should be of interest in pathology, since an antithrombotic action for FN has been postulated, mediated by the limitation of platelet-collagen interaction[2].

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1 Koteliansky, V. E., Leytin, V. L., Sviridov, D. D., Repin, V. S., and Smirnov, V. N. FEBS Lett. *1* (1981) 59.
2 Moon, D. G., Kaplan, J. E., and Mazurkewicz, J. E., Blood *67* (1986) 450.
3 Santoro, S. A., Biochem. biophys. Res. Commun. *116* (1983) 135.
4 Eynard, A. R., Galli, G., Tremoli, E., Maderna, P., Magni, F., and Paoletti, R., J. Lab. clin. Med. *107* (1986) 73.
5 Tangen, O., Berman, H. J., and Marfey, P., Thromb. Diath. Haemorrh. *25* (1971) 268.
6 Rovasio, R. A., Delouvée, A., Yamada, K. M., Rimpl, R., and Thiery, J. P., J. Cell Biol. *96* (1983) 462.
7 Bensusan, H. B., Koh, Th., Henry, K. G., Murray, B. A., and Culp, L. A., Proc. natl Acad. Sci. USA *75* (1978) 5864.
8 Hynes, R. O., and Yamada, K. M., J. Cell Biol. *95* (1982) 369.
9 Ginsberg, M. H., Forsyth, J., Lightsey, A., Chediak, J., and Plow, E. F., J. clin. Invest. *71* (1983) 619.
10 Leung, L., and Nachman, R., A. Rev. Med. *37* (1986) 179.
11 Mosesson, M. W., and Umfleet, R. A., J. biol. Chem. *245* (1970) 5728.
12 Santoro, S. A., and Cunningham, L. W., Proc. natl Acad. Sci. USA *76* (1979) 2644.
13 Kleinman, H. K., Wilkes, C. M., and Martin, G. R., Biochemistry *20* (1981) 2325.
14 Legrand, Y. J., Fauvel, F., Arbeille, B., Leger, D., Mouhli, H., Gutman, N., and Muh, J. P., Lab. Invest. *54* (1966) 566.
15 Yamada, K. M., Hasegawa, T., Hasegawa, E., Kennedy, D. W., Hirano, H., Hayashi, M., Akiyama, S. K., and Olden, K., Prog. clin. Biol. Res. *151* (1984) 1.

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Immunocytochemical demonstration of contractile cells in the human ovarian follicle[1]

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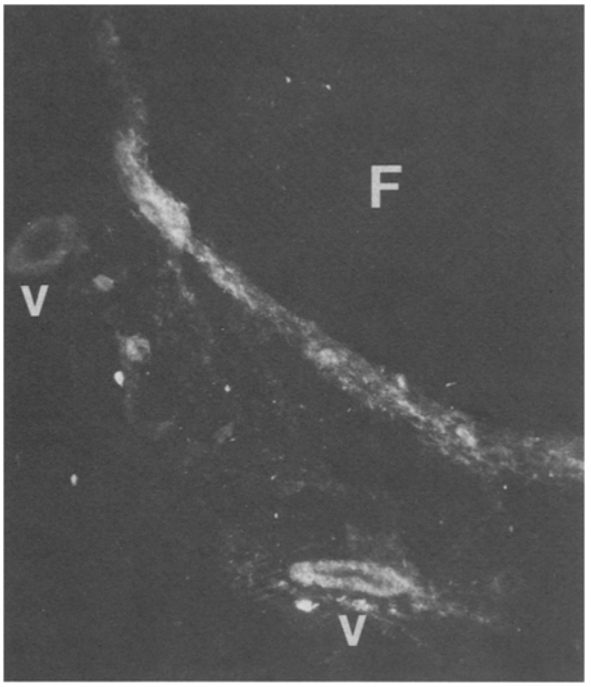
Summary. Actin- and myosin-like immunoreactivity is found in cells located in the theca externa of the follicle wall of the human ovary, and corresponding to previously observed myoid cells. The immunocytochemical observation provides direct structural evidence that non-vascular contractile cells are also present in the follicle wall in humans. As expected, perifollicular blood vessels showed a positive immunoreaction for actin and myosin in their smooth muscle walls.
Key words. Immunoreactivity; actin; myosin; ovary; contractile cells.

The ability of the human follicle wall to contract has been established in pharmacological experiments in vitro[2]. However, the morphological basis for the motor activity has been a matter of dispute[3]. At the ultrastructural level cells have been found in the theca externa layer with several characteristics of smooth muscle cells, i. e. filaments, dense bodies and micropinocytotic vesicles. Since the amount of filaments is somewhat lower than in 'classical' smooth muscle cells, and since transitional forms between fibroblasts and smooth muscle-like cells are present in the follicle wall, it has been questioned whether these ovarian cells are in fact contractile.

