Cervical mucus and their functions

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Summary

In the 1940s, it was generally believed that the cyclic changes of the cervical mucus were due to varying proportions of water. In the following decades, the author found experimental evidence with NMR and other methods indicating that the cyclic changes were instead due to secretion of different types of mucus in a certain succession.

Microscopic examination of mucus samples carefully spread out on slides and dried (no fixation, no staining, no mounting) has helped to identify these mucus types. The four main types are denoted S, L, G and P and have some important subtypes. Some other components have also been identified.

New simple noninvasive techniques have been developed which may assist in future investigations on mucus biosynthesis and function at the levels of cell and molecular biology.

Introduction

Following the clinical observations by Séguy and Simmonet[13] and Séguy and Vimeux[14] and the histological studies by Wollner[16-18] and Sjövall,[15] it became generally believed that the secreting elements in the endocervical mucosa all worked in synchronism. The proliferation occurring around the middle of the cycle and culminating at ovulation was supposed to explain the occurrence of the transparent, watery, abundant secretion at that time.

In the beginning of the 1950s, a cyclic occurrence of mycoplasma in cervical mucus was reported.[2] It appeared difficult to explain this finding on the basis of a homogenous mucus only varying its water contents. By 1957 it became possible to investigate mucus with NMR. In a homogenous hydrogel, the proton NMR relaxation curve should be a straight line in a logarithmic diagram (Figure 1A), changing its inclination with varying viscosity when the water content was altered. The measured relaxation curves behaved differently (Figure 1B) and could be explained by the presence of two different types of hydrogels, a less viscous replacing a more viscous one as time approached ovulation. These observations made it also possible to estimate the approximate size of the components present in the mucus. They must be larger than

$$2\sqrt{D\,T_1}$$

(D being the water diffusion constant at T_1 the spin-lattice relaxation time) unless diffusion would blur out the effect. The estimated minimum size should be about 100 µm.

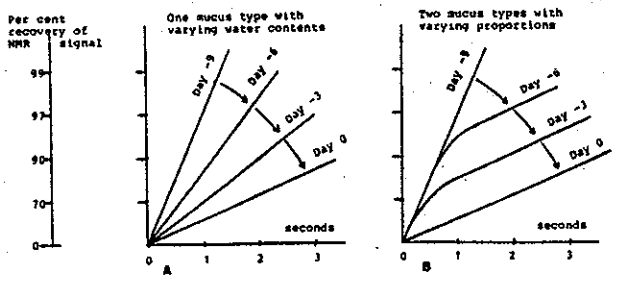

Figure 1: Recovery of proton NMR signals. The NMR signal is suppressed by a radiofrequency pulse. Then it grows to its original size (relaxation). This recovery is presented on a logarithmic ordinate. Abscissa = time in seconds (linear). For a single-component hydrogel the recovery becomes linear, steeper for higher viscosity. For a two- (or multi-) component hydrogel the recovery follows curved lines.

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Microscopic observations on wet mucus in the microscope supported NMR, and it was proposed[3,4] that the cervical mucus is a mixture of 'rods' of different composition, probably emanating from different crypts. Later, this was supported by direct observations of mucus aspirated from individual crypts[5] by means of micro-NMR magnetic resonance on the micro-scale.

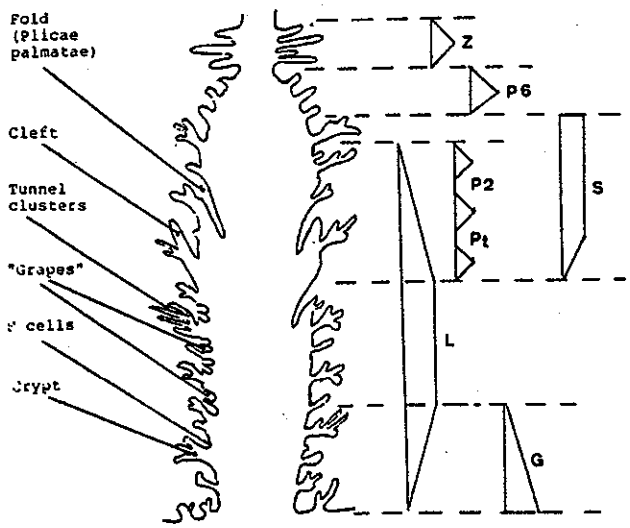

Figure 2: The principal anatomic structures of the cervix. Early epithelial growth gives rise to folds and clefts (the plicae palmatae). Later growth processes end up with 'grape-like' structures and crypts and tunnel clusters.

