Binucleate and polyploid cells in the decidua of the mouse

By J. D. ANSELL1, P. W. BARLOW2 and ANNE MCLAREN3

From the Department of Genetics and the Agricultural Research Council
Unit of Animal Genetics, University of Edinburgh

SUMMARY

The DNA contents of mouse decidual nuclei were estimated by microdensitometry and ploidies of up to 64 times the haploid DNA content were found. Electrophoretic studies, using the enzyme marker glucose phosphate isomerase (GPI-1), on decidual giant and multinucleate cells from a mouse chimera, revealed that cell fusion was not the mechanism of formation of such cells.

The development of the decidua in rodents is associated with the production of multinucleate and giant cells (Fig. 1A). In the rat, binucleate cells appear throughout the secondary or antimesometrial zone of the decidua by the eighth day of pregnancy (Krehbiel, 1937), while in the mouse this region is characterized by large bi-, tri-, or tetranucleate cells (Snell & Stevens, 1966). All decidual cells may eventually enlarge, containing nuclei of a size consistent with a DNA content of four, eight and possibly even higher multiples of the haploid complement (Sachs & Shelesnyak, 1955). In the white rat spectrophotometric determination of DNA levels in decidual cells indicates that by the tenth day of gestation ploidy levels have reached 16 C–32 C, with maximal levels of 64 times the haploid DNA content (Zybina & Grishchenko, 1972). The mechanism of nuclear enlargement in decidual tissue is as yet unknown.

In the work described below the DNA content of individual nuclei from the decidual tissue of the mouse was examined by microdensitometry. Artificially induced decidua (Finn & Hinchliffe, 1964) were dissected from the uterus on the ninth day of pseudopregnancy, loosely homogenized, fixed in three parts ethanol to one part acetic acid and the nuclei air-dried on to a microscope slide (Fig. 1B). Slides bearing nuclei of both liver (which serves as an internal DNA standard) and decidua were stained as described by Barlow & Sherman (1972). Nuclear DNA measurements were made with a Vickers M 85 scanning micro-

1 Author's address: Agricultural Research Council, Unit of Reproductive Physiology and Biochemistry, 307 Huntingdon Road, Cambridge CB3 0JQ, U.K.
2 Author's address: Agricultural Research Council Unit of Development Botany, University of Cambridge, 181A Huntingdon Road, Cambridge CB3 0DY, U.K.
3 Author's address: Agricultural Research Council Unit of Animal Genetics, University of Edinburgh, West Mains Road, Edinburgh EH9 3JN, U.K.
Fig. 1(A). Histological section of part of a mouse decidua on the eighth day of gestation stained with haematoxylin and congo red. Large binucleate cells containing giant nuclei are arrowed. ×1800.

(B) Feulgen stained, air-dried decidual nuclei from a nine-day decidua showing a range of nuclear sizes. ×660.
Polyploidy in mouse deciduomata

Fig. 2. The distribution of DNA values of 222 randomly selected nuclei prepared from decidual cells. The abscissa shows the range of DNA values (2C–32C).

densitometer at 565 nm. Fig. 2 shows the DNA content of nuclei in a population of decidual cells from two animals. Nuclei from another four animals were also measured and showed a similar distribution of DNA values (not shown here). Most (ca. 86 %) of the nuclei have DNA values in the range 2C to 8C (where C is the haploid value), but nuclei with a 64C DNA content were also found when measurements were made on the largest nuclei in the population. However, such large 64C nuclei were infrequent (< 0.2 %) in the population on which DNA measurements were made.

The morphology of nuclei from decidual cells is similar to those from liver, the heterochromatin forming a shell around the nucleolus ('Nukleolen schalenartig umgebend' of Tschermak-Woess, 1963).

In order to test the hypothesis that giant and multinucleate cells could be formed from fusion of diploid decidual cells, we examined decidual tissue from a mouse, chimeric for the two electrophoretic variants of the enzyme glucose phosphate isomerase (GPI-1), for the expression of the heteropolymeric form of the enzyme. Isozyme variants which produce heteropolymeric forms in heterozygotes have been used to detect the formation of functional heterokaryons in vitro (Ruddle & Nichols, 1971) and in vivo, demonstrating the fusion of myoblasts in chimeras to form the syncitial heterokaryons of skeletal muscles (Mintz & Baker, 1967; Baker & Mintz, 1969). Using the GPI-1 marker system, heterokaryon formation can be detected in a tissue where fusion of cells comprises only 1 % of the total tissue sample (Chapman, Ansell & McLaren, 1972).

A female mouse was derived from aggregation of embryos (Bowman & McLaren, 1970) of the two strains homozygous for the isozymes GPI-1 A and GPI-1 B, namely C3HBi/McL (GPI-1 B) and a randomly bred multiple recessive strain (GPI-1 A). The mouse was overtly chimeric for coat colour and in two previous pregnancies by a multiple recessive male had produced a total of 15 progeny, all homozygous recessive in phenotype. It was subsequently mated
with a male homozygous for GPI-1A, to avoid any heterozygosity in the embryonic tissues which might confound the enzyme phenotype of the decidua, and killed on the eighth day of pregnancy. Twelve decidua were dissected from the uterus, freed of embryonic and trophoblast tissue, homogenized, and frozen and thawed individually in distilled water. GPI-1 enzyme phenotypes of the decidual homogenates were determined by the technique of starch gel electrophoresis described by Chapman, Whitten & Ruddle (1971) and Chapman et al. (1972). The results are shown in Fig. 3.

A double-banded phenotype corresponding to a mixture of GPI-1A and GPI-1B was observed for all of the twelve decidual samples (Fig. 3, B–G and J–O). Blood samples taken from the chimera prior to dissection (Fig. 3, H and P) also correspond to mixtures of the two isozyme variants, but predominantly of the GPI-1B type. Samples A and I in Fig. 3 show the expression of the heteropolymeric form of the enzyme (GPI-1AB) in a liver sample from a male (multiple recessive × C57BL/C3H F1) heterozygous for the GPI-1 isozymes.

The electrophoretic patterns of GPI-1 in the decidua indicate that both components of the chimera contribute to the formation of every decidua, though in different proportions. No evidence of the formation of the heteropolymer GPI-1 AB was found in any of the decidual samples analysed.

From these findings we conclude that cell fusion is not the mechanism for the formation of multinucleate or giant cells in the mouse decidua. A similar conclusion was reached for the trophoblast giant cells of mouse placentae (Chapman et al. 1972; Gearhart & Mintz, 1972).

The observed increase in nuclear DNA content in mouse decidual tissue probably therefore occurs as a result of endoreduplication, and multinucleate cells arise from mitoses without subsequent cell division.
A. McL. is grateful to the Ford Foundation for financial support. J. D. A. is in receipt of a Ministry of Agriculture, Fisheries and Food Research Studentship.

REFERENCES


(Received 21 June 1973)