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INCORPORATION OF 3H-PROLINE BY MOUSE DECIDUAL CELLS: AN ULTRASTRUCTURAL RADIOAUTOGRAPHIC STUDY

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The endometrial stroma of the virgin mouse is formed by a typical connective tissue in which the fibroblasts are the most frequent cell type. On occasion of blastocyst implantation this stroma undergoes a remarkable remodelling of both cellular and extracellular components (R. H. Krehbiel, 1939, *Physiol. Zoology, 10*: 212-233; Lobel et al., 1967, *Acta Endocrinol., 56* (Suppl. 23): 7-109; C. A. Finn, 1967, *J. Ultrastruct. Res., 20*: 321-324; P. A. Abrahamsohn, 1983, *Anat. Embryol., 166*: 263-274; 1989, Morphology of the Decidua, p. 127-133. In K. Yoshinaga, *Blastocyst Implantation*, Adams, Boston). These modifications result in the formation of a new structure denominated decidua.

Recent results on decidual extracellular matrix showed that the remodelling of this stroma involves collagen phagocytosis (T. M. T. Zorn et al., 1989, *Anat. Rec., 225*: 96-100; 1990, *J. Struct. Biol., 103*: 23-33) and an accumulation of thick collagen fibrils in the extracellular space (T. M. T. Zorn et al., 1986, *Cell Tissue Res., 244*: 443-448). The thickening of these fibrils is progressive and directly related with decidualization as no thick fibrils are observed in the extracellular space of the interimplantion sites and nondecidualized regions of the pregnant endometrium and virgin animals (Alberto-Rincon et al., 1989, *Am. J. Anat., 1986*: 417-429).

Although these results suggest an acumulation of collagen fibrils, it is not known yet if these thick fibrils result from aggregation of old thin fibrils present before decidualization, whether they are formed "de novo" or result from both mechanisms.

In the present experiment, female Swiss mice on the 6th day of pregnancy were injected with 3H-proline (L-2, 3, 4, 5, 3-H) proline s.a. 100-130 Ci/mMol, (Amersham, England). Each animal was injected via the inferior cava vein with 1.5 mCi of 3H-proline, around 8 AM. The animals were killed 1, 2, 6 and 24 hours after the injection. The uteri were fixed by perfusion with 10 ml of Tyrode solution followed by fixative consisting of 2% glutaraldehyde, 2% paraformaldehyde in 0,1M sodium cacodilate buffer pH 7.3. Thereafter the implantation and interimplantation sites were postfixed with 1% osmium tetroxide, dehydrated in an ethanol series and embedded in araldite.

Sections (80 nm thick) were picked up onto parlodion coated grids and were coated with carbon. Thereafter they were submitted to radioautographic technique (T. Nagata, 1982, *Saibo, 14*: 40-45). The development of the radioautographs was carried out according to Murata et al., (1979, *Acta, Histochem. Cytochem., 12*: 443-450) after a time exposure of 12 months. The radioautographs were stained with uranyl acetate and lead citrate, and observed with Zeiss EM9 and JEOL CXII electron microscopes.

The qualitative analysis of the radioautographs showed that after 1h there was a great amount of silver grains concentrated over decidual cells. After 2h some of the grains could be found on the extracellular space, most of them on thick collagen fibrils. From 6h on, a considerable amount of grains was observed over bundles of collagen fibrils especially over the thick ones. The interimplantation regions, where decidualization does not occur, as well as the nondecidualized region of the implantation sites, showed a low incorporation when compared with decidualized areas.

These observations strongly suggest that decidual cells of the mouse are able to synthesize collagen despite their condition of transformed fibroblasts. This results are in accordance with Kisalus et al., (1987, *Anat. Rec., 218*: 402-415), who showed by *in vitro* experiments of incorporation of 3H-proline that explants of human decidual cells produce different collagen types.

Our results also show that the thick collagen

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Electron microscopic radioautogram of labeled decidual cells 1 h after 3H-proline injection. Silver grains are observed over the cytoplasm and on the extracellular space. A bundle of thick collagen fibrils is labeled (arrows). Bar = 1μm.

fibrils results at least in part from association of collagen molecules synthesized "de novo" by decidual cells.

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