The endocervix has a rather complicated anatomy (Figure 2), with exophytic structures (folds and 'grapes') and endophytic structures (clefts, crypts, 'tunnels'). The word 'crypt' would more adequately be replaced with 'SU'/secretory unit,[12] because an assembly of epithelial cells producing a certain type of mucus is confined to an anatomical crypt only in about 70 per cent. However, the word 'crypt' has been so widely accepted that it will be used here instead of SU.

G, L, S, and P, the main types of cervical mucus

Two distinctly different types of single crypt mucus were characterised:[6] G (crypts reacting on gestagenic stimulation) and E (crypts reacting on oestrogenic stimulation). Following continued studies, the E-type was later divided into L-type and S-type mucus,[7] the letters L and S referring to the shapes of the 'rods', L = loaf-shaped, S = string-shaped, but S also stands for 'sperm conveying' mucus.

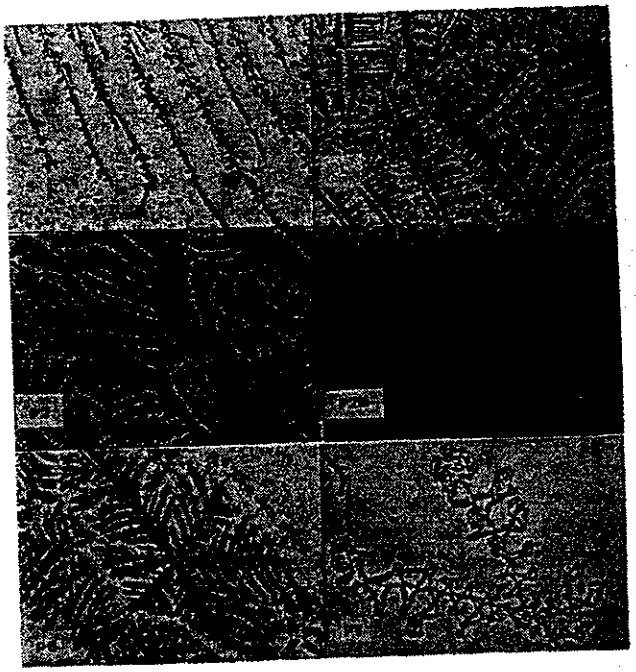

Figure 3: Microphotos of spread-out and dried mucus specimens. No fixing, no staining, no mounting. S mucus, parallel arrays of small crystals, 280 X. L mucus, rectangular crystal branching (arborisation), 100 X. P2 mucus, acute angle branches, 280 X. P2M shows P2 mucus with areas of mucolysis, 400 X. P6, hexagonal star of crystals, 350 X. PT shows secretion Pt with triangular formations along linear arrays in a very thin spread-out specimen, 1040 X.

Further studies with microscopy showed that the G mucus always contained varying amounts of cells, usually much more after ovulation (G+) than before (G–). Also, it was found that the L mucus was responsible for the typical 'ferning' pattern, while S mucus showed much more tiny crystals, often long, thin needle-like structures, see Figure 3. Some of these tiny crystals showed, however, a branching, not the 90-degree type of the L mucus, but more acute angles, usually about 60 degrees. Also, this type of crystal showed a cyclic variation different from both L and the needle-shaped S [illegible] most often had a maximum on the days of peak fertility and, therefore, was denoted P (Figure 3). It appeared that the P mucus was present in various subtypes with different crystal symmetry, often a two-fold symmetry (P2) or a six-fold symmetry (P6). All these distinctions became possible just by smearing out cervical mucus gently on a slide in all directions, so that the various mucus types separated from each other. The 'spread-out technique' was used for many years before it was published.[9]

Methods for obtaining cervical mucus

Two principles for harvesting cervical mucus have already been mentioned: the aspiration of mucus from single crypts and removing the bulk cervical mucus and spreading it out on slides. The bulk mucus may be removed using glass tubes or plastic tubes or forceps of various kinds. As described in a recent article, Odeblad and colleagues,[12] a third method makes use of specially designed cotton swabs. Just a few layers of cotton are used, enough to adhere the mucus, but still so small that no appreciable amount of the liquid phase of the mucus is sucked in. A further development of these 'miniswabs' will be described later.

