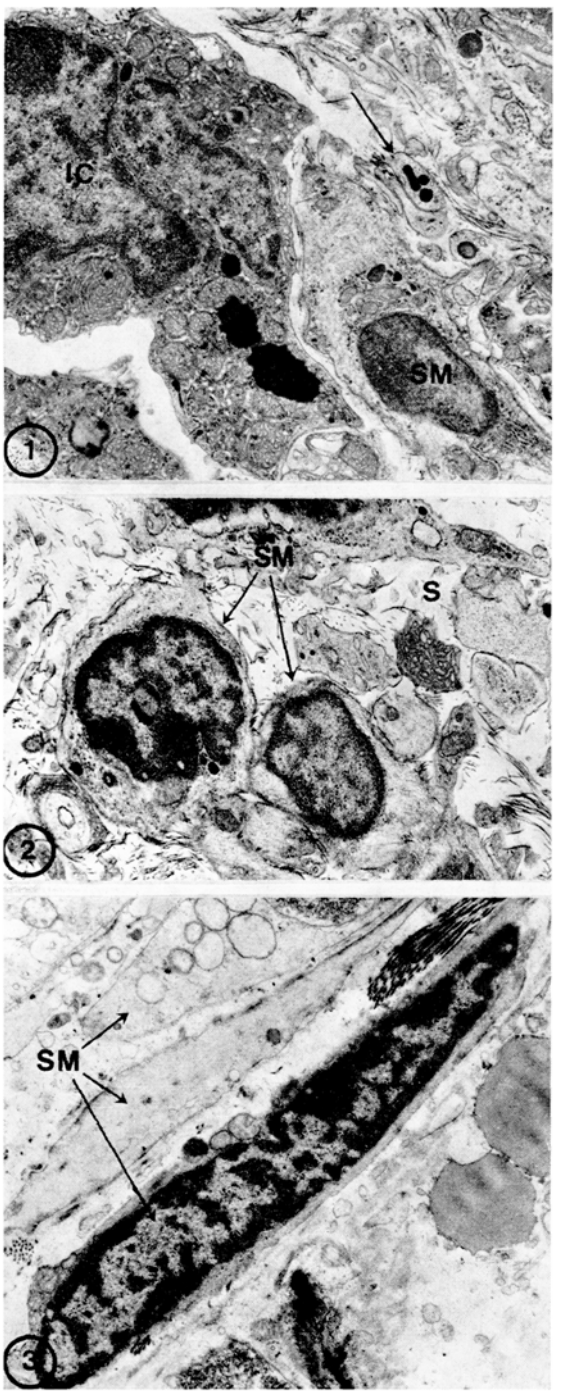
The Presence of Smooth Muscular Cells in the Ovary of Several Mammals as Seen Under the Electron Microscope[1]

Many classical authors described in the past the presence of smooth muscle tissue in the ovary of different mammals[2–4]. But the real existence of this tissue, its distribution and significance was denied or discussed by others[5,6].

In this report the real presence of smooth muscle cells was observed with the electron microscope in different areas of the ovaries of rabbits, cats and mice, and the ultrastructural features of these cells are described.



The ovaries of rabbits, cats and mice were removed, reduced to small pieces and fixed in 5% glutaraldehyde in phosphate buffer at pH 7.2 for 2–5 h and post-fixed in 1% OsO_4 in phosphate buffer[7] for 2–3 h. The blocks were enbedded in araldite and the sections cut with a Porter-Bloom MT_1 ultramicrotome were stained with Pb acetate[8] or with Pb citrate[9] and observed with a Zeiss EM 9 electron microscope. In addition, 0.5–1 μm thick sections were stained with basic fuchsin for light microscopy.

Many smooth muscular cells were observed in different areas of the ovaries of rabbits, cats and mice with the aid of the electron microscope. Often these cells are arranged in fascicles, or in small groups, but sometimes appear isolated. They were found scattered in the ovarian stroma, related to interstitial cells, in the periphery of the corpus luteum and sometimes between luteal cells in the middle of the gland. The smooth muscular cells are seldom noticed between cells of the theca interna and externa or in the areas of atretic follicles.