With the introduction of immunocytochemical methods for specific demonstration of the smooth muscle proteins, actin and myosin[4,5], it became possible to study whether the follicle wall contains contractile cells. In a study on rat ovaries, contractile proteins were demonstrated in elongated cells forming concentric layers in the theca externa of the Graafian follicle[6]. The present study was performed to answer the question whether the human ovarian follicle also contains cells with actin and myosin.

Preovulatory Graafian follicles were taken from fertile women, who were subjected to hysterectomy and had given their permission for the ovarian biopsy. The tissue was frozen and sectioned in a cryostat at −30 °C. 5-µm-thick sections were cut and placed under a hairdryer for 1–2 h. They were then incubated for 30 min at room temperature with specific γ-globulin enriched rabbit antibodies and their corresponding controls: 1) antiserum (1 mg/ml) raised against purified chicken gizzard smooth muscle myosin, 2) antiserum (1–2 mg/ml) to actin purified from an acetone powder of chicken gizzard smooth muscle, which showed a single band on 10% sodium dodecyl sulfate-acrylamide gel electrophoresis, corresponding to a molecular weight of 42,000, 3) antibody to gizzard smooth muscle myosin previously adsorbed to chicken gizzard myosin, 4) the same antibody adsorbed to striated muscle (pectoralis) myosin and 5) normal non-immune rabbit γ-globulin. After washing, the sections were incubated for 30 min in a solution of fluorescein-labelled immunoglobulin (1 mg/ml) raised in sheep against rabbit. Control sections were incubated with this second antibody alone. The sections were then washed in phos-



Cryostat section from human ovary incubated with myosin antibodies and fluorescein-labelled anti-γ-globulin. Intense fluorescence in the wall of a Graafian follicle (F). In the ovarian stroma the media layer of blood vessels (V) shows strong myosin immunoreactivity.

phate-buffered saline and mounted in a glycerol-0.1 M-glycine buffer (7:3 v/v), pH 8.6. The sections were examined in a Zeiss fluorescence microscope equipped with Schott filters BG 12 and OG 4. Sections of 15-μm thickness were also taken for visualization of adrenergic nerves by the glyoxylic acid technique (for further details see Walles et al. [6]).

The cryostat sections from the human follicles, which were incubated with myosin antibodies (1) showed an intense fluorescence of cells in the theca externa of the follicle wall (fig.). Intense myosin fluorescence was seen also in the walls of blood vessels beneath the follicle in the ovarian cortical stroma. When ovarian sections were incubated with myosin antibodies previously adsorbed to striated muscle myosin (4) the cells in the theca externa showed a fluorescence of similar intensity, though the background fluorescence was reduced. Previous adsorption to smooth muscle myosin (3) completely extinguished the fluorescence. Anti-actin incubated sections (2) showed a fluorescence pattern comparable to that seen with myosin antibodies (1). Sections incubated with non-immune control globulin or with the fluorescein-labelled antiserum alone were completely devoid of fluorescence.

The results show that the theca externa layer of the human ovarian follicle includes populations of cells containing actin- and myosin-like immunoreactivity. The presence of both these proteins is a criterium for the contractile nature of the cells. In all probability these cells are identical with those previously demonstrated by electron microscopy and classified as smooth muscle-like (myoid) cells or smooth muscle cells [2,3,6]. Also autonomic nerve terminals have been shown in the theca externa layer of the human follicle wall, where they form close contacts (about 100 μm) with the smooth muscle-like cells [6]. By electrical stimulation of the adrenergic nerves in vitro it has been possible to induce contractile activity in strips dissected from the human Graafian follicle wall, which confirms the structural evidence for a neuromuscular complex [2]. The possibility that neuromuscular mechanisms might be involved in the development and/or rupture of the follicle is supported also by in vivo experiments showing that the number of ovulations is markedly altered in rat ovaries after exposure to sympathomimetic or sympatholytic agents [7].

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2 Owman, Ch., Sjöberg, N.-O., Svensson, K.-G., and Walles, B., J. Reprod. Fert. *45* (1975) 553.

3 Owman, Ch., and Stjernquist, M., Handbook of Chemical Neuroanatomy, vol. 6: The Peripheral Nervous System, p. 445. Elsevier, Amsterdam 1988.

4 Gröschel-Stewart, U., Schreiber, J., Mahlmeister, C., and Weber, C., Histochemistry *46* (1976) 229.

5 Gröschel-Stewart, U., Ceurremans, S., Lehr, I., Mahlmeister, C., and Paar, E., Histochemistry *50* (1977) 271.

6 Walles, B., Gröschel-Stewart, U., Owman, Ch., Sjöberg, N.-O., and Unsicker, K., J. Reprod. Fert. *52* (1978) 175.

7 Kannisto, P., Owman, Ch., and Walles, B., J. Reprod. Fert. *75* (1985) 357.

0014-4754/90/070682-02$1.50 + 0.20/0