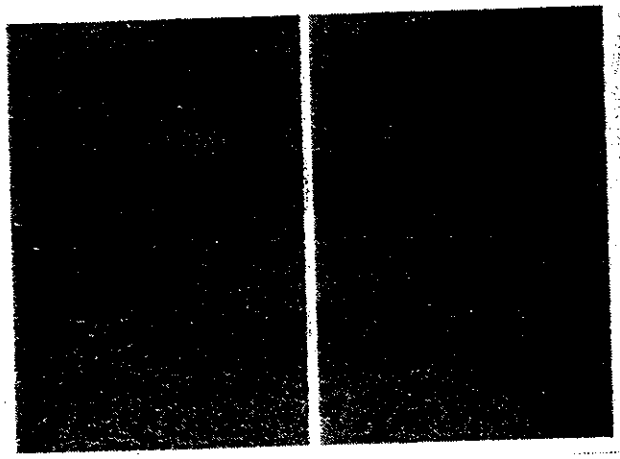

Figure 4: Photos showing granules, both 400 X. Left a droplet of isthmus secretion, 'grain drop', + S and P2 mucus. Right, granules aggregating to form star- or ring-like structures.

Further findings in cervical mucus

Several previously undefined structures in cervical mucus were identified in thinly spread-out mucus specimens. Small granules (Figure 4) about 1 µm in diameter have been identified[8,10] probably emanating from the isthmus glands. The granules probably contain several enzymes, one of them has a mucolytic action. The grains have an affinity for P2 mucus and probably also L mucus, which are slowly degraded by its action. Figure 3 shows the effect of this mucolytic activity. Because of this effect, the P2 mucus has also been denoted Pa ('a' for activity).

Another observation in well-spread-out specimens is the presence of assemblies of nonciliated epithelial cells without presence of leucocytes or lymphocytes. These assemblies are more common in early menarcheal and postmenopausal samples than in women of fertile age. This circumstance sug-

Table 1: Sites of biosynthesis, microscopic characteristics and hormonal stimulation of various mucus types

Type and subtype	Main area of biosynthesis	Presence during cycle	Cells			Granules	Crystals			Stimulated by
			Ep. cells	Leucocytes	Lymphocytes		Rectangular	Linear	Hexagonal	
G G-	Lower cx	1-st infert. phase	+	+	+	+	0	0	0	Low gestagen
G G+	Lower cx	2-nd infert. phase	+++	+++	+++	+	0	0	0	High gestagen
L	All cx	Fertile phase	0	(+)	0	+	+++	0	0	Medium oestrogen
S	Upper $^1/_2$ cx	Last half of fertile phase	0	0	0	+	0	+++	0	High oestrogen Noradrenalin
P P2=Pa	Upper $^1/_2$ cx	First half of fertile phase	(+)	0	(+)	+++	0	0	+++	Incr. oestrogen?
P P6	Upper $^1/_6$ of cx	Peak fert. day	(+)	0	0	+	0	0	+++	Decr. oestrogen? Noradrenalin?
P Pt	Upper $^1/_2$ cx	Peak fert. day	++	0	0	+	0	0	+++	Decr. oestrogen?
F	All cx	Infert. phases	+++	(+)	(+)	0	0	0	0	?
Z	Isthmus Upper cx	All phases	0	0	0	++++	0	0	0	?

gests that they do not have a direct fertility-promoting function. They may be cells of more fundamental importance, such as the stem cells from which G, L, S and P secretory cells develop. These cells have, therefore, been given the notation, F cells.

Finally, another group of cells, apparently producing mucus of type Pt and similar varieties of P mucus, has been observed in extremely thin samples. They will be described later in this paper. See also Table 1 and Figures 3 and 9.

