomes of both nuclei combine to a common metaphase plate. In 90% of the binucleated cells in prophase, the two nuclei show the same degree of chromosome contraction, but in 10% one nucleus is found in interphase while the other is in prophase.