Each smooth muscular cell presents typical filaments, numerous free ribosomes in the perinuclear area of cytoplasm, lipid droplets and at times glycogen particles (200–250 Å in diameter). Mitochondria are not abundant and often exhibit long cristae. The Golgi vescicular complex is almost always present and related with 1–2 centrioles, sometimes in the process of ciliogenesis. The rough endoplasmic reticulum is moderately developed. The plasma membrane shows many invaginations caveolae intracellulares and a few opaque areas of condensation. Sections of nerve endings (myoneural junctions) are occasionally seen.

The plasma membrane is covered with a dense lamina. An amorphous or fibrillar material adherens to the external aspect of the dense lamina. The nucleus is elongated and its envelope forms wide perinuclear cisternae.

The present findings on the occurrence of a smooth muscle tissue in the ovary of rabbits, cats and mice are generally in accord with some other recent observations on the rat and monkey ovaries[10,11]. In addition some smooth muscular cells, however, may display features other than those just described. Some have a cytoplasm with rare filaments and appear generally indifferentiated and resemble fibroblasts. Others have more lipid droplets, membranes of the rough endoplasmic reticulum, ribosomes and pinocytosis vesicles. The second type of smooth muscular cells is in close contact with steroidogenic cells (luteal or interstitial cells). These observations suggest that ovarian smooth muscular cells have a cycle of differentiation similarly to the other ovarian cells[12,13] and may be influenced by the hormonal action of the hypophysis and by the local action of the ovarian steroids as well[14,15].

Fig. 1. Mouse ovary. Smooth muscle cell (SM) in relation with some interstitial cells (IC). The cytoplasm of the smooth muscle cell is filled with filaments and contains mitochondria and rough membranes. The arrow indicates a transverse section of a fine unmyelinated nerve fiber. ×7,400.

Fig. 2. Mouse ovary. Transverse section of a group of smooth muscle cells (SM) in the ovarian stroma (S). ×7,000.

Fig. 3. Cat ovary. Longitudinal section of some smooth muscle (SM) cells of a voluminous atretic follicle. ×6,700.

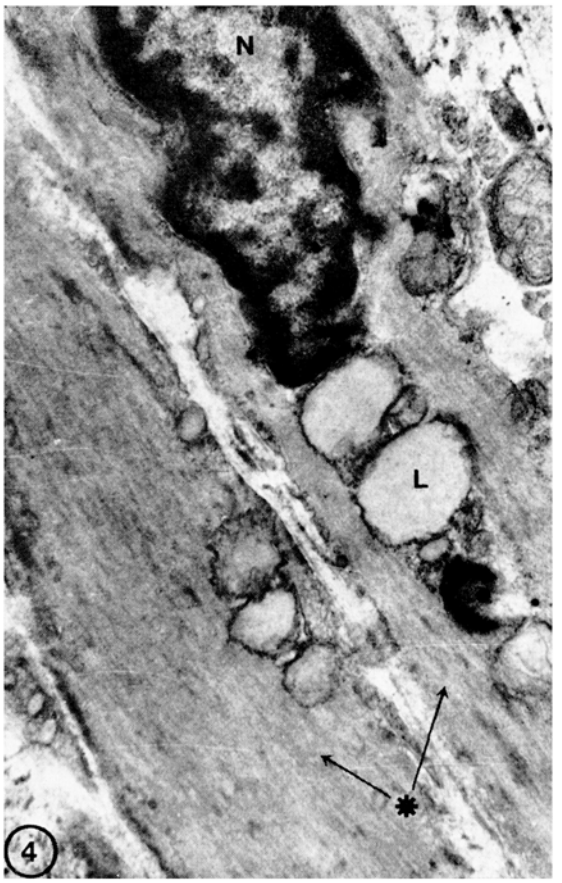


Fig. 4. Rabbit ovary. High magnification of 2 modified smooth muscle cells in the theca interna of an antral follicle. The cytoplasm contains a big nucleus (N), numerous myofibrils (*→) with scattered clear lipid droplets (L). ×22,700.

Owing to the distribution and significance of the smooth muscular tissue in the ovary, their direct role in the follicular dehiscence and atusia still remains debatable.