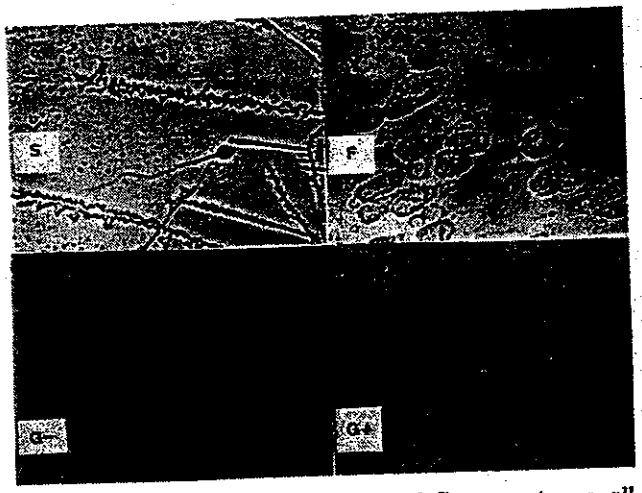

Figure 5: Photos illustrating S, F and G mucus types, all 1000 X S = sperm swimming in mucus. F = F-secretion with cell nuclei which are relatively large and have a loose structure, no leucocytes, no lymphocytes. G- = preovulatory G mucus with a few leucocytes and lymphocytes (round) and a few epithelial cells with elongated nuclei. G+ shows postovulatory G mucus with plenty of leucocytes and lymphocytes, epithelial cells are also present but more difficult to identify.

The integrated function of the various mucus types (Figure 6)

The G mucus is present in the cervical canal in all phases of the menstrual cycle except during the fertile period and menstruation. It is a natural barrier to sperm, and the presence of leucocytes and lymphocytes and gamma globulins suggest that the G mucus is a protective substance. Its high viscosity and glue-like character make it also

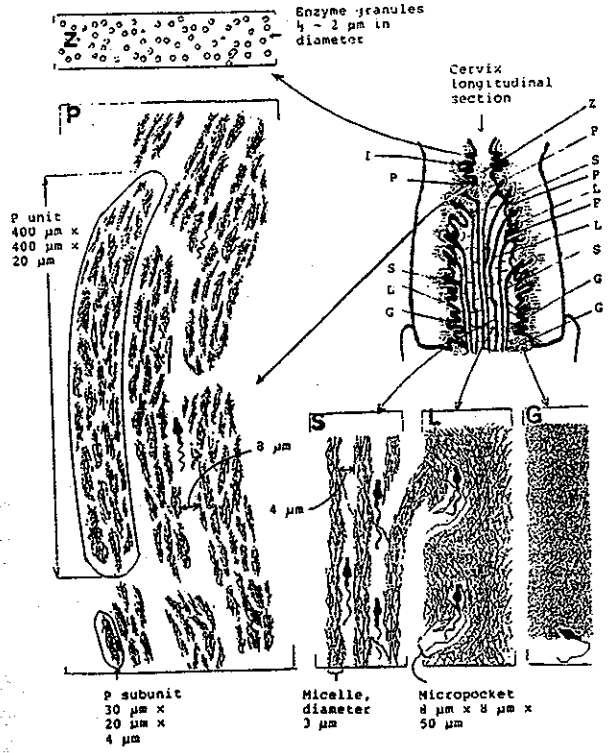

Figure 6: A longitudinal schematic section of the cervix. Close to its left side some important anatomical structures are indicated, I (= isthmus) and crypts for P, S, L and G biosynthesis. On its right side are important secretions indicated, z (from isthmus), P, S, L and G from respective crypts and F secretion from nondifferentiated ('fundamental') cervical mucosa. Simplified gel structures for Z, P, S, L and G secretions are also presented. Note that the gels are three-dimensional and any two-dimensional picture implies a simplification.

a mechanical plug closing the cervical canal, which is also narrowed during the infertile phases by the fibromuscular system in the cervix.

The L mucus has an intermediate viscosity and is secreted during the whole fertile period. An L crypt becomes finally filled with L mucus during the process of secretion, and then this 'pearl' or 'loaf' of the transparent mucus is expelled. Probably 30 or more such units are produced during the fertile period, forming rows of 'pearls'. These arrays of L mucus units form a flexible mechanical

support for the more fluid S mucus which appears 1-3 days later than the L mucus. Another function of the L mucus is to capture sperm which deviate from the S mucus during sperm ascent. When sperm enter into the L mucus, they have difficulty escaping. In this way, the L mucus act as a filter for sperm with irregular or curved swimming trajectories. Certain regions of the 'loafs' seem more apt to perform this capture function. They are called micropockets.

The S mucus is usually secreted in crypts lying close to the L crypts. There is usually a contact between the upper end of a filament of the S mucus and L mucus, while the lower end of the filament is free floating. The filaments, also called micelles, are made up of glycoproteins and/or proteoglycans and are about 0.5 µm thick. Their lengths are approximately logarithmically normal, distributed around a mean of about 0.5 mm at the time of peak fertility. Normally the filaments may be a little branched in their upper portions. Excessive branching seems to reduce fertility. Rapidly swimming sperm appear to propagate between the filaments and may reach an S crypt within 4-15 minutes. There may be a downward flow of about 15 µm/sec. within a 'string' of S mucus. This flow is essential because it gives an upwards orientation of the swimming sperm.[1]

Accordingly, both L and S mucus cooperate to bring about an optimal sperm propagation to a crypt.[11]

It was originally believed that the P mucus occurred only close to ovulation. However, after the characterisation of the various subtypes it became evident that P2 could be present as early as at the beginning of the fertile phase (4-8 days before ovulation, Figure 7). It was also found that P2 often showed absorption of granules and mucolytic activity. Not only did it decay, but it also induced mucolysis in adjacent mucus of other types. Its physiological role may be to liquify the mucus obliterating the S crypts with sperm cells in order to allow their further ascent.

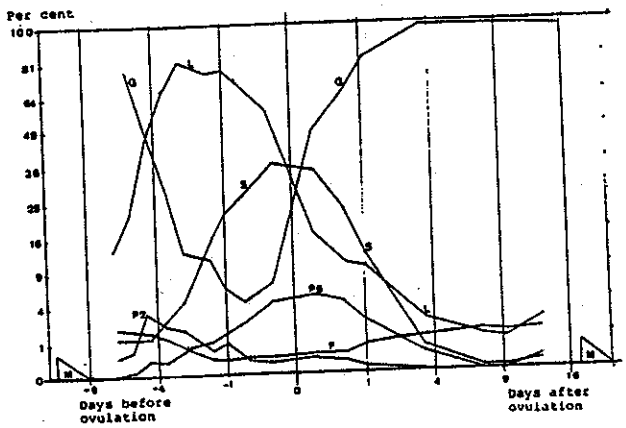

Figure 7: Cyclic variation s of secretions G, L, S, P2, P6 and F in a healthy 24 year-old woman. Note that both coordinates have squared scales in order to more clearly show the periovulatory changes for the scanty secretions P2, P6 and F. Ovulation at day 0. M = menstruation.

The subtype P6 is mostly confined to the uppermost part of the cervix and probably conveys the sperm in the chinks between the 'plates' of mucus, each such 'plate' appearing as a hexagonal star in the spread-out and dried mucus specimens. P6 is present mainly at and shortly after ovulation.

Pt is one member of a family of P subtypes (Figure 3) visible in very thin mucus specimens. This family embraces at least four members with distinctly different crystallisation patterns. We do not know, presently, if they have different physiological functions. Pt will be discussed later in this paper.

In regularly spread-out mucus specimens, P2 and P6 are the most common varieties of P mucus. However, there were also members with other mirror symmetries. We are uncertain about their possible physiological importance, perhaps they do not have any special significance.

Sperm migration in the cervix

Sperm invade the S mucus strings at the external os and propagate along the strings. A few of them may find the way directly to the uterine cavity, but the majority of sperm is conveyed to the S crypts. In the S crypts they seem to reduce or loose their movements, and they may 'hibernate' for a few hours to some days. The average time is about 20 hours. When the sperm come into the crypt, the S mucus secretion rate seems to diminish or disappear. Some women who want to conceive have even noted a temporary decrease of the slippery secretion during the day after intercourse. The mechanism(s) which lead to the sperm immobilisation and inhibition of secretion are not known, but some kind of neural factor can be expected. After a day or so, spermatozoa are released from the crypt and begin to propagate and find their way to the uterine cavity between the disk-shaped units of P6 mucus.