Riassunto. Mediante l'impiego del microscopio elettronico viene dimostrata la reale esistenza di piccoli gruppi di cellule muscolari lisce, non in rapporto con vasi, in differenti zone dell'ovaio (stroma, corpo luteo, teca, ghiandola interstiziale) del gatto, del coniglio e del topo. Le cellule muscolari subiscono caratteristiche modificazioni in relazione al ciclo ovarico.

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[1] This paper was presented to the 28th Italian Congress of Anatomy in Naples (24–28 October 1969).
[2] C.-J. Rouget, Physiology *1*, 320 (1858).
[3] H. Winiwarter and G. Sainmont, Archs Biol. *24*, 627 (1909).
[4] G. Motta, Riv. ital. Ginec. *10*, 1 (1929).
[5] R. Schroder, *Handbuch der mikroskopischen Anatomie des Menschen* (W. V. Möllendorf, Berlin 1930), vol. VII, p. 329.
[6] L. Claesson, Acta Anat. *3*, 295 (1947).
[7] G. Millonig, J. appl. Physics *32*, 1637 (1961).
[8] G. Millonig, J. Biophys. Biochem. Cytol. *9*, 409 (1961).
[9] E. S. Reynolds, J. Cell Biol. *17*, 208 (1963).
[10] L. Osvaldo-Decima, J. Ultrastruct. Res. *29*, 218 (1970).
[11] J. D. O'Shea, Anat. Rec. *1967*, 127 (1970).
[12] P. Motta Z. Zellforsch. *98*, 233 (1969).
[13] P. Motta, Biol. Lat. *18*, 107 (1966).
[14] R. Laguens, J. Ultrastruct. Res. *10*, 578 (1964).
[15] R. Ross and S. J. Klebanoff, J. Cell Biol. *32*, 155 (1967).

Differentiation of Endodermal Tissues in Homografts of Primitive Ectoderm from Two-Layered Rat Embryonic Shields

Recently Nicolet[1] showed that in chick embryonic shields the definitive endoderm (presumptive gut epithelium) arises by invagination of cells from the epiblast in the anterior part of the primitive streak. The possibility that an analogous mechanism exists during the germ layer formation in mammalian embryos was indicated by Grobstein[2] in 1952. He mechanically removed the outer cell layer (primitive endoderm) of mouse embryonic shields on the 7th day of pregnancy (variable cylinder length and developmental stage) and grafted the precultured clusters of primitive endoderm-deprived shields into the anterior chamber of the eye of adult mice for 30 days. The epithelium of the gut differentiated within these grafts at a high incidence. The author concluded that 'the inner cell layer, or primitive ectoderm, of the mouse embryonic shield cannot be regarded as a germ layer with capacities sharply limited to ectodermal differentiation'.

In a recent communication we showed that particular germ layers of presomite rat embryos can be separated from one another by treatment with proteolytic enzymes. This procedure does not affect the viability of embryonic cells and their ability to differentiate into normal tissues after transplantation[3].

In the experiment we are reporting here the albino rats of the inbred Fischer strain were used. The embryonic shields were all at the pre-primitive streak stage, and the outer cell layer was removed following pre-treatment with enzymes. The clustering and pre-culturing of embryonic shields were avoided and the grafts were transferred underneath the kidney capsule of adult rats.

Pregnant females were killed by ether 8 days after mating and the embryos were isolated in sterile Tyrode's saline. Only the egg cylinders belonging to the stage 12 of Nicholas[4] (pre-primitive streak) were selected for the experiment. They consisted only of an inner (primitive ectoderm) and an outer cell layer (primitive endoderm). The ectoplacental cone and the Reichert's membrane were removed and the embryonic shields with their extraembryonic parts were treated with enzymes[3]. The action of enzymes was blocked by a mixture of saline and a

[1] G. Nicolet, Experientia *23*, 576 (1967).
[2] C. Grobstein, J. exp. Zool. *119*, 355 (1952).
[3] B. Levak-Švajger, A. Švajger and N. Škreb, Experientia *25*, 1311 (1969).
[4] J. S. Nicholas and D. Rudnick, J. exp. Zool. *75*, 205 (1938).