The BRAMS principle

This is an important principle which seems to regulate, in general, the behaviour of all mucus types of the cervix. The word 'BRAMS' stands for the following:

B = biosynthesis of mucus in the epithelial cells

R = release of mucus from the cell
There is evidence (e.g., in the S cells) that oestrogens stimulate the biosynthesis and noradrenalin stimulates the release.

A = activity or activation (e.g., biological activity such as sperm conduction or polymerisation of mucin molecules to mucus substance).

M = mucolysis (degradation, depolymerisation), partial or complete, with or without the cooperation of mucolytic enzyme.

S = sensation or mucus and/or its degradation products, the perception usually occurs at the vulva.

The BRAMS principle stresses the importance of a balance between mucus production and degradation, a kind of homeostasis. The BRAMS principle

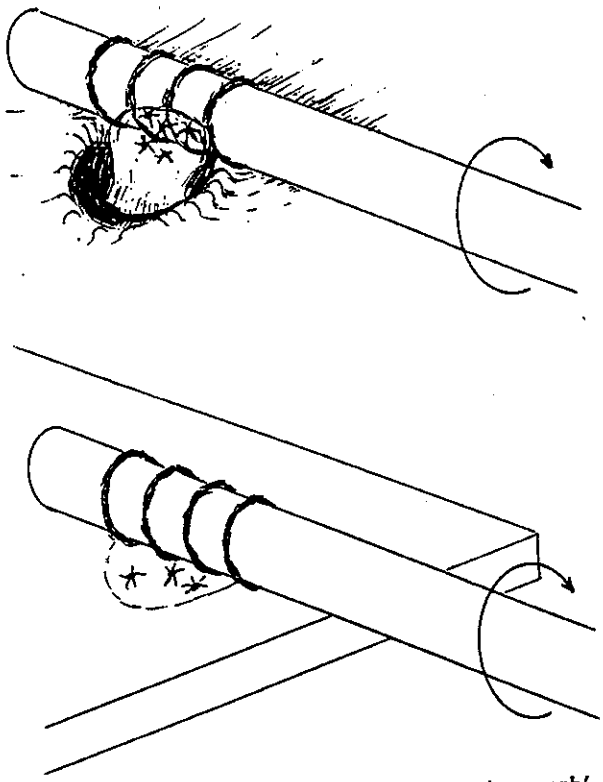

Figure 8: The design and use of a 'cotton-ring swab'. It is shown (top) how it is used to remove Pt mucus from a cervical crypt and (bottom) how the mucus is rolled on a slide. The cotton rings implies that the cells are not crushed between the wooden stick and the glass.

aids in thinking in a rational way when analysing cases of cervical functional pathology and the symptoms experienced by the patient.

Apply the BRAMS principle to normal L mucus: it is biosyntisised in the L crypt, it polymerises and is released and forms the 'loafs' or 'pearls' which help to detach low-quality sperm from the main stream of swimming sperm in the S mucus. It begins to depolymerise when passing the vagina and gives the typical wet sticky sensation at the vulva.

The Pt secretion

The Pt secretion can most easily be identified in very thin specimens of spread-out bulk mucus. It is characterised by a remarkable pattern in the dried (not fixed, not stained) specimens (Figure 3). It requires high magnification to be clearly observed, and it seems to permit interesting possibilities for studies on the secretion mechanisms. First observed some years ago, efforts have been made to recover Pt secretion in a more reliable way; a method using 'cotton ring swabs' has been developed. The sampling is performed as follows. Most of the bulk mucus is removed from the cervical canal by suction (and spread-out for common microscopic inspection). A cotton ring swab is now introduced. This swab has 3-4 thin rings of cotton fibres (Figure 8) instead of a lump of cotton. The swab is rolled on the endocervical surface, and some material (including Pt mucus) adheres on the rings. The wooden stick with the cotton rings is now transferred to a slide, and the material is rolled on the slide. The spaces between the rings are large enough so that the fragile epithelial cells are not crushed but remain together with their newly-formed secretion (Figure 9). The epithelial cells are those normally shed from the endocervical mucosa (including the crypts). We do not yet know if these cells are 'normal', but we can obtain cells, apparently in different secretory phases, for study.

One might say that we have several steps of the BRAMS principle illustrated for Pt secretion:

Figure 9: Microphotos of Pt mucus on a slide sampled according to Figure 8. The mucus is not fixed, not stained and not mounted, just dried. A cell, presumably non-active (N) is shown. Cell B contains large vacuoles, and is presumably in a state of biosynthesis. R shows a cell presumably in a state of release of Pt secretion. M shows Pt mucus apparently being fragmented i.e. undergoing mucolysis. The pictures are unsharp for two reasons, (1) the samples have an irregular thickness which prevents good focusing on all details and (2) the limited resolution of the optical microscope at this magnification (2400 X).

Biosynthesis, Figure 9 B
Release, Figure 9 R
Action or activity (possibly) Figure 3 PT
Mucolysis, Figure 9 M

The cells will later be studied with electron microscopy. Also, because we can obtain cells in various stages of secretion, it might be possible to retract the pertinent mRNA for various steps in the biosynthesis and release. Indications exist that the Pt cells might play a role in immunological processes in the cervix, something which remains to be investigated.

References

[1] Höglund, A., Odeblad, E. Sperm penetration in cervical mucus: a biophysical and group-theoretical approach. In: Insler V, Bettendorf, G. eds. The Uterine Cervix in Reproduction, Workshop conference in Rottach-Egern 1977: 129-134.

[2] Melén, B., Odeblad, E. Pleuropneumonia-like organisms in the genito-urinary tract of healthy women. *Acta Derm.-Venerol Scand* 1951; 30: 74-76.

[3] Odeblad, E. The physics of the cervical mucus. *Acta Obstet Gynecol Scand* 1959; 38 (Suppl 1): 44-58.

[4] Odeblad, E. Discussion to the preceding paper. *Acta Obstet Gynecol Scand* 1959; 38 (Suppl 1): 126-127.

[5] Odeblad, E. Micro-NMR in high permanent magnetic fields. *Acta Obstet Gynecol Scand* 1966; 45: Suppl. 2.

[6] Odeblad, E. Biophysical investigations in oral contraception. *Acta Obstet Gynecol Scand* 1968; 47 (Suppl. 8): 7-19.

[7] Odeblad, E. Physical properties of cervical mucus. In: Elstein, M., Parke, D.V. eds. Mucus in health and disease. *Adv. Exp Med and Biol* 1977; 89: 217-225.

[8] Odeblad, E. The discovery of different types of cervical mucus and the Billings ovulation method. *Bull Nat Fam Plann Council Victoria* 1994; 21, iii: 3-35(a).

[9] Odeblad, E. The spread-out technique, advantages, pitfalls and interpretation. In: Rutllant, M. ed. IV Symp. Int sobre Regulación Natural de la Fertilidad. *Colegio Oficial de Medicos Barcelona* 1994; 295-303.

[10] Odeblad, E., Menarguez, M. Cell and molecular biology of the cervix. In: Rutlland, M. ed. IV Symp Int sobre Regulación Natural de la Fertilidad. *Colegio Oficial de Medicos Barcelona* 1994; 121-129.

[11] Odeblad, E., Ingelman-Sundberg, A., Hallström, L., *et al.* The biophysical properties of the cervical-vaginal secretions. *Int Rev. Nat Fam Plan* 1983; 7: 1-56.

[12] Odeblad, E., Menarguez, M., Slettvoll, J., Temprano, H. How to study the cervical secretory system and its role in Natural Family Planning. *Bull Ovul. Method Res and Ref Centre Austalia* 1996; 23, ii: 3-20.

[13] Séguy, J., Simmonet, H. Recherche de signes directs d'ovulation chez la femme. *Gynécol et Obstet* 1993; 28: 657-663.

[14] Séguy, J., Vimeux, J. Contribution à l'étude des sterilités in-explicées. 1933; 27: 346-358.

[15] Sjövall, A. Untersuchungen überdie Schleimhaut der Cervix Uteri. *Acta Obstet Gynecol Scand* 1938; 18: Suppl. 4.

[16] Wollner, A. A preliminary study of the cyclic histologic changes in the human cervical mucosa in the intermenstrual period. *Am J Obstet Gynecol* 1936; 32: 365-374.

[17] Wollner, A. The physiology of the human endocervical mucosa. *Surg Gynecol Obstet* 1937; 64: 758-764.

[18] Wollner, A. The histologic correlationship of endometrial and cervical biopsies. *Am J Obstet Gynecol* 1938; 36: 